EVALUATION OF BIOACTIVE COMPOUNDS FROM SOME MEDICINAL PLANTS AND ITS ACTIVITY IN *ALLIUM CEPA* WITH SPECIAL REFERENCE TO GENOTOXICITY AND ANTIMUTAGENICITY

THESIS

SUBMITTED TO THE
SANT GADGE BABA AMRAVATI UNIVERSITY, AMRAVATI
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE FACULTY OF SCIENCE (BOTANY)

BY

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Certificate

This is to certify that the thesis entitled **"Evaluation of Bioactive Compounds from Some Medicinal Plants and Its Activity in *Allium cepa* with Special Reference to Genotoxicity and Antimutagenicity"** submitted for the award of degree of **DOCTOR OF PHILOSOPHY IN BOTANY** in the faculty of science to **Sant Gadge Baba Amravati university, Amravati** embodies the bonafide research work carried out by **MISS. SARIKA SUDAM KHANDARE** under my guidance and supervision. No part of the Thesis has been submitted for any degree. All the assistance and help availed of during the course of this investigation and source of literature have been duly acknowledged.

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Declaration

I hereby declare that the work presented in this thesis entitled, **"Evaluation of Bioactive Compounds from Some Medicinal Plants and Its Activity in *Allium cepa* with Special Reference to Genotoxicity and Antimutagenicity"** has not been submitted earlier for the award of Degree of Diploma to any other university.

The present work is completely original and has been carried out at Cytology and Genetics Laboratory, Post-Graduate Department of Botany, Govt. Vidarbha Institute of Science and Humanities, Amravati.

December 31st, 2012

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Date:

Ms. Sarika S. Khandare

DEDICATED
TO

MY BELOVED PARENTS &

MY RESPECTED GUIDE

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List of Abbreviations

%	- Percentage
&	- and
α–BHC	- Alpha hexachloro cyclohexane
µL/ml	- Microliter per millilitre
^{0}C	- Degree Celsius
A	- Anaphase
ADA	- Adenosinedeaminase
AICAR	- Aminoimidazolecarboxamidoribonucleotide
AICAside	- Aminoimidazolecarboxamidoribonucleoside
AK	- Adenosine kinase
AMI	- Active Mitotic Index
AMPDA	- AMP deaminase
AT	- Arrested Telophase
CA	- Chromosome aberration
CB	- Chromatid Bridges
CM	- Clumped Metaphases
Conc.	- Concentration
CPK	- Cell proliferation kinetics
CRL 1675	- Melonocytes
DDT	- Dichlorodiphenyltrichloroethane
DHF	- Dihydrofolate
DHFglu	- Dihydrofolatepolyglutamate
DHFR	- Dihydrofolatereductase
DMBA	- 9, 10-dimethyl-1, 2-benzanthracene

DNA	- Deoxyribo Nucleic Acid
ecto-5′NT	- ecto-5′nucleotidase
EM	- Electromagnetic
EMS	- Ethyl methanesulfonate
FAICAR	- Formyl- AICAR
$FeCl_3$	- Ferric chloride
FH_2	- Dihydrofolate
FH_4	- Tetrahydrofolate
FTIR	- Fourier Transform Infrared Spectroscopy
g/L	- gram per litre
HaCaT	- Keratinocytes
HCl	- Hydrochloric acid
$HgCl_2$	- Mercury chloride
HPLC	- High Performance Liquid Chromatography
HPTLC	- High Performance Thin Layer Chromatography
hrs	- Hours
IMP	- Inosine monophosphate
IR	- Infrared
LAE	- Leaf acetone extracts
LDE	- Leaf aqueous extracts
LPE	- Leaf petroleum ether extracts
M	- Metaphase
Mi	- Mitosis
mg/kg	- Milligram per kilogram
mg/L	- Milligram per litre
mg/plate	- Milligram per plate

MI - Mitotic index

MMS - Methyl Methanesulfonate

MNNG - N-methyl-N'-nitro-N-nitrosoguanidine

MTX - Methotrexate

MTXglu - Methotrexate polyglutamate

n_1 - first sample

n_2 - second sample

nm - nanometer

no. - Number

OC - Organic carbon

P - Prophase

ppb - Part per billion

ppm - Part per million

PT - Precocious chromosome at Telophase

RAE - Root acetone extracts

RDE - Root aqueous extracts

R_f - Relative frequency

RFC1 - Reduced folate carrier 1

RNA - Ribonucleic Acid

RPE - Root petroleum ether extracts

S.D. - Standard deviation

S.E. - Standard error

SA - Sodium azide

SAM - S-adenosylmethionine

SCE - Sister Chromatid Exchange

T - Telophase

THF - Tetrahydrofolate

THP-1+A23187 - Dendritic cells

TLC - Thin layer chromotagraphy

Trad MCN - *Tradescantia* pollen mother cells

UDS - Unscheduled DNA synthesis

UF - Unsaponifiable fraction

ug/ml - Microgram per millilitre

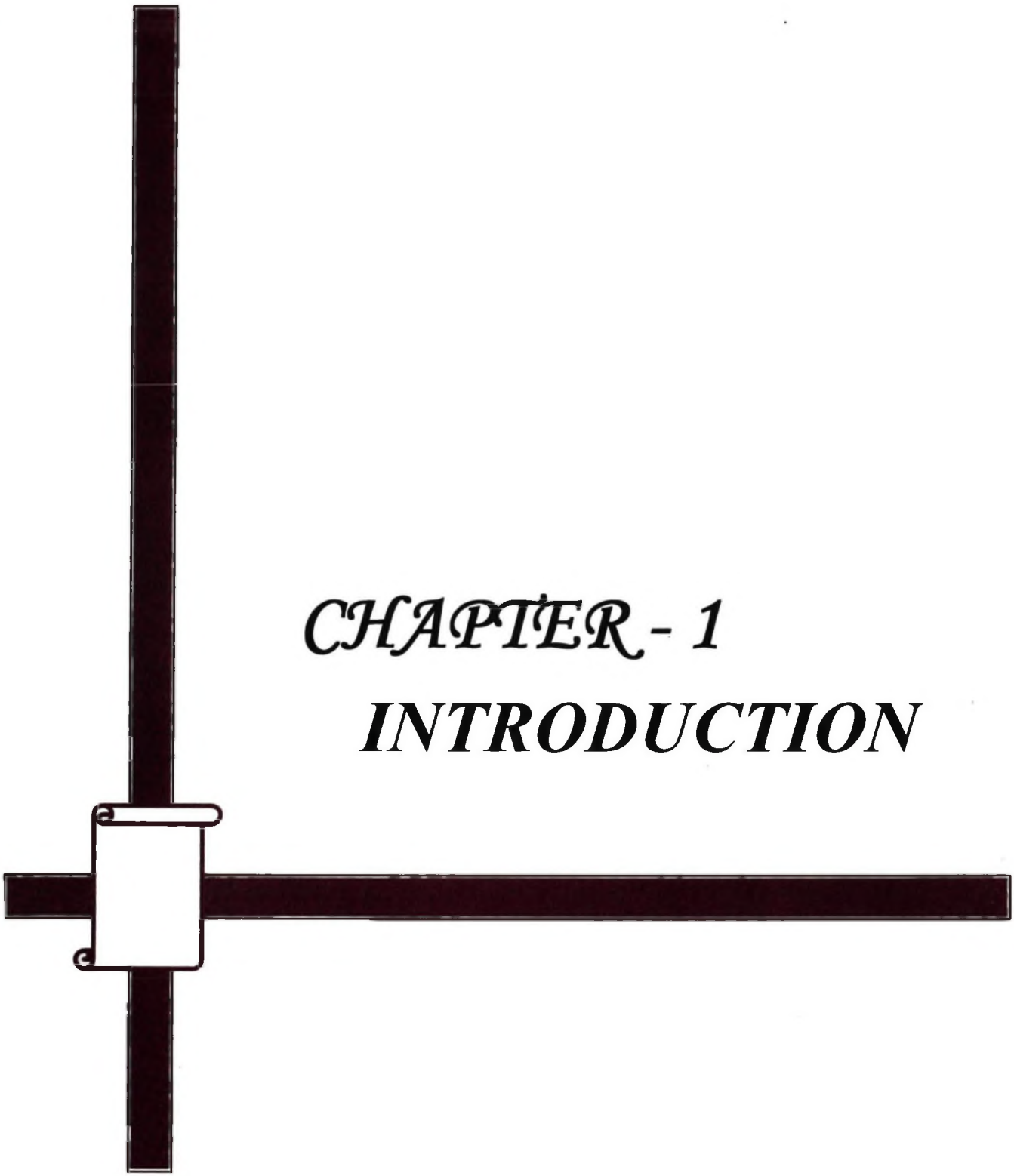
UV-Vis - Ultraviolet and visible absorption spectroscopy

X_i - No. of observation of first sample

$\overline{X}$ - Arithmetic mean of first sample

Y_j - No. of observations of second sample

$\overline{Y}$ - Arithmetic mean of second sample



CHAPTER - 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Natural products, especially plants, have been used for the treatment of various diseases for thousands of years. Herbal medicines are being used by about 80% of the world population, primarily in developing countries for routine health care and are also entering the therapeutic in the developed countries (Kamboj, 2000). The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history, but has increased substantially in the last decades (Woods, 1999 and WHO, 2002). The popularity of herbal medicines is connected with their easy access, therapeutic efficacy, relatively low cost and assumed absence of toxic side effects. Widespread public opinion is that being natural products, herbal medicines are harmless and free from adverse effects and it is believed that even if the expected medical effects are not achieved, their consumption is not dangerous (Stewart et al., 1999; Ernst, 2002 and Veiga-Junior et al., 2005).

Natural plant based chemicals; crude compounds are gaining importance in medicine and pharmaceutical industry. Such plant based compounds are being used all over the world. Secondly, these compounds are considered as safer to use and ecofriendly in nature. Physiological actions of these crude compounds are multidirectional in nature but its action is still not clear. Some compounds act as cytotoxic, genotoxic, mutagenic and anticarcinogenic there by affecting plants and animals. Many of the antibiotics and anticancer drugs of the future will come from soil and plants around us. The plants were found to contain chemicals with strong anticancerous activity. These chemicals were then extracted, isolated and there chemical composition structures were determined.

1.1. Genotoxicity:

Genotoxicity of chemical agents is an intrinsic chemical character based on the agent's electrophilic potential to bind with such nucleophilic sites in the cellular macromolecules such as deoxyribonucleic acid (DNA); it is the carrier of hereditary information. Genotoxicity is thus toxicity manifested in the genetic material of cells (Encyclopedia, 2007) includes both direct and indirect effects in DNA.

1) The induction of mutations (Genechromosomal, Genomial, Recombinational) that at the molecular level are similar to events known to be involved in carcinogenesis.
2) Indirect surrogate events associated with mutagenesis e.g. unscheduled DNA synthesis (UDS) and sister chromatid exchange (SCE) or DNA damage (e.g. the formation of adducts) which may eventually lead to mutations.

Genotoxicity is the effect of various types of toxic substances on cell and its organization. Most of them are complex organic substances knowingly or unknowingly they enter in the living system and affect the body functions. Intensity of their hazardous effect depend on their degree of concentration, higher the concentration more will be the harmful effect of the chemical when a foreign chemicals comes in contact with cells, first it may attach to the outer membrane or otherwise enter in the cytoplasm and finally may get entry into the nucleus. The prominent hazardous effect on living system are observed to be,

a) Change in form and size of the cell in general and nuclei in particular.
b) Cracking of nuclei.
c) Vacuolization of the interphasic nucleus.
d) Retardation in cell division that hinders the growth.

e) Despirallizing the genetic material there by showing effect on stainability of the chromosome.

f) Heavy disturbances on normal activities of the cell organells and

g) Decrease in rate of metabolic activities.

Most significant part of genotoxicity is mutagenicity. Mutagenesis refer to different types of physical and chemical agents, study of their mutagenic potential types of mutations induced by them and lastly their impact on environment in general and living organisms. In nature mutation whether chromosomal or genic, constitutes on the primary sources of variation on which the natural selection operates. Whereas, induced mutation by which the direct hit of the gene or through DNA repair mechanism. Large numbers of environmental chemicals are wide spread in nature. They are food additives, pesticides, insecticides, industrial pollutants and vast array of chemical compounds used as drugs in medicines. All this contributes to the increasing frequency of mutations in all types of organisms. Well-defined changes that have been brought about by chemical mutagens are chromosomal mutations. They can be recorded in mitosis and meiosis. Mutagenic chemicals appear to exert by forming adducts with DNA. Their potential as mutagen will be detected only by mutagenic assay that show the induction of chromosomal aberrations at mitosis. The mechanisms whereby these changes are brought about are unknown but it postulated that they may be due to free radicals and chemicals that interfere with the integrity, function or configuration of chromatid or DNA.

Mutations are the cause of innate metabolic defects in cellular system, triggering the mortality in living organisms. Mutagens are involved in the initiation and promotion of several human diseases including cancer, the significance of novel bioactive phytocompounds in counteracting the promutagenic and carcinogenic effects are

gaining credence. Such chemicals that reduce the mutagenicity of physical and chemical mutagens are called as antimutagens. The antimutagens have been first reported almost four decades ago and since then numerous studies have been carried out in order to identify compounds, which might protect humans against DNA-damage and its consequences.

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. There are several ways in which the action of mutagen metabolism can be effective antimutagens (Ferguson, 1994). Natural antimutagens from edible and medicinal plants are of particular importance because they may be useful for human cancer prevention and have no undesirable xenobiotic effect on living organisms.

1.2. Genotoxic Substance:

Genotoxic substances are a type of carcinogen, specifically those capable of causing genetic mutation and contributing to the development of tumors. This includes both chemical compounds and certain types of radiations. Typical genotoxins like aromatic amines are believed to cause mutations because they are electrophilic and form strong covalent bonds with DNA, preventing accurate replication. Genotoxic agents which are capable of inducing chromosome structural changes or chromosome mutation is direct measure of genetic damage. Pesticides cause several other cytotoxic effects such as stickiness, spindle abnormalities, laggard etc. It has been recognized that genotoxic effects are not necessarily affecting DNA directly result in chromosomal segregation errors and therefore should be considered genetically significant (Grover et al., 1985). The time of exposure must be considered carefully since the activity of many chemicals is influenced by the stage of cell division cycle.

1.3. Unscheduled DNA Synthesis (UDS):

Mutagenic chemicals appear to exert their effect by forming adducts with DNA. Their potential as mutagens will be detected only by mutagenesis assays that show chromosomal aberration of mitosis. Chromosomal numerical changes (aneuploidy), change in chromosome morphology (sister chromatid exchange, SCE) and point mutations. The mechanisms whereby these changes are brought about are unknown free radicals and chemicals that can be interfere with the integrity, function and configuration of chromatin by physical effects. This damage to DNA is recognized and repaired by group of enzymes that are concerned with the replication repair and normal functioning of the genome.

Toxic substances are those chemicals that cause any of a wide number of adverse effects in living organisms. Toxicants exert their effect at the cellular levels in three ways:

1) They can affect enzymes, the cellular proteins that regulate many important chemical reactions. A disturbance of enzymatic activity can seriously alter the functioning of an organ of tissue e.g. Mercury and Arsenic both bind to certain enzymes, blocking their activity.
2) Toxins may bind directly to cells or molecules within the cells thereby upsetting the chemical balance within the body e.g. carbon monoxide binds to the hemoglobin in the blood, interferes within the transport of oxygen and can led to death if levels are high enough.
3) Toxin may cause the release of other naturally occurring substances that have an adverse effect on cellular system e.g. toxin carbon tetrachloride stimulates certain nerve cells to release large quantities of adrenaline believed to cause liver damage.

Although the human body is extremely complex, its relationship to a genotoxin can be described in fairly simple terms. In any living organism, the smallest unit capable of independent existence is the cell and each cell has specific functions that it must maintain to sustain life.

In the present study all these seven plants were selected on the basis of preliminary characteristics features like medicinal, therapeutic importance and easy accessibility. These plants also may have cytotoxic, genotoxic or anticarcinogenic potential so, the study especially pointed towards the effects of different extracts (petroleum ether, aqueous and acetone) of these plant parts (leaf and root) on gene, chromatin and DNA level using *Allium cepa* as a test system.

1.4. *Boerhavia diffusa* Linn. (Syn. *B. repens* Linn.):

A genus of herb belonging to Nyctaginaceae family distributed throughout the tropical and subtropical regions and grows as common weed. It is diffusely branched and creeping perennial herb. Roots are fusiform, stout and woody. Stems are 60-90 cm long, slender, prostrate, swollen at nodes and minutely hairy. The leaves are opposite, ovate, oblong, green, glabrous, sub undulate margins and long petiole. Flowers are pink, small, borne in small umbels which are arranged axillary and terminal panicles.

According to Ayurveda *Boerhavia* is bitter, anemia, cooling heat diseases, astringent to bowels useful in biliousness, blood impurities, leucorrhoea, asthma alternatives etc. The leaves are useful in dyspepsia, tumors, abdominal pains and spleen enlargement. According to Unani system of medicine the leaves are appetizers, alexiteric useful in ophthalmic and in joint pains. Seeds are tonic expectorant, carminative useful in lumbago, scabies. The seeds are considered as promising blood purifier. Roots are used to treat gonorrhea, all internal inflammation and edema. Roots stimulate the employing of the gall bladder, as a diuretic for all types of liver disorders

(including jaundice and hepatitis), gall bladder pain, stones, urinary tract disorders, renal disorders, kidney stones, cystitis and nephritis. The decoction of the root is taken as a remedy in rheumatism, impure blood, cough, asthma, hernia, dropsy, chest-pain, piles and swellings (The wealth of India, 2004).

1.5. *Withania somnifera* Dunal:

A genus is a small, woody shrub in the Solananceae family found throughout the drier parts of India in waste places and on bunds. It is an erect and much branched perennial undershrub is an erect, evergreen and tomentose shrub. Roots are stout fleshy, whitish brown; leaves simple, ovate, glabrous and opposite; flowers inconspicuous, greenish or lubrid-yellow, in axillary, umbellate cymes; berries small, globose and orange-red when mature.

Withania somnifera Dunal, also Known as Ashwagandha, it has been an important herb in the Ayurvedic and Indigenous medical systems for over 3000 years. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia. Clinical trials and animal research support the use of *Withania somnifera* for anxiety, cognitive, neurological disorders, inflammation, hyper lipidemia and Parkinson's disease. Its chemo preventive properties make it a potentially useful adjunct for patients undergoing radiation and chemotherapy (The wealth of India, 2003).

1.6. *Calotropis procera* (Ait) Ait.f.:

A genus of tomentose shrubs 2 - 4m in height distributed throughout the tropical and subtropical regions. An erect, perennial shrub commonly found in way sides and waste places throughout the India (Kirthikar and Basu, 1933 and The Wealth of India, 1959). This plant is soft - wooded, evergreen and shrub. It has one or few stems, few

branches and relatively few leaves. It has a very deep, stout taproot with lateral roots. The leaves are opposite, oblong and acute. Inflorescence is covered with woolly tomentum. The flowers are umbelliform cyme, purplish-red and silvery outside with odor.

C. procera (Ait.) Ait. F. is a useful indigenous medicinal plant. The latex is used as wound healing agent. It is also used as an abortificient in folk medicines. Calotropin isolated from latex and roots of *C. procera* inhibit spermatogenesis in male and induce abortion in female rats and rabbits. It is also used as a remedy for black scars of face, boils, cold cough, asthma, ear ache, eczema, skin eruptions and inflammatory lesions, pains of the body, rheumatism, syphilis, leprosy and oedema. The leaves are roasted and are applied to painful joints or swelling (The wealth of India, 2004).

1.7. *Asclepias curassavica* Linn.:

A. curassavica is belonging to the family Asclepiadaceae. An erect, glabrous, perennial sometimes annual herb, 30 to 120 cm tall with a milky sap distributed throughout the tropical and subtropical regions is grows as roadside weed. It is an erect and simple or branched perennial herb about 80-120 cm high, stem smooth, round, dull green or suffused with dull red; leaves are opposite, lanceolate to oblong-lanceolate, acute at base and acuminate at apex. Flowers are bright red or orange with yellow centers, numerous and borne in umbelliform cymes. Corolla is reflexed, 5- petals, erect and spoon shaped.

The plant is used medicinally in the tropics for the anodyne properties of its roots. Extract of the root is used as an emetic and laxative. Other uses employed are against warts, fever, vomiting and as an expectorant. A decoction of the plants is used as an abortifacient. The roots are known as pleurisy root and used as an expectorant. It is used in abdominal tumors and in tuberculosis (The wealth of India, 2003).

1.8. *Vitex negundo* Linn.:

A genus belonging to the family Verbenaceae, distribution naturally found along coastlines. This is a shrub or small tree growing from 1 to 4 meters in height. It is some times prostate or ascending in habit, covered by soft hairs (tomentose). Stems quadrangular, whitish tomentose. Leaves are petiolate, leaflet variable in size, terminal leaflet 3-10× 1.5-3 cm, acute. Flowers are blue or white, in terminal cymose panicles. Drupes ovoid, 4-seeded, black and Seeds were oblong (The wealth of India, 2003).

The leaves are used to treat female ailments, to relieve fever and dried leaves are burned to control mosquitoes. Powdered leaves are also used as a febrifuge. *Vitex negundo* L. is prescribed in headache, catarrh and watery eyes and are said to promote the growth of the beard. Cancer of the breast is also treated with it. The leaves are vermifuge, aromatic, tonic (Chopra et al., 1956), alternative anodyne, antiparasitic, discutient, anti-inflammatory and antirheumatic (Chatterjee and Pakrashi, 1995).

1.9. *Hyptis suaveolens* (Linn.) Poit.:

A genus is belonging to the Labiatae, distributed throughout the tropical and sub-tropical regions. It grows as common weed on road side and overgrazed areas around cattle yards. It is resistant to fire. Herbs, sub shrubs or shrubs. It is an erect and strongly aromatic annual or perennial herb with densely patent hairy stem. Leaves are ovate-lanceolate with a rounded-cordate base, obtuse or acute, crenate- serrate to dentate and thinly short above and densely hairy on lower surface. Flowers are borne in 1-5-flowered verticillasters. Nutlets are oblong and glabrous.

Decoction of leaves is used to cleanse wounds. The plant is used in the antilles as a stimulant. The plant is considered stimulant, carminative, sundorific and lactagogue (Agrawal, 1986) and it is pounded and applied to parasitical cutaneous diseases.

1.10. *Antirrhinum majus* Linn.:

The genus belonging to family Scrophulariaceae, perennial herb distributed throughout tropical and subtropical regions. Leaves are spirally arranged, broadly lonceolate, flowers are produced on a tall spike, zygomorphic, with two lips closing the corolla tube, pink to purple flowers often with yellow lips. The fruit is an avoid capsule (Blamey and Grey-Wilson, 1989).

The leaves and flowers are anti-phlogistic, bitter, resolvent and stimulant (Clapham et al., 1962). They have been employed in poultices on tumors and ulcers (Grieve, 1984). It is effective in the treatment of all kinds of inflammation and is also used on hemorrhoids.

1.11. Methotrexate (MTX):

Methotrexate is formerly known as amethopterin, is an antimetabolite and antifolat drug used in treatment of cancer and autoimmune diseases. Methotrexate (MTX) is a commonly used chemotherapeutic agent but like most of these agents, it has a nonselective toxicity and a high occurrence of drug resistance. It acts by inhibiting the metabolism of folic acid. MTX is a cell cycle phase specific drug, whereby prolonged exposure of the drug to the cancer cells is necessary for optimum therapeutic effect. Methotrexate was originally used as part of combination chemotherapy regimens to treat many kinds of cancers. Treatment of many neoplastic disorders which including acute lymphoblastic leukemia, lymphocytic leukemia and some autoimmune diseases, including ankylosing, spondylitis.

1.12. *Allium* test system:

Allium test system has advantages over other test system because of the following reasons:

1. Germination by bulb is very simple.

2. Somatic chromosome number is very limited (2n = 16).
3. Chromosomes are medium to long in absolute length.
4. Genome is sensitive to toxic chemicals.
5. Root tips radially treated with aqueous solution provide large cell area.
6. Treatment periods are short.
7. Fixation and squashing are usually made within 48 hrs.
8. Chromosomes are get stained intensively by aceto-carmine or aceto-orecin.

The test system used was *Allium cepa* 2n = 16. It is one of the reliable model for screening drugs, chemical pollutants and contaminants because root growth inhibition and adverse effects on chromosomes (Fiskejo, 1988). Secondly it is the excellent indicator of cytotoxic and antimutagenic effect to study the different chemicals. It has well known relatively simple, quick, inexpensive and easy protocol. Therefore, the effect of leaf and root extracts of *Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens* and *Antirrhinum majus* on genotoxicity and antimutagenecity was investigated. In this study aqueous, acetone and petroleum ether extracts were used to investigate the differential response of these compounds to metabolic / nuclear state.

1.13. Ultraviolet and visible absorption spectroscopy:

UV-Vis is the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from a sample surface. Absorption measurements can be at a single wavelength or over an extended spectral range. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels and UV-Vis spectroscopy is usually applied to molecules or inorganic complexes in solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of

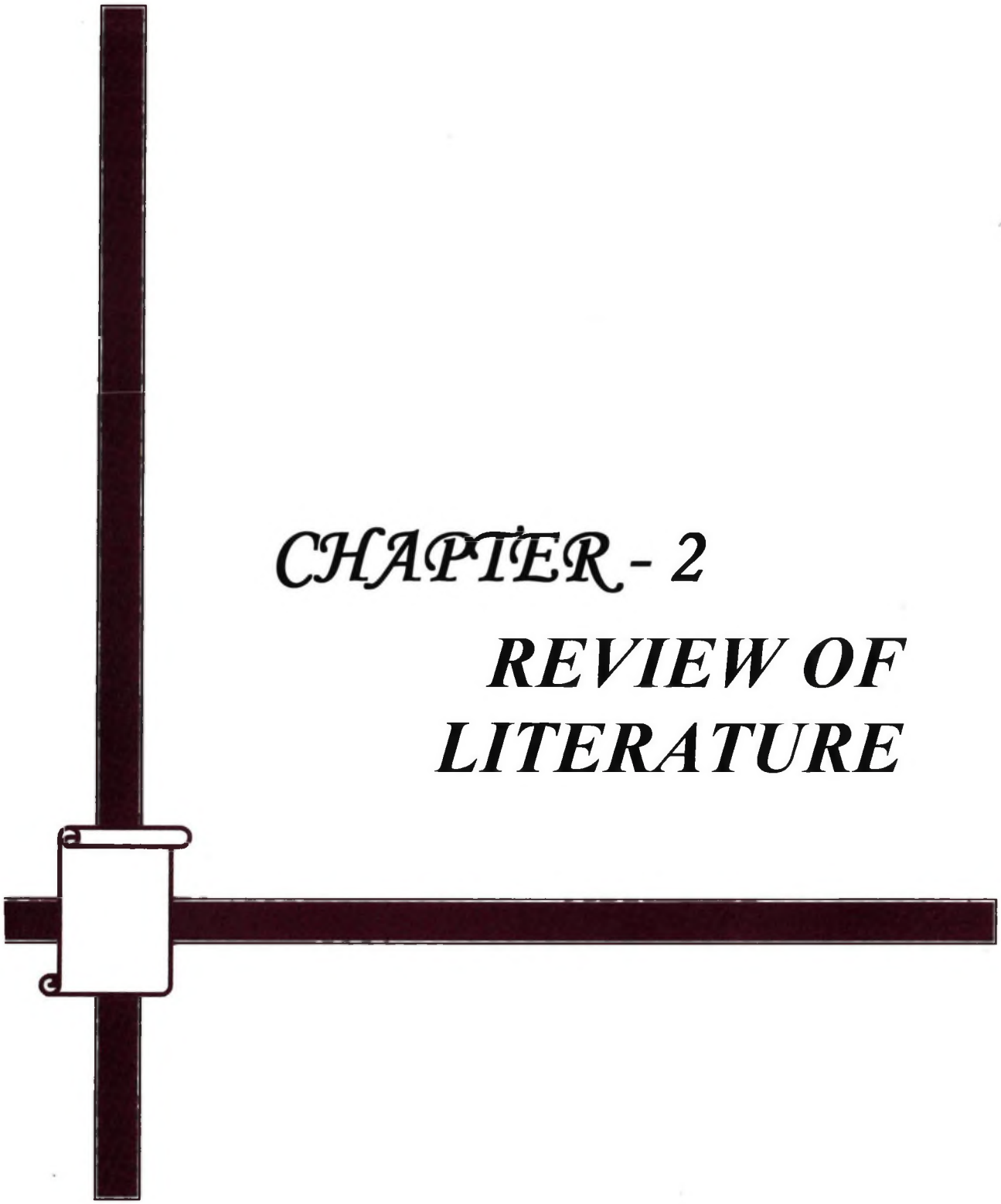
an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert law. Since the UV-Vis range spans the range of human visual acuity of approximately 190 - 1100nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission and reflectivity of a variety of technologically important materials, such as pigments, coatings, windows and filters. This more qualitative application usually requires recording at least a portion of the UV-Vis spectrum for characterization of the optical or electronic properties of materials. Hence, these crude compounds can be measured at precise wavelengths by UV-Vis spectrophotometer for quantitative measurement.

1.14. Fourier transform infrared spectrophotometer:

FTIR is most useful for identifying chemicals that are either organic or inorganic. It can be utilized to quantitate some components of an unknown mixture. It can be applied to the analysis of solids, liquids and gasses. The term Fourier Transform Infrared Spectroscopy (FTIR) refers to a fairly recent development in the manner in which the data is collected and converted from an interference pattern to a spectrum. An invaluable tool in organic structure determination and verification involves the class of electromagnetic (EM) radiation with frequencies between 4000 and 400 cm^{-1} (wavenumbers). The category of EM radiation is termed infrared (IR) radiation and its application to organic chemistry known as IR spectroscopy. Radiation in this region can be utilized in organic structure determination by making use of the fact that it is absorbed by interatomic bonds in organic compounds. Chemical bonds in different environments will absorb varying intensities and at varying frequencies. Thus IR spectroscopy involves collecting absorption information and analyzing it in the form of a spectrum. The frequencies at which there are absorptions of IR radiation ("peaks" or "signals") can be correlated directly to bonds within the compound.

The chemical structures of the isolated compounds were established by spectroscopic techniques such as FTIR in analytical chemistry used for determining action of group of compounds of a sample and its structure. It is often used to identify drugs, contaminants and adulterants. It is also used to resolve plant extract and many other biochemical preparations.

Hence, the present study attempt and investigation were carried out to evaluate the genotoxic and carcinogenic/anticarcinogenic effect of these isolated crude compounds extracted from leaf and root of these seven plants using *Allium cepa* and the chemical structures of the isolated compounds were established by spectroscopic techniques such as UV-Vis spectrophotometer and FTIR.



CHAPTER - 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1. *Boerhavia diffusa* Linn. (Syn. *B. repens* Linn.):

2.1.1. Chemical constituants:

A genus of herb belonging to Nyctaginaceae family, distributed throughout the tropical and subtropical regions. Roots contains 2-glucopyrano-4-hydroxy -5-(p-hydroxyphenyl) -propionyldiphen methane (Jain and Khanna, 1989). The main constituants in the plants include, hentriacontane, β-sitosterol, ursolic acid, myricyl alcohol, myristic acid, oxalic acid, D-glucose, punarnavoside, punarnavine-1, punarnavine-2, proteins, carbohydrates, β-ecdysone, triaconitanol, 5-7- dihydroxy-3′, 4′-dimethoxy-6, 8-dimethyl flavone and hypoxanthin 9-L- arabinofuranoside and three protenoid analogues, boeravinones A, B and C (Chatterjee and Pakrashi,1991).

2.1.2. Medicinal uses:

According to Nadkarni (1976), Charak, used it in the form of an ointment in leprosy and skin diseases and it is also said to be effective in urethritis. Its roots are expectorant, diuretic and laxative are used in oedema, jaundice, ascites, strangury, gonorrhea and internal inflammation (Chatterjee and Pakrashi, 1991). The leaves of the plant are used in eye wounds, muscular pains, dropsy and in gonorrhea. Dry powder of its leaves mixed with mustard oil is used externally on itches and eczema. The leaves boiled with rice, garlic and water are rubbed on body in rheumatism (Asolkar et al., 1992). The powder of the dried plant is used in abdominal cancer and tumor. The seeds and flowers are considered contraceptive in Ayurveda.

2.2. *Withania somnifera* Dunal:

2.2.1. Chemical constituants:

A genus is a small, woody shrub in the Solananceae family found throughout the drier parts of India in waste places and on bunds. The leaves contain withanone, many withanolides, somnirol, somnitol, other alkaloids and withaferins. The root possesses anhygrine, withasomine and C-28 steroid lactones. Besides, this plant contains withaniol, nicotine, somniferine, somniferinine, withanine, pseudowithanine, isopelletierine, tropine, anaferine, anahygrine and visamine (Behl et al., 1993).

2.2.2. Medicinal uses:

The brushed leaves are applied in carbuncles, scabies, ulcers and painful swellings and fermentation of its leaves is used for sore eyes and swollen hands, feet (Dastur, 1962). A decoction of root is used with long pepper, ghee and honey in scrofula (Dastur, 1962). The root is also used for toning up the uterus of women who is habitual of miscarriage.

The roots are said to be adaptogenic, aphrodisiac, deobstruent, alterative, diuretic and tonic (Chatterjee and Pakrashi,1995) and used in cough, dropsy, leucorrhoea, hiccough, menstrual problems, to restore loss of memory, in nervous exhaustion, spermatorrhoea and seminal debility. The powder of root is given daily with sugar, honey and long pepper in the doses of 1.7gm in spermatorrhoea and debility. *Withania somnifera* is widely used in the ayurvedic system of medicine to treat tumors. Chemical investigation of the roots and leaves of this plant has yielded bioactive withanolids. Jayaprakasam et al., 2003, studied the withanolids which inhibit cyclooxygenase enzymes, lipid peroxidation and proliferation of tumor cells.

2.3. *Calotropis procera* (Ait.) Ait.f.:

2.3.1. Chemical constituants:

A genus of tomentose shrubs 2-4m in height distributed throughout the tropical and subtropical regions. The leaves contain ascorbic acid, calactin, calotoxin, calatropagenin, calatropin, polysaccharide containing D-arabinose, D-glucose, D-glucosamine, L-rhamnose and 3-proteinase. The root has benzolisolieolone, benzollineolone, long-chain fatty acids and C (18) isoursane. Besides, this plant also contains, calactinic acid, choline, O-pyrocatechuic acid, β-sitosterol, taraxasterol, isovalerate and taraxasteryl acetate (Chatterjee and Pakrashi, 1995). Gupta et al., 1996, reported the presence of vrsa-13 (18), 19 (29) - dien-3 α - yi - acetate, 18aH-urs-19 (29) - en - 3 - one, 18α H-ursa-12, 20 (30) - dien-3α-yi acetate and 18αH-urs-12-en-3α-ol, four new ursane type triterpines from the root bark.

2.3.2. Medicinal uses:

C. procera found to have anti-cancer activity against human epidermal carcinoma of the nasopharynxtissue culture (Dhar et al., 1968). Bhakuni et al., 1969 suggested ethyl alcohol (50%) extracts of root and leaves are anticancerous in nature and are used in cardiac arrhythmia. Root bark is antidysenteric, antispasmodic, diaphoretic, emetic, expectorant and purgative and is also used in piles, syphilis and their paste is applied in hydrocele and elephantiasis. Mossa et al., 1991 investigated the ethanolic extract of *C. procera* for its antipyretic, analgesic, anti- inflammatory, anti-bacterial, purgative and muscle relaxant activities. The powder of leaves boiled in sweet oil is used in skin eruption, eczema, toothache, ulcers and wounds. The

tinctures of the leaves are given in intermittent fever (Chatterjee and Pakrashi, 1995).

2.4. *Asclepias curassavica* Linn.:

2.4.1. Chemical constituants:

A genus is belonging to the family Asclepiadaceae. An erect, glabrous, perennial sometimes annual herb, 30 to 120 cm tall with a milky sap distributed throughout the tropical and subtropical regions. The leaves contain ascurogenin, calotropagenin, corotxigenin, clepogenin, coroglaucigenin, curassavogenin, uzarigenin and uzarin. In root, vincetoxin is found, Asclepin and its four glycosides, calactin, calotropin, calotropagenin, curassavicine, oleanolic acids and β-sitosterol and its glucosides have also been reported from the plant (Chatterjee and Pakrashi, 1995).

2.4.2. Medicinal uses:

Dhar et al. (1968) suggested ethyl alcohol extract of the plant possesses cardio tonic properties. An alcohol extract of *Asclepias curassavica* L., a plant widely used in folk medicine for treating cancer and warts; systematic fractionation of the extract has led to isolation and characterization of calotropin as a cytotoxic principle. Nadkarni (1976), used the roots as a good remedy for piles, gonorrhea and warts. The juice of leaves is anthelmintic, antidycenteric, sudorific, useful in haemorrhage and gonorrhea. The roots are astringent, cathartic, emetic, purgative and styptic (Chatterjee and Pakrashi, 1995).

2.5. *Vitex negundo* Linn.:

2.5.1. Chemical constituants:

A genus is belonging to the family Verbenaceae, distribution naturally found along coastlines. This is a shrub or small tree growing from 1 to 4 meters in height. Leaves contain an alkaloid nishindin, flavonoids like flavones, luteolin-7-glucoside, casticin, iridoid glycosides, an essential oil and other constituents like vitamin C, carotene, gluco-nonital, benzoic acid, β-sitosterol and C-glycoside (Husain et al., 1992). The leaves yield 5- hydroxyisophthalic acid, 3, 4-dihydroxybenzoic acid, 2-p-hydroxy-benxoylmussainosidic acid, citral, camphene, iso-oreintin, oreintin, agnuside, nishindaside and negundoside. The root contains β-sitosterol, 3-formyl 4,5-di-methyl-8- oxo- 5H-6, 7-dihydronaphtho (2,3-b) furan and acetyl oleanlic acid (Chatterjee and Pakrashi,1995).

2.5.2. Medicinal uses:

A smoke of dried leaves is taken in headache and catarrh. The decoction of the leaves with long pepper is given in catarrh fever with heaviness of head and dullness of hearing. The juice of the leaves is used for removing foetid discharges and worms from ulcers (Nadkarni, 1976 and Das and Das, 1994). Chatterjee and Pakrashi, 1995, stated that the whole plant is considered as astringent, cephalic and stomachic. This is used in asthma, bronchitis, consumption, eye diseases, leucoderma and painful teething.

2.6. *Hyptis suaveolens* (Linn.) Poit.:

2.6.1. Chemical constituants:

A genus is belonging to the Labiatae, distributed throughout the tropical and sub-tropical regions. It grows as common weed on road side and

overgrazed areas around cattle yards. It is resistant to fire. Herbs, sub shrubs or shrubs.The plant yields essential oil containing menthol (Chopraet al., 1956). Besides, the leaves and flowers contain campesterol and fucosterol. The roots contain β-sitosterol, oleanolic acid and a-peltoboykinolic acid (Asolkar et al., 1992).

2.6.2. Medicinal uses:

Decoction of leaves is used to cleanse wounds. The plant is used in the antilles as a stimulant. The plant is considered stimulant, carminative, sundorific and lactagogue (Agrawal, 1986) and it is pounded and applied to parasitical cutaneous diseases. An infusion of the herb is given in catarrh and uterus infections and a paste of the plant in water is used as snuff in nose bleeding. An extract of root is taken as appetizer and the roots are also chewed with betel nuts as stomachic. The leaves are used in cancers and tumors. An infusion of the leaves is applied on headache and to boils and their decoction is used for piles and retention of pregnancy (Asolkar et al., 1992). The juice of the leaves is also used as eye lotion, nose- drop, colic and stomachache.

2.7. *Antirrhinum majus* Linn.:

2.7.1. Chemical constituants:

The genus belonging to family Scrophulariaceae, perennial herb distributed throughout tropical and subtropical regions. The plant contains choline and four tertiary alkaloids of which 4-methyl-2, 6-nophthyridine ($C_9H_8N_2$, mp 78^0c) the major one and two iridoid (cyclopentanoid or methyl cyclopentanoid monoterpene) glucosides, namely, antirrhinoside and 5-0-β-D-glucosylantirrhinoside. Iridoids are known for their varied biological activity and *A. majus*in all probability owes its medicinal and therapeutic properties to

their presence. The aerial parts of the flowering plant contain sixteen amino acids including γ-aminobutyric acid (GABA). The leaves yield a fat rich in linolenic acid and also containing hexdeca trans-3-enoic and cis-7, cis-10, cis-13-trienoic acids , β-sitosterol as major sterol and alkanes (0.18%) with n-C_{16}-C_{35} and branched C_{25}-C_{35} (The wealth of India, 2003).

2.7.2. Medicinal uses:

The leaves and flowers are anti-phlogistic, bitter, resolvent and stimulant (Clapham et al., 1962). They have been employed in poultices on tumors and ulcers (Grieve, 1984). It is effective in the treatment of all kinds of inflammation and is also used on hemorrhoids. Selection of plants was made on the basis of amount and purity of bioactive compounds.

2.8. Methotrexate (MTX):

Methotrexate is a novel xenobiotic inducer of rat liver and intestinal sulfotransferase. MTX therapy is associated with liver damage, both acute and more seriously in chronic cases (Zachariae, 1980; Doroshow et al., 1981 and Klaber, 1982).

Methotrexate is an antimetabolite widely used in cancer chemotherapy, which can cause intestinal mucosal injury. The antimitotic effect of MTX is known to give rise to malabsorption syndrome. It inhibits the enzyme dihydrofolatereductase (DHFR) which is required for DNA synthesis and cell division (Jolivet, 1983). Owing to the requirement of deoxynucleotide in DNA synthesis, methotrexate can impair tumor cell growth and induce cell death by secondary genotoxic effects or apoptosis (Lorico et al., 1988; Kinsell et al., 1997).

Combination therapies including methotrexate are currently used to treat several tumor types, including osteosarcomas and lymphomas (De Vita et al., 2001). However, MTX is less effective against many other tumor types: in the case of colorectal cancer, e.g. the tumors are generally resistant in the case of head and neck squamous cell carcinoma a proportion of tumors are initially responsive but rapidly become resistant to MTX therapy.

Methotrexate is an inhibitor of dihydrofolatereductase (DHFR) and as such limits the formation of nucleotides and thus inhibits DNA replication. It has a significant role in the treatment of breast cancer, acute lymphocyte leukemia and lymphomas (McGuire, 2003). Methotrexate is an antineoplastic agent used to fight a number of different cancers. Substantial evidence supports the concept that methotrexate was mutagenic and carcinogenic in animals (Chowdhury and Palo, 2004).

2.9. Genotoxicity/ Cytotoxicity:

Genotoxicity describes a deleterious action on a cell's genetic material affecting its integrity. This includes both certain chemical compounds and certain types of radiation. Genotoxic substances are all that with affinity to interact with DNA - which is not proof of their dangerousness to humans, but does render them potentially mutagenic or carcinogenic.

At higher concentration cyproheptadine has been shown to suppress cell division and exhibit cytotoxic effects of non-specific nature (Zang et al., 1975).Adriamycin induced genetic toxicity in *Allium cepa* test. The most important effect of the drug was the drastic lowering of the mitotic index, interphasic cell death, nuclear lesion, nuclear dissolution, nuclear polymorphism and somatic reduction of chromosome at concentration and

durations ranging from 0.1 to 10.0% and 3 to 24hrs respectively. Mitotic abnormalities encountered at metaphase were C-metaphase, clumping, abnormal equatorial plate and chromatid bridges, sticky bridges, chromosome fragments unequal distribution of chromosomes with paired chromatids, acute fragmentation leading to chromatin globules etc. were frequent in treated cells, studied by Mercykutty and Stephen (1980).When sodium azide is dissolved in water it forms a toxic hydrogen azide gas, with generation of azide ions being the possible reason for its genotoxicity and cytotoxicity in *Allium cepa* test systems (Arenaz et al., 1983).

It was reported in the earlier studies that high doses of *Pueraria mirifica* caused a variety of toxicities in animals including genotoxicity by inducing micronuclei formation in the polychromatic erythrocytes per animal of mice (Pongdam et al., 1987; Manoruang, 1996 and Aritajat et al., 2001).

Agar and Uysal (1997) investigated the effects of $HgCl_2$ on *A. Cepa* root meristem cells. $HgCl_2$ applied to *A. cepa* root cells at different dosages caused chromosomal defects to occur in the cells. Chromosomal anomalies demonstrated differences due to division phases. These anomalies were irregular distribution of chromatin and granulation of prophase and clustering of chromatids, lagging chromosomes and chromatids breakage at metaphase. Irregular distributions between sister chromatids and bridge formation were observed at anaphase. The cytotoxic effects of aspirin, an anti-inflammatory drug, are mainly due to its ability to induce DNA fragmentation and proteolytic cleavage. It is also known to active caspases through cyclooxygenase in dependent mechanism (Bellosillo et al., 1998).

Steinkellner et al. (1998) was investigated the potential use of micronucleus assays in plants for the detection of genotoxic effects of heavy-metal ions. Three different plant systems in micronucleus with *Tradescantia* pollen mother cells (Trad MCN) and with meristematic root tip cells of *Allium cepa* and *Vicia faba* (*Allium*/MCN). As^{3+}, Pb^{2+}, Cd^{2+} and Zn^{2+} caused a dose dependent increase of MCN frequencies in all three-test systems. Cu^{2+} gave consistently negative responses, Zn^{2+} caused only moderate. The ranking of genotoxic potencies in all three tests was in the descending order: As^{3+}> Pb^{2+}> Cd^{2+}> Zn^{2+}> Cu^{2+}. Increase of the exposure levels caused toxic effects, reduction of root growth, cell division delays and decrease of MCN frequencies. Inceer and Beyozoglu (2000) reported that $CuCl_2$ and Al^{3+} reduced the mitotic efficiency of the root meristem of *Vicia hirsute*, *A. cepa* and *A. sativum* L.

Genotoxicity and mutagenicity of pesticides for non-target organisms and their influence on ecosystems are of worldwide concern (Pimental et al., 1998). Florenda and Ruth (1999) studied the cytogenetic effects of malathion, an organophosphate pesticide were investigated in *Allium cepa*. Root tips of*A. cepa* were exposed at 2850mg/L malathion for 24 and 48hrs. The mitotic index of root tip cells exposed to malathion was significantly decreased compared to control. The numbers of chromosomal aberrations were chromosomal stickiness, laggards, anaphase bridges and forward chromosomes.

Magda, 2001, studied the genotoxic effects of aqueous extracts of neem, *Azadirachta indica* A. Juss, meliaceae leaves, kernels and seed coats was evaluated using *Allium cepa* a chromosome aberration assay. Neem

extracts suppressed the mitotic activity of *Allium* root meristems after 24 and 48hrs treatment with all concentrations. Neem seed coat extract was the least effective in its ability to inhibit cell division, whereas kernel extract was the most effective. Analysis of the data of phase index indicates that all treatments reduced prophase percentage, meanwhile the percentage of metaphases and anaphases rose over those of control. Also, the extracts caused different kinds of chromosome aberrations in dividing and non-dividing cells of *Allium cepa* such as micronucleus and multinucleated cells observed in the interphase stage; bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed anaphase-telophase observed in dividing cells. Bridges was the most frequent kind of aberrations in dividing cells. Neem seed kernel extract proved to be inducing more chromosomal aberrations than Neem leaf extract and Neem seed coat extract.

Elzbieta et al. (2002) studied on evaluation of the risks posed by the herbicide stomp 330 EC using *Allium* assay. Onion bulbs with 3-5 cm roots grown in dark at room temperature were placed in 0.01%; 0.02%; 0.03%; 0.04% and 0.08% solutions of tested herbicide for 24 or 48hrs and subsequently post incubated in water for 24 or 48 hours. A decrease in mitotic index, karyokinetic spindle abnormalities, lagging chromosomes during anaphase and telophase bridges, displaced phragmoplasts, incomplete cytokinesis, micronuclei and lobed interphase nuclei were all noted. Moreover macroscopic and histological irregularities of the root were observed. Macroscopic irregularities were local swellings, disturbances of cell plate formation or abnormalities in cell shape. Histological abnormalities observed multinucleate cells and absence of statolite starch grains in root tip cells.

Results indicated strong cytotoxic and genotoxic irreversible effects of very low concentrations of stomp 330 EC herbicide.

Chromium (VI) produced various types of cytotoxic and chromo toxic effects in the test plant. In higher concentrations of Chromium (VI), clastogenic effect was prominent causing different types of abnormalities like clumping, laggard, despiralisation of chromosome, delayed anaphase and formation of anaphase bridge from which it may be concluded that the effect of Chromium (III) ions, on germination, physiology and cytological behavior is less as compared to Chromium VI ions (Cervantes et al., 2001; Mukherji and Sharma, 2002; Zayed and Terry, 2003).

Inceer et al., 2003 studied cytogenetic effects of copper chloride on root tip cells of *Helianthus annuus* were investigated. Seeds were treated with 10, 25, 50 and 100mg/L (ppm) of copper chloride for 24hrs. It was found that copper chloride has a marked mitodepressive action on mitosis. Mitotic abnormalities were increased and the mitotic index was decreased depending on the concentration of the copper chloride applied.

Biscardi et al. (2003) studied genotoxic effects of pesticide-treated vegetables extracts can cause adverse health effects and many pesticides are mutagenic/carcinogenic. The presence of pesticides and genotoxic compounds extracted from 21 treated vegetables and 8 types of grapes samples from the markets of a region in Southern Italy. The extracts were analyzed for pesticides by gas-chromatography and HPLC and for genotoxicity with plant tests in *A. cepa* root, the micronucleus test and the chromosomal aberration test, found 33 pesticides, some of which are outlawed. Genotoxicity was found in some of the vegetables and grapes tested. The micronucleus test in

interphase cells gave much higher mutagenicity than the chromosomal aberration test in anaphase-telophase cells.

Slapsyte and Sukackaite, (2003) studied genotoxic activity of sodium selenite and its effect in combination with methyl methanesulfonate (MMS) using chromosome aberration (CA) and sister chromatid exchange (SCE) tests in human peripheral blood lymphocytes in vitro. Only high doses of sodium selenite were found to be genotoxically active. A dose-related response in the SCE induction was determined. A decrease of MMS - induced chromosome aberrations was found in cultures treated concurrently with MMS and sodium selenite. However, the combined effect of MMS and sodium selenite resulted in a higher SCE rate as compared to that induced by MMS alone.

Yuzbasioglu (2003) studied the cytogenetic effects of afugan were investigated in root tip cells of *A. cepa* L. EC_{50} value of this fungicide was determined as 40ppm using root growth inhibition test and then the roots of *A. cepa* were treated with 10, 20, 40 and 60ppm concentrations for 12, 24 and 48hrs. Mitotic index, mitotic phase frequencies and mitotic abnormalities were determined for each concentration and treatment period. The most common types of abnormalities are stickiness, C-mitosis, bridges, lagging chromosomes, fragments, multipolarity and micronucleated.

Barbara et al., 2004, examines the acute and chronic dose - responses of arsenic in established human cell lines using keratinocytes (HaCaT), melonocytes (CRL 1675) and dendritic cells (THP-1+A23187). Chronic conditions were established by treating the three cell lines with at least 8 passages in 0.2ug/ml arsenic trioxide. Cytotoxicity was assessed using the fluorisceindiacetate assay after 72hrs of exposure. Single cell gel

electrophoresis (comet assay) was used to measure DNA damage. Acute exposure to arsenic has LD_{10} and LD_{25} values of 0.38ug/ml and 3.0ug/ml for keratinocytes; 0.19ug/ml and 0.38ug/ml for dendritic cells. Cytotoxicity assays for chronically exposed cells resulted in LD_{10} and LD_{25} values of 0.4ug/ml and 0.8ug/ml for keratinocytes; 0.10ug/ml and 0.20ug/ml for melanocytes and 0.10ug/ml and1.0ug/ml for dendritic cells. The comet assay showed that arsenic was highly genotoxic to the 3 cell lines. No significant differences ($P > 0.05$) in DNA cleavage were observed between acute and chronic exposures. In conclusion, this research shows that arsenic is dermatotoxic, showing a high degree of genotoxicity and cytotoxicity to skin cells.

Bidau et al. (2004) studied the cytotoxicity of aqueous extracts of *Ilex paraguariensis* in *Allium* test and *Artemia salina* Microwell test. The extracts were prepared from commercial and laboratory and were tested at concentrations of 5-40g/L. Both extracts were greater for the commercial material for which concentrations ≥ 10g/L virtually abolished mitosis. The disturbance of mitotic behavior was significant at 5-10g/L of the laboratory product and included C-mitosis, disorganized metaphases, sticky metaphases, arrested anaphases and binucleated interphases in *Allium* test. None of the extracts were cytotoxic in the *Artemia salina* test.

Adams et al., 2004, concluded that *Curcuma longa* exhibit a high degree of cytotoxicity. But the compounds cytotoxicity may arise from the presently unknown mechanism.

Rao et al. (2005) investigated the effects of 3 synthetic pyrethroid insecticides viz., Cypermethrin, Alphamethrin and Fenvalerate on the mitotic activity and mitotic chromosomes in the root meristems of *A. cepa.* Root

meristems were exposed to concentrations of each test compound for one hour pulse treatment and allowed to different recovery period. Cytological analysis revealed test compounds elicited varying degrees of cytotoxic, toxicity to spindle and clastogenic effects. In the ultimate analysis Cypermethrin and Alphamethrin have more toxicity to spindle and weak clastogenic activity whereas fenvalerate has relatively strong clastogenic activity in vitro.

Genotoxins are a broader category of substances that induce changes to the structure or number of genes via chemical interaction with DNA and/or non-DNA targets (Maurici et al., 2005). Gadano et al., 2006 evaluated the genetic damage induced by decoction and infusion of *Chenopodium ambrosoides* L. and *Chenopodium multifidum* L. (Chenopodiaceae) which ware assayed in different concentrations (1, 10, 100, 1000µL extract/ml culture), by addition of the extract to human lymphocyte cell cultures, negative controls were included. The end points evaluated were (CA) chromosomal aberrations, (SCE) sister chromatid exchanges, (CPK) cell proliferation kinetics and (MI) mitotic index. The results showed (a) statistical increase in the percentage of cells with CA and in the frequency of SCE when cultures were exposed to both aromatic plants, (b) a decrease in MI of both assayed, although no modification in the CPK values was observed, (c) no effect was noticed in the analysis of *Chenopodium album* L. which was used as negative control of the essential oil. These results suggest a cyto and genotoxic effect of *Chenopodium ambrosoides* and *Chenopodium multifidum* aqueous extracts related to the essential oil of the plant.

Thepouyporn et al., 2006 were tested mutagenicity and antimutagenicity potentials using 'Ames' test in crude distilled water and

absolute ethanol extracts from the stems and leaves of *Peperomia pellucida* (Linn.) Kunth, *Eichhornia crassipes*, Solms, *Colocasia esculenta* Schott and *Brachiaria mutica* (Forssk.) Stapf and the stem of *Musa sapientum* Linn.no mutagenic effect was found in any of the 10mg/plate crude extracts of these plants for either TA98 or TA100 of *Salmonella typhimurium*, in a direct test and a mutagenic induced test by 5 - 9 mix. Both distilled water and absolute ethanol extract of 0.5-10mg/plate *B. mutica* showed strong antimutagenicity to AFB1, B(a)P and 4NQO in two tester strains. Ethanol extract of 0.1 - 0.5 mg/plate *C. esculenta* also showed antimutaginicity to AFB1, B(a)P and 4 NQO in two tester strains, but the 0.5-10mg/plate water extract and an antimutagenic effect only for B(a)P in TA98.The ethanol extracts of 5mg/plate *B. mutica* and 0.5mg/plate *C. esculenta* are cytotoxic, as indicated by their partial killing effect.

Akinboro and Bakare (2007) studied the cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants: *Azadirachta indica* (A. Juss), *Morinda lucida* (Benth.), *Cymbopogon citratus* (DC Stapf.), *Mangifera indica* (Linn.) and *Carica papaya* (Linn.) using the *Allium cepa* assay. The extracts were prepared with tap water. Onion bulbs were exposed to 1, 5, 10, 25 and 50% and 1, 2.5, 5, 10 and 20% concentrations (v/v). The EC_{50} obtained for decoctions of *Azadirachta indica*, *Cymbopogan citratus*, *Mangifera indica* and *Carica papaya* were 0.6, 3.0, 1.4 and 0.8% respectively. It was 2.6 and 0.8% for the squeezed extracts of *Azadirachta indica* and *Morinda lucida* respectively. All the extracts tested were observed to have mitodepressive effects on cell division and induced mitotic spindle disturbance in *A. cepa.*

Haripyaree et al., 2007 studied the effect of *Mimosa pudica, Vitex trifolia, Centella asiatica; Leucas aspera* and *Plantago major* to determine whether aqueous extracts of the plants interact with genetic material, mammalian cytogenetic assay was used. Mice which received interperitonial injections of different doses of the extract of *Mimosa pudica* showed dose dependent increase in chromosomal aberrations, SC damages and micronucleus frequency, while other plant extracts did not induce significantly higher frequency of chromosome aberrations.

Qari (2008) studied the antimutagenic and genotoxic potential of *Origanum majorana* was evaluated in *Vicia faba* root meristem cells. So for there is no report on the biological properties of *Origanum majorana* in plant test systems. The root tip cells were treated with sodium azide at 250 and 350ug/ml for 6hrs and *Origanum majorana* was given at 50, 100 and 200 µg/ml for 20hrs, prior to sodium azide at treatment. The tips were squashed after colchicine treatment and the cells were analyzed for chromosome aberration and mitotic index. *Origanum majorana* induces chromosomal aberration in *Vicia faba* root tip cells in an insignificant manner, when compared with untreated control. Sodium azide alone induces chromosomal aberrations significantly with increasing concentrations.

Boswellia serrata was subjected to genotoxicity studies in order to ascertain an aspect of the safety of the drug. Dry extracts of *B. serrata* showed no mutagenicity up to 5 mg/plate when tested with *Salmonella typhimurium* TA97a, TA98, TA100, TA102 and TA1535 strains with or without metabolic activation. In addition, the extract showed significant protective effect against mutagenicity induced by mutagen in *S. typhimurium* TA98 and TA100 strains

with or without metabolic activation. Similarly, in vitro chromosomal aberration assay did not reveal any significant alterations up to 5 ml culture as compared to the negative control both in the presence and absence of metaboilic activation (S9 mix). The results of these studies indicate that *B. serrata* is non-mutagenic in Ames test and is protective against the mutagenicity induced by 4-nitrogquinolene -1-oxide, sodium azide and 2-aminaoflourene in TA98 and TA100 strains (Mangesh et al., 2008).

The Pesticides, chlorophyrifos, Alpha-thrin, Efektovirikop and springbok were assessed for cytotoxicity and genotoxicity were studied by Asita and Makhalemele, 2008 in the onion root tip assay. Cytotoxicity was determined by comparing the mitotic index (MI) of treated cells with that of the negative control. The MI of cells treated with chloropyrifos, Alpha-thrin or springbok was half or less, that of the control at one or more doses and adjudged cytotoxic. Efekto virikop was not cytotoxic. Genotoxicity was measured by comparing the number of cells/1000 in aberrant division stages at each dose with the negative control using the Mann whitney test. Chloropyrifos was genotoxic ($P < 0.05$), inducing chromosome lagging and bridges, pulverized and sticky chromosomes, multipolar anaphase and telophase. Efekto virikop and springbok induced lagging chromosomes. Alpha thrin was not genotoxic.

Asita and Makhalemele (2009) studied the effect of three doses each of the pesticides, dithane (0.196, 0.391, 0.782% solution), malathion (0.034, 0.069, 0.137% solution) and garden ripcord (1.015, 2.030, 4.060% solution) were assessed for cytotoxicity and genotoxicity to onion tip cells. The doses represented the LC_{50} (does that inhibited 50% of seeds from germinating), 1/2

LC_{50} and 1/4 LC_{50} for each pesticide that were determined in dose selection experiment. The mitotic index of the negative control group was reduced to half or less, at the two, highest doses of all three pesticides.All three pesticides were therefore cytotoxic to the onion root tip cells. Genotoxicity was measured by comparing the number of cells/1000 in the aberrant division stages at each does of each pesticide with the number of such cells in the negative control group using the Mann-Whitney statistical test. Dithane was genotoxic ($P < 0$ 0.5), inducing multipolar anaphases and telophases and lagging chromosomes. Malathion was genotoxic ($P < 0.05$), inducing lagging chromosomes, Garden ripcord was not genotoxic.

Silva et al., 2009, evaluated the clastogenic and /or genotoxic potential of Fruit pulp extract of *Tamarindus indica, in vivo* in peripheral blood and liver cells of wistar rats, using the comet assay and in bone marrow cells of swiss mice, using the micronucleus test. The extract was administered by gavage at doses at 1000, 1500 and 2000mg/kg body weight. The extract made with *T. indica* was devoid of clastogenic and genotoxic activities in the cells of the rodents, when administered orally of these three acute doses.

Ukaegbu and Odeigah in 2009 investigated the genotoxicity of sewage effluent using both morphological and root chromosome assay. The mean root lengths of onions exposed to different concentrations of the effluent were determined from the growth curve as 47%. The result of mean root length was statistically evaluated by the analysis of variance and least significant difference. There was a significant decrease in root length of the experiment. Also the mitotic index decreased as concentration increased. Total aberrations increased significantly as concentration increased ($p < 0.05$).

The genotoxicity and the antigenotoxicity of commercial *Argania spinosa* seed oil and its unsaponifiable fraction (UF) against *Drosophila melanogaster* was evaluated using wing spots enumeration. Results showed that the argan oil (at 20% dosage) and the UF (at 0.112g/l) were not genotoxic. Results on the effects of argan oil and UF on the mutagenic properties of methyl methanesulfonate (MMS) and ethyl carbonate (Urethane) showed an inhibition rate of 54 and 43% against MMS and urethane respectively, by argon oil and 56 and 75% against urethane and MMS respectively, by UF. The result of the present study suggests that argan oil prevent mutations induced by urethane and MMS in *D. melanagaster*, as a consequence consumption of argan oil can prevent human DNA lesion induced by some environmental mutagens was studied by Dalouh et al., 2010.

Fruits of *Piper longum*, *Semecarpus anacardium* and whole plant of *Achyranthus aspera* have been screened for their cytotoxic constituents. Petroleum ether and chloroform extracts of *Piper longum* fruits showed greater cytotoxicity with LC50 values of 0.104 and 5.719ug/ml and various fractions of petroleum ether extract of *Piper longum* fruits showed the significant cytotoxicity. The activity guided isolation of the active principles from *Piper longum* Fruits have given us with five biologically active, cytotoxic fractions one of them was identified as B-sitosterol (Hullatti and Murthy, 2010).

Similar observations have been reported by Badr, 1983, Da et al., 1994 and Minija et al., 1999. It is possible that concentration of any chemical may have an inhibitory or stimulatory effect on the cell cycle. In recent studies suggested that *Lavandula stoechas*, aqueous extracts (AskinCelik and

Aslanturk, 2007) and ethanolic extracts of *Citrus lemon* and *Citrus sinensis* (Ozmen and AskinCelik, 2007), petroleum ether and aqueous extracts of *Achyranthes aspera* (Malode and Khandare, 2009), petroleum ether leaf extracts of *Calotropis procera* (Malode and Khandare, 2010) and using *Allium cepa* root tip meristem model to evaluate the genotoxic activity of petroleum ether leaf extract of *Vitex negundo* L. distilled water served as control. The percentage of mitotic index has decreased at 50µg and 100µg/ml concentrations whereas, in 150µg and 200µg showed no significant changes for 3 hours treatment. Chromosomal aberrations were increased with increase in concentrations of extracts. Chromosomal aberrations like chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases were seen in dividing cells. Further the crude extract was analyzed by spectroscopic techniques such as UV-Vis spectrophotometer and FTIR (Khandare and Malode, 2012) have low cytotoxic and genotoxic effects.

2.10. Antimitotic:

Reddy and Rao (1969) scored mitotic and meiotic irregularities on treating *Vicia faba* root tips with dimecron–100 and rogor–40 from the percent frequency of chromosomal aberration. Beu et al., (1976) have also showed that exposure of root tips of *Vicia faba* to high concentration of herbicide parquet has led to inhibition of DNA synthesis.

Badr and Elkington (1982) studied the effect of isoproturon on mitosis in *Allium cepa* and *Hordeum vulgare*. Isoproturon reduced the mitotic activity to a greater extent and had a disruptive action on spindle apparatus resulting in C-mitosis, lagging chromosome and multipolar anaphases and telophases. It

was observed that *A.cepa* was more susceptible to mitodepressive and chromotoxic action of the herbicide.

Kabarity and Mallallah, 1980; Pandita, 1986; Badr and Ibrahim, 1987 studied effect of Chromium attributed to the less proportion of interphase cells enters into prophase from which more proportion of cells become arrested at metaphase, anaphase and telophase with the increase in the concentration of the chemical in a continuous cell cycle. Hence, chemical might have inhibited the DNA synthesis at S phase of the cell cycle and prolonged the duration of metabolic activities during interphase cells entering into prophase. At the same time, the mitodepression would have caused due to the prolonged G2 period and blockage of biosynthesis of DNA, RNA and protein.

Effect of detergent Sodium Lauryl Sulphate on meiotic nuclei of *A.cepa* was studied. The meiotic index decreased with rise in concentration of the detergent and with prolonged exposure the meiotic activity also decreased. Different types of cytological abnormalities were observed in detergent treated cells, such as sticky metaphase, chromatin fragmentation, shrinkage of chromatin material, sticky chromatin bridge of anaphase. Abnormalities increased with the increase in concentration of detergent studied by Datta et al. (1988).

Potassium metabisulphide has decreased mitotic index in the treatment groups compared with the control at all concentrations and treatment periods. Reduction in the mitotic activity could be due to inhibition of DNA synthesis (Schneiderman et al., 1971; Sudhakar and Venu, 2001).

Alkaloids isolated from periwinkle plant (*Vinca rosea*; *Catharanthus roseus*), in which two compounds i.e. vinblastine sulfate and vincristine

sulfate have been shown to be useful in the chemotherapy of malignancy (Bruce, 1996). These compounds act by inhibiting mitosis. Further they bind to tubulin and act on the mitotic spindle.

Borboa and De La Torre (1996) investigated that the application of 5 $\times 10^{-4}$ M Zn (II) and 3 $\times$ 10^{-3} M Cd (II) depressed but did not block root growth in *Allium cepa.*, both heavy metals were indirect clastogens at these moderate concentration, they induced chromosomal bridges, but no breaks were detected in the first ana-telophases. On the other hand Zn and Cd potentiated six and eleven times the direct clastogenic effect of the β-irradiation produced by [^{3}H] thymidine incorporation into DNA. The chromosomal breaks observed at mitosis. The whole cycle experienced 1-5 and 2-0 fold increases in the presence of Zn and Cd in relation to that in control meristems (15h).

Saenz et al. (1998) studied the effects of hexane extracts of *Viscum cruciatum* Sieber (II) and *Crataegus monogyna* Jacq. (I), *Crataegus monogyna* Jacq., parasitized with *Viscum cruciatum* Sieber (II) and *Crataegus monogyna* Jacq. (Rosaceae) non-parasitized (III) and a triterpenic fraction isolated from II and III were screened on mitotic division in *Allium cepa* L. A mitodepressive effect of the hexane extracts (I, II, III) was observed. The results obtained with the triterpenic fractions showed higher activity than the hexane extracts II and III. Triterpenic fractions isolated from the hexane extract of *Crataegus monogyna* non-parasitized showed complete inhibition of cell division at 48 hrs of treatment.

Badria et al. (2001) studied the effect of Gossypol was isolated from root bark of *Gossypium barbadense* L. var. Giza 86. Its 1, 4-binaphthoquinone

derivative, gossypolone was prepared by oxidation of gossypol with $FeCl_3$ in acetic acid/acetone. The mitotic rate was markedly decreased after 3, 6 and 24hrs of incubation with 500, 250 and 125ppm gossypol respectively. With gossypolone, the decrease in mitotic rate was noticed after 6, 12 and 24 hrs incubation with 500, 250 and 125ppm, respectively. The decrease was irreversible in root tips incubated with 500 and 250ppm gossypol or gossypolone. In the other hand root tips incubated with 125ppm gossypol or gossypolone regained normal mitotic activity after post incubation in water. Both gossypol and gossypolone markedly decreased the growth rate of *Allium cepa* L. bulbs in dose-dependent manner.

Muhsin et al. (2007) investigated the effect of Boron on the mitotic index of *Allium cepa* root meristematic cells. By using the growth inhibition test LD_{50} value was determined first and then different doses of varied concentrations of Boron were introduced to onion tuber roots. Distilled water was used as control. Since *Allium cepa* cell cycle is 24hrs, application process was carried out at 12, 24 and 48hrs. The most observed abnormalities were C-metaphase, prometaphase and disturbed anaphase-telophases. In addition to these, anaphasebridge, polyploidy and late chromosome particulates were also observed.

Mitotic inhibitors are derived from natural substances such as plant alkaloids and prevent cells from undergoing mitosis by disrupting microtubule polymerization, thus preventing cancerous growth. Microtubules are long, ropelike proteins that extend through the cell and move cellular components around. Microtubules are long polymers made of smaller units (monomers) of the protein tubulin. Microtubules are created during normal

cell functions by assembling (polymerizing) tubulin components, and are disassembled when they are no longer needed. One of the important functions of microtubules is to move and separate chromosomes and other components of the cell for cell division (mitosis). Mitotic inhibitors interfere with the assembly and disassembly of tubulin into microtubule polymers. This interrupts cell division, usually during the mitosis (Mi) phase of the cell cycle when two sets of fully formed chromosomes are supposed to separate into daughter cells (Definition of mitotic inhibitor, 2007; Treatment Options: Mitotic Inhibitors, 2007).

2.11. Mutagenic / antimutagenic:

The mutagenicity, antimutagenic / anticarcinogenic and cytological effects have been reviewed by several workers Wuu and Grant (1966); Upadhyay and Swaminathan (1969); Warfield (1972); Ames et al. (1975); Khalatkar and Kashikar (1980) and Somashekar et al. (1984). Many Thai plant species have been proven to have antimutagenic activity, for instance, *Cymbopogon citratus* Stapf (Vinitketkumnuen et al., 1994) and *Hibiscus sabdariffa* Linn. (Chewonarin et al.,1999).

Malode and Khalatkar (1994) induced gene block for low glucosinolate with EMS and SA treatments in *Brassica juncea.* They also suggested that glucosinolate content was under the influence of gene and different alleles contribute to the total content. The data demonstrate the induction of low glucosinolate mutations with EMS and SA.

Zehra et al. (1996) studied the water samples were collected form the river Ganga at Narora (U.P.). Analysis of water indicated the presence of several pesticides such as DDT, α–BHC, aldrin, endrin and dieldr in at

concentrations of 1.36, 1.38, 0.95, 0.61 and 0.41ppb respectively. The organophosphorus pesticides such as dimethoate and methyl parathion also appear to be present concentrations of 0.20 and 0.41ppb, respectively. The liquid-liquid extracted water samples were assayed for mutagenic potential by the Ames *Salmonella* / microsome test.

Kusamran et al., 1998, studied the antimutagenicity and anticarcinogenic potential of some Thai vegetables. They found the methanol extract of neem leaves contained weak antimutagen inhibiting the mutagenicity of direct-acting mutagens, 2-(2-furyl)-3-(5-nitro-2-Furyl) acrylamide (AF-2) and sodium azide (NaN_3). They also demonstrated that Thai bitter gourd fruits markedly exhibited some inhibitory effects on 9, 10-dimethyl-1, 2-benzanthracene (DMBA) induced mammary gland carcinogenesis in animal experiments.

Nakahara et al. (2002) induced remarkable antimutagenicity for the rhizomes of finger root (*Boesenber giapandurata),* a common Thai spice in the ginger family and they also found antimutagenicity and antibacterial activity in the twigs, fruits and flower of *Oroxylum indicum* (Bignoniaceae).

Mutations are induced at chromosome levels and are the probable causes of cancer related diseases; the inhibition of chromosomal damage by curcumin suggests the antimutagenic and anticarcinogenic activity, (Shukla et al. 2002). Malode (2004) induced zero erucic and low glucosinolate level in *Brassica juncea* cv. pusa bold through induced mutation. He further stated that mutagen sodium azide looks promising in generating variations in glucosinolate and erucic acid level.

Cortes-Eslava et al. (2004) found strong antimutagenicity for coriander juice on mutagenesis produced by 4-nitro-o-phenylenediamine and 2-aminofluorene. The term mutagen refers to a substance that induces transmissible changes in DNA structure (Maurici et al., 2005) involving a single gene or a group of genes.

Saenphet et al., 2005 evaluated effect of *Thunberia laurifolia* Linn., against the mutagenicity induced by aqueous extracts from *Pueraria mirifica* Airy shaw and Suvatabandhu in male rats. The formation of micronuclei in polychromatic erythrocytes was induced by oral administration of an aqueous extract of *P. mirifica* at the doses of 400, 600 and 800mg/kg to the rats for 30 days. The results were that the extracts of *P. mirifica* at doses of 600 and 800mg/kg acted as a mutagenic agent by inducing higher frequencies of micronuclei as compared to the controls. For the antimutagenic test, *P. mirifica* extract at a dose of 600mg/kg was mixed with fresh and dried extractof *T. laurifolia* in proportions of 7:3 and 1:1 respectively. The results of 4-week treatment indicated that aqueous extracts of *T. laurifolia,* prepared by both fresh and dry methods, could significantly inhibit the induction of micronuclei as induced by *P. mirifica.* They concluded from the results that, under certain circumstance *T. laurifolia* exhibits a significant antimutagenic activity. They used of *P. mirifica* and *T. laurifolia* as a fusion in herbal medicine.

Eryngium creticum L., *Nigella sativa* L. and *Teucrium polium* L., the antimutagenic activity of these plant species was tested in rat hepatocyte primary cultures by treatment with N-methyl-N'-nitro-N-nitrosoguanidine

(MNNG), a directly acting mutagen, which was shown to induce massive chromosomal damage in hepatocytes, Khader et al. (2006).

Verschaeve and Van Staden, 2008 investigated mutagenic and antimutagenic properties of extract from South African traditional medicinal plants. Investigation on methanol and dichloromethane extracts of selected plants were conducted with Bacterial Ames, UMC–C and VITOTOX and with cytocalacene B- micronucleus test and alkaline Comet assay in human white blood cells. They further stated that methanol extracts of *Helichrysum simillium* DC (Asteraceae) is genotoxic in properties whereas *Bauhinia galpinii* and *R. myricoides* may contain antimutagenic natural substances are able to lower cancer risk from everyday exposure from environmental mutagens as well as to mutagenic pharmaceuticals.

Methanolic extracts of *Acorus calamus* (Rhizome), *Hemidesmus indicus* (stem), *Holarrhena antidysenterica* (Bark) and *Plumbago zeylanica* (Root), were tested for their antimutagenic potential. These extracts, at tested concentrations showed no sign of mutagenicity of *Salmonella typhimurium* tester strains. The extracts of the plants exhibited varying level of antimutagenicity. At a dose of 100µg/plate, the extracts to exhibited the inhibition of His+ revertants from 18.51% to 82.66% against direct acting mutagens, methyl methanesulfonate (MMS) and sodium azide (NaN_3) induced mutagenicity in *salmonella* tester strains TA97a, TA100, TA102 and TA104. However at lower concentrations (25 and 50ug/plate) of the plant extracts, a decrease in antimutagenic activity was recorded. Dose dependent antimutagenic activity of the extracts is also evident from linear regression analysis of the data. The overall antimutagenic potential of above four extracts

was found to be in order of *A. calamus*>*H. indicus*> *H. antidysenterica*> *P. zeylanica* (Aqil et al., 2008).

Sultan and Celik (2009) evaluates the genotoxic and antimutagenic effects of *Capparis spinosa* L. flower buds aqueous extract on the *A. cepa* L. root the meristem cells. The results of this study suggest that *C. spinosa* buds aqueous extract is non-genotoxic. However, the study reveals that *C. spinosa* aqueous extract has antimutagenic potential against EMS induced chromosomal aberrations in *A. cepa*root meristem cells and the antimutagenic potential of *C. spinosa* flower buds extract is effective at 30g/L concentration.

Hence, environmental biologist and geneticist throughout the world are showing great concern over the indiscriminate use of synthetic chemicals cause mutagenicity, antimutagenic/anticarcinogenic effects have been reviewed by various investigators Zatyko et al. (1965); Warfield (1972); Ames et al. (1975); Khalatkar and Kashikar (1980); Malode and Khalatkar (1994); Johnson (1997); Malode (2004); Khader et al. (2006) and Kavitha et al. (2006).

2.12. *Allium* test system:

Allium cepa somatic chromosomes are as sensitive as those of human chromosome to various categories of environmental chemicals which directly or indirectly act upon genetic system and hazardous effect are depending on their cytotoxic and mutagenic potential. *A. cepa* system now-a-days is extensively used as plant test system all over the world. Since 1938 when Levan used *Allium* test for the first time, it has been the standard test for studying the effect of various chemicals, pollutants and pharmaceutical agents on the chromosome. (Buiatti and Nutironchi, 1969; Fiskesjo, 1969; Kak et al.,

1973; Sabharwalet al., 1975; Malode and Khandare, 2009; Malode and Khandare, 2010 and Khandare and Malode, 2012).

Allium cepa root tip meristems have been widely used for the evaluation of cytotoxic and anti-mitotic activity of various compounds (Shehab, 1980; Al-Meshal, 1987; Williams and Omoh, 1996).In the *Allium* test, inhibition of rooting and the appearance of stunted roots indicate retardation of growth and cytotoxicity, while root wilting explains toxicity (Grant, 1982, Odeigah et al., 1997 a).

Allium test combines two test targets: toxicity and genotoxicity. Furthermore, important fact is that it has low cost and it shows good correlation with mammalian test systems (Fiskesjo, 1993; Grant, 1994, 1999; Cabrera and Rodriquez, 1999; Jovtchv et al., 2002 and Li and Meng, 2002). Aqueous extracts from lead-contaminated soils before and after remediation were examined for genotoxic effect in *Allium* test, Chang et al. (1997).

The *Allium* test has many advantages as genotoxicity screening assay, one being that *Allium* root cells possess the mixed function oxidase system which is capable of activating promutagens or genotoxic chemicals (Odeigah et al., 1997b). The higher plants *A. cepa, Tradescantia paludosa* and *Vicia faba* have relatively large monocentric chromosomes in reduced numbers and are accepted as suitable test organisms for the study of environmental mutagenesis (Rank and Nielsen, 1998; Grover and Kaur, 1999; Kong and Ma, 1999; Moraes and Jordao, 2001: Patra and Sharma, 2002).

Parameters such as root shape and growth, frequencies of mitosis and abnormal cell division can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of environmental pollutant (Nielson and Rank, 1994; Amin,

2002). The *Allium cepa* assay is an efficient test for chemical screening and *in-situ* monitoring for genotoxicity of environmental contaminants and has been widely used to study genotoxicity of many pesticides revealing that these compounds can induce chromosomal aberrations in root meristems of *A. cepa* (Cabrera et al., 1994; Thais et al., 2007).

2.13. Thin layer chromatography:

Thin layer chromatography (TLC) studies were carried out for the presence of different phyto-consitituents in the extracts. TLC is a mode of liquid chromatography, in which the extract is applied as a small spot or bond at the origin of thin silica gel GF 254 (activated) layer supported on glass plate. The mobile phase migrates through the stationary phase by capillary action. The mobile phase used for *Mimysop selengi* extract was petroleum ether: Acetone (7:3) Stahl, 1969; Wagner et al., 1984; Harborne, 1984.

Thin layer chromatography (TLC) isagain, gaining popularity as an important analytical tool for analysis of pesticides (U.S. National Library of Medicine, 1995).Use of additional, more polar solvent system todevelop TLC plates in future work an individual plants will allow better separation on these zones. Since the antimicrobial activity in species of Piperaceae family has been found with the amides, alkaloids, neoliganans and chromene (Dorman and Deans, 2000 and Costantin et al., 2001) it is possible that these compounds could be responsible for the antimicrobial properties.

An efficient HPTLC-UV/FTIR coupling procedure is presented for the separation and rapid identification of flurazepam by dichloride and its related substances in bulk powder and capsules. The optimized mobile phase for HPTLC-FTIR *in situ* measurement is containing a proportion of 50%

magnesium tungstate. The proposed procedure shows several advantages to the related compound procedure shows several advantages to the related compound test of the pharmacopoeia, e.g. baseline separation of the known impurities and detection of the substances as peaks in the UV, Gram-Schmidt or window chromatograms. Quantification of the related compounds was carried out densitometrically. This method was showed that the current resurgence of interest in modern instrumental TLC is rightful based on the flexibility and efficiency of this analytical method (Silke et al., 2001).

Nalina and Rahim, 2003 have been reported to *Piper betle* (sireh) have antibacterial and antimitotic properties. The purpose of this study was to develop a fast and simplified thin layer chromatography technique in the separations of the active antibacterial compound which can be used in bio autography. This study suggests that a suitable mobile phase for the separation is a mixture of non-polar and polar organic solvents.

Tikare et al., 2011 studied the effect of shade dried fruits of *Vitis vinifera* Linn. (Vitaceae) were subjected to successive solvent extraction with 70% ethanol. The extracts revealed the presence of carbohydrates, proteins, cardiac glycosides, alkaloids and flavonoids when subjected to chemical tests. TLC was run for their confirmation of flavonoids in *Vitis vinifera* and then subjected to isolation by preparative TLC method and analyzed by UV, FTIR and HPTLC. In thePharmacological screening, the alcoholic extract was used for evaluation of cognitive enhancing activity using elevated plus maze and passive avoidance task method with Mentat as standard by using parameters of step down and transfer latency. Induction was carried out by MES and scopolamine for 7 days. On 7th day the brain was isolatedfor evaluation of

acetyl cholinesterase enzyme activity. The alcoholic extracts (200mg/Kg) showed significant effect when compare to control, there was significant increase in step down latency and decrease in the transfers latency and also decrease in acetyl cholinesterase enzyme activity, which was an effective as that of standard drug. From this study it is concluded that fruits of *Vitis vinifera* contain flavonoids and hence possess significant cognitive enhancing activity.

2.14. Ultraviolet and visible absorption spectroscopy:

The intense UV absorption at 200nm was attributable to the n-n transition of the carbonyl group and other values at 210, 230 and 250nm correlated to the presence of benzene ring (Silverstein et al., 1981).

The UV-Vis spectrum of compound 3 showed a typicalpattern with a max of 506nm (Felix et al., 2008) and a shoulder at 400-500nm, indicating the presence of pelargonidinaglycone in the structure of this compound inagreement with the literature (Hong and Wrolstad, 1990). Thus, compound 3 was identified as pelargonidin 3-o-rutinoiside. Several authors have recently characterized this anthocyanin, in Moraceae fruits (Pawlowska et al., 2008) and in *Rucus aculeatus* berries (Longo and Vasapollo, 2005).

2.15. Fourier transform infrared spectrophotometer:

The FTIR bond at 3369cm^{-1} indicated the presence of N-H group and the two other prominent peaks at 1719 and 1650cm^{-1} confirmed the presence of two carbonyl groups (Bellamy, 1957). The C-O-C and C-N vibrations were observed at 1026, 1111 and 1276cm^{-1} respectively. The three bands at 1451, 1489 and 1537cm^{-1} favoured the presence of benzene ring (Raphael, 1969).

FTIR also used in the natural product chemistry for the identification of new compounds isolated from various plant species (Sati and Pant, 1985; awat et al., 1989; Purohit et al., 1991; Joshi et al., 1996 and Prasad et al., 1997).

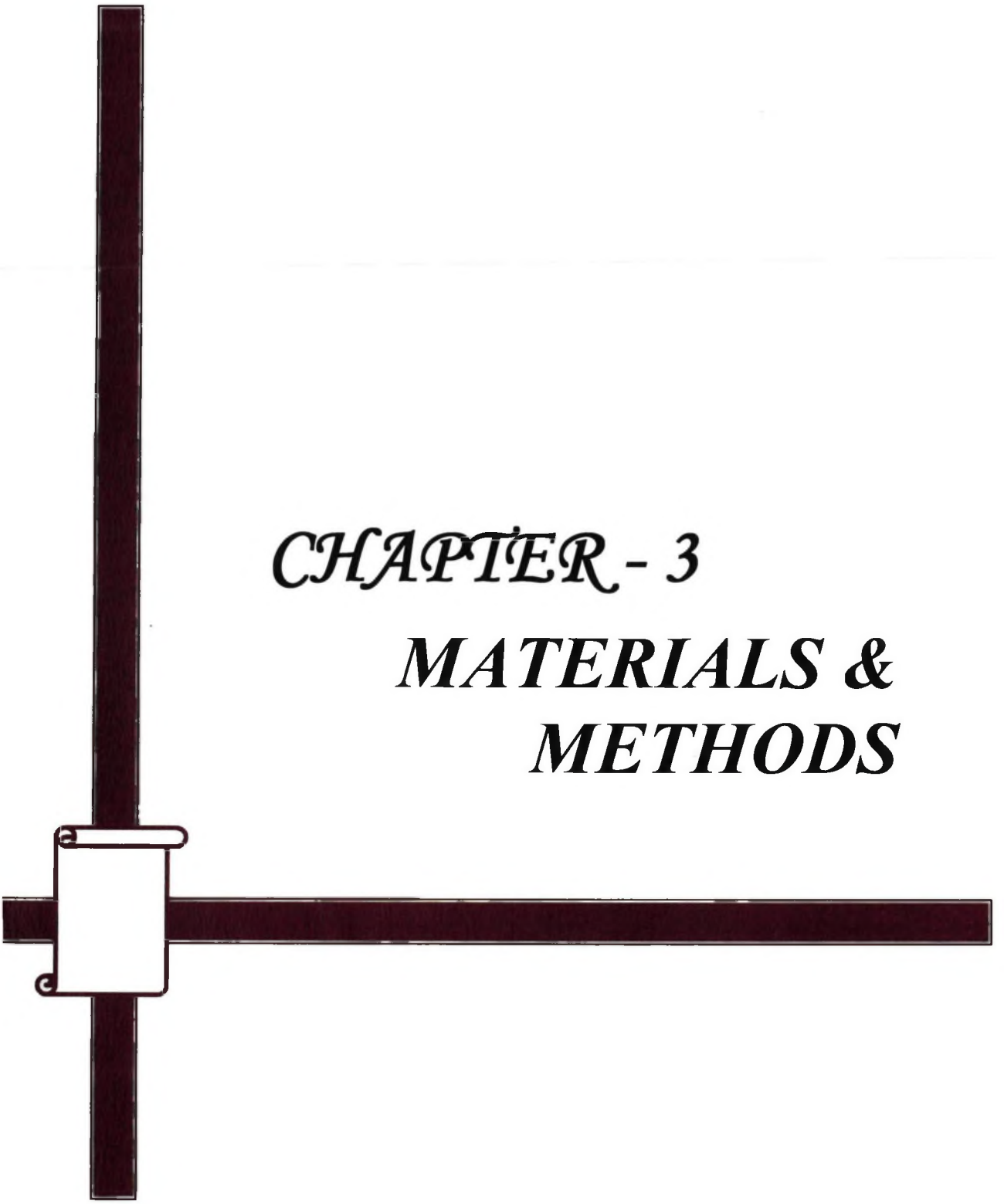
FTIR can be used to identify the structure of unknown composition or its chemical group and intensity of the absorption spectra associated with the molecular compositions or content of the chemical group (McCann et al., 1992; Surewicz et al., 1993). By acquiring IR spectra from plant samples, it could detect the minor changes of macromolecule compounds such as, carbohydrate, protein, lipid and cell wall proteins.

At present particularly in plant physiology research FTIR has been used to identify concrete structures of certain plant secondary metabolites (Yang and Yen, 2002; Ivanova and Singh, 2003 and Stehfest et al., 2005).

FTIR is one of the most widely used methods to identify chemical constituents and elucidation the compounds structures and has been used as a requisite method to identify medicines in pharmacopoeia of many countries. Owing to the fingerprints characters and extensive applicability to the samples, FTIR has played an important role in pharmaceutical analysis (Gough et al., 2003 and Vlachos et al., 2006).

One of the great advantages of FTIR is that any sample in any state can be studied and short time to obtain data. Liquid, solutions, pastes, powders, films, fibers, gases and surfaces can all be examined. FTIR has been recognized as a powerful analytical technique in the food industry (Schultz et al., 2002) and it has been employed to measure some quality parameters of oils (Schultz et al., 2004). It allows the qualitative determination of organic

compounds as the characteristics vibrational mode of each molecular group causes the appearance of bands in the infrared spectrum at a specific frequency, which is further influenced by surrounding functional groups (Schultz et al., 2003).



CHAPTER - 3

MATERIALS & METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1. Plant Materials:

Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens and *Antirrhinum majus* plants parts were collected from different locations of Amravati. Leaves and roots of these plants were used as the experimental material in the present investigation (Plate no. 1 and 2).

Initially leaves and roots of these plants were dried in sunlight, when materials were completely dried, then leaf and root powder prepared using grinder. Dried powdered material was used for the extraction of crude compound by Soxhlet method (Sadasivam and Manickam, 1996). For extraction of crude compounds 25gm of leaf powder and 12.5gm of root powder was used for extraction process. All the experiments were carried out in triplicates.

3.2. Preparation of Plant Extract:

Soxhlet method was used with petroleum ether, distilled water and acetone as solvent systems for extraction of crude compounds.

3.2.1. Soxhlet Method:

Thimbles were prepared using piece of Whatman filter paper no. 1 in such a way to hold the 25gm leaf and 12.5gm root powder respectively in a separate Soxhlet apparatus. Wrap around a second filter paper, which is left open at the top. A cotton wool is placed at the top for even distribution the solvent as it drops on the sample during extraction. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus. Extract with petroleum ether, distilled water and acetone (150 drops / min) for 24hrs without interruption by gentle heating at 40^0C for the petroleum ether, at 100^0C for the distilled water and at 30^0C for acetone. Allowed to

A) *Boerhavia diffusa* Linn.

B) *Withania somnifera* Dunal

C) *Calotropis procera* (Ait) Ait.f.

D) *Asclepias curassavica* Linn.

Plate No. 1: Vegetative / flowering twigs of selected plants.

E) *Vitex negundo* Linn.

F) *Hyptis suaveolens* (Linn.) Poit.

G) *Antirrhinum majus* Linn.

Plate No. 2: Vegetative / flowering twigs of selected plants.

cool and dismantled the extraction flask. The petroleum ether, distilled water and acetone were evaporated on a steam or water bath until no odor of petroleum ether, acetone and no content of distilled water remains and cooled at room temperature. Carefully removed the dirt or moisture outside the flask and weighed the flask. Heating for three times till constant weight is recorded.

3.2.2. Calculations:

$$\% \text{ Extract in Leaf and Root Powder} = \frac{\text{Weight of extract (g)}}{\text{Weight of sample}} \times 100$$

3.3. Doses selections and Preparation of Solutions:

For each extract, the crude compounds obtained from leaves and roots were first dissolved in 1 ml acetone and make up 100ml with distilled water. Selections of doses were made on the basis of preliminary experiments on *Allium cepa* system and doses were selected on the basis of LD_{50}. Doses concentrations used were 50, 100, 150 and 200µg/ml. Distilled water was used as a control and methotrexate was used as a standard control. Freshly prepared solutions were used for giving treatments.

3.4. *Allium* test system:

Allium test system was used for treatments. *Allium* test is one of the reliable model for screening drugs, chemical, pollutants and contaminants because root growth inhibition and adverse effects on chromosomes provide likely indication of toxicity. Since, the cells possess important activation enzymes; the *Allium* test has a wide area of application (Fiskesjo, 1988).

3.4.1. Germination of Onion bulb:

The onions were descaled, leaving the root primordial portion intact. Tap water was taken in coupling jar and descaled onion was placed on the top of each coupling jar

with root primordial region dipping in the water at room temperature 28 ± 0.5^{0}C for germination of root tips.

3.4.2. Method of Treatments:

To study the effects of leaf and root extracts. The germinated onion bulb with 2-3 cm long were treated with 50, 100, 150 and 200µg / ml solution of leaf petroleum ether extracts (LPE), leaf aqueous extracts (LDW) and leaf acetone extracts (LAE) for leaf and root petroleum ether extracts (RPE), root aqueous extracts (RDW) and root acetone extracts (RAE) for root. The treatment duration was three hours. Five germinated bulbs were directly dipped in solutions of leaf and root at 8^{0}C in Remi incubator. After the treatments the root tips were thoroughly washed under tap water for 5-6 times to remove excess of chemical sticking to roots. An individual control in distilled water and standard control in methotrexate were also maintained. Root tips of different length were taken and freshly prepared Carnoy's fluid I (3: 1), absolute ethyl alcohol and glacial acetic acid at different times to determine the frequency of highest mitotic activity for 24hrs For further studies the materials were preserved in 70% alcohol at 8^{0}C.

3.5. Cytological Studies:

3.5.1. Fixative:

Carnoy's Fluid I

It is a fixing mixture of glacial acetic acid (1 part) and absolute alcohol (3 parts).

3.5.2. Hydrolyzing agent:

1 N HCl

Take Conc. HCl 8.3 ml and add distilled water to make up the volume 100 ml.

3.5.3. Nuclear Stains (Appendix – I):

a. Aceto - carmine (2%):

45% acetic acid was prepared by adding 45 ml glacial acetic acid to 55 ml distilled water. 100 ml of 45% acetic acid was taken in beaker. The solution was heated to boil. 2gm carmine powder was added into it and let the solution simmer for 5 minutes. Let it settle unit it cool, filtered with circular Whatman filter paper no. 1 and stored in smoked glass bottle.

b. Aceto – Orcein (2%):

100 ml 45% glacial acetic acid was prepared; the solution was heated in conical flask to boiling. 2gm of Orcein powder was added slowly to the boiling solution, stirred with glass rod. Boil gently till the dye was dissolved completely (2 minutes), then cooled down at room temperature, filtered and stored in glass bottles with stopper.

3.5.4. Hydrolysis, Staining and Squashing:

Preserved root tips about 8-10 in number were taken in cavity block and hydrolyzed in 1N HCl at 60^0C in oven for 10 to 12 minutes and stained in 2% acetocarmine or 2% acetoorcein (Plate no.3) for 25 to 30 minutes. Deeply stained meristematic region of root tips i.e. 0.5 to 1mm in length were squashed on slide by applying very little pressure over cover glass. Squashes were made according to the method of Darlington and La Cour (1962). Sealed the edges of coverslip with paraffin bee wax mixture and observations were made from semi-permanent preparations. After observations photographs were taken with CCD camera and slides which were important cytologically were made permanent with N-butanol, acetic acid series.

For cytological observations, about 25 slides per concentrations were scored and results were recorded. The slides of control were observed for normal mitosis in which prophase having chromosomes become visibly distinct as long thin threads divided into

chromatids, each sister chromatid is attach to each other in region of the centromere. Nucleolus and nucleolar membrane are present. In metaphase disappearance of nuclear membrane and nucleolus occurred, spindle formation takes place which results into movement of chromosome at equatorial regions. In anaphase centromere divides, become functionally double, chromatid converted into independent chromosomes that separates and move to opposite poles and in telophase spindle disappears and reconstruction of nuclear envelopes about the two groups of offspring chromosomes begins, chromosomes uncoiled to become like slender thread and nucleoli reappears.

The leaf and root extracts of *Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens* and *Antirrhinum majus* treated slides were observed for abnormalities in mitosis shows clumped metaphase in which induction of cell cycle arrest at metaphase and subsequent apoptosis occur. In clumped metaphase chromosomes are intermingled with each other. In single and multiple chromatid bridges one or more chromatid become fragmented during anaphase and centromere of chromosome goes to the opposite pole and central position remains in between the two poles forming a bridge, precocious chromosome at telophase occur and reconstruction of nuclear envelop about the two groups of offspring chromosomes was arrested because of arrested telophase.

The duration of genotoxicity test is three to four weeks, including initial toxicity testing, scoring of aberrations statistics. All experiments were carried out thrice. Statistical analysis was performed using't' test for independent samples.

3.6. Parameters Studied:

Following parameters have been selected to study the genotoxic effects of leaf and root extracts of these plants in solvent petroleum ether, distilled water and acetone.

1) Mitotic Index:

Mitotic index in terms of percentage frequency of dividing cells was taken into consideration. Active mitotic index was calculated by scoring only metaphase and from the total dividing cells. It was computed as follows.

$$\text{Active mitotic index} = \frac{\text{No. of metaphase} + \text{No. of anaphase}}{\text{Total No. of cells observed}} \times 100$$

2) Chromosomal Aberrations:

Chromosomal aberrations in mitosis were calculated by scoring about 1200 to 1400 meristematic cells for each treatment.

a. Aberrations in various metaphases such clumped metaphase was scored.
b. Aberrations in various anaphases such as bridge / bridges were scored.
c. Aberration in various telophases such as arrested telophases and precocious chromosome at telophases were also scored.

Percentage of aberrant metaphases, anaphases and telophases of each type of irregularity were calculated by using formula.

$$\text{Percentage of aberrant stages} = \frac{\text{Total No. of aberrant stages}}{\text{Total No. of dividing cells}} \times 100$$

Microscopic examination allows assessment of chromosome damage and cell division disturbances, thus providing additional information as to the severity or mechanism of the toxic effect or potential mutagenicity.

3.7. Statistical Analysis:

Pooled data of three independent replicates of each treatment were used for determining the standard error (Panse and Sukhatme, 1954). Statistical analysis was carried out applying the t-test (independent samples) to test the significance of the difference between the two sample means by the following formula.

When we have given the two random samples of sizes n_1 and n_2 respectively and we want to test the average effect of these two sample means.

$$t = \frac{\overline{X} - \overline{Y}}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

For the $(n_1 + n_2 - 2)$ degree of freedom.

$$\text{Where, } S^2 = \frac{1}{n_1 + n_2 - 2}\left[\sum(Xi - \overline{X})^2 + \sum(Yj - \overline{Y})^2\right]$$

Where. $\mathbf{n_1}$ - first sample,

$\mathbf{n_2}$ - second sample,

$\overline{\mathbf{X}}$ - Arithmetic mean of first sample

$\overline{\mathbf{Y}}$ - Arithmetic mean of second sample

$\mathbf{X_i}$ - No. of observation of first sample,

$\mathbf{Y_j}$ - No. of observations of second sample.

3.8. UV-Vis Spectrophotometer:

Sample preparation by Thin Layer Chromatography:

Silica gel plates were prepared by mixing the adsorbent, silica gel and water (1:2). This mixture was spread as thick slurry on glass. The resultant plate was dried and activated by heating in an oven for thirty minutes at 110°C. The thickness of the adsorbent layer is typically around 0.1 - 0.25 mm for chromatography. The petroleum ether, distilled water and acetone 1mg of leaf and root extracts were dissolved in 100µl of acetone. A small spot of solution of the sample is applied to a plate, about 1.5cm from the bottom edge. Methanol as a solvent was used for separation of various compounds. Different compounds in the sample mixture travel at different rates due to the differences in R_f values of the chemical compound and affinity to the stationary phase.The developed chromatograms were air dried and the bands were scraped off

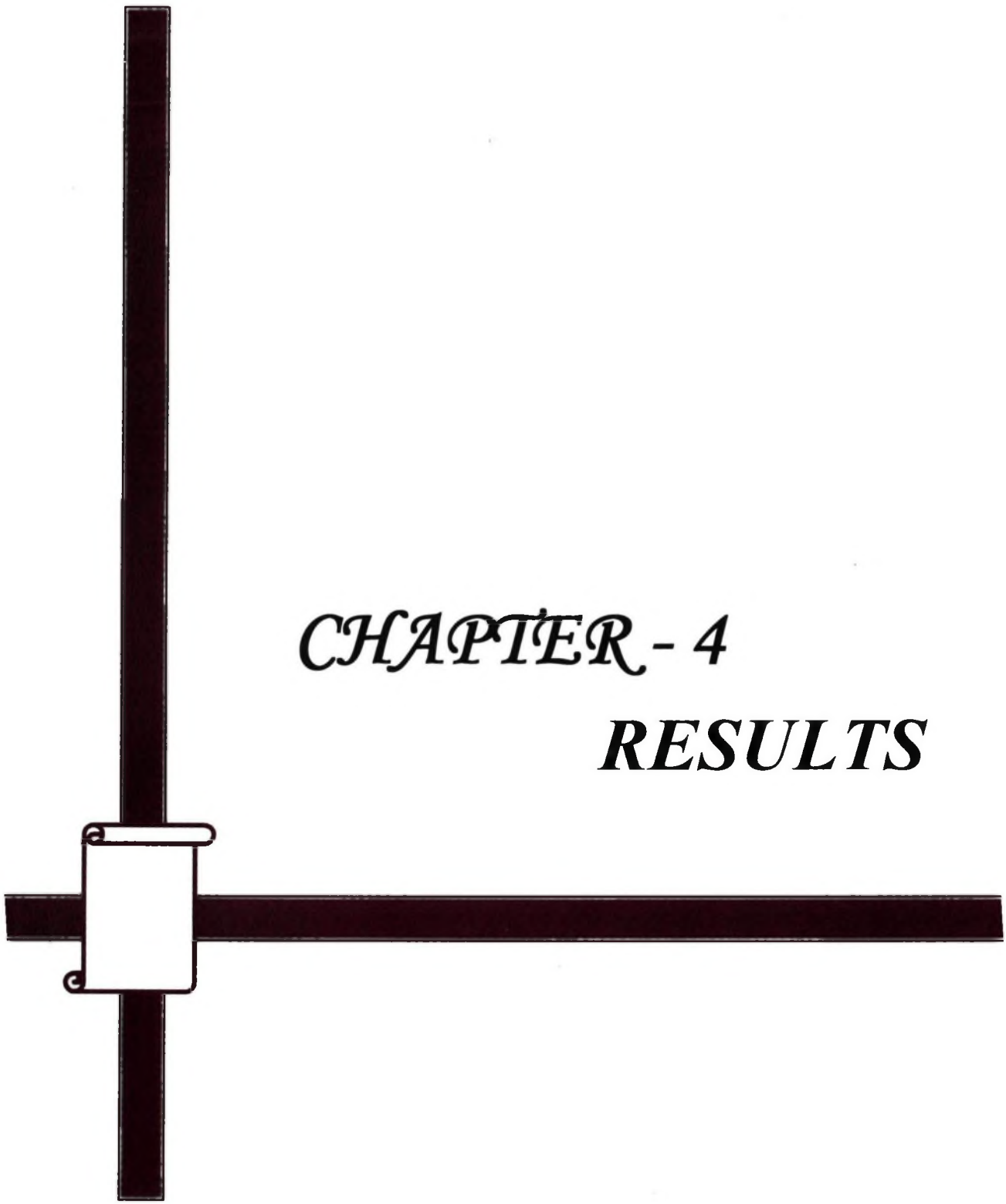
separately and dissolved in 10ml of acetone and methanol for examined under UV-Vis spectrophotometer region at 190-1100nm.

3.9. Fourier transform infrared spectrophotometer (FTIR):

Prepare a concentrated solution of leaf and root extracts in a suitable solvent i. e. acetone and distilled water dissolve it in a small test tube first and transfer this solution with a pipet onto the IR plates. FTIR spectrophotometer was used for the analysis of leaf and root extracts. The spectrum was focused in mid IR region of 400-4000cm^{-1}.

Appendix – I: Stains used, their formulae, molecular weights and sources

S.N.	Chemicals	Formula	Molecular Weight	Source
1	Carmine	$C_{22}H_{20}O_{13}$	492.38	SELKROM
2	Orcein	$C_{28}H_{24}N_2O_7$	500.488	BDH



CHAPTER - 4

RESULTS

CHAPTER 4

RESULTS

The inhibitory effects of leaf and root extracts of *Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens* and *Antirrhinum majus* in petroleum ether, distilled water and acetone as solvent was evaluated on the mitotic activity of *Allium cepa* root meristems and the effect was compared with water as a control and methotrexate as standard control. The mitotic cells were counted in above groups at 3 hours of treatment with each concentrations i.e. leaf and root extracts in petroleum ether, distilled water and acetone as solvent i.e. 50µg, 100µg, 150µg and 200µg per ml concentrations. Leaf and root of each plant produced a significant decrease in mitotic index that was dose and time dependent.

Table 1: Quantification of extracts from leaf and root powder of seven plants (by Soxhlet method).

Plant Samples	Weight of sample (gm)	Extraction solvent	Wt. of extract (gm)
1.***Boerhavia diffusa*** Leaf powder	25	Petroleum ether	1.077
	25	Aqueous (D.W.)	0.340
	25	Acetone	0.797
Root powder	12.5	Petroleum ether	0.154
	12.5	Aqueous (D.W.)	1.118
	12.5	Acetone	0.099
2. ***Withania somnifera*** Leaf powder	25	Petroleum ether	0.615
	25	Aqueous (D.W.)	10.624
	25	Acetone	1.166
Root powder	12.5	Petroleum ether	0.081
	12.5	Aqueous (D.W.)	1.996
	12.5	Acetone	0.308
3. ***Calotropis procera*** Leaf powder	25	Petroleum ether	0.874
	25	Aqueous (D.W.)	11.240
	25	Acetone	0.986

Table Contd.....

Plant Samples	Weight of sample (gm)	Extraction solvent	Wt. of extract (gm)
3. *Calotropis procera* Root powder	12.5	Petroleum ether	0.270
	12.5	Aqueous (D.W.)	1.895
	12.5	Acetone	0.397
4. *Asclepias curassavica* Leaf powder	25	Petroleum ether	0.228
	25	Aqueous (D.W.)	0.411
	25	Acetone	0.256
Root powder	12.5	Petroleum ether	0.186
	12.5	Aqueous (D.W.)	0.122
	12.5	Acetone	0.066
5.*Vitex negundo* Leaf powder	25	Petroleum ether	1.911
	25	Aqueous (D.W.)	6.23
	25	Acetone	3.142
6. *Hyptis suaveolens* Leaf powder	25	Petroleum ether	0.711
	25	Aqueous (D.W.)	3.593
	25	Acetone	0.725
Root powder	12.5	Petroleum ether	0.071
	12.5	Aqueous (D.W.)	0.899
	12.5	Acetone	0.382
7. *Antirrhnum majus* Leaf powder	25	Petroleum ether	0.228
	25	Aqueous (D.W.)	14.545
	25	Acetone	1.696
Root powder	12.5	Petroleum ether	0.106
	12.5	Aqueous (D.W.)	2.453
	12.5	Acetone	0.422

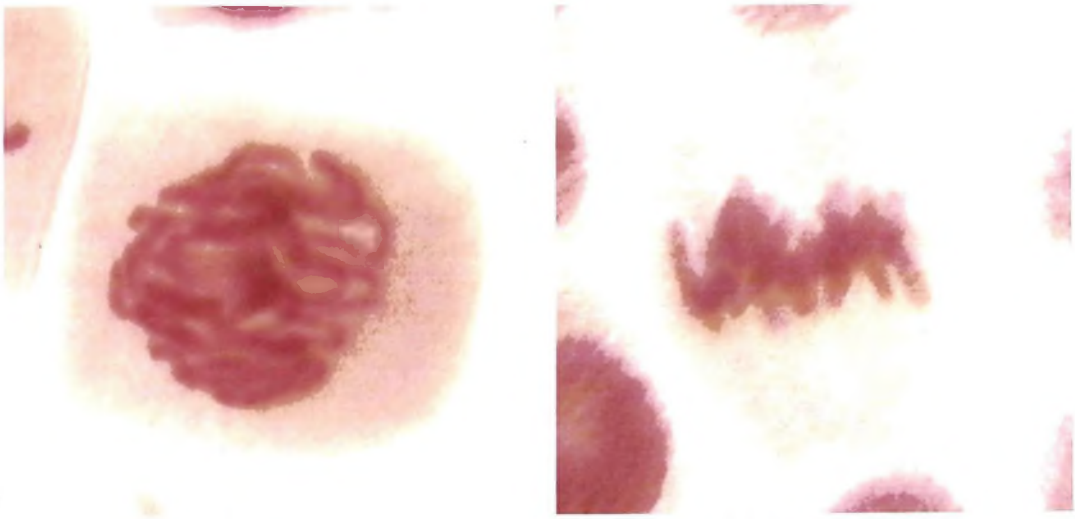
4.1. *Boerhavia diffusa*:

4.1.1. Quantification:

The data on quantification of petroleum ether, distilled water and acetone is given in Table 1. From 25gm of leaf powder 1.077gm, 0.340gm and 0.797gm of crude compounds were obtained whereas, from 12.5gm of root powder 0.154gm, 1.118gm and 0.099gm of crude compounds were obtained by using leaf petroleum ether, distilled water and acetone as solvent, respectively.

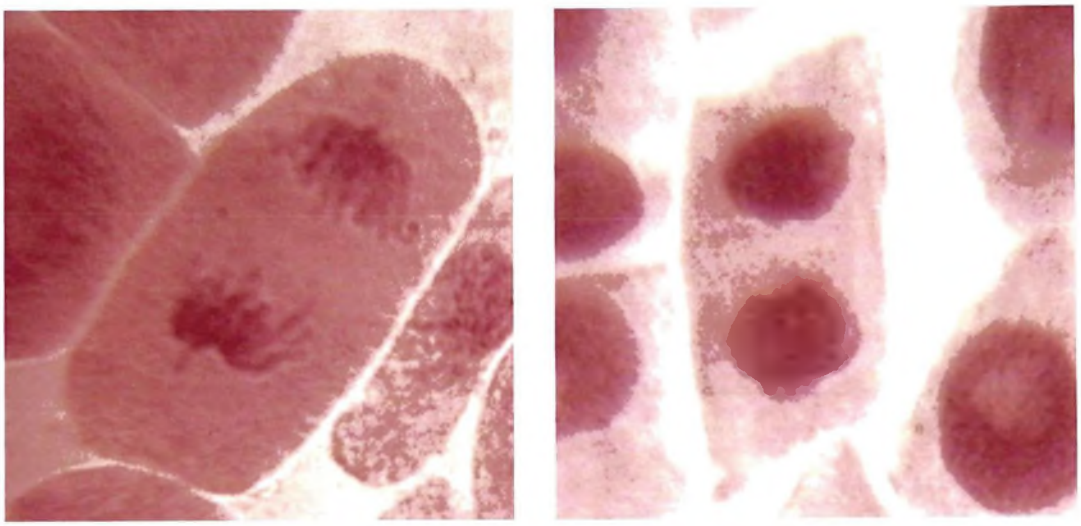
4.1.2. Mitotic index:

The data on effect of leaf and root extracts of *B. diffusa* on mitotic index and active mitotic index is tabulated in Table 2 and 3. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase, metaphase, anaphase



Prophase

Metaphase



Anaphase

Telophase

Plate No. 3: Microphotograph showing Normal Mitosis – *Allium cepa* (2n=16)

and telophase were recorded (Plate no. 3). The mitotic index frequency in control was 11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively for 3hrs of methotrexate treatment.

Table 2: Effect of leaf extracts of *Boerhavia diffusa* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	1889	36	39	17	24	115	6.03 ± 0.53	2.96	1.20
	100	2098	47	34	34	30	145	6.91 ± 0.28	3.24	0.63
	150	1938	49	40	24	32	145	7.48 ± 0.29	3.30	0.65
	200	1822	42	41	23	31	137	7.51 ± 0.18	3.51	0.41
LDE	50	2167	38	33	29	23	123	5.67 ± 0.31	2.86	0.70
	100	1721	39	37	19	20	115	6.68 ± 0.37	3.25	0.84
	150	1697	30	34	24	26	114	6.78 ± 0.29	3.41	0.66
	200	2071	43	49	30	30	152	7.33 ± 0.36	3.81	0.81
LAE	50	2190	27	40	20	26	113	5.15 ± 0.14	2.73	0.33
	100	2223	47	45	32	24	148	6.65 ± 0.37	3.46	0.84
	150	1930	38	38	23	31	130	6.73 ± 0.27	3.16	0.61
	200	1850	29	42	29	28	128	6.91 ± 0.25	3.83	0.57

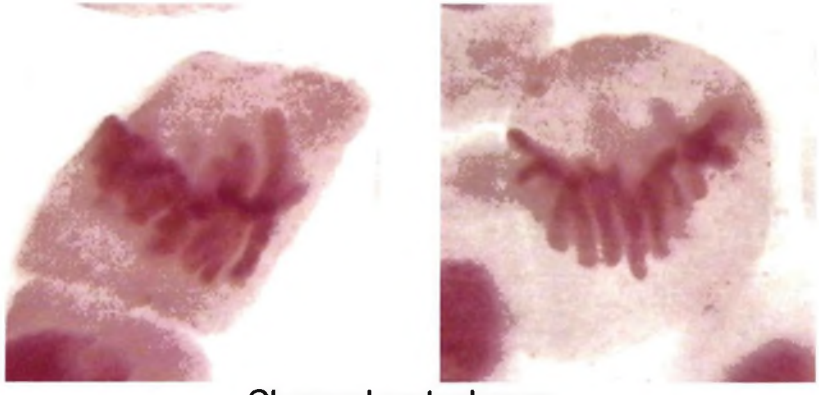
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root showed mitotic index frequencies were 6.03%, 6.91%, 7.48% and 7.51% (LPE); 5.67%, 6.68%, 6.78% and 7.33% (LDE); 5.15%, 6.65%, 6.73% and 6.91% (LAE); 5.52%, 6.31%, 6.52% and 6.67% (RPE); 5.15%, 5.40%, 6.38% and 7.57% (RDE) and 4.95%, 5.81%, 5.87% and 6.00% (RAE) in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

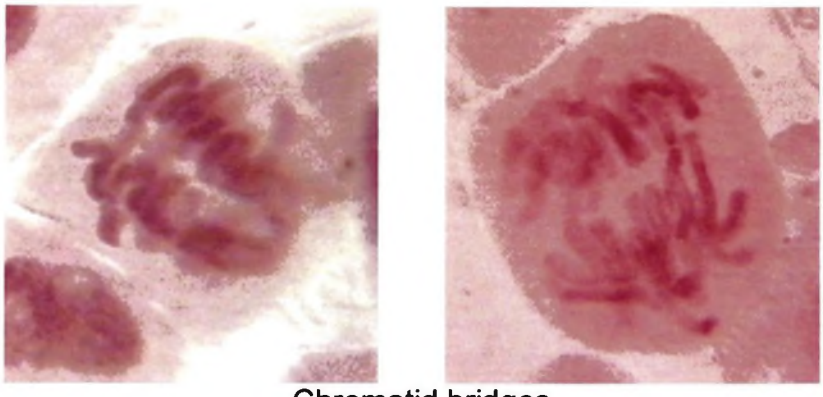
Table 3: Effect of root extracts of *Boerhavia diffusa* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	1935	36	34	17	20	107	5.52 ± 0.35	2.62	0.79
	100	1804	40	34	16	24	114	6.31 ± 0.18	2.77	0.41
	150	2083	48	46	21	21	136	6.52 ± 0.30	3.21	0.68
	200	1812	34	45	17	25	121	6.67 ± 0.46	3.42	1.04
RDE	50	2135	35	28	19	28	110	5.15 ± 0.26	2.20	
	100	2109	36	41	19	18	114	5.40 ± 0.38	2.84	0.85
	150	2035	41	43	13	33	130	6.38 ± 0.16	2.75	0.36
	200	1967	49	52	30	18	149	7.57 ± 0.58	4.16	1.31
RAE	50	1979	28	30	19	21	98	4.95 ± 0.27	2.47	0.62
	100	2097	44	35	21	22	122	5.81 ± 0.16	2.67	0.37
	150	1924	33	39	17	24	113	5.87 ± 0.22	2.91	0.50
	200	1933	37	37	19	23	116	6.00 ± 0.28	2.89	0.63

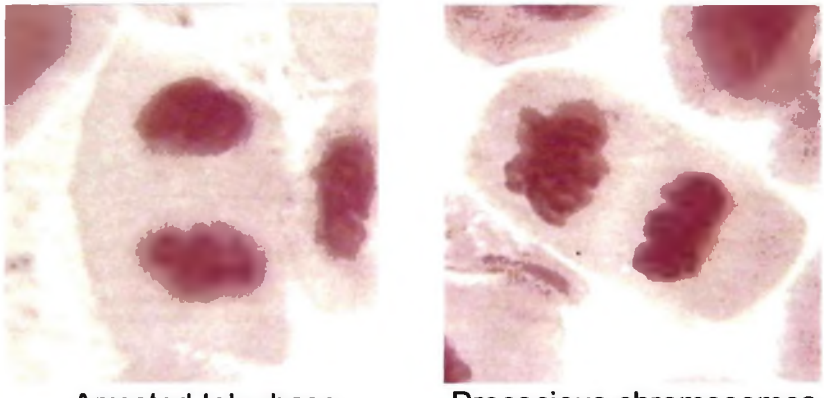
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.



Clumped metaphases



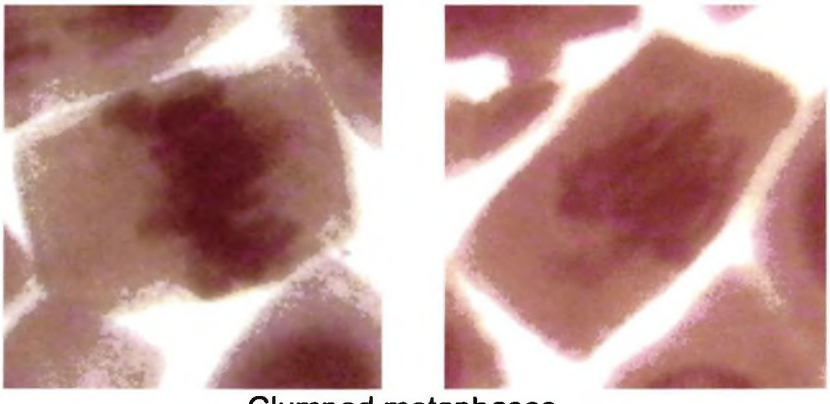
Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No. 4: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of Methotrexate.



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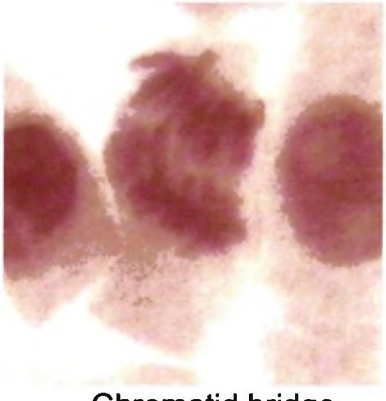
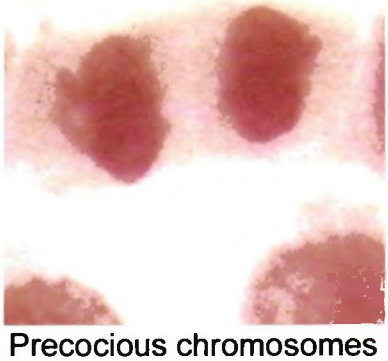
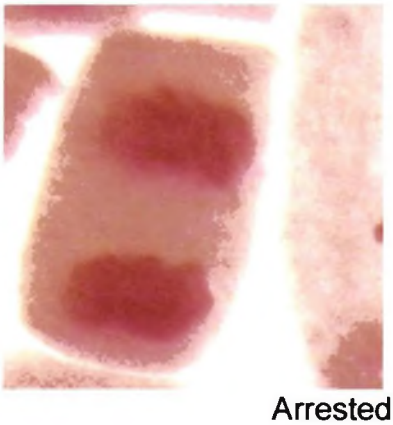
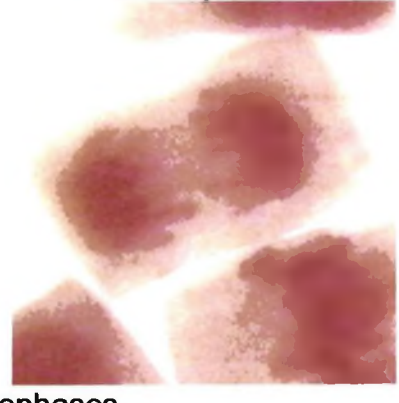


Chromatid bridge



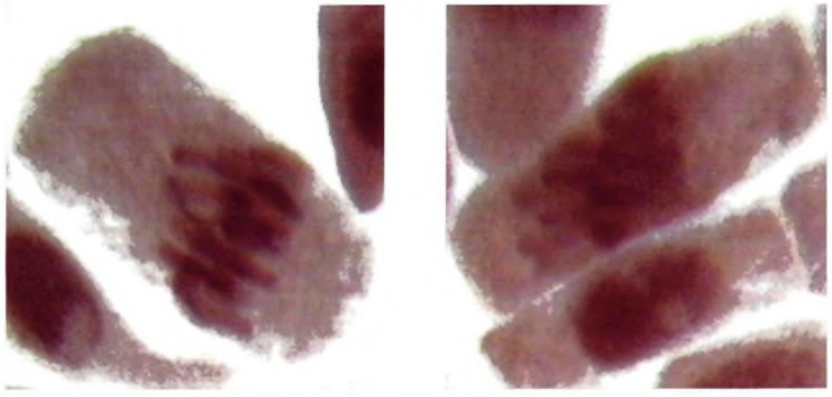
Precocious chromosomes at telophase



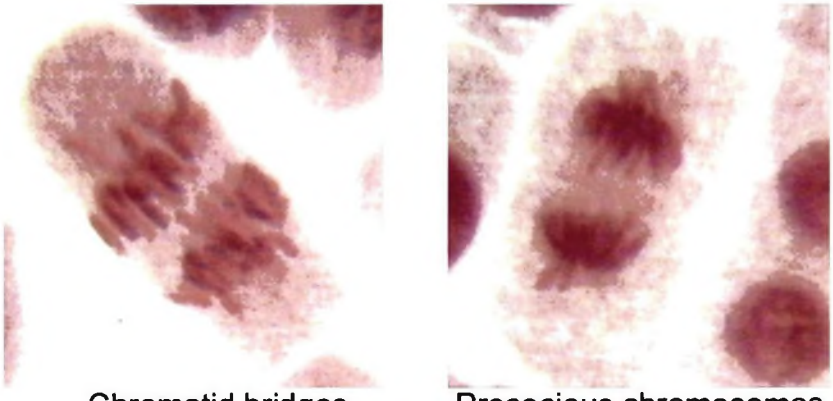


Arrested telophases

Plate No. 5: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* leaf petroleum ether extract.

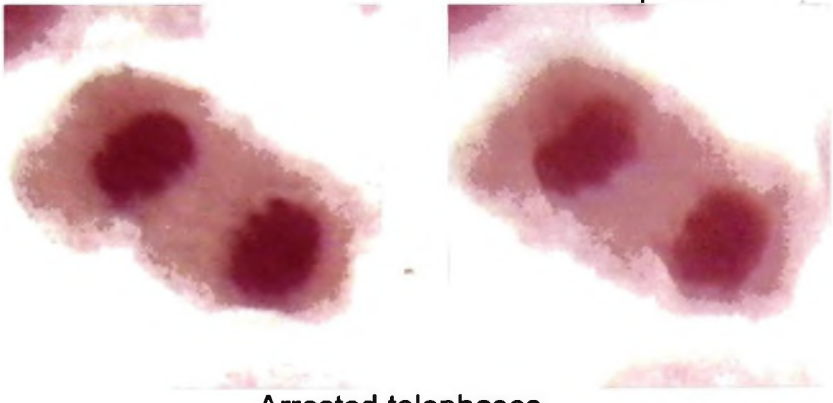


Clumped metaphases



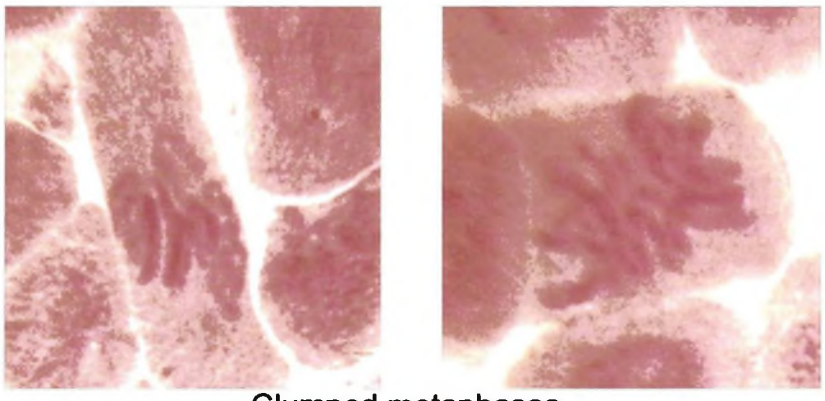
Chromatid bridges

Precocious chromosomes at telophase

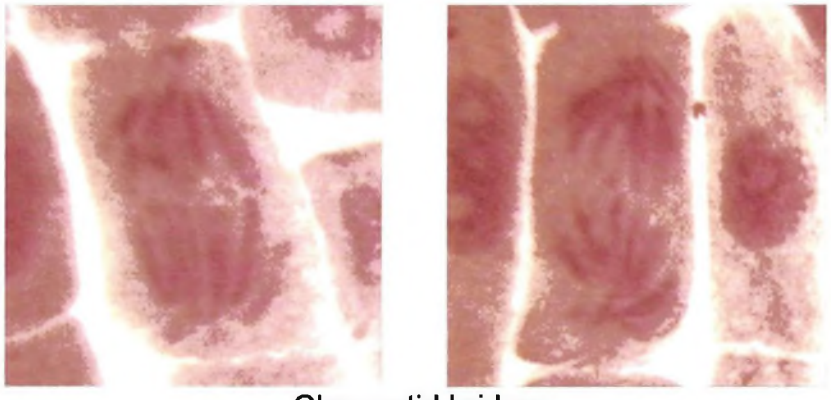


Arrested telophases

Plate No. 6: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* leaf aqueous extract.

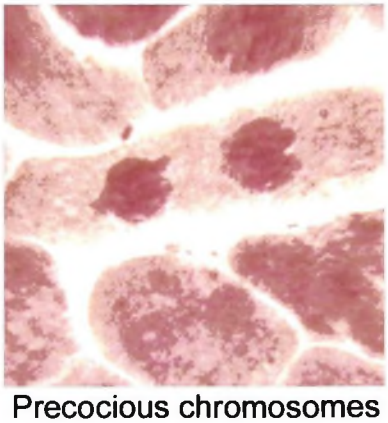


Clumped metaphases



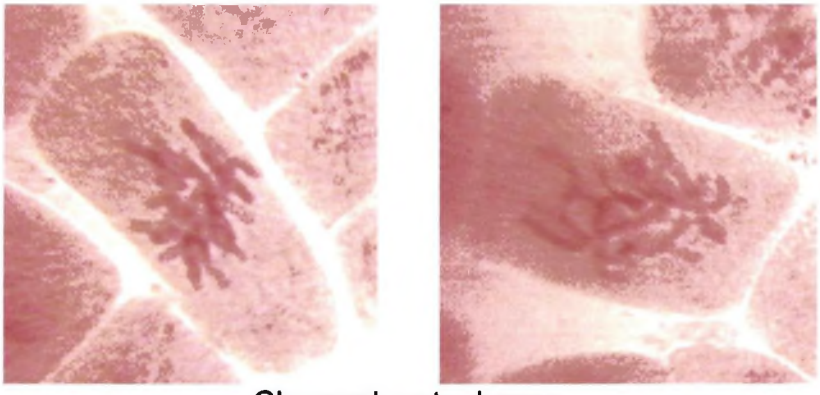
Chromatid bridges

Arrested telophase

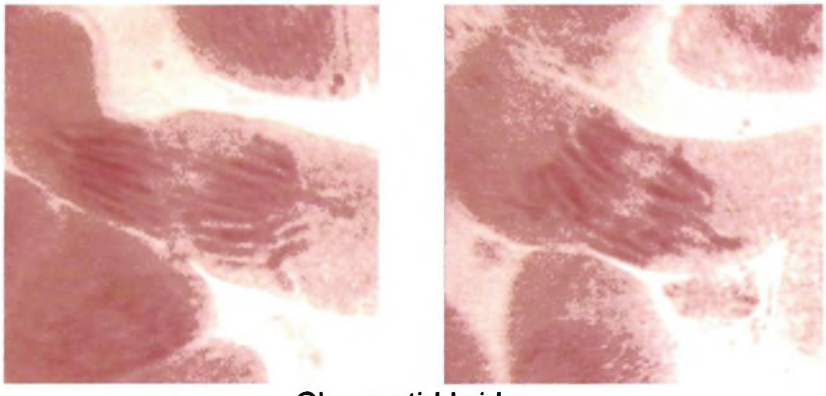


Precocious chromosomes at telophase

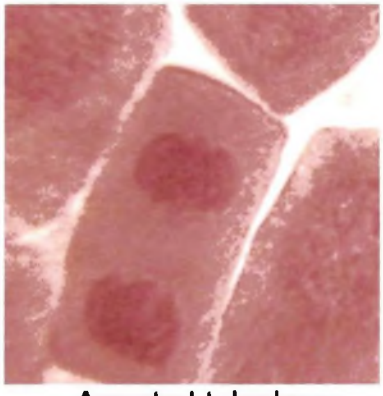
Plate No. 7: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* leaf acetone extract.



Clumped metaphases



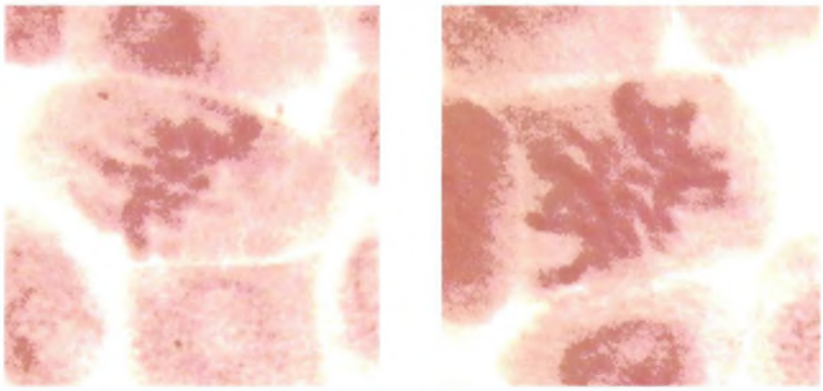
Chromatid bridges



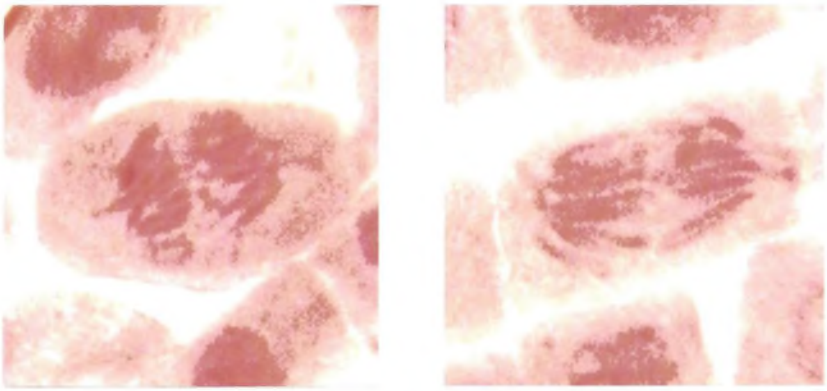
Arrested telophase

Precocious chromosomes at telophase

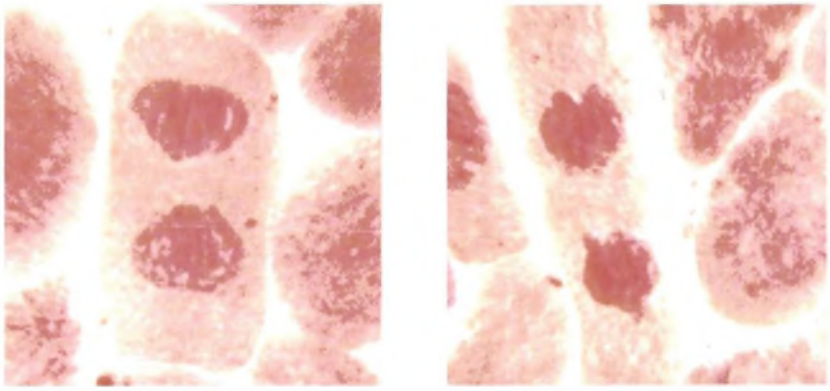
Plate No. 8: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* root petroleum ether extract.



Clumped metaphases



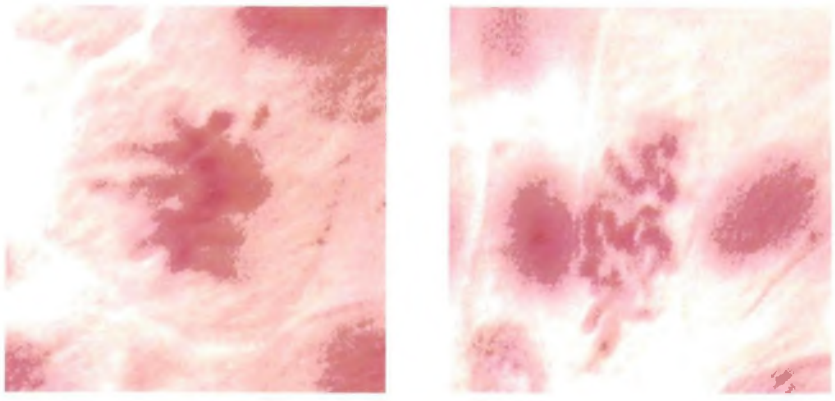
Chromatid bridges



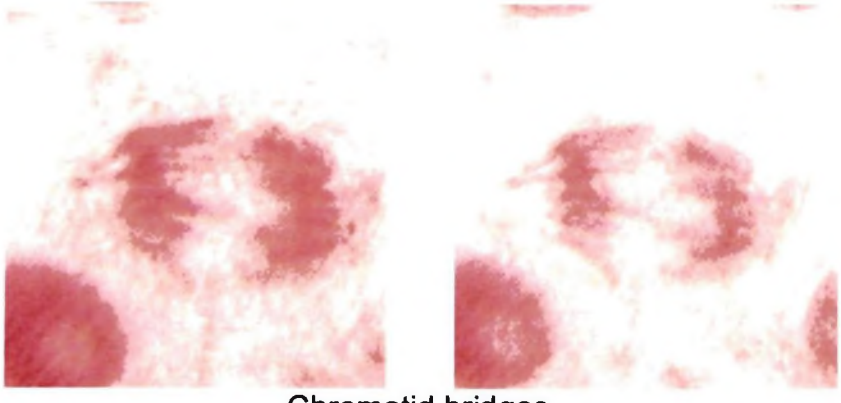
Arrested telophase

Precocious chromosomes at telophase

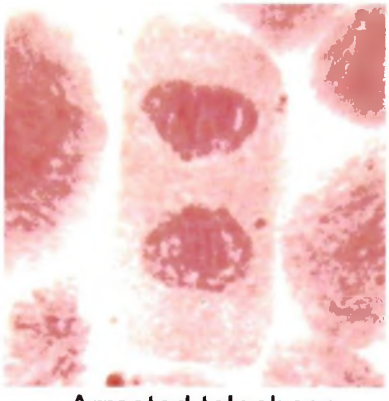
Plate No. 9: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* root aqueous extract.



Clumped metaphases



Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No.10: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Boerhavia* root acetone extract.

4.1.3. Active mitotic index:

Active mitotic index frequencies in 50µg, 100µg, 150µg and 200µg/ml concentrations were 2.96%, 3.24%, 3.30% and 3.51% (LPE); 2.86%, 3.25%, 3.41% and 3.81% (LDE); 2.73%, 3.46%, 3.16% and 3.83% (LAE); 2.62%, 2.77%, 3.21% and 3.42% (RPE); 2.20%, 2.84%, 2.75% and 4.16% (RDE) and 2.47%, 2.67%, 2.91% and 2.89% (RAE) respectively for 3 hours of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

4.1.4. Cytological abnormalities:

Table 4: Effect of leaf extracts of *Boerhavia diffusa* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosonal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	1889	11	39	13	10	73	3.90
	100	2098	18	34	17	14	83	3.91
	150	1938	14	40	18	13	85	4.38
	200	1822	12	41	19	11	83	4.55
LDE	50	2167	19	33	12	14	78	3.59
	100	1721	12	37	10	10	69	4.00
	150	1697	16	34	11	12	73	4.36
	200	2071	24	49	14	16	103	4.97
LAE	50	2190	15	40	11	15	81	3.69
	100	2223	15	45	14	10	84	3.77
	150	1930	14	38	17	14	83	4.30
	200	1850	18	42	16	12	88	4.75

CB – Chromatid Bridges, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

The cytological abnormalities are scored in mitotic cells and results are shown in Table 4 and 5. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into observable cytological changes (Plate no. 5-10). These were chromatid bridges, clumped metaphases, arrested telophases and precocious

chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 4, 5 and Plate no. 4) for 3hrs in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 5: Effect of root extracts of *Boerhavia diffusa* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosonal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
RPE	50	1935	11	34	12	12	69	3.56
	100	1804	11	34	15	11	71	3.93
	150	2083	15	46	6	13	80	3.84
	200	1812	14	45	11	13	83	4.58
RDE	50	2135	12	28	17	16	73	3.41
	100	2109	18	41	6	12	77	3.65
	150	2035	11	43	18	13	85	4.17
	200	1967	24	52	14	14	104	5.28
RAE	50	1979	13	33	15	15	76	3.84
	100	2097	14	35	14	10	73	3.48
	150	1924	10	39	11	13	73	3.77
	200	1933	13	37	9	14	73	3.79

CB – Chromatid Bridges, **CM** – Clumped metaphases, **AT** = Arrested Telophases, **PT** = Precocious chromosomes at Telophase.

The frequencies of chromosomal abnormalities in 50µg, 100µg, 150µg and 200µg per ml concentrations in leaf and root extracts treatments were 3.90%, 3.91%, 4.38% and 4.55% (LPE); 3.59%, 4.00%, 4.36% and 4.97% (LDE); 3.69%, 3.77%, 4.30% and 4.75% (LAE); 3.56%, 3.93%, 3.84% and 4.58% (RPE); 3.41%, 3.65%, 4.17% and 5.28% (RDE) and 3.84%, 3.48%, 3.77% and 3.79% (RAE), respectively for 3hrs treatments. No mitotic aberrations were recorded in control. The above data indicated that the chromosomal aberrations were increased with increase in concentration except in root acetone extract.

4.1.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by 't' test (Table 6 and 7). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg, 150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Table 6: *Boerhavia diffusa* leaf extracts:- Calculated value of t-test (an independent Sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	6.13
Control and 100µg / ml Leaf petroleum ether extracts	6.43
Control and 150µg / ml Leaf petroleum ether extracts	5.50
Control and 200µg / ml Leaf petroleum ether extracts	5.93
Control and 50µg / ml Leaf aqueous extracts	8.11
Control and 100µg / ml Leaf aqueous extracts	6.24
Control and 150µg / ml Leaf aqueous extracts	6.55
Control and 200µg / ml Leaf aqueous extracts	5.38
Control and 50µg / ml Leaf acetone extracts	10.01
Control and 100µg / ml Leaf acetone extracts	6.28
Control and 150µg / ml Leaf acetone extracts	6.76
Control and 200µg / ml Leaf acetone extracts	6.56

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 6.13, 6.43, 5.50 and 5.93

(LPE); 8.11, 6.24, 6.55 and 5.38 (LDE); 10.01, 6.28, 6.76 and 6.56 (LAE); 8.02, 7.90, 6.89 and 5.74 (RPE); 9.29, 7.99, 7.89 and 4.06 (RDE) and 9.47, 8.83, 8.38 and 7.83 (RAE) respectively. These calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore null hypothesis is rejected i.e. the values of $|t|$ are significant hence samples are dependent on each other when treated with control.

Table 7: *Boerhavia diffusa* root extracts: - Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.02
Control and 100µg / ml Methotrexate	7.90
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	8.02
Control and 100µg / ml Root petroleum ether extracts	7.90
Control and 150µg / ml Root petroleum ether extracts	6.89
Control and 200µg / ml Root petroleum ether extracts	5.74
Control and 50µg / ml Root aqueous extracts	9.29
Control and 100µg / ml Root aqueous extracts	7.99
Control and 150µg / ml Root aqueous extracts	7.89
Control and 200µg / ml Root aqueous extracts	4.06
Control and 50µg / ml Root acetone extracts	9.47
Control and 100µg / ml Root acetone extracts	8.83
Control and 150µg / ml Root acetone extracts	8.38
Control and 200µg / ml Root acetone extracts	7.83

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

4.1.6. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. The results of UV-Vis showed that the intense absorption at 220, 255 and 296nm (LPE), 219, 252 and 294nm (LDE) 220, 255 and 294nm (LAE)

and 220, 255 and 295nm (RPE), 215, 252 and 296nm (RDE) and 220 and 299 (RPE) using acetone and at 220, 255 and 298nm (LPE), 220, 255 and 292nm (LDE) , 220, 255 and 294nm (LAE), 254nm (RPE), 257 and 296nm (RDE) and 218 and 292nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 237nm (LPE), 238nm (LDE), 235nm (LAE), 237nm (RPE), 237nm (RDE) and 241nm (RAE) in acetone and 235nm (LPE), 236nm (LDE), 239nm (LAE), 273nm (RPE), 235nm (RDE) and 263nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 8 and 9).

Table 8: UV- Vis and FTIR bands in the spectra of leaf extracts of *Boerhavia diffusa.*

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		**Leaf aqueous extract**		**Leaf acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
220	220	219	220	220	220
255	255	252	255	255	-
296	298	294	292	294	296
237	235	238	236	235	239

FTIR ANALYSIS (cm^{-1})			
Functional groups	**Leaf petroleum ether extract**	**Leaf aqueous extract**	**Leaf acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3433.89	3448.84, 1592.29	3485.49
Alkane	2928.52	2924.66	2928.04
C-H	2859.56	2866.32, 1451.48	2857.15, 1451.48
C=O	1734.55	-	1731.65
C-O	1219.05	1245.09	1219.53
C-H (phenyl ring)	772.03	773.98	835.69
C=C	1642.92	-	1636.65
Std. Taxol	1079.69 to 3498.99	1067.15 to 3448.84	1077.76 to 3485.49
NO_2	-	-	1367.58
C,H (aromatic bends)	1079.69	1067.15	1077.76

The FTIR band at 3433.89cm^{-1} (LPE), 3448.84 and 1592.29cm^{-1} (LDE), 3485.49 and 1557.57cm^{-1} (LAE), 3429.55, 1551.3cm^{-1} (RPE), 3439.19, 1597.11cm^{-1} (RDE) and 3410.74cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O,

C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2928.52, 2859.56, 1734.55, 1219.05, 772.03, 1642.92 and 1079.69cm^{-1}(LPE), 2924.66, 2866.32, 1451.48, 1245.09, 773.98 and 1067.15cm^{-1}(LDE), 2928.04, 2857.15, 1451.48, 1731.65, 1219.53, 835.69, 1636.65 and 1077.76cm^{-1} (LAE), 2926.59, 2864.87, 1451, 1727.79, 1219.53, 1610.13 and 1079.69cm^{-1} (RPE), 2926.11, 2861.49, 1427.85, 1725.87, 1242.68, 766.25 and 1067.64cm^{-1} (RDE) and 2927.08, 2859.57, 1451, 1720.56, 1220.02, 772.03, 1604.35 and 1075.83cm^{-1} (RAE) respectively whereas, C=O and C=C groups were absent in leaf aqueous extract, C-H (phenyl ring) in root petroleum ether extract and C=C in root aqueous extract.

Table 9: UV- Vis and FTIR bands in the spectra of root extracts of *Boerhavia diffusa*.

UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		**Root aqueous extract**		**Root acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
220	-	215	-	220	218
255	254	252	257	-	-
295	-	296	296	299	292
237	273	237	235	241	263

FTIR ANALYSIS (cm^{-1})			
Functional groups	**Root petroleum ether extract**	**Root aqueous extract**	**Root acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3429.55, 1551.3	3439.19, 1597.11	3410.74
Alkane	2926.59	2926.11	2927.08
C-H	2864.87, 1451	2861.49, 1427.85	2859.57, 1451
C=O	1727.79	1725.87	1720.56
C-O	1219.53	1242.68	1220.02
C-H (phenyl ring)	-	766.25	772.03
C=C	1610.13	-	1604.35
Std. Taxol	1079.69 to 3476.32	1067.64 to 3439.19	1075.83 to 3488.86
NO_2	1365.16	-	1366.13
C,H (aromatic bends)	1079.69	1067.64	1075.83

Peaks at 1079.69 to 3498.99cm-1 (LPE), 1067.15 to 3448.84cm^{-1}(LDE), 1077.76 to 3485.49cm^{-1} (LAE), 1079.69 to 3476.32cm^{-1} (RPE), 1067.64 to 3439.19cm^{-1} (RDE)

and 1075.83 to 3488.86cm^{-1} (RAE) favoured the presence of standard taxol group. At 1367.58cm^{-1} indicated presence of NO_2 (nitro compounds) in leaf acetone extract and absent in leaf petroleum ether and aqueous extracts and at 1365.16cm^{-1} and 1366.13cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract.

4.2. *Withania somnifera:*

4.2.1. Quantification:

The data on quantification of petroleum ether, distilled water and acetone is given in Table. 1. From 25gm of leaf powder 0.615gm, 10.624gm and 1.166gm of crude compounds were obtained whereas, from 12.5gm of root powder 0.081gm, 1.996gm and 0.308gm of crude compounds were obtained by using petroleum ether, distilled water and acetone as solvent, respectively.

4.2.2. Mitotic index:

The data on effect of leaf and root extracts of *W. somnifera* on mitotic index and active mitotic index is tabulated in Table 10 and 11. The cell divisions were differentiated into number of cells in each phase of cell division i.e. prophase, metaphase, anaphase and telophase were recorded.

The mitotic index frequency, in control was 11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively for 3hrs of methotrexate treatment.

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root in 50µg, 100µg, 150µg and 200µg per ml concentrations showed mitotic index

frequencies were 5.51%, 6.50%, 7.61% and 8.62% (LPE); 7.99%, 8.41%, 9.47% and 10.12% (LDE); 4.94%, 5.69%, 6.00% and 6.71% (LAE); 5.48%, 5.51%, 6.01% and 8.26% (RPE); 4.34%, 5.30%, 6.98% and 7.12% (RDE) and 5.34%, 6.58%, 6.74% and 6.88% (RAE), respectively.

Table 10: Effect of leaf extracts of *Withania somnifera* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	1985	30	41	22	18	111	5.51 ± 0.32	3.17	0.72
	100	2066	35	57	24	20	136	6.50 ± 0.34	3.92	0.77
	150	2024	48	51	31	26	156	7.61 ± 0.49	4.05	1.11
	200	1935	48	65	29	27	169	8.62 ± 0.18	4.85	0.41
LDE	50	2152	60	58	30	24	172	7.99 ± 0.52	4.08	1.18
	100	2211	59	60	32	35	186	8.41 ± 0.30	4.16	0.68
	150	2016	60	63	34	34	191	9.47 ± 0.72	4.81	1.61
	200	1778	58	58	33	31	180	10.12±0.40	5.11	0.91
LAE	50	2083	30	31	19	23	103	4.94 ± 0.29	2.40	0.66
	100	2159	43	47	16	17	123	5.69 ± 0.21	2.91	0.47
	150	1964	35	36	13	34	118	6.00 ± 0.20	2.49	0.46
	200	2026	40	45	23	28	136	6.71 ± 0.22	3.35	0.50

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

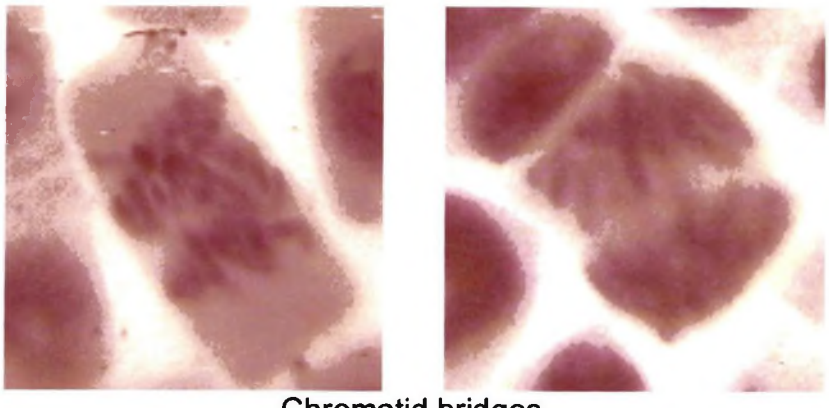
4.2.3. Active mitotic index:

Active mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 3.17%, 3.92%, 4.05% and 4.85% (LPE); 4.08%, 4.16%, 4.81% and 5.11% (LDE); 2.40%, 2.91%, 2.49% and 3.35% (LAE); 2.43%, 2.81%, 2.71% and 3.99% (RPE); 2.08%, 2.56%, 3.35% and 3.01% (RDE) and 2.75%, 3.33%, 3.85% and 3.48% (RAE), respectively for 3 hours of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

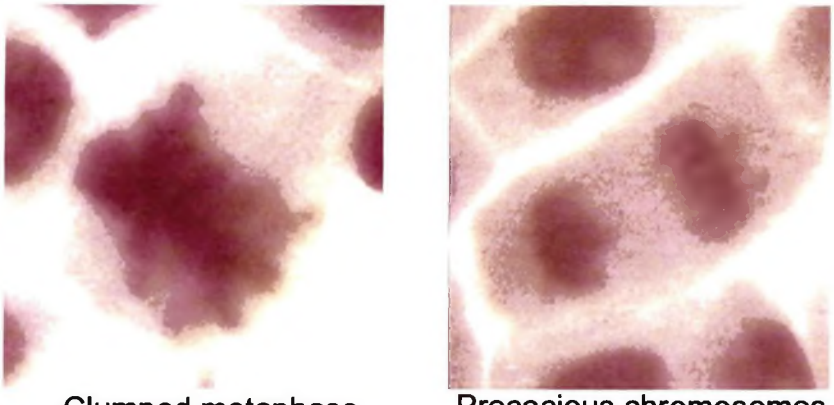
Table 11: Effect of root extracts of *Withania somnifera* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	1934	37	36	11	22	106	5.48 ± 0.33	2.43	0.75
	100	2027	39	44	13	16	112	5.51 ± 0.43	2.81	0.96
	150	1928	41	34	19	22	116	6.01 ± 0.36	2.71	0.82
	15	22	40	43	12	19	114	8.26 ± 0.41	3.99	0.93
RDE	50	2346	32	35	14	21	102	4.34 ± 0.34	2.08	0.77
	100	2260	39	39	19	23	120	5.30 ± 0.34	2.56	0.76
	150	1790	37	43	17	28	125	6.98 ± 0.28	3.35	0.63
	200	2022	49	40	21	34	144	7.12 ± 0.07	3.01	0.17
RAE	50	2356	45	48	17	16	126	5.34 ± 0.11	2.75	0.25
	100	2070	42	49	20	24	135	6.58 ± 0.41	3.33	0.93
	150	1840	40	54	17	11	122	6.74 ± 0.52	3.85	1.17
	200	2065	42	52	20	26	140	6.88 ± 0.51	3.48	1.15

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

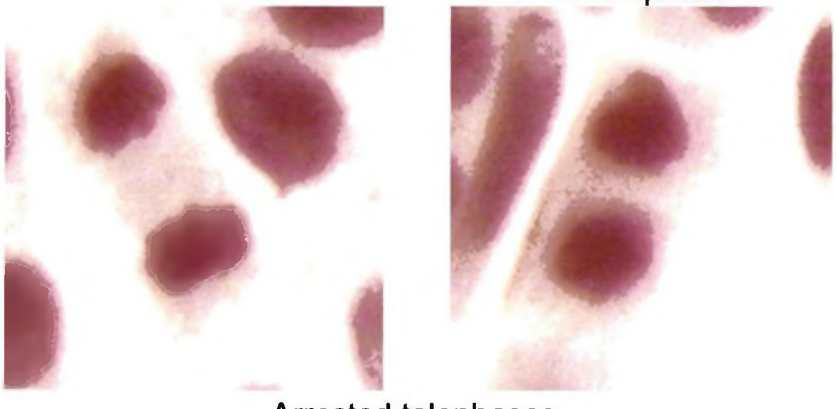


Chromatid bridges



Clumped metaphase

Precocious chromosomes at telophase



Arrested telophases

Plate No.11: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* leaf petroleum ether extract.

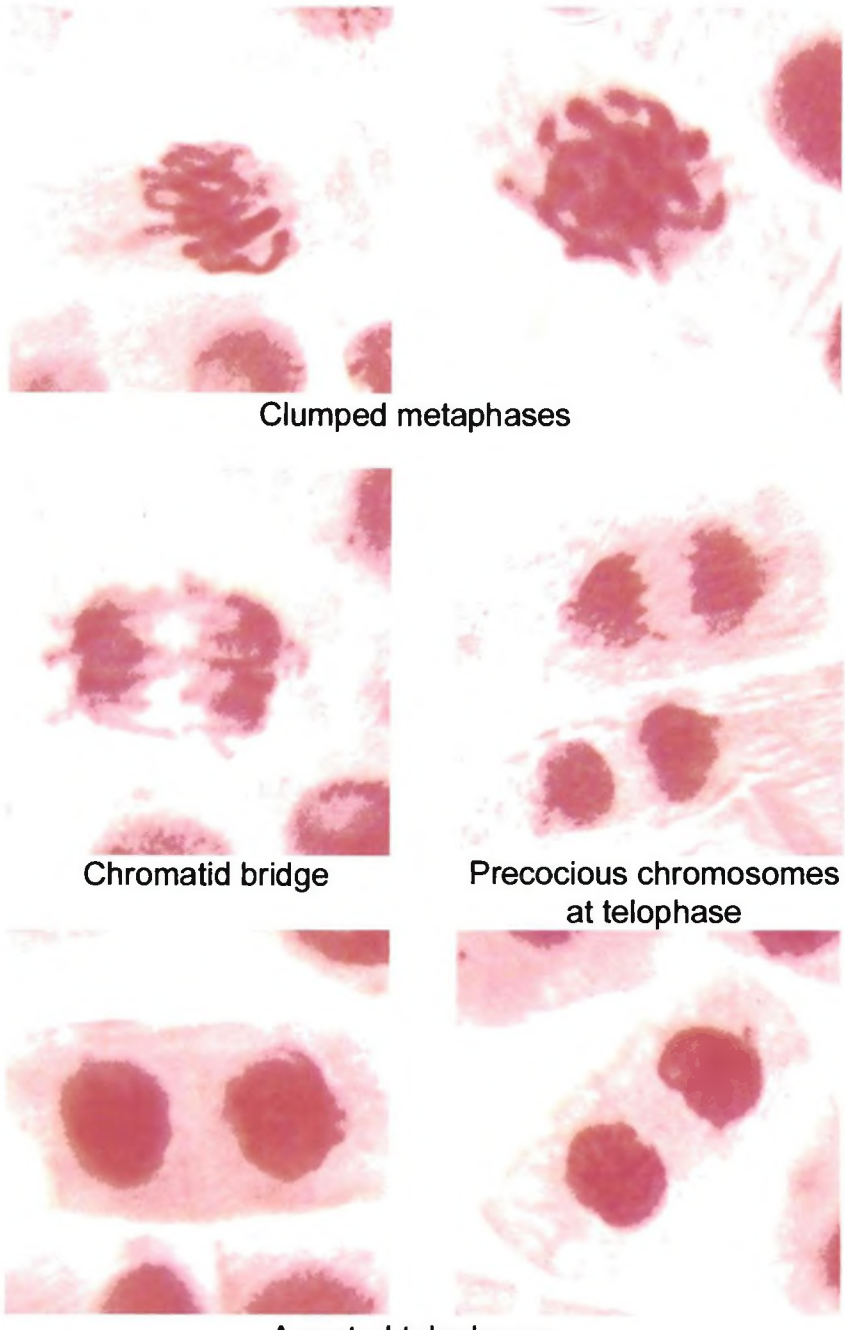
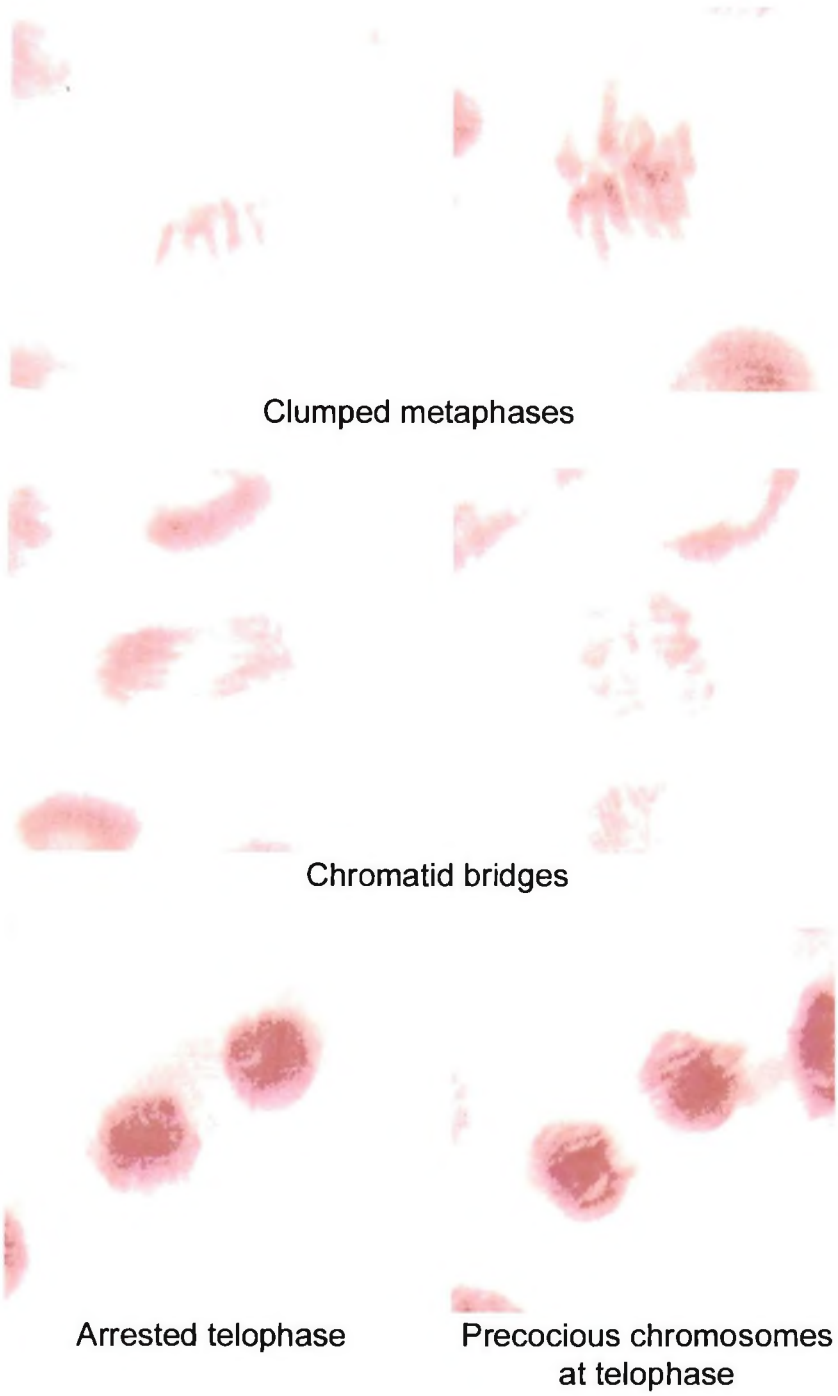


Plate No.12: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* leaf aqueous extract.



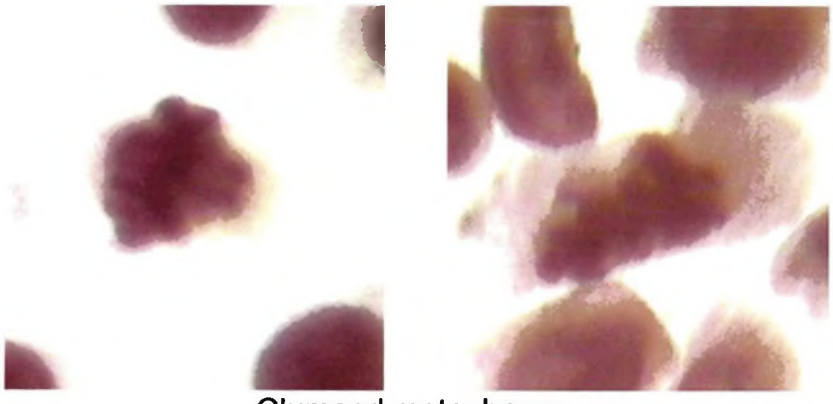
Clumped metaphases

Chromatid bridges

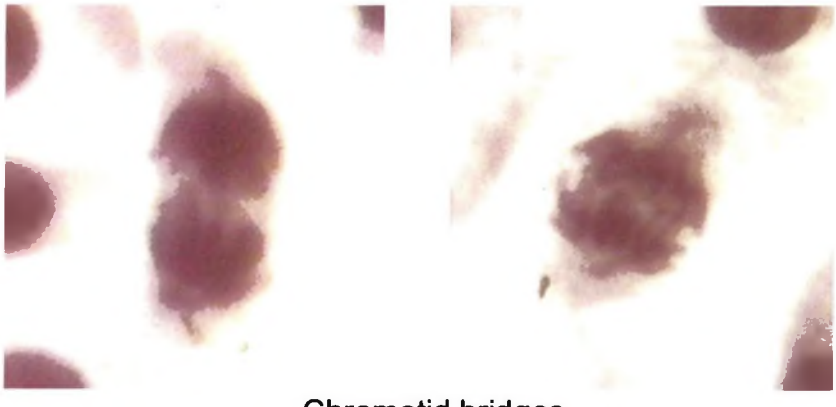
Arrested telophase

Precocious chromosomes at telophase

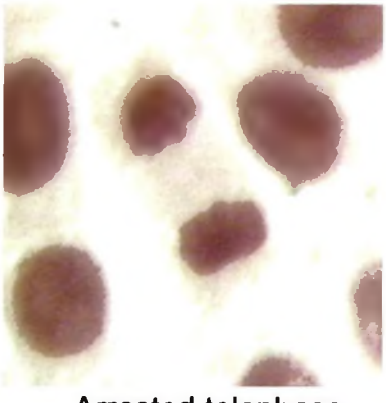
Plate No.13: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* leaf acetone extract.



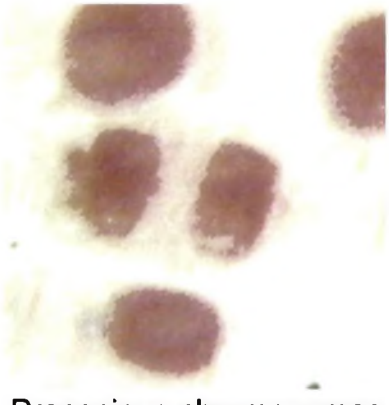
Clumped metaphases



Chromatid bridges

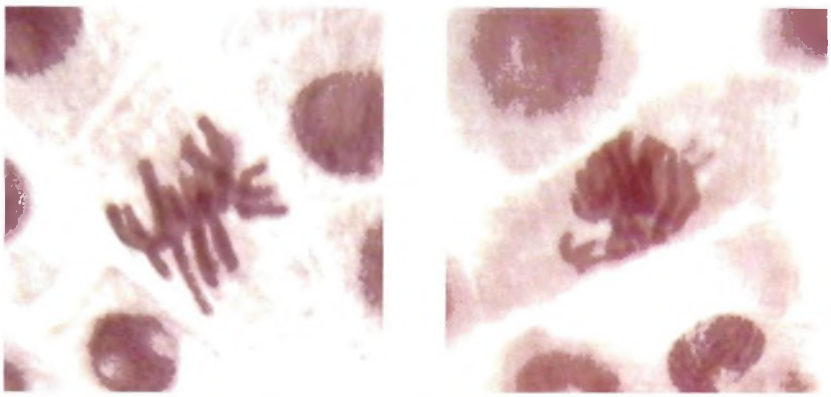


Arrested telophase

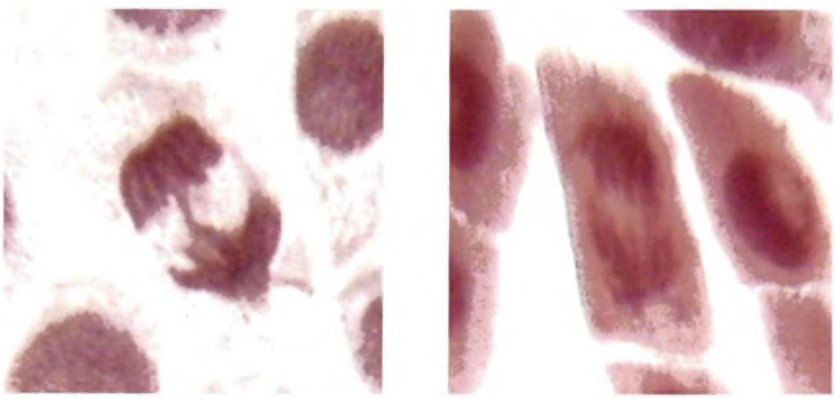


Precocious chromosomes at telophase

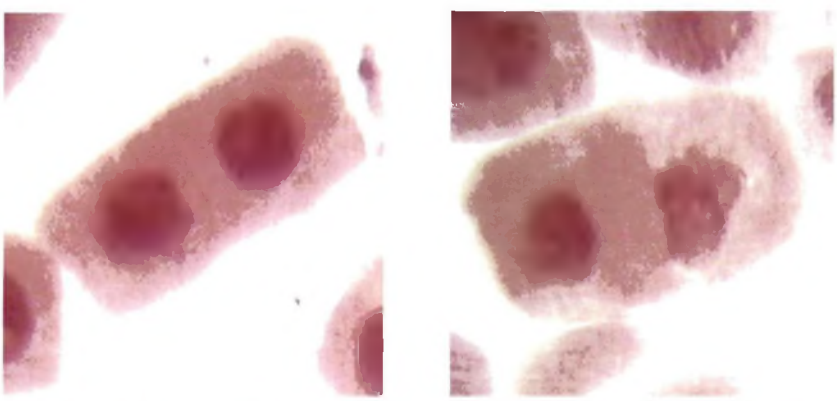
Plate No.14: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* root petroleum ether extract.



Clumped metaphases



Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No.15: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* root aqueous extract.

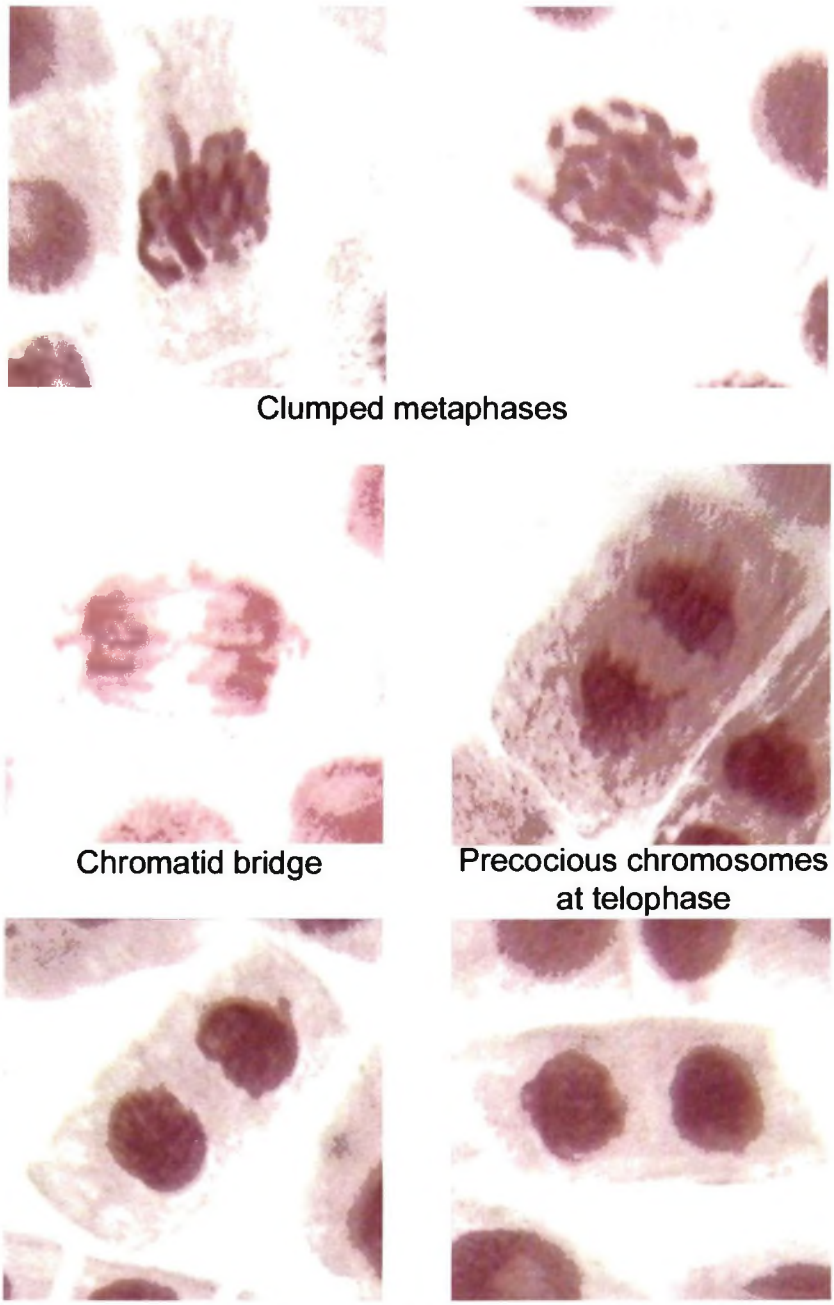


Plate No.16: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Withania* root acetone extract.

4.2.4. Cytological abnormalities:

The cytological abnormalities in mitotic cells and results are tabulated in Table 12 and 13. The treatment with standard control (MTX) Plate no. 4, leaf and root extracts resulted into observable cytological changes (Plate no. 11-16). These were chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 12, 13 and Plate no. 4) for 3hrs in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 12: Effect of leaf extracts of *Withania somnifera* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	1985	7	41	13	4	65	3.27
	100	2066	11	57	14	6	88	4.17
	150	2024	12	51	20	5	88	4.31
	200	1935	12	65	20	7	104	5.29
LDE	50	2152	17	58	11	12	98	4.52
	100	2211	20	60	20	15	115	5.20
	150	2016	22	63	15	19	119	5.90
	200	1778	24	58	14	15	111	6.24
LAE	50	2083	15	31	11	12	69	3.31
	100	2159	12	47	14	13	86	3.98
	150	1964	11	36	15	18	80	4.07
	200	2026	12	45	16	12	85	4.19

CB – Chromatid Brides, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

The frequencies of chromosomal abnormalities in 50µg, 100µg, 150µg and 200µg per ml in leaf and root extracts treatments were 3.27%, 4.17%, 4.31% and 5.29% (LPE); 4.52%, 5.20%, 5.90% and 6.24% (LDE); 3.31%, 3.98%, 4.07% and 4.19% (LAE); 3.56%, 3.35%, 3.68% and 5.58% (RPE); 2.77%, 3.27%, 4.69% and 4.54%

(RDE) and 3.48%, 4.34%, 5.00% and 4.45% (RAE) respectively for 3hrs treatments. No mitotic aberrations were recorded in control (DW).

Table 13: Effect of root extracts of *Withania somnifera* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
RPE	50	1934	11	36	9	13	69	3.56
	100	2027	8	44	6	10	68	3.35
	150	1928	15	34	8	14	71	3.68
	200	1378	9	43	11	14	77	5.58
RDE	50	2346	9	35	8	13	65	2.77
	100	2260	13	39	7	15	74	3.27
	150	1790	13	43	10	18	84	4.69
	200	2022	18	40	16	18	92	4.54
RAE	50	2356	13	48	8	13	82	3.48
	100	2070	17	49	7	17	90	4.34
	150	1840	14	54	10	14	92	5.00
	200	2065	15	52	10	15	92	4.45

CB – Chromatid Brides, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

Different types of chromosomal abnormalities were increased with increase in concentrations whereas; in root distilled water chromosomal abnormalities were increased at 150µg as compared to 200µg extract.

4.2.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by 't' test (Table 14 and 15). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg,

150µg and 200µg per ml dosage which represented its mutagenic /genotoxic actions in *Allium cepa.*

Table 14: *Withania somnifera* leaf extracts: - Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	8.60
Control and 100µg / ml Leaf petroleum ether extracts	6.38
Control and 150µg / ml Leaf petroleum ether extracts	4.30
Control and 200µg / ml Leaf petroleum ether extracts	4.57
Control and 50µg / ml Leaf aqueous extracts	3.78
Control and 100µg / ml Leaf aqueous extracts	4.04
Control and 150µg / ml Leaf aqueous extracts	1.64
Control and 200µg / ml Leaf aqueous extracts	1.34
Control and 50µg / ml Leaf acetone extracts	9.33
Control and 100µg / ml Leaf acetone extracts	8.75
Control and 150µg / ml Leaf acetone extracts	8.30
Control and 200µg / ml Leaf acetone extracts	7.12

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (MTX); 8.60, 6.38, 4.30 and 4.57 (LPE); 3.78, 4.04, 1.64 and 1.34 (LDE); 9.33, 8.57, 8.30 and 7.12 (LAE); 14.71, 7.48, 7.23 and 3.83 (RPE); 9.81, 8.46, 6.31 and 6.90 (RDE) and 9.86, 6.12, 5.28 and 5.29 (RAE) respectively.

These calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore, null hypothesis is rejected i.e.

the values of |t| are significant hence samples are dependent on each other when treated with control whereas in 150µg (1.64) and 200µg (1.34) the calculated values i.e. of 't' are less than tabulated value (2.3) therefore null hypothesis is accepted and samples are not significant as compared with control.

Table 15: *Withania somnifera* root extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	14.71
Control and 100µg / ml Root petroleum ether extracts	7.48
Control and 150µg / ml Root petroleum ether extracts	7.23
Control and 200µg / ml Root petroleum ether extracts	3.83
Control and 50µg / ml Root aqueous extracts	9.81
Control and 100µg / ml Root aqueous extracts	8.46
Control and 150µg / ml Root aqueous extracts	6.31
Control and 200µg / ml Root aqueous extracts	6.90
Control and 50µg / ml Root acetone extracts	9.86
Control and 100µg / ml Root acetone extracts	6.12
Control and 150µg / ml Root acetone extracts	5.28
Control and 200µg / ml Root acetone extracts	5.19

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

4.2.6. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. . The results of UV-Vis showed that the intense absorption at 220 and 297nm (LPE), 222, 252 and 293nm (LDE) 221, 255 and 293nm (LAE) and 220, 254 and 297nm (RPE), 219 and 252 nm (RDE) and 216, 252 and 296 (RAE) using acetone and at 220 and 255nm (LPE), 255 and 296nm (LDE) , 220 and

255nm (LAE), 221, 254 and 295nm (RPE), 219, 254 and 296nm (RDE) and 223 and 252nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 235nm (LPE), 236nm (LDE), 239nm (LAE), 237nm (RPE), 235nm (RDE) and 236nm (RAE) in acetone and 236nm (LPE), 235nm (LDE), 239nm (LAE), 272nm (RPE), 236nm (RDE) and 235nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 16 and 17).

Table 16: UV- Vis and FTIR bands in the spectra of leaf extracts of *Withania somnifera.*

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		**Leaf aqueous extract**		**Leaf acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
220	220	222	225	221	220
-	255	252	-	255	255
297	-	293	296	294	-
235	236	239	239	235	235

FTIR ANALYSIS (cm^{-1})			
1Functional groups	**Leaf petroleum ether extract**	**Leaf aqueous extract**	**Leaf acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3443.05	3446.43, 1598.08	3448.36, 1595.67
Alkane	2925.15	2923.7	2926.11
C-H	1451.48	2861.01	2859.08
C=O	1730.21	-	-
C-O	1220.5	1246.06	1224.36
C-H (phenyl ring)	611.94	830.38	833.28
C=C	1643.89	-	-
Std. Taxol	1084.03 to 3443.05	1075.83 to 3488.38	1079.21 to 3490.79
NO_2	1365.65	-	1370.47
C,H (aromatic bends)	1084.03	1075.83	1079.21

The FTIR band at 3443.05cm^{-1} (LPE), 3446.43 and 1598.08cm^{-1} (LDE), 3448.36 and 1595.67cm^{-1} (LAE), 3434.85cm^{-1} (RPE), 3443 and 1596cm^{-1} (RDE) and 3474.4 and 1552.27cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2925.15, 1451.48, 1730.21, 1220.5, 611.94, 1643.89 and 1084.03cm^{-1} (LPE), 2923.7,

2861.01, 1246.06, 830.38 and 1075.83cm^{-1}(LDE), 2926.11, 2859.08, 1224.36, 833.28 and 1079.21cm^{-1} (LAE), 2860.05, 1445.21, 1722.01, 1220.98, 826.53, 1645.82 and 1069.08 cm^{-1} (RPE), 2860, 1719.6, 1247.99, 619.65 and 1076cm^{-1} (RDE) and 2928.52, 2865.83, 1453.89, 1709.95, 1220.5, 778.3, 1644.85 and 1082.58cm^{-1} (RAE) respectively whereas C=O and C=C groups were absent in leaf aqueous and acetone extracts, alkane in root petroleum ether and aqueous extract and C=C in root aqueous extract.

Table 17: UV- Vis and FTIR bands in the spectra of root extracts of *Withania somnifera.*

UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		**Root aqueous extract**		**Root acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
220	221	219	219	216	223
254	254	252	254	252	252
297	295	-	296	296	-
237	272	235	236	236	235

FTIR ANALYSIS (cm $^{-1}$)			
Functional groups	**Root petroleum ether extract**	**Root aqueous extract**	**Root acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3434.85	3443, 1596	3474.4, 1552.27
Alkane	-	-	2928.52
C-H	2860.05, 1445.21	2860	2865.83, 1453.89
C=O	1722.01	1719.6	1709.95
C-O	1220.98	1247.99	1220.5
C-H (phenyl ring)	826.53	619.65	778.3
C=C	1645.82	-	1644.85
Std. Taxol	1069.08to 3434.85	1076 to 3443	1082.58 to 3473.4
NO_2	1365.16	-	1367.09
C,H (aromatic bends)	1069.08	1076	1082.58

Peaks at 1084.03 to 3443.05cm^{-1}(LPE), 1075.83 to 3488.38cm^{-1}(LDE), 1079.21 to 3490.79cm^{-1} (LAE), 1069.08to 3434.85cm^{-1} (RPE), 1076 to 3443cm^{-1} (RDE) and 1082.58 to 3473.4cm^{-1} (RAE) favoured the presence of standard taxol group. Generated

peaks at 1365.65cm^{-1} and 1370.47cm^{-1}indicated presence of NO_2 (nitro compounds) in leaf petroleum ether and acetone extracts and absent in leaf aqueous extract and at 1365.16cm^{-1} and 1367.09cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract.

4.3. *Calotropis procera:*

4.3.1. Quantification:

The data on quantification of petroleum ether, distilled water and acetone is given in Table 1. From 25gm of leaf powder 0.874gm, 11.24gm and 0.986gm of crude compounds were obtained whereas, from 12.5gm of root powder 0.270gm, 1.895gm and 0.397gm of crude compounds were obtained using petroleum ether, distilled water and as solvent, respectively.

4.3.2. Mitotic index:

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 5.45%, 5.92%, 5.96% and 6.88% (LPE); 7.56%, 8.09%, 8.14% and 9.75% (LDE); 5.45%, 6.53%, 7.54% and 7.62% (LAE); 4.17%, 5.00%, 5.32% and 5.44% (RPE); 4.52%, 4.98%, 5.25% and 5.59% RDE) and 4.36%, 4.50%, 4.77% and 4.97% (RAE) respectively.

4.3.3. Active mitotic index:

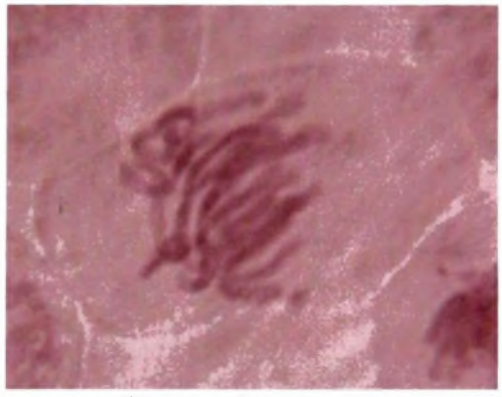
Active mitotic index frequencies were 3.10%, 3.12%, 3.21% and 3.46% (LPE); 4.34%, 4.11%, 4.29% and 5.47% (LDE); 2.33%, 3.29%, 4.05% and 3.52% (LAE); 1.91%, 2.16%, 2.04% and 2.24% (RPE); 1.85%, 2.41%, 2.62% and 2.70% (RDE) and 2.03%, 2.22%, 2.45% and 1.98% (RAE) respectively in 50µg, 100µg, 150µg and 200µg

per ml concentrations for 3hrs of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

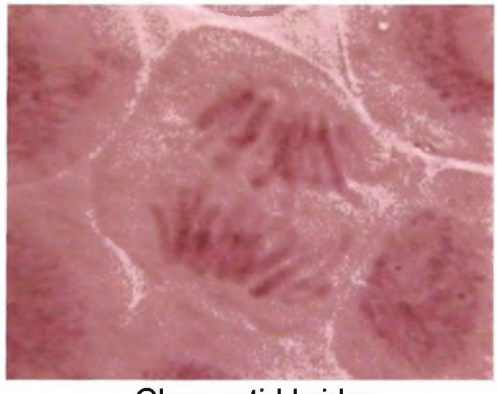
Table 18: Effect of leaf extracts of *Calotropis procera* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	1870	33	46	12	11	102	5.45 ± 0.53	3.10	1.17
	100	1822	35	39	18	16	108	5.92 ± 0.28	3.12	0.56
	150	1929	35	36	26	18	115	5.96 ± 0.29	3.21	0.58
	200	2019	49	47	23	20	133	6.88 ± 0.18	3.46	0.40
LDE	50	2325	47	79	22	28	176	7.56 ± 0.29	4.34	0.65
	100	2136	51	54	34	34	173	8.09 ± 0.56	4.11	1.26
	150	2026	51	63	24	27	165	8.14 ± 0.27	4.29	0.61
	200	2101	62	87	28	28	205	9.75 ± 0.18	5.47	0.41
LAE	50	2145	41	31	19	26	117	5.45 ± 0.58	2.33	1.31
	100	2157	44	42	29	26	141	6.53 ± 0.38	3.29	0.85
	150	1948	42	53	26	26	147	7.54 ± 0.24	4.05	0.55
	200	2296	62	52	29	32	175	7.62 ± 0.30	3.52	0.68

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.



Clumped metaphase

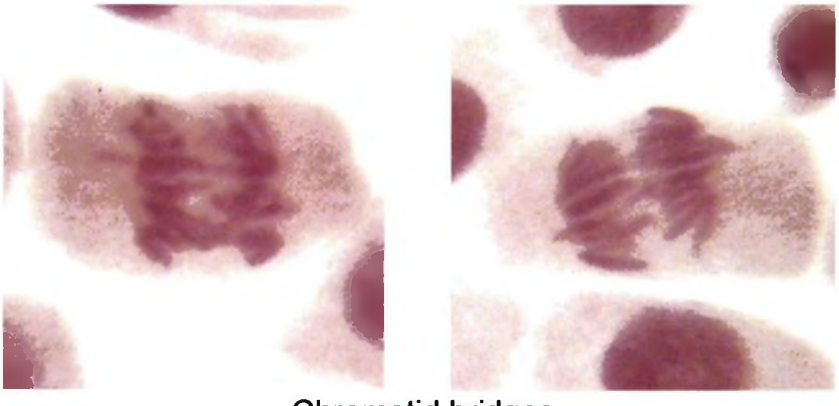


Chromatid bridge

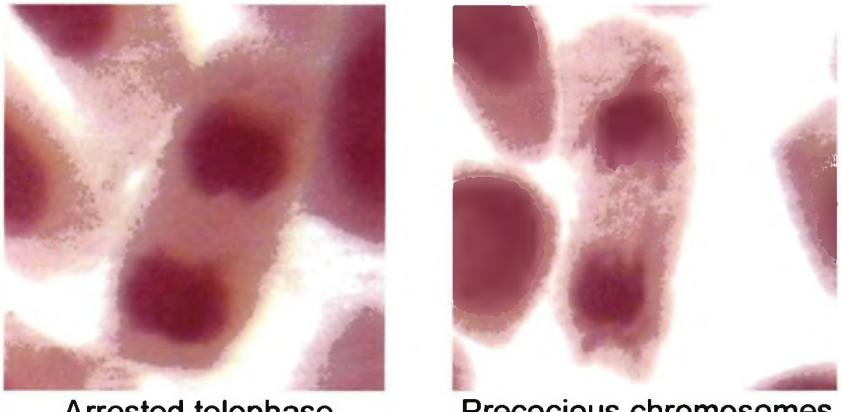
Arrested telophase

Plate No. 17: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* leaf petroleum ether extract.

Clumped metaphases



Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No.18: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* leaf aqueous extract.

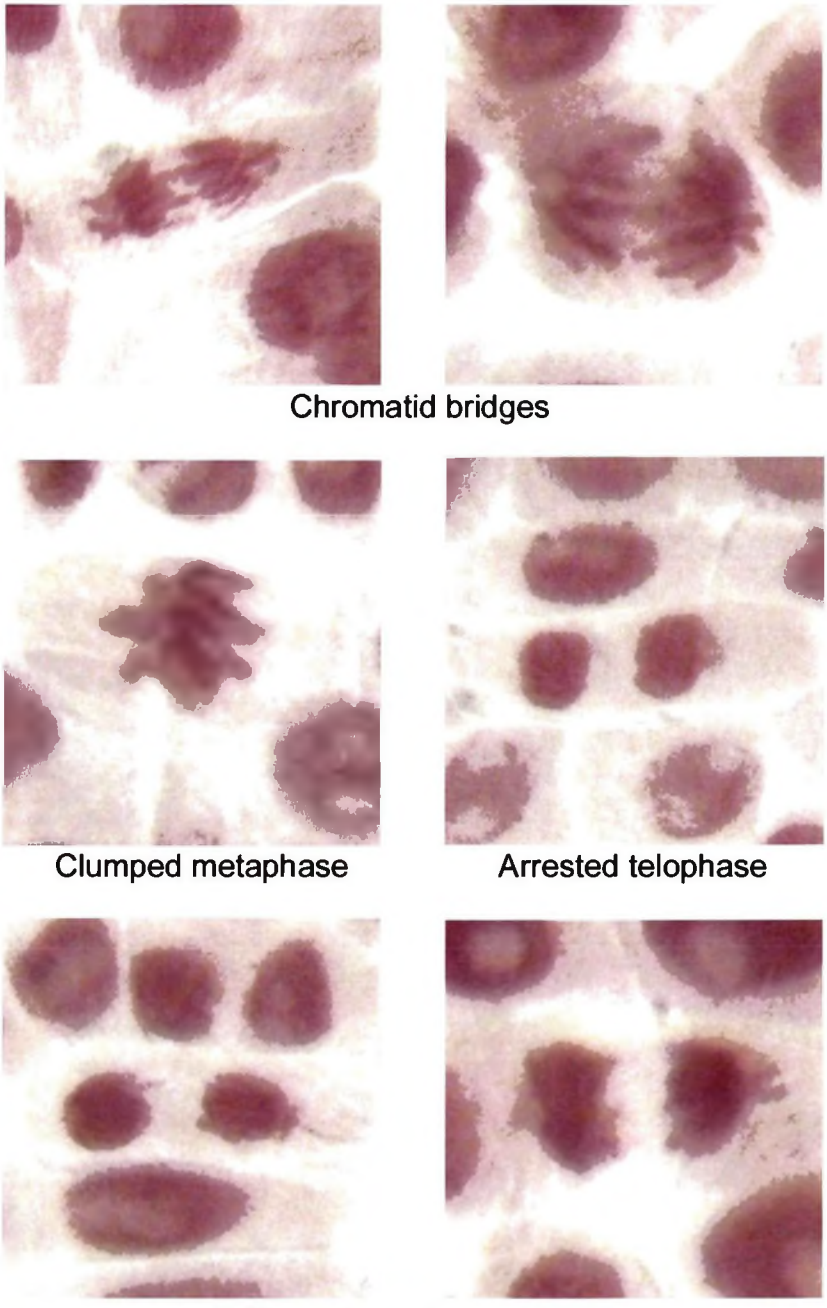
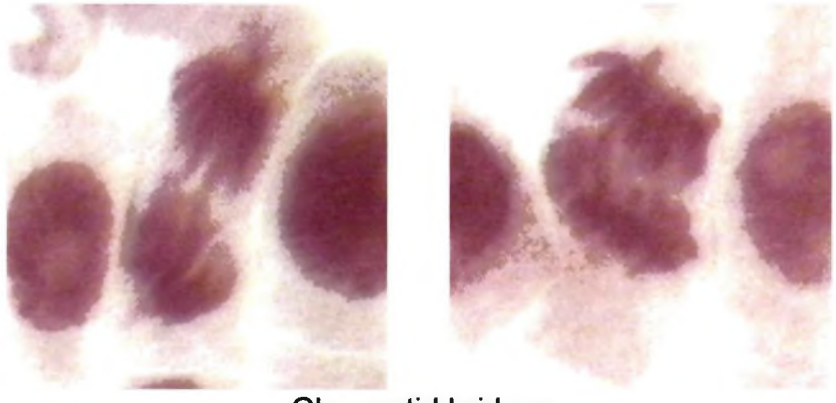
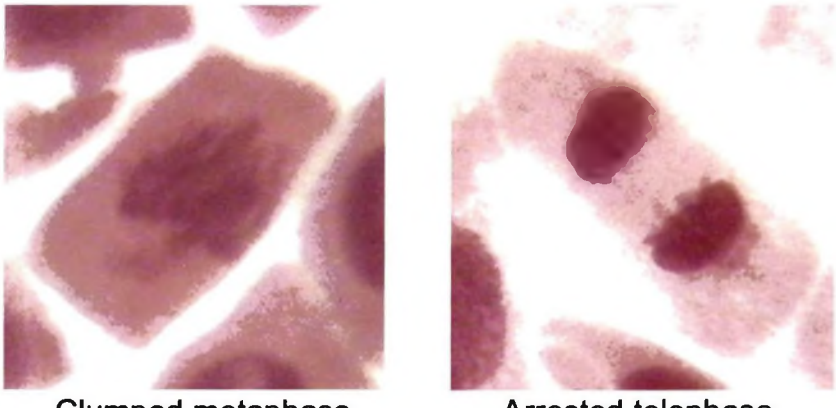


Plate No. 19: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* leaf acetone extract.

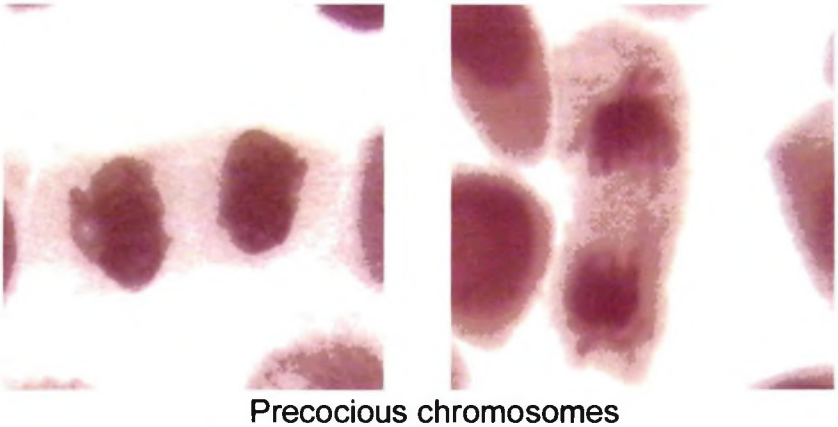


Chromatid bridges



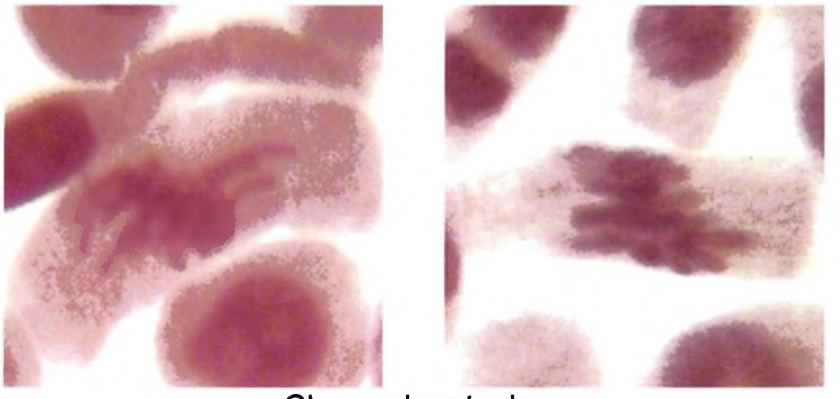
Clumped metaphase

Arrested telophase

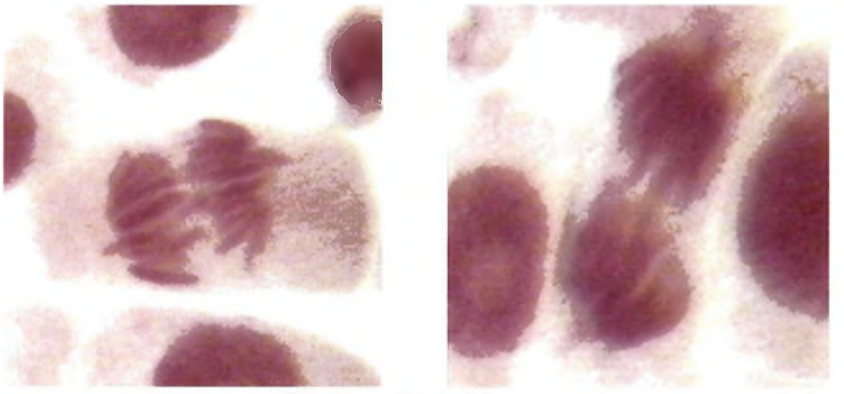


Precocious chromosomes
at telophases

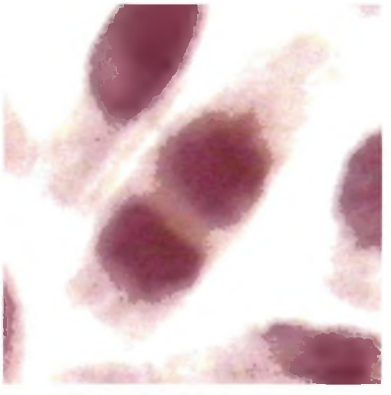
Plate No. 20: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* root petroleum ether extract.



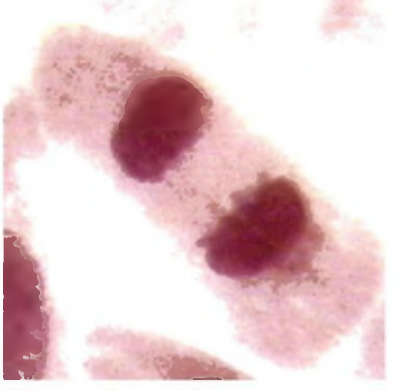
Clumped metaphases



Chromatid bridges

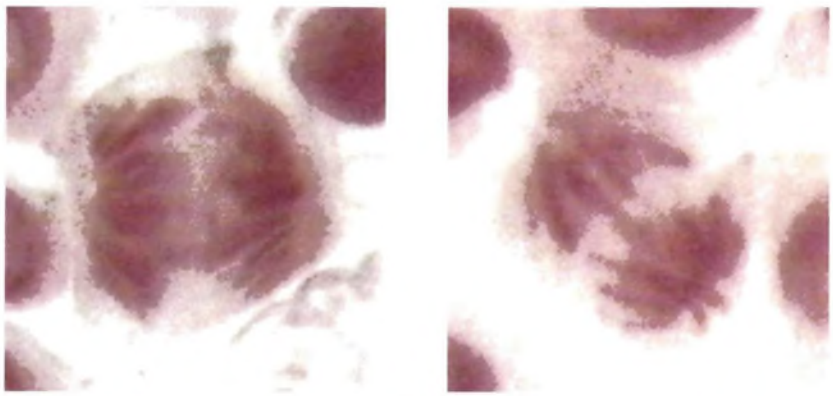


Arrested telophase



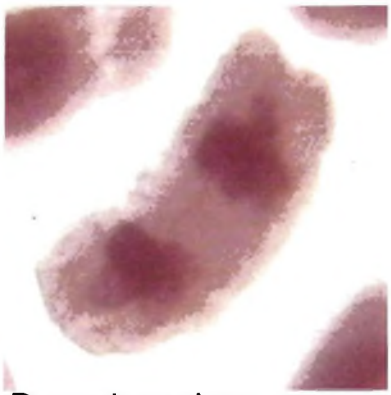
Precocious chromosomes at telophase

Plate No. 21: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* root aqueous extract.

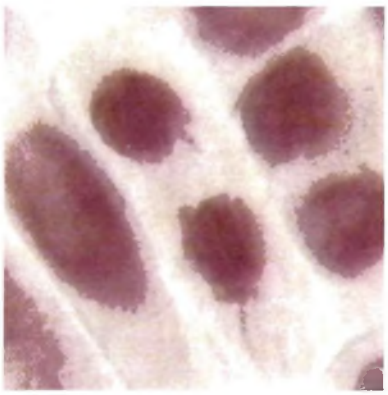


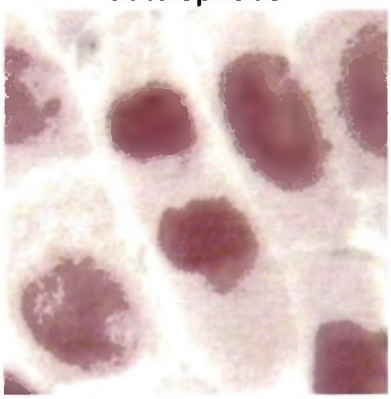
Chromatid bridges

Clumped metaphase



Precocious chromosomes at telophase





Arrested telophases

Plate No. 22: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Calotropis* root acetone extract.

Table 19: Effect of root extracts of *Calotropis procera* on mitosis in *Allium cepa.*

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	2300	27	28	16	25	96	4.17± 0.18	1.91	0.41
	100	1940	30	26	16	25	97	5.00± 0.27	2.16	0.61
	150	2009	35	27	14	31	107	5.32± 0.32	2.04	0.72
	200	1874	33	24	18	27	102	5.44± 0.17	2.24	0.40
RDE	50	1991	34	22	15	19	90	4.52± 0.13	1.85	0.32
	100	2027	31	37	12	21	101	4.98± 0.37	2.41	0.85
	150	1904	28	36	14	22	100	5.25± 0.29	2.62	0.61
	200	2036	34	39	16	25	114	5.59± 0.36	2.70	0.84
RAE	50	2015	29	26	15	18	88	4.36± 0.13	2.03	0.31
	100	2065	27	30	16	20	93	4.50± 0.26	2.22	0.58
	150	2158	29	31	22	21	103	4.77± 0.22	2.45	0.50
	200	1968	38	26	13	21	98	4.97± 0.33	1.98	0.75

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

4.3.4. Cytological abnormalities:

The cytological abnormalities are scored in mitotic cells and results are shown in Table 20 and 21. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into an observable cytological changes (Plate no. 17-22) these were chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases. The frequencies of chromosomal abnormalities after the

treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 20, 21 and Plate no. 4) for 3hrs at 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 20: Effect of leaf extracts of *Calotropis procera* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	1870	3	46	12	00	61	3.26
	100	1822	8	39	16	00	63	3.45
	150	1929	8	36	26	00	70	3.62
	200	2019	9	47	20	00	76	3.76
LDE	50	2325	16	79	15	13	123	5.29
	100	2136	27	54	19	15	115	5.38
	150	2026	13	63	14	13	103	5.08
	200	2101	18	85	15	14	132	6.28
LAE	50	2145	11	31	16	10	68	3.17
	100	2157	17	42	14	12	85	3.94
	150	1948	17	53	16	10	96	4.92
	200	2296	16	52	20	15	103	4.48

CB – Chromatid Brides, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

The frequencies of chromosomal abnormalities in leaf and root extracts treatments were 3.26%, 3.45%, 3.62% and 3.76% (LPE); 5.29%, 5.38%, 5.08% and 6.28% (LDE); 3.17%, 3.94%, 4.92% and 4.48% (LAE); 2.78%, 3.35%, 3.23% and 3.46% (RPE); 2.56%, 3.35%, 3.62% and 3.73% (RDE) and 2.77%, 2.90%, 2.96% and 2.89% (RAE) respectively at 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs treatments. No mitotic aberrations were recorded in control.

In leaf petroleum ether and distilled water root extracts, chromosomal abnormalities were increased with increase in concentrations whereas, in leaf distilled water and root petroleum ether extracts showed more chromosomal abnormalities in

100µg as compare to 150µg concentration and leaf acetone and root acetone extracts at 150µg showed more chromosomal abnormalities as compared to 200µg.

Table 21: Effect of root extracts of *Calotropis procera* on somatic chromosomes in *Allium cepa.*

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
RPE	50	2300	11	28	13	12	64	2.78
	100	1940	14	26	15	10	65	3.35
	150	2009	9	27	11	18	65	3.23
	200	1874	16	24	10	15	65	3.46
RDE	50	1991	11	22	6	12	51	2.56
	100	2027	10	37	8	13	68	3.35
	150	1904	11	36	10	12	69	3.62
	200	2036	12	39	12	13	76	3.73
RAE	50	2015	11	26	8	11	56	2.77
	100	2065	10	30	12	8	60	2.90
	150	2158	12	31	8	13	64	2.96
	200	1968	11	26	10	10	57	2.89

CB – Chromatid Brides, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

4.3.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by 't' test (Table 22 and 23). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg, 150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone 50µg, 100µg, 150µg and 200µg per ml

concentrations extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 4.5, 4.52, 4.03 and 2.82 (LPE); 5.30, 3.54, 4.58 and 2.22 (LDE); 6.50, 6.42, 5.62 and 5.23 (LAE); 11.43, 9.44, 8.57 and 9.38 (RPE); 11.11, 9.44, 8.87 and 8.38 (RDE) and 11.40, 10.31, 10.17 and 8.97 (RAE) respectively.

Table 22: *Calotropis procera* leaf extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	4.5
Control and 100µg / ml Leaf petroleum ether extracts	4.52
Control and 150µg / ml Leaf petroleum ether extracts	4.03
Control and 200µg / ml Leaf petroleum ether extracts	2.82
Control and 50µg / ml Leaf aqueous extracts	5.30
Control and 100µg / ml Leaf aqueous extracts	3.54
Control and 150µg / ml Leaf aqueous extracts	4.58
Control and 200µg / ml Leaf aqueous extracts	2.22
Control and 50µg / ml Leaf acetone extracts	6.50
Control and 100µg / ml Leaf acetone extracts	6.42
Control and 150µg / ml Leaf acetone extracts	5.62
Control and 200µg / ml Leaf acetone extracts	5.23

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level)

These calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore null hypothesis is rejected i.e. the values of $|t|$ are significant hence samples are dependent on each other when treated with control except in 200µg of leaf aqueous extra

Table 23: *Calotropis procera* root extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	11.43
Control and 100µg / ml Root petroleum ether extracts	9.44
Control and 150µg / ml Root petroleum ether extracts	8.57
Control and 200µg / ml Root petroleum ether extracts	9.38
Control and 50µg / ml Root aqueous extracts	11.11
Control and 100µg / ml Root aqueous extracts	9.44
Control and 150µg / ml Root aqueous extracts	8.87
Control and 200µg / ml Root aqueous extracts	8.38
Control and 50µg / ml Root acetone extracts	11.40
Control and 100µg / ml Root acetone extracts	10.31
Control and 150µg / ml Root acetone extracts	10.17
Control and 200µg / ml Root acetone extracts	8.97

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

4.3.6. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. . The results of UV-Vis showed that the intense absorption at 220, 255 and 294nm (LPE), 219, 252 and 294nm (LDE) 218, 255 and 296nm (LAE) and 219, 255 and 295nm (RPE), 220, 254 and 292nm (RDE) and 220, 256 and 296 (RAE) using acetone and at 220, 256 and 296nm (LPE), 225, 255 and 296nm (LDE) , 224, 255 and 296nm (LAE), 220, 254 and 295nm (RPE), 220, 255 and 296nm (RDE) and 220, 255 and 297nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 241nm (LPE), 238nm (LDE), 235nm (LAE), 239nm (RPE), 236nm (RDE) and 239nm (RAE)

in acetone and 237nm (LPE), 239nm (LDE), 235nm (LAE), 235nm (RPE), 236nm (RDE) and 235nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 24 and 25).

Table 24: UV- Vis and FTIR bands in the spectra of leaf extracts of *Calotropis procera*.

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		Leaf aqueous extract		Leaf acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
220	220	219	225	218	224
255	256	252	255	255	255
294	296	294	296	296	296
241	237	238	239	235	235

FTIR ANALYSIS (cm^{-1})			
Functional groups	Leaf petroleum ether extract	Leaf aqueous extract	Leaf acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3449.32, 1554.68	3444.98	3478.74, 1553.71
Alkane	2926.11	2924.66	2928.52
C-H	2865.83, 1453.41	2859.08	2861.01, 1455.82
C=O	1734.55	1720.56	1735.03
C-O	1219.53	1247.5	1220.02
C-H (phenyl ring)	-	-	772.52
C=C	1644.37	-	-
Std. Taxol	1083.55 to 3449.32	1065.75 to3444.98	1077.76 to 3478.78
NO_2	1366.13	-	1365.16
C,H (aromatic bends)	1083.55	1065.75	1077.77

The FTIR band at 3449.32 and 1554.68cm^{-1} (LPE), 3444.98cm^{-1} (LDE), 3478.74 and 1553.71cm^{-1} (LAE), 3442.09cm^{-1} (RPE), 3463.79 and 1593.25cm^{-1} (RDE) and 3454.62cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2926.11, 2865.83, 1453.41, 1734.55, 1219.53, 1644.37 and 1083.55 cm^{-1}(LPE), 2924.66, 2859.08, 1720.56, 1247.5 and 1065.75cm^{-1}(LDE), 2928.52, 2861.01, 1455.82, 1735.03, 1220.02, 772.52 and 1077.77cm^{-1} (LAE), 2926.59, 2868.24, 1445.21, 1734.06, 1219.05, 834.24, 1644.37 and 1073.9cm^{-1} (RPE), 2926.59, 2859.08, 1442.8, 770.59 and 1079.69cm^{-1} (RDE) and 2927.08, 2858.12, 1419.66, 1731.17, 1221.46, 764.34 and

1079.21cm^{-1} (RAE) respectively whereas C=O and C=C groups were absent in leaf aqueous extract, C-H (phenyl ring) in root petroleum ether extract and C=C in root aqueous extract.

Table 25: UV- Vis and FTIR bands in the spectra of root extracts of *Calotropis procera*.

UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		Root aqueous extract		Root acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
219	220	220	220	220	220
255	254	254	256	256	255
295	295	292	296	296	297
239	235	236	239	239	235

FTIR ANALYSIS (cm $^{-1}$)			
Functional groups	Root petroleum ether extract	Root aqueous extract	Root acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3442.09	3463.79, 1593.25	3454.62
Alkane	2926.59	2926.59	2927.08
C-H	2868.24, 1445.21	2859.08, 1442.8	2858.12, 1419.66
C=O	1734.06	-	1731.17
C-O	1219.05	-	1221.46
C-H (phenyl ring)	834.24	770.59	764.34
C=C	1644.37	-	-
Std. Taxol	1073.9 to 3442.09	1079.69 to 3463.79	1079.21 to 3561.68
NO_2	1365.65	-	1365.65
C,H (aromatic bends)	1073.9	1079.69	1079.21

Peaks at 1083.55 to 3449.32cm-1 (LPE), 1065.75 to3444.98cm^{-1}(LDE), 1077.76 to 3478.78cm^{-1} (LAE), 1073.9 to 3442.09cm^{-1} (RPE), 1079.69 to 3463.79cm^{-1} (RDE) and 1079.21 to 3561.68cm^{-1} (RAE) favoured the presence of standard taxol group. At 1366.13cm^{-1} and 1365.16cm^{-1} indicated presence of NO_2 (nitro compounds) in leaf petroleum ether and acetone extract and absent in leaf aqueous extracts and at 1365.65cm^{-1} and 1365.65cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract.

4.4. *Asclepias curassavica:*

4.4.1. Quantification:

The data on quantification of leaf petroleum ether, distilled water and acetone was given in Table 1. From 25gm of leaf powder 0.228gm, 0.411gm and 0.256gm of crude compounds were obtained whereas, from 12.5gm of root powder 0.186gm, 0.122gm and 0.066gm of crude compounds were obtained respectively.

4.4.2. Mitotic index:

Table 26: Effect of leaf extracts of *Asclepias curassavica* on mitosis in *Allium cepa.*

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	2047	26	16	21	29	92	4.49 ± 0.20	1.80	0.45
	100	1657	34	20	16	22	92	5.55 ± 0.19	2.17	0.43
	150	1890	33	37	13	28	111	5.87 ± 0.56	2.64	1.27
	200	1977	34	36	27	25	122	6.17 ± 0.21	3.18	0.48
LDE	50	1931	36	37	29	25	127	6.57 ± 0.34	3.41	0.78
	100	2028	35	42	27	31	135	6.65 ± 0.39	3.40	0.89
	150	2199	42	42	34	30	148	6.73 ± 0.37	3.45	0.83
	200	1849	30	41	32	21	124	6.78 ± 0.26	3.94	0.58
LAE	50	1936	36	32	15	19	102	5.26 ± 0.21	2.42	0.48
	100	2077	29	43	16	22	110	5.29 ± 0.18	2.84	0.41
	150	1959	34	47	16	20	117	5.97 ± 0.63	3.21	1.42
	200	1921	38	42	25	24	129	6.71 ± 0.24	3.48	0.55

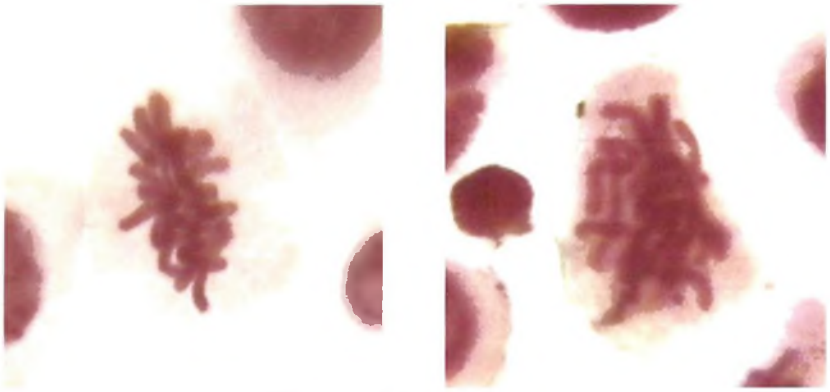
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

Table 27: Effect of root extracts of *Asclepias curassavica* on mitosis in *Allium cepa*.

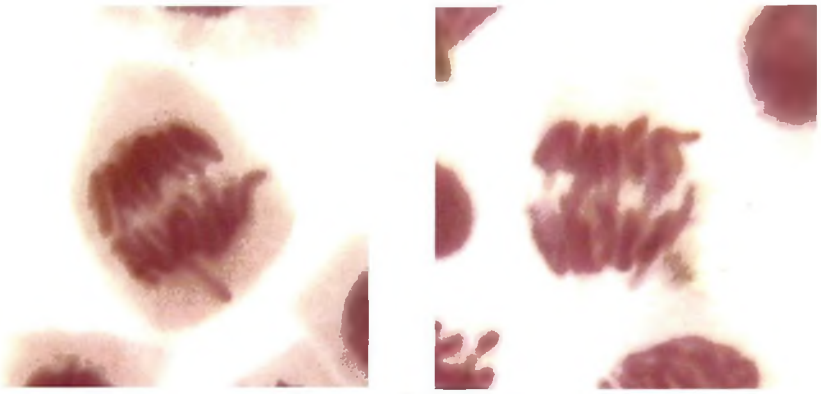
Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	1886	28	22	10	22	82	4.35 ± 0.12	1.69	0.27
	100	1863	35	33	11	20	99	5.34 ± 0.26	2.36	0.60
	150	1869	34	29	19	24	106	5.67 ± 0.20	2.56	0.46
	200	2036	41	42	17	30	130	6.38 ± 0.25	2.89	0.57
RDE	50	2078	26	25	14	14	79	3.80 ± 0.25	1.87	0.57
	100	1854	34	24	16	12	86	4.63 ± 0.34	2.15	0.77
	150	1733	33	28	15	28	104	6.00 ± 0.39	2.48	0.87
	200	1957	35	50	18	26	129	6.59 ± 0.22	3.47	0.51
RAE	50	2160	26	19	10	16	71	3.28 ± 0.40	1.34	0.90
	100	1883	30	40	12	16	98	5.20 ± 0.20	2.76	0.46
	150	1833	35	34	15	18	102	5.55 ± 0.18	2.67	0.42
	200	1606	30	22	12	25	89	5.56 ± 0.33	2.11	0.74

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

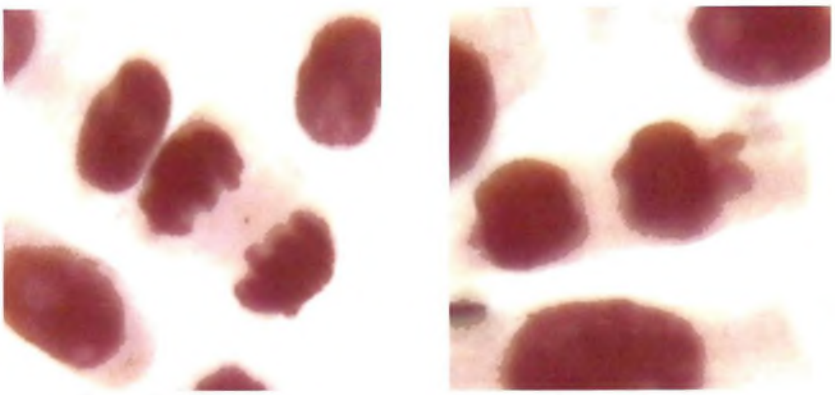
The data on effect of leaf and root extracts of A. *curassavica* on mitotic index and active mitotic index is tabulated in Table 26 and 27. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase, metaphase, anaphase and telophase were recorded (Plate no. 3). The mitotic index frequency, in control was 11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies in 50µg, 100µg,



Clumped metaphases



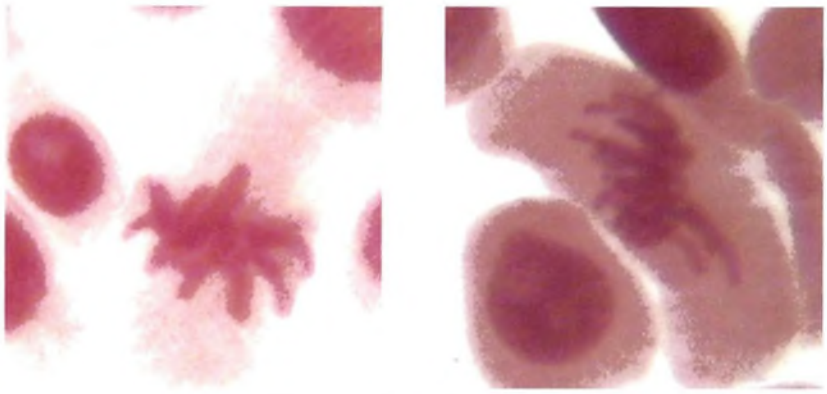
Chromatid bridges



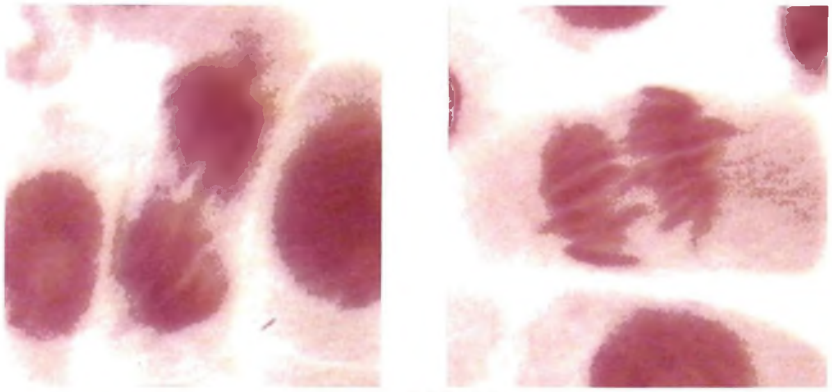
Arrested telophase

Precocious chromosomes at telophase

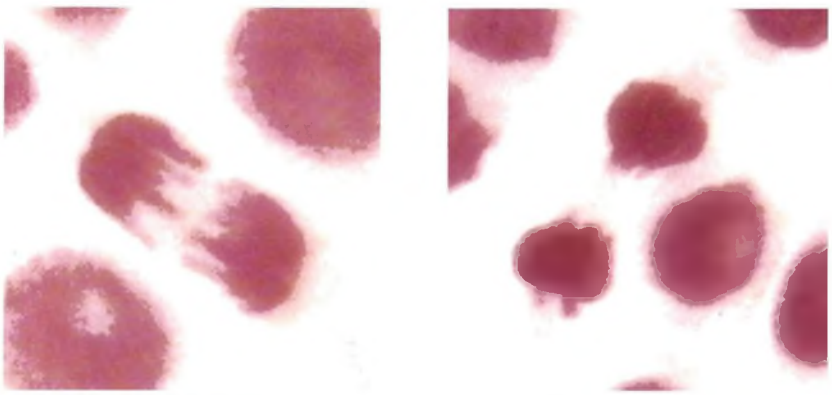
Plate No. 23: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* leaf petroleum ether *extract.*



Clumped metaphases



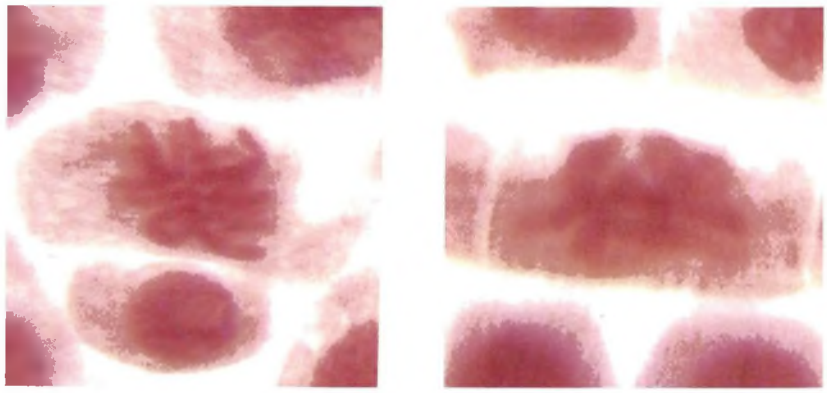
Chromatid bridges



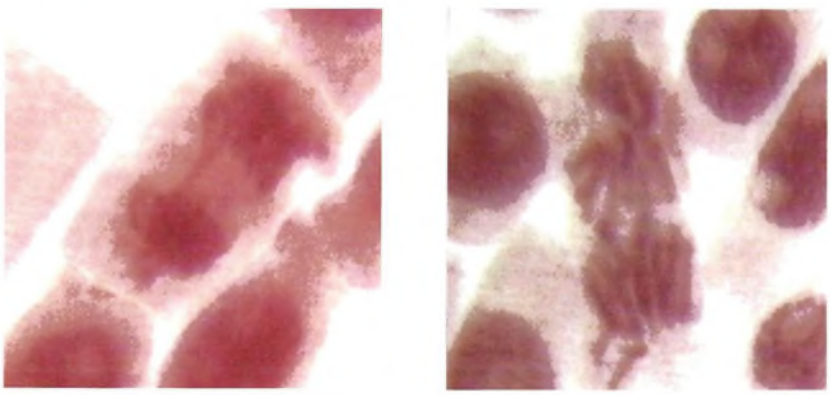
Arrested telophase

Precocious chromosomes at telophase

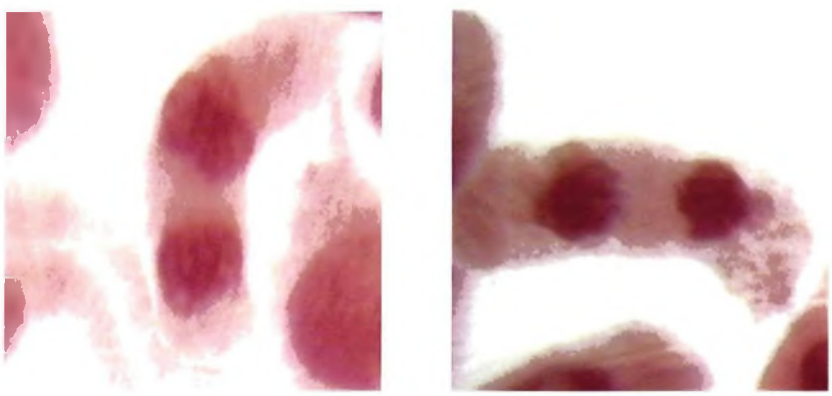
Plate No. 24: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* leaf aqueous extract.



Clumped metaphases



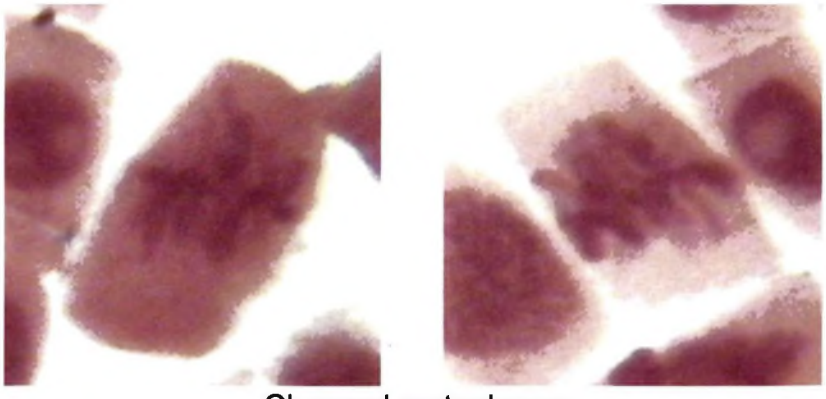
Chromatid bridges



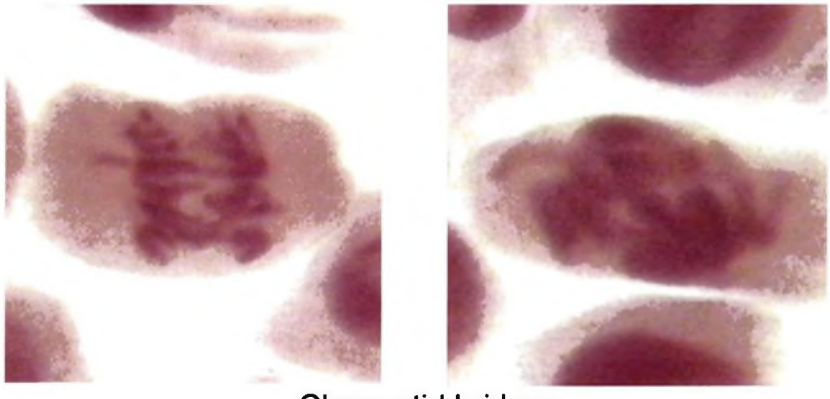
Arrested telophase

Precocious chromosomes at telophase

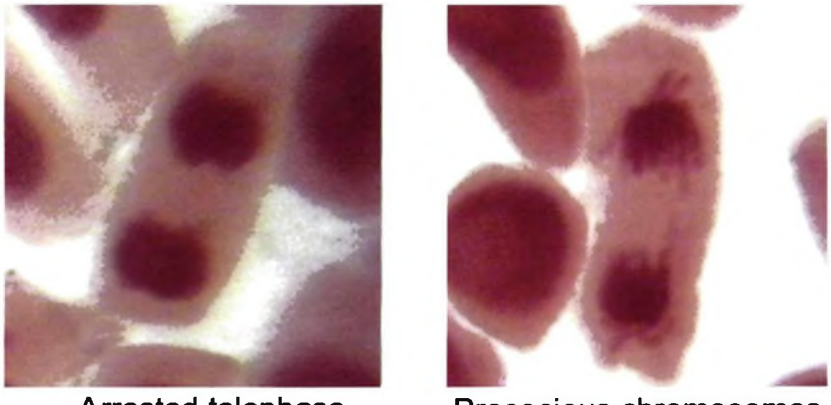
Plate No. 25: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* leaf acetone extract.



Clumped metaphases



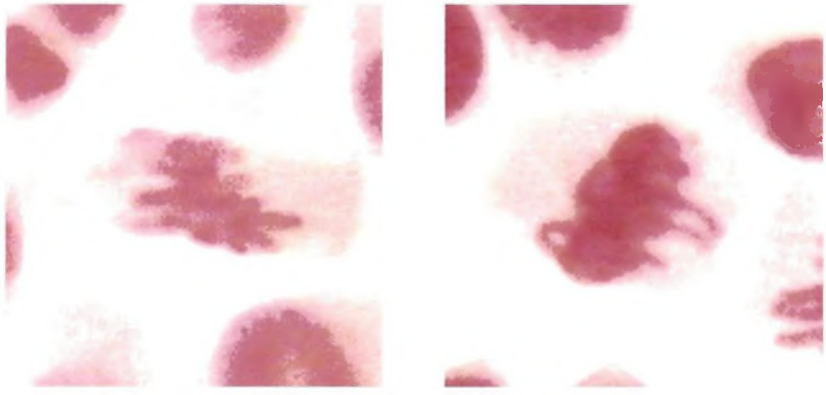
Chromatid bridges



Arrested telophase

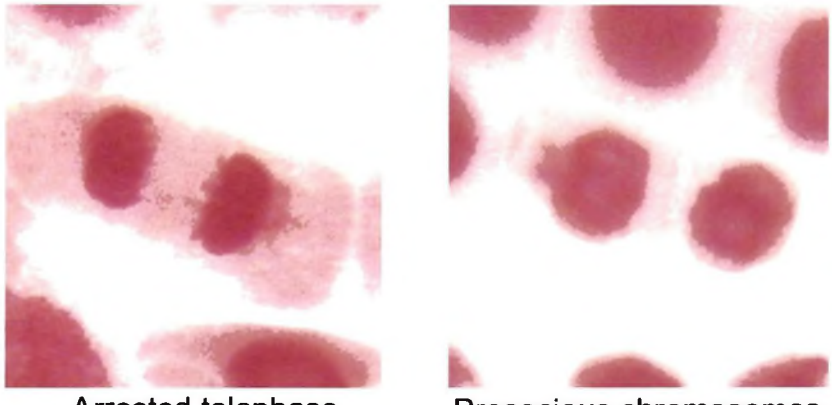
Precocious chromosomes at telophase

Plate No. 26: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* root petroleum ether extract.



Clumped metaphases

Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No. 27: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* root aqueous extract.

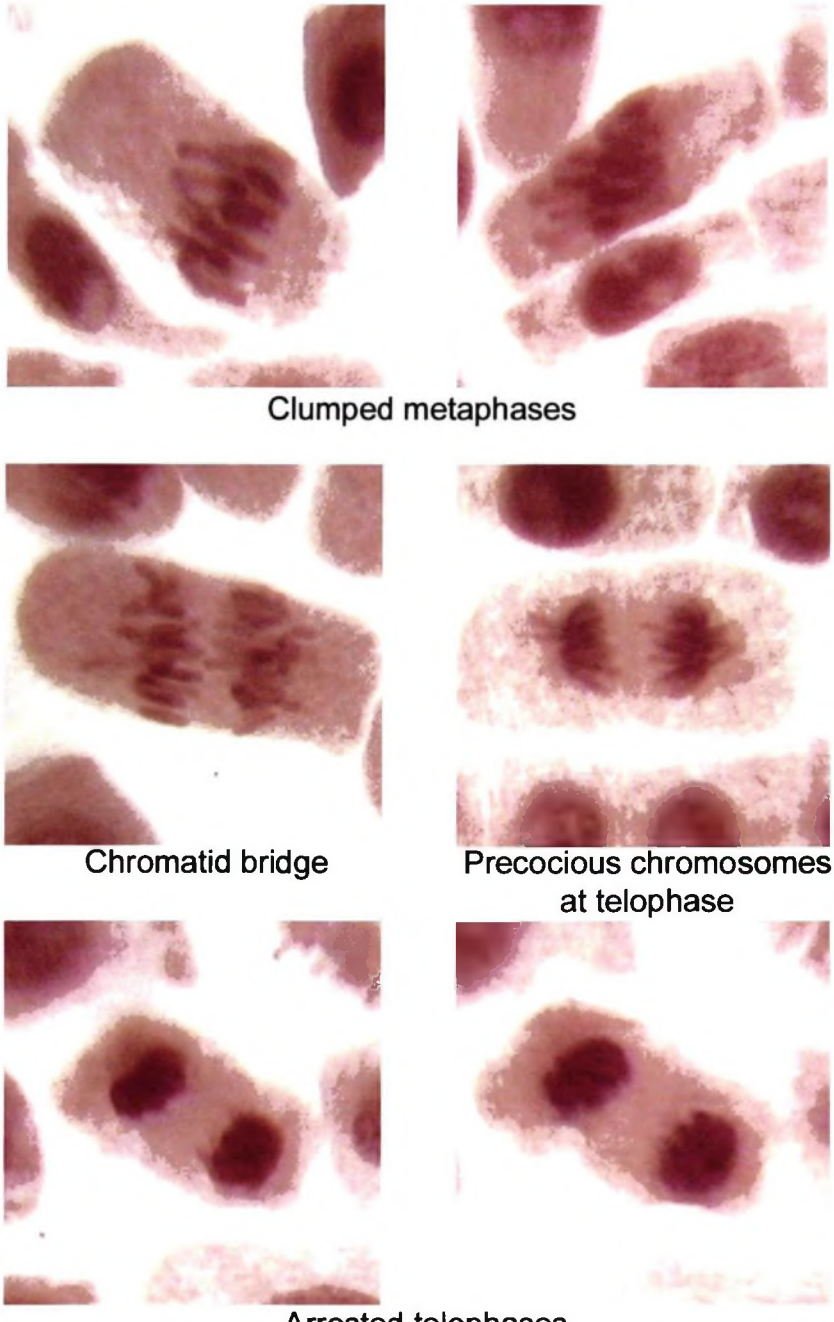


Plate No. 28: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Asclepias* root acetone extract.

150µg and 200µg per ml concentrations were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively for at 50µg, 100µg, 150µg and 200µg per ml concentrations 3hrs of methotrexate treatment.

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 4.49%, 5.55%, 5.87% and 6.17% (LPE); 6.57%, 6.65%, 6.73% and 6.78% (LDE); 5.26%, 5.29%, 5.97% and 6.71% (LAE); 4.35%, 5.34%, 5.67% and 6.38% (RPE); 3.80%, 4.63%, 6.00% and 6.59% (RDE) and 3.28%, 5.20%, 5.55% and 5.56% (RAE) respectively.

4.4.3. Active mitotic index:

Active mitotic index frequencies were 1.80%, 2.17%, 2.64% and 3.18% (LPE); 3.41%, 3.40%, 3.45% and 3.94% (LDE); 2.42%, 2.84%, 3.21% and 3.48% (LAE); 1.69%, 2.36%, 2.56% and 2.89% (RPE); 1.87%, 2.15%, 2.48% and 3.47% (RDE) and 1.34%, 2.76%, 2.67% and 2.11% (RAE) respectively at 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

4.4.4. Cytological abnormalities:

The cytological abnormalities are scored in mitotic cells and results are shown in Table 28 and 29. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into observable cytological changes (Plate no. 23-28). These were chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 28, 29 and

Plate no. 4) for 3hrs at 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 28: Effect of leaf extracts of *Asclepias curassavica* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	2300	11	28	13	12	64	2.78
	100	2047	11	16	18	12	57	2.78
	150	1657	7	20	9	13	49	2.95
	200	1890	11	37	13	10	71	3.75
LDE	50	1977	10	36	16	11	73	3.69
	100	1931	22	37	9	16	84	4.35
	150	2028	18	42	17	14	91	4.48
	200	2199	15	42	18	12	87	3.95
LAE	50	1849	14	41	12	10	77	4.16
	100	1936	15	32	11	9	67	3.46
	150	2077	13	43	13	11	80	3.85
	200	1959	12	47	11	9	79	4.03

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

The frequencies of chromosomal abnormalities in leaf and root extracts in 50µg, 100µg, 150µg and 200µg per ml concentrations were 2.78%, 2.95%, 3.75% and 3.69% (LPE); 4.35%, 4.48%, 3.95% and 4.16% (LDE); 3.46%, 3.85%, 4.03% and 4.42% (LAE); 2.91%, 3.48%, 3.47% and 4.07% (RPE); 2.50%, 2.80%, 3.80% and 4.49% (RDE) and 1.99%, 3.66%, 3.54% and 3.48% (RAE) respectively for 3hrs treatments. No mitotic aberrations were recorded in control. Chromosomal abnormalities were also increased with increase in concentrations whereas, in 100µg and 150µg leaf petroleum ether, leaf distilled water and root petroleum ether extracts were showed lower frequencies than 200µg extract.

Table 29: Effect of root extracts of *Asclepias curassavica* on somatic chromosomes in *Allium cepa.*

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	1886	10	22	9	14	55	2.91
	100	1863	10	33	8	14	65	3.48
	150	1869	13	29	12	11	65	3.47
	200	2036	13	42	9	19	83	4.07
LDE	50	2078	13	25	2	12	52	2.50
	100	1854	12	24	4	12	52	2.80
	150	1733	12	28	11	15	66	3.80
	200	1957	14	48	12	14	88	4.49
LAE	50	2160	8	19	7	9	43	1.99
	100	1883	11	40	6	12	69	3.66
	150	1833	12	34	7	12	65	3.54
	200	1606	9	22	9	16	56	3.48

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

4.4.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by't' test (Table 30 and 31). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg, 150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 10.78, 9.11, 6.11 and 7.95 (LPE); 6.54, 6.14, 6.19 and 6.76 (LDE); 9.43, 9.60, 5.61 and 6.92 (LAE); 11.53, 8.93,

8.82 and 7.39 (RPE); 11.42, 9.39, 7.11 and 7.22 (RDE) and 10.77, 9.60, 9.14 and 8.12 (RAE) at 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 30: *Asclepias curassavica* leaf extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	10.78
Control and 100µg / ml Leaf petroleum ether extracts	9.11
Control and 150µg / ml Leaf petroleum ether extracts	6.11
Control and 200µg / ml Leaf petroleum ether extracts	7.95
Control and 50µg / ml Leaf aqueous extracts	6.54
Control and 100µg / ml Leaf aqueous extracts	6.14
Control and 150µg / ml Leaf aqueous extracts	6.19
Control and 200µg / ml Leaf aqueous extracts	6.76
Control and 50µg / ml Leaf acetone extracts	9.43
Control and 100µg / ml Leaf acetone extracts	9.60
Control and 150µg / ml Leaf acetone extracts	5.61
Control and 200µg / ml Leaf acetone extracts	6.92

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

These calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore null hypothesis is rejected i.e. the values of $|t|$ are significant hence samples are dependent on each other when treated with control.

Table 31: *Asclepias curassavica* root extracts: - Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	11.53
Control and 100µg / ml Root petroleum ether extracts	8.93
Control and 150µg / ml Root petroleum ether extracts	8.82
Control and 200µg / ml Root petroleum ether extracts	7.39
Control and 50µg / ml Root aqueous extracts	11.42
Control and 100µg / ml Root aqueous extracts	9.39
Control and 150µg / ml Root aqueous extracts	7.11
Control and 200µg / ml Root aqueous extracts	7.22
Control and 50µg / ml Root acetone extracts	10.77
Control and 100µg / ml Root acetone extracts	9.60
Control and 150µg / ml Root acetone extracts	9.14
Control and 200µg / ml Root acetone extracts	8.12

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

4.4.6. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. . The results of UV-Vis showed that the intense absorption at 220 and 255 (LPE), 220, 255 and 296nm (LDE) 222 and 295nm (LAE) and 222, 253 and 295nm (RPE), 220, 254 and 296nm (RDE) and 222, 255 and 294 (RAE) using acetone and at 219 and 294nm (LPE), 219, 255 and 294nm (LDE) , 222 and 295nm (LAE), 224, 252nm (RPE), 258 and 294nm (RDE) and 222 and 294nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 235nm (LPE), 238nm (LDE), 235nm (LAE), 236nm (RPE), 239nm (RDE) and 236nm (RAE) in acetone and 236nm (LPE), 240nm

(LDE), 235nm (LAE), 273nm (RPE), 237nm (RDE) and 242nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 32 and 33).

Table 32: UV- Vis and FTIR bands in the spectra of leaf extracts of *Asclepias curassavica.*

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		**Leaf aqueous extract**		**Leaf acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
220	219	220	219	222	219
255	-	255	255	-	-
-	294	296	294	295	296
235	236	238	240	235	235

FTIR ANALYSIS (cm^{-1})			
Functional groups	**Leaf petroleum ether extract**	**Leaf aqueous extract**	**Leaf acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3431,1571.07	3443.53, 1591.81	3443.05, 1599.04
Alkane	2926.59	2926.11	2924.66
C-H	1420.62	1451.96	1424.96
C=O	1732.62	-	1732.62
C-O	1221.46	1243.16	1220.98
C-H (phenyl ring)	-	775.41	774.45
C=C	-	-	-
Std. Taxol	1084.99 to 3508.15	1082.58 to 3490.31	1078.24 to 3492.72
NO_2	1367.09	-	1366.61
C,H (aromatic bends)	1084.99	1082.58	1078.24

The FTIR band at 3431 and 1571.07cm^{-1} (LPE), 3443.53 and 1591.81cm^{-1} (LDE), 3443.05 and 1599.04cm^{-1} (LAE), 3426.17cm^{-1} (RPE), 3471.5, 1597.59cm^{-1} (RDE) and 3444.02, 1598.56cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2926.59, 1420.62, 1732.62, 1221.46 and 1084.99 cm^{-1}(in leaf petroleum ether extract), 2926.11, 1451.96, 1243.16, 775.41 and 1082.58cm^{-1}(in leaf aqueous extract), 2924.66, 1424.96, 1732.62, 1220.98, 774.45 and 1078.24cm^{-1} (in leaf acetone extract), 2927.56, 2865.35, 1448.11, 1715.74, 1219.53 and 1081.14cm^{-1} (root petroleum ether extract), 2929, 2861.01, 1405.04, 1718.63, 1242.2, 765.28 and 1070.05cm^{-1} (root aqueous extract) and 2926.11, 2859.56, 1730.69, 1218.57, 770.11

and 1077.28cm^{-1} (root acetone extract) respectively whereas, C-H (phenyl ring) in leaf and root petroleum ether extract and C=C group in all leaf and root extract were absent.

Table 33: UV- Vis and FTIR bands in the spectra of root extracts of *Asclepias curassavica*.

UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		**Root aqueous extract**		**Root acetone extract**	
Solvent (Acetone)	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**	**Solvent (Acetone)**	**Solvent (Methanol)**
222	224	220	-	222	222
253	252	254	258	255	-
295	-	296	294	294	294
236	273	239	237	236	242

FTIR ANALYSIS (cm^{-1})			
Functional groups	**Root petroleum ether extract**	**Root aqueous extract**	**Root acetone extract**
	Solvent (Acetone)	**Solvent (Distilled water)**	**Solvent (Acetone)**
N-H	3426.17	3471.5, 1597.59	3444.02, 1598.56
Alkane	2927.56	2929	2926.11
C-H	2865.35, 1448.11	2861.01, 1405.04	2859.56
C=O	1715.74	1718.63	1730.69
C-O	1219.53	1242.2	1218.57
C-H (phenyl ring)	-	765.28	770.11
C=C	-	-	-
Std. Taxol	1081.14 to 3492.72	1070.05 to 3471.5	1077.28 to 3444.02
NO_2	1364.68	-	1365.16
C,H (aromatic bends)	1081.14	1070.05	1077.28

Peaks at 1084.99 to 3508.15cm-1(in leaf petroleum ether extract), 1082.58 to 3490.31cm^{-1}(in leaf aqueous extract), 1078.24 to 3492.72cm^{-1} (in leaf acetone extract), 1081.14 to 3492.72cm^{-1} (root petroleum ether extract), 1070.05 to 3471.5cm^{-1} (root aqueous extract) and 1077.28 to 3444.02cm^{-1} (root acetone extract) favoured the presence of standard taxol group. At 1367.09cm^{-1} and 1366.61 indicated presence of NO_2 (nitro compounds) in leaf petroleum ether and acetone extract and absent in leaf aqueous extracts and at 1364.68cm^{-1} and 1365.16cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract .

4.5. *Vitex negundo:*

4.5.1. Quantification:

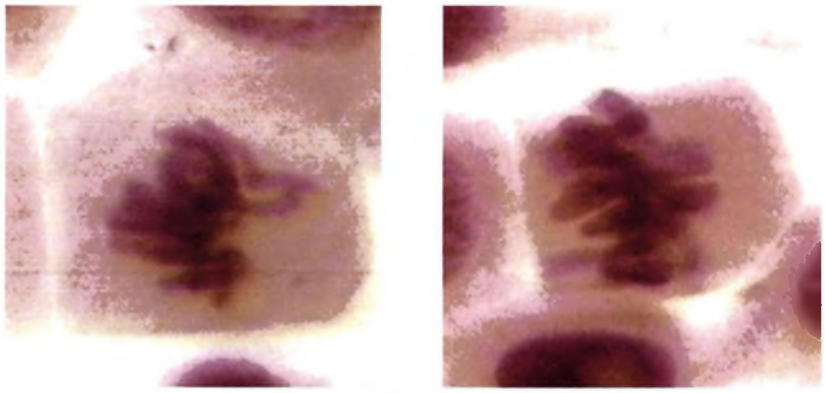
The data on quantification of petroleum ether, distilled water and acetone is given in Table 1. From 25gm of leaf powder 1.911gm, 6.23gm and 3.142gm of crude compounds were obtained using petroleum ether, distilled water and acetone as solvent.

4.5.2. Mitotic index:

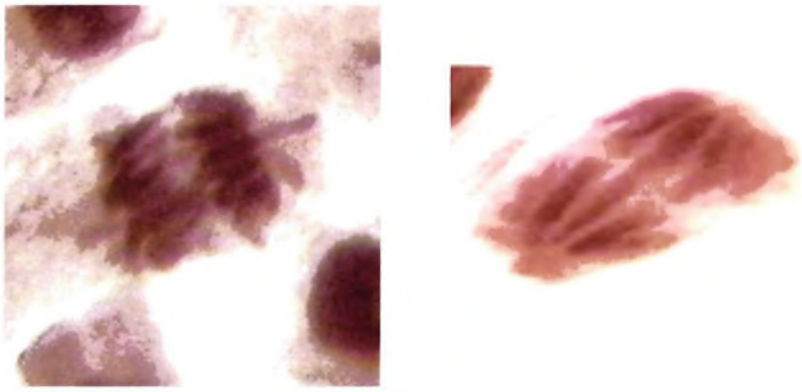
Table 34: Effect of leaf extracts of *Vitex negundo* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	2169	49	58	29	23	159	7.33 ±0.25	4.01	0.56
	100	2152	53	74	30	33	190	8.82 ± 0.34	4.83	0.75
	150	2156	56	91	41	39	229	10.62±0.14	6.12	0.33
	200	2080	57	93	37	44	231	11.10±0.19	6.25	0.44
LDE	50	2520	57	38	18	25	138	5.47 ± 0.19	2.22	0.43
	100	2466	52	57	29	25	163	6.60 ± 0.25	3.48	0.57
	150	2013	48	44	21	31	144	7.15 ± 0.33	3.22	0.74
	200	2173	55	72	24	37	188	8.65 ± 0.45	4.41	1.01
LAE	50	1887	35	29	20	21	105	5.56 ± 0.47	2.59	1.07
	100	2024	40	51	14	24	129	6.37 ± 0.41	3.21	0.93
	150	2217	43	56	24	26	149	6.72 ±0.57	3.60	1.29
	200	1802	45	56	21	23	145	8.04 ± 0.63	4.27	1.42

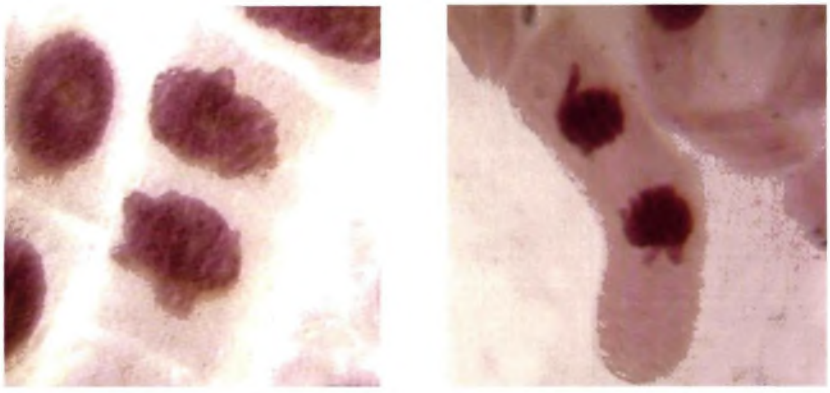
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.



Clumped metaphases



Chromatid bridges

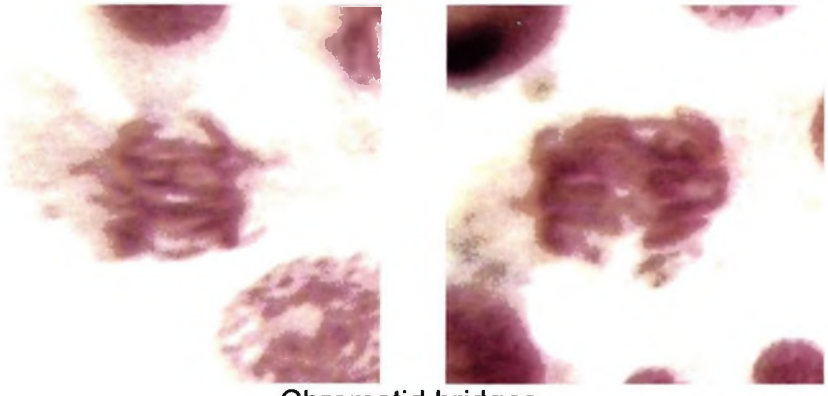


Arrested telophases

Precocious chromosomes at telophase

Plate No. 29: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Vitex* leaf petroleum ether extract.

Clumped metaphases

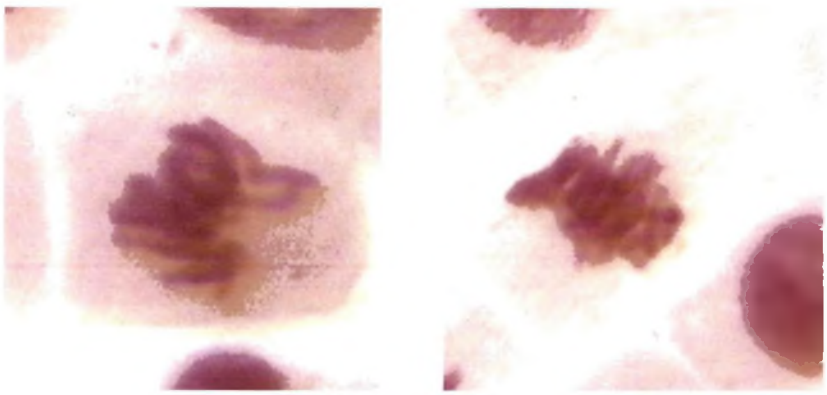


Chromatid bridges

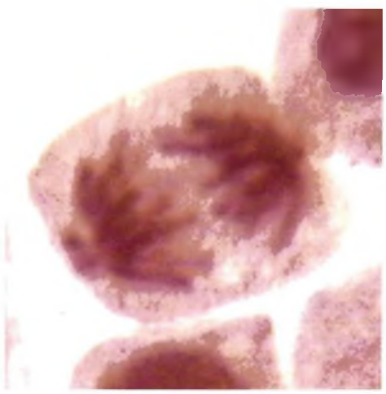
Arrested telophase

Precocious chromosomes at telophase

Plate No. 30: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Vitex* leaf aqueous extract.

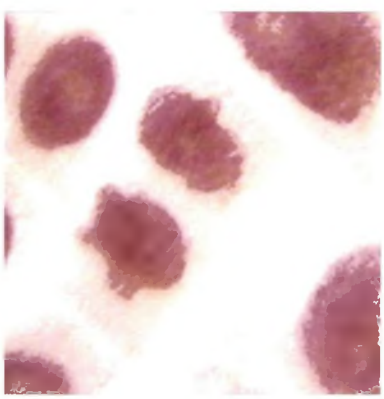


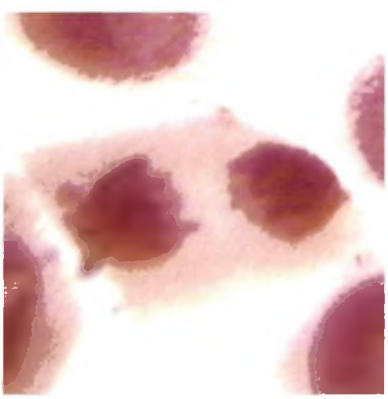
Clumped metaphases



Chromatid bridge

Arrested telophase





Precocious chromosomes
at telophases

Plate No. 31: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Vitex* leaf acetone extract.

The data on effect of leaf extracts of *V. negundo* on mitotic index and active mitotic index is tabulated in Table 34. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase, metaphase, anaphase and telophase were recorded (Plate no.3). The mitotic index frequency, in control was 11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively in 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs of methotrexate treatment.

The treatment with petroleum ether, distilled water and acetone extracts of leaf showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 7.33%, 8.82%, 10.62% and 11.10% (LPE); 5.47%, 6.60%, 7.15% and 8.65% (LDE); 5.56%, 6.37%, 6.72% and 8.04% (LAE) respectively.

4.5.3. Active mitotic index:

Active mitotic index frequencies were 4.01%, 4.83%, 6.12% and 6.25% (LPE); 2.22%, 3.48%, 3.22% and 4.41% (LDE); 2.59%, 3.21%, 3.60% and 4.27% (LAE) respectively in 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

4.5.4. Cytological abnormalities:

The cytological abnormalities are scored in mitotic cells and results are shown in Table 35. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into an observable cytological changes (Plate no. 29, 30 and 31). These were chromatid bridges, clumped metaphases, arrested telophases and precocious

chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 35 and Plate no. 4) for 3hrs in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

Table 35: Effect of leaf extracts of *Vitex negundo* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	2169	25	58	7	13	104	4.79
	100	2152	17	71	10	18	116	5.39
	150	2156	21	86	15	18	140	6.49
	200	2080	19	73	16	18	126	6.57
LDE	50	2520	12	38	14	11	75	2.97
	100	2466	14	57	13	12	96	3.89
	150	2013	16	44	18	13	91	4.52
	200	2173	17	72	20	17	126	5.79
LAE	50	1887	14	29	10	11	64	3.39
	100	2024	7	51	15	9	82	4.05
	150	2217	13	56	11	15	95	4.28
	200	1802	16	56	12	13	97	5.38

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase

The frequencies of chromosomal abnormalities in leaf extracts in 50µg, 100µg, 150µg and 200µg per ml concentrations were 4.79%, 5.39%, 6.49% and 6.57% (LPE); 2.97%, 3.89%, 4.52% and 5.79% (LDE); 3.39%, 4.05%, 4.28% and 5.38% (LAE) respectively for 3hrs treatments. No mitotic aberrations were recorded in control. The above data indicated that the chromosomal aberrations were increased with increase in concentration.

4.5.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations,

mitotic index and active mitotic index has been analysed by't' test (Table 36). The reduction in mitotic index and number of aberrations induced by extracts of leaf in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg, 150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Table 36: *Vitex negundo* leaf extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	6.10
Control and 100µg / ml Leaf petroleum ether extracts	3.33
Control and 150µg / ml Leaf petroleum ether extracts	0.80
Control and 200µg / ml Leaf petroleum ether extracts	0.016
Control and 50µg / ml Leaf aqueous extracts	9.24
Control and 100µg / ml Leaf aqueous extracts	7.06
Control and 150µg / ml Leaf aqueous extracts	8.58
Control and 200µg / ml Leaf aqueous extracts	3.20
Control and 50µg / ml Leaf acetone extracts	6.42
Control and 100µg / ml Leaf acetone extracts	7.09
Control and 150µg / ml Leaf acetone extracts	5.07
Control and 200µg / ml Leaf acetone extracts	3.35

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and 5% level).

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 6.10, 3.33, 0.80 and 0.016 (LPE); 9.24, 7.06, 8.58 and 3.20 (LDE); 6.42, 7.09, 5.07 and 3.35 (LAE) in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively. These calculated values are greater than tabulated value therefore null hypothesis is rejected and samples differs

significantly as compared to control however, at 150µg and 200µg leaf petroleum ether extracts showed lower calculated values hence samples are not significant.

4.5.6. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. The results of UV-Vis showed that the intense absorption at 220 and 297nm (LPE), 220nm (LDE) and 220 and 296nm (LAE) using acetone and at 220 and 255 (LPE), 255 and 296nm (LDE), 255 and 296m (LAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 235nm (LPE), 273nm (LDE) and 237nm (LAE) in acetone and 235nm (LPE), 273nm (LDE) and 235nm (LAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 37).

The FTIR band at 3438.71cm^{-1} (LPE), 3442 (LDE) and 3445.46cm^{-1} (LAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2926.11, 2857.64, 1457.75, 1735.99, 1220.5, 772.52, 1645.82 and 1086.44 (LPE), 2922, 1451, 1735 and 1076.32cm^{-1}(LDE) and2927, 2859, 1222, 1646.3 and 1078.24cm^{-1} (LAE) respectively whereas C=O and C=C groups were absent in leaf aqueous extract, C-H (phenyl ring) in root petroleum ether extract and C=C in root aqueous extract.

Peaks at 1086.44 to 3438.71cm-1(LPE), 1076.32 to 3493.68cm^{-1} (LDE) and 1078.24 to 3445.46cm^{-1} (LAE) favoured the presence of standard taxol group. At 1366.61cm^{-1} and 1364.2cm^{-1} indicated presence of NO_2 (nitro compounds) in leaf petroleum ether and acetone extract and absent in leaf aqueous extracts (Spectra. 25, 26 and 27).

Table 37: UV- Vis and FTIR bands in the spectra of leaf extracts of *Vitex negundo*.

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		Leaf aqueous extract		Leaf acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
220	220	220	-	220	220
-	255	-	255	-	-
297	-	-	296	296	296
235	235	273	243	237	235

FTIR ANALYSIS (cm^{-1})			
Functional groups	Leaf petroleum ether extract	Leaf aqueous extract	Leaf acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3438.71	3442	3445.46
Alkane	2926.11	2922	2927
C-H	2857.64, 1457.75	1451	2859
C=O	1735.99	1735	-
C-O	1220.5	-	1222
C-H (phenyl ring)	772.52	-	-
C=C	1645.82	-	1646.3
Std. Taxol	1086.44 to 3438.71	1076.32 to 3493.68	1078.24 to 3445.46
NO_2	1366.61	-	1364.2
C,H (aromatic bends)	1086.44	1076.32	1078.24

4.6. *Hyptis suaveolens:*

4.6.1. Quantification:

The data on quantification of petroleum ether, distilled water and acetone is given in Table 1. From 25gm of leaf powder 0.711gm, 3.593gm and 0.725gm of crude compounds were obtained whereas from 12.5gm of root powder 0.071gm, 0.899gm and 0.382gm of crude compounds were obtained using petroleum ether, distilled water and acetone as solvent, respectively.

4.6.2. Mitotic index:

The data on effect of leaf and root extracts of *H. suaveolens* on mitotic index and active mitotic index is tabulated in Table 38 and 39. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase,

metaphase, anaphase and telophase were recorded (Plate no. 3). The mitotic index frequency, in control was 11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively in 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs of methotrexate treatment.

Table 38: Effect of leaf extracts of *Hyptis suaveolens* on mitosis in *Allium cepa.*

Treatments	Cone. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	2033	34	42	21	08	105	5.16 ± 0.36	3.09	0.81
	100	1823	31	58	24	22	135	7.38 ± 0.26	4.48	0.59
	150	1762	30	35	21	17	103	5.84 ± 0.36	3.17	0.82
	200	1925	27	47	21	16	111	5.76 ± 0.39	3.53	0.89
LDE	50	2139	51	66	30	30	177	8.27 ± 0.37	4.48	0.84
	100	2154	48	58	24	38	168	7.79 ± 0.67	3.80	1.50
	150	2531	61	10	38	33	237	9.36 ± 0.37	5.64	0.84
	200	1676	45	80	29	25	179	10.68±0.67	6.50	1.50
LAE	50	2080	51	47	31	34	163	7.83 ± 0.41	3.75	0.92
	100	2319	43	42	27	26	138	5.95 ± 0.30	2.97	0.69
	150	2007	46	67	27	30	170	8.47 ± 0.35	4.68	0.80
	200	1897	35	60	25	30	150	7.90 ± 0.80	4.48	1.79

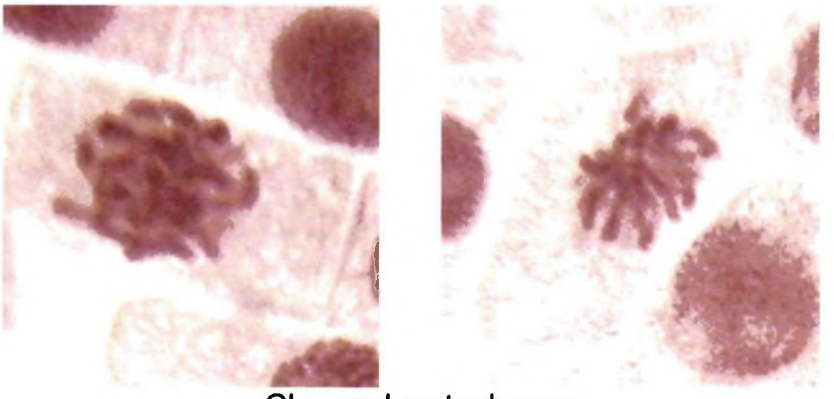
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

Table 39: Effect of root extracts of *Hyptis suaveolens* on mitosis in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	2032	37	35	13	18	103	5.06 ± 0.18	2.36	0.41
	100	1964	31	31	18	20	100	5.09 ± 0.10	2.49	0.24
	150	1828	36	33	19	22	110	6.01 ± 0.25	2.84	0.57
	200	1736	37	29	17	21	104	6.12 ± 0.65	2.64	1.45
RDE	50	1850	32	22	13	16	83	4.48 ± 0.32	1.89	0.73
	100	1628	36	27	16	24	103	6.32 ± 0.20	2.64	0.46
	150	1681	30	48	16	20	114	6.78 ± 0.32	3.80	0.72
	200	2243	47	63	21	28	159	7.08 ± 0.33	3.74	0.74
RAE	50	2349	28	41	17	13	99	4.21 ± 0.36	2.46	0.81
	100	1860	40	30	17	18	105	5.64 ± 0.17	2.52	0.38
	150	1526	24	32	15	18	89	5.83 ± 0.28	3.07	0.63
	200	1716	38	48	19	25	130	7.57 ± 0.45	3.90	1.01

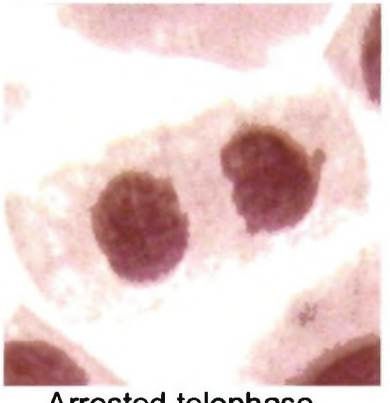
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root in 50µg, 100µg, 150µg and 200µg per ml concentrations showed mitotic index frequencies were 5.16%, 7.38%, 5.84% and 5.76% (LPE); 8.27%, 7.79%, 9.36% and 10.68% (LDE); 7.83%, 5.95%, 8.47% and 7.90% (LAE); 5.06%, 5.09%, 6.01% and 6.12% (RPE); 4.48%, 6.32%, 6.78% and 7.08% (RDE) and 4.21%, 5.64%, 5.83% and 7.57% (RAE) respectively.

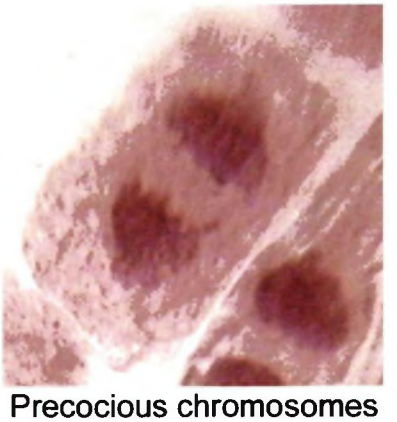


Clumped metaphases

Chromatid bridges



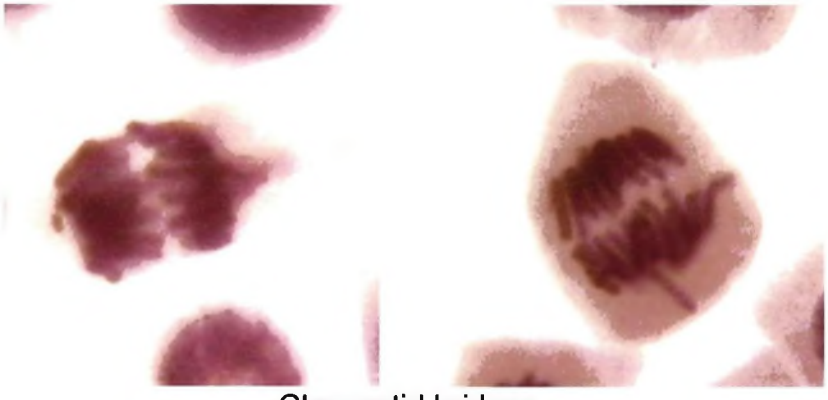
Arrested telophase



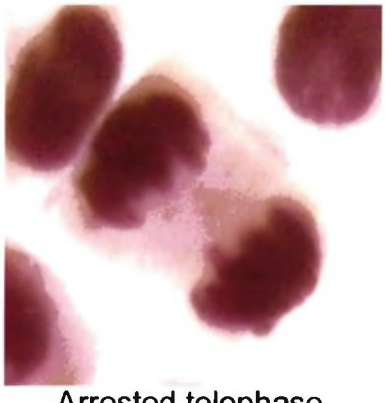
Precocious chromosomes at telophase

Plate No. 32: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* leaf petroleum ether extract.

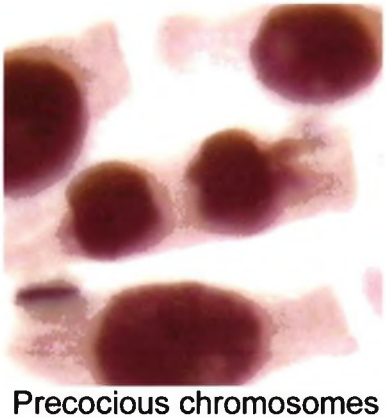
Clumped metaphases



Chromatid bridges

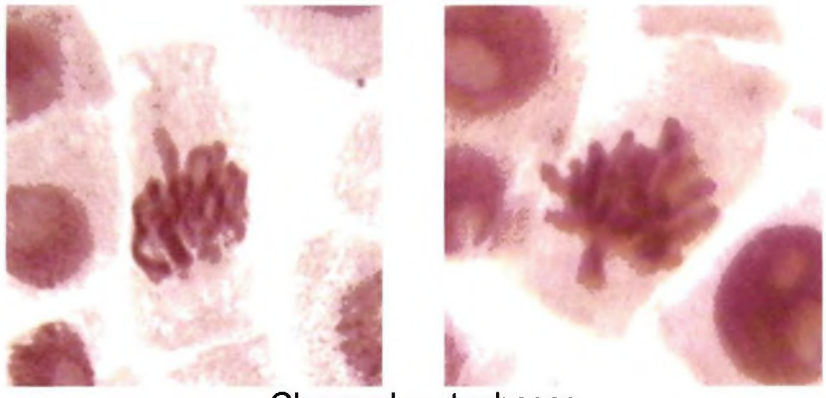


Arrested telophase

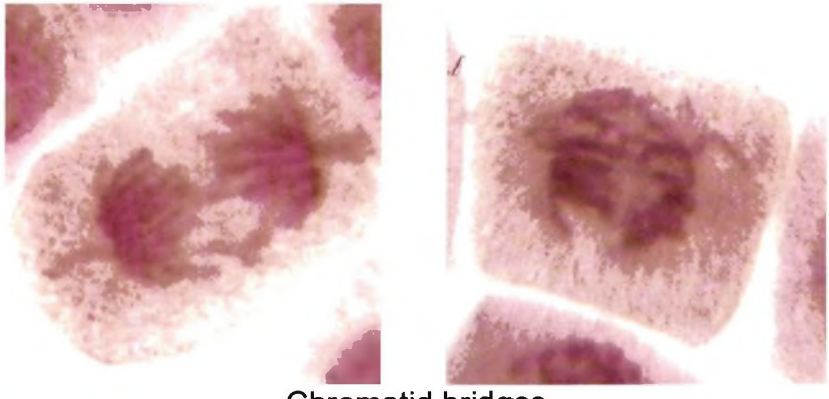


Precocious chromosomes at telophase

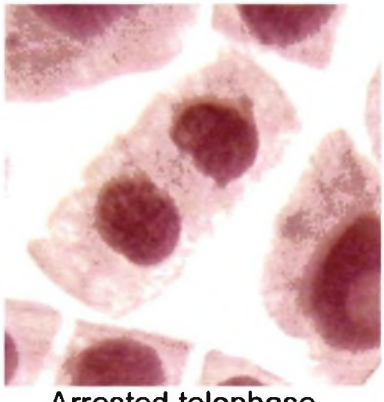
Plate No. 33: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* leaf aqueous extract.



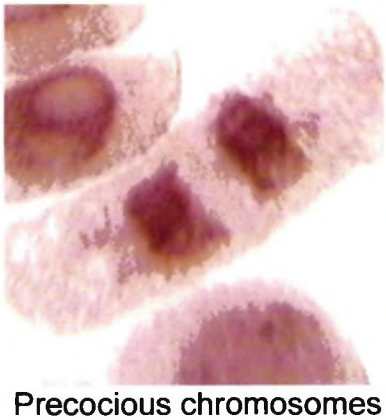
Clumped metaphases



Chromatid bridges

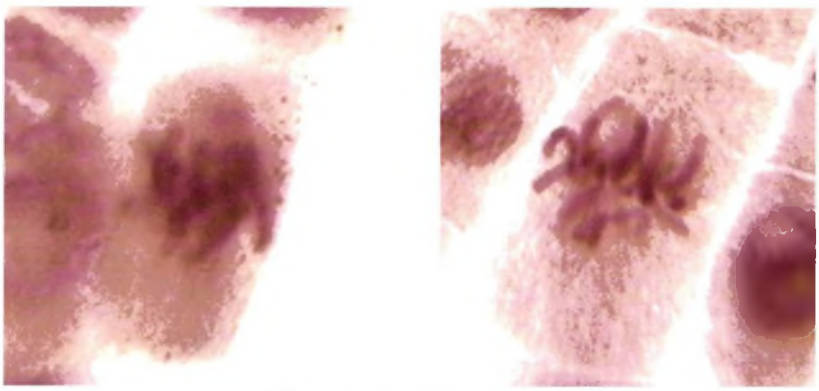


Arrested telophase

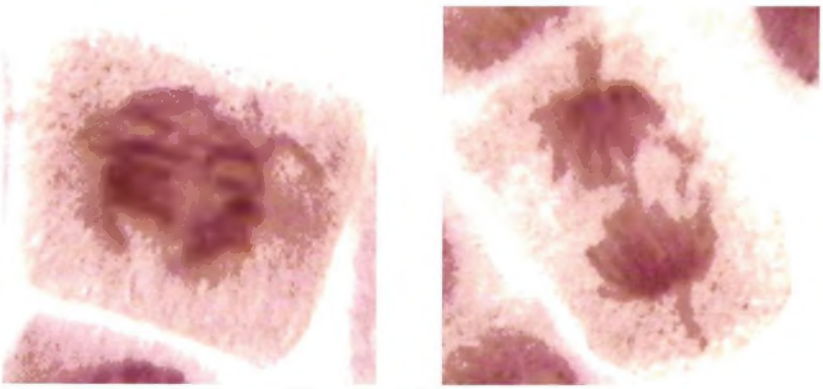


Precocious chromosomes at telophase

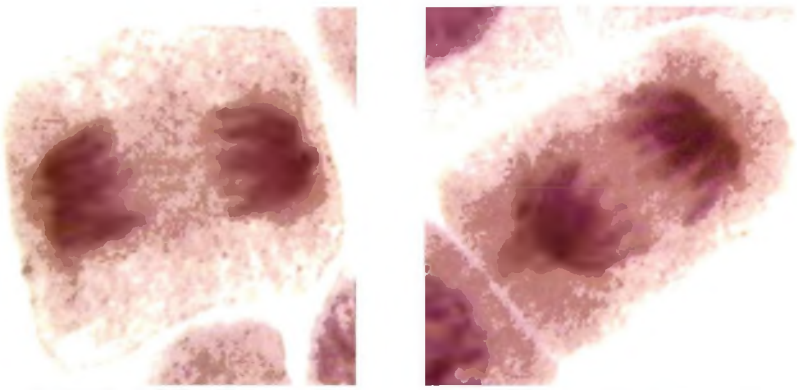
Plate No. 34: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* leaf acetone extract.



Clumped metaphases



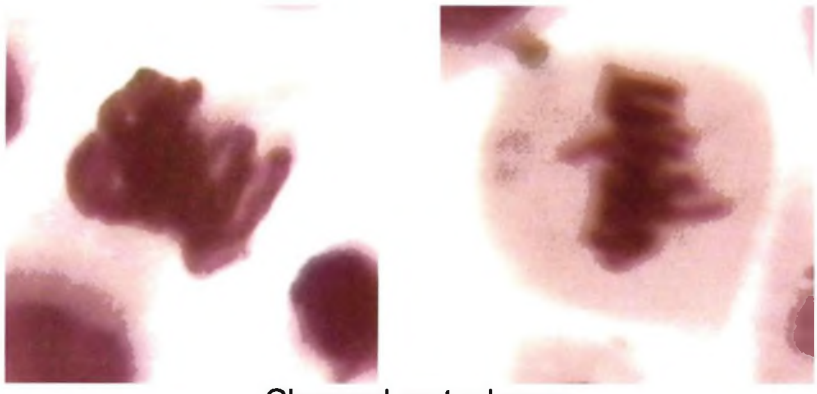
Chromatid bridges



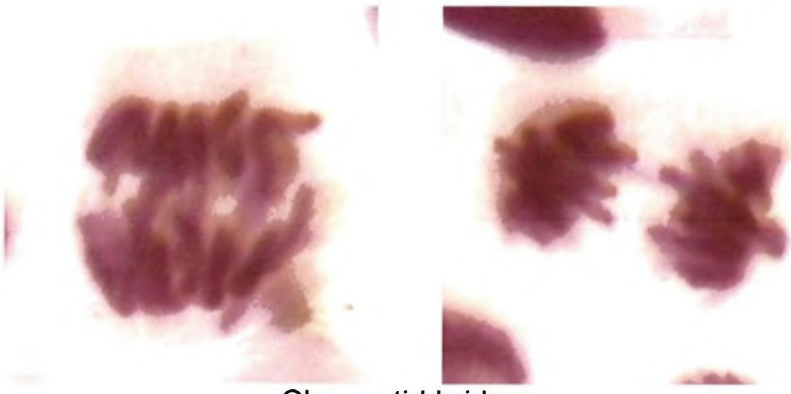
Arrested telophase

Precocious chromosomes at telophase

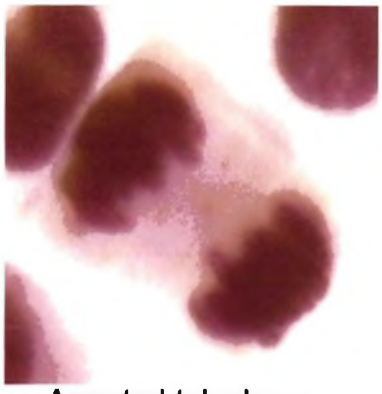
Plate No. 35: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* root petroleum ether extract.



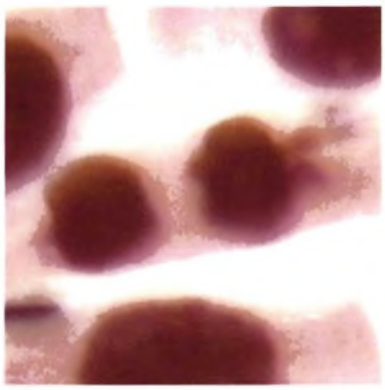
Clumped metaphases



Chromatid bridges

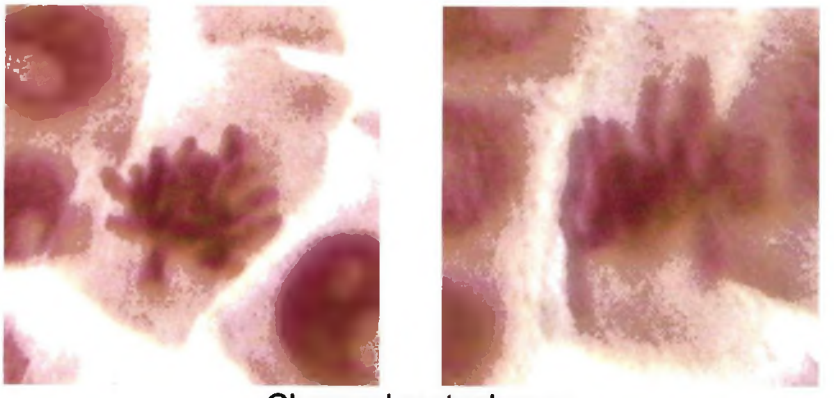


Arrested telophase

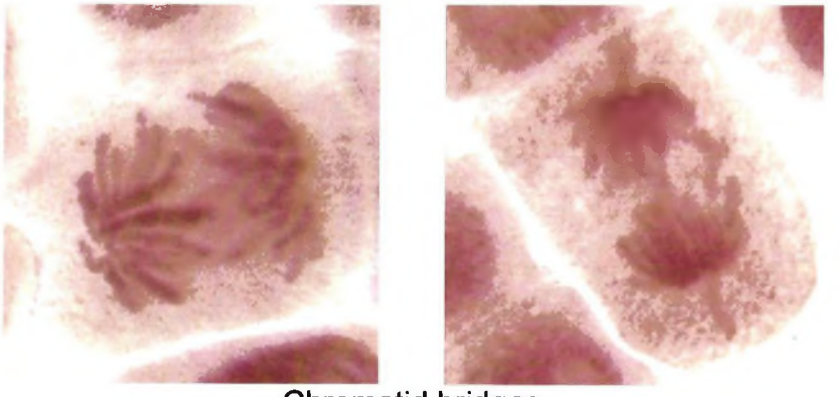


Precocious chromosomes at telophase

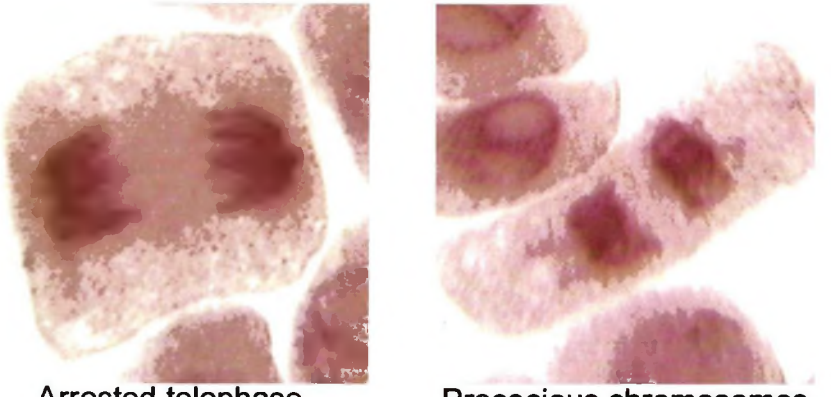
Plate No. 36: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* root aqueous extract.



Clumped metaphases



Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No. 37: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Hyptis* root acetone extract.

4.6.3. Active mitotic index:

Active mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 3.09%, 4.48%, 3.17% and 3.53% (LPE); 4.48%, 3.80%, 5.64% and 6.50% (LDE); 3.75%, 2.97%, 4.68% and 4.48% (LAE); 2.36%, 2.49%, 2.84% and 2.64% (RPE); 1.89%, 2.64%, 3.80% and 3.74% (RDE) and 2.46%, 2.52%, 3.07% and 3.90% (RAE) respectively for 3hrs of treatments. Mitotic index was increased with increase in concentrations whereas in leaf petroleum ether extract mitotic index at 100µg was more as compared to other concentrations and in leaf acetone extract at 100µg it was less than other concentrations.

4.6.4. Cytological abnormalities:

The cytological abnormalities are scored in mitotic cells and results are shown in Table 40 and 41. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into observable cytological changes (Plate no. 32-37). These were chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 40, 41 and Plate no. 4) for 3hrs in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

The frequencies of chromosomal abnormalities at 50µg, 100µg, 150µg and 200µg per ml concentrations in leaf and root extracts treatments were 2.60%, 4.59%, 3.23% and 3.58% (LPE); 5.28%, 4.87%, 4.74% and 6.92% (LDE); 4.79%, 3.75%, 5.72% and 5.37% (LAE); 3.10%, 3.15%, 3.77% and 3.57% (RPE); 2.74%, 3.86%, 4.75% and 4.81% (RDE) and 2.97%, 3.26%, 4.25% and 5.01% (RAE) respectively for 3hrs treatments. No mitotic aberrations were recorded in control.

Table 40: Effect of leaf extracts of *Hyptis suaveolens* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	2033	3	42	5	3	53	2.60
	100	1828	4	58	12	10	84	4.59
	150	1762	5	35	10	7	57	3.23
	200	1925	6	47	9	7	69	3.58
LDE	50	2139	19	63	23	8	113	5.28
	100	2154	15	53	19	18	105	4.87
	150	2531	26	61	17	16	120	4.74
	200	1676	19	72	9	16	116	6.92
LAE	50	2080	18	47	14	20	99	4.79
	100	2319	19	42	12	14	87	3.75
	150	2007	18	67	16	14	115	5.72
	200	1897	15	60	13	14	102	5.37

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

Table 41: Effect of root extracts of *Hyptis suaveolens* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
RPE	50	2032	10	35	8	10	63	3.10
	100	1964	11	31	6	14	62	3.15
	150	1828	14	33	8	14	69	3.77
	200	1736	12	29	8	13	62	3.57
RDE	50	1850	12	22	6	11	51	2.74
	100	1628	12	27	12	12	63	3.86
	150	1681	12	48	9	11	80	4.75
	200	2243	17	63	9	19	108	4.81
RAE	50	2349	16	41	4	9	70	2.97
	100	1860	13	30	7	11	61	3.26
	150	1526	15	32	6	12	65	4.25
	200	1716	14	48	11	13	86	5.01

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

In leaf petroleum ether extract chromosomal abnormalities were higher in 100µg whereas lower in 100µg leaf aqueous and leaf acetone extracts and in root extracts chromosomal abnormalities were increased with increase in concentrations except in root petroleum ether extract.

4.6.5. Statistical analysis:

Table 42: *Hyptis suaveolens* leaf extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	4.5
Control and 100µg / ml Leaf petroleum ether extracts	3.03
Control and 150µg / ml Leaf petroleum ether extracts	4.91
Control and 200µg / ml Leaf petroleum ether extracts	4.24
Control and 50µg / ml Leaf aqueous extracts	3.99
Control and 100µg / ml Leaf aqueous extracts	3.50
Control and 150µg / ml Leaf aqueous extracts	2.45
Control and 200µg / ml Leaf aqueous extracts	0.44
Control and 50µg / ml Leaf acetone extracts	4.46
Control and 100µg / ml Leaf acetone extracts	7.73
Control and 150µg / ml Leaf acetone extracts	3.77
Control and 200µg / ml Leaf acetone extracts	3.00

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by't' test (Table 42 and 43). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg,

150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Table 43: *Hyptis suaveolens* root extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	9.96
Control and 100µg / ml Root petroleum ether extracts	10.30
Control and 150µg / ml Root petroleum ether extracts	7.99
Control and 200µg / ml Root petroleum ether extracts	4.63
Control and 50µg / ml Root aqueous extracts	9.77
Control and 100µg / ml Root aqueous extracts	7.77
Control and 150µg / ml Root aqueous extracts	6.4
Control and 200µg / ml Root aqueous extracts	5.92
Control and 50µg / ml Root acetone extracts	9.84
Control and 100µg / ml Root acetone extracts	9.08
Control and 150µg / ml Root acetone extracts	14.47
Control and 200µg / ml Root acetone extracts	4.62

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level.

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 4.5, 3.03, 4.91 and 4.24 (LPE); 3.99, 3.50, 2.45 and 0.44 (LDE); 4.46, 7.73, 3.77 and 3.00 (LAE); 9.96, 10.30, 7.99 and 4.63 (RPE); 9.77, 7.77, 6.4 and 5.92 (RDE) and 9.84, 9.08, 14.47 and 4.62 (RAE) respectively. Calculated value of 200µg (0.44) leaf distilled water extract was less than tabulated value (2.3) hence null hypothesis is accepted and sample is not differ significantly. However, in remaining leaf and root extracts were showed higher

calculated value therefore null hypothesis is rejected and samples are differing significantly when compared with untreated control.

4.6.6. Spectroscopic analysis:

Table 44: UV- Vis and FTIR bands in the spectra of leaf extracts of *Hyptis suaveolens*.

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		Leaf aqueous extract		Leaf acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
220	220	220	220	220	224
258	255	252	255	255	255
295	294	295	299	295	296
236	235	236	236	235	235

FTIR ANALYSIS (cm^{-1})			
Functional groups	Leaf petroleum ether extract	Leaf aqueous extract	Leaf acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3400	3431.96	3452.21, 1550.34
Alkane	2927.56	2925.15	2928.04
C-H	2864	2860.53, 1436.05	2865.83, 1443.28
C=O	1735.51	1725.87	1736.96
C-O	1219.53	1008.32	1219.05
C-H (phenyl ring)	-	-	766.25
C=C	-	1672.82	1639.55
Std. Taxol	1078.24 to 3460.41	1008.32 to3482.11	1090.3 to 3452.21
NO_2	-	-	1366.61
C,H (aromatic bends)	1078.24	1071.01	1090.3

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. . The results of UV-Vis showed that the intense absorption at 220, 258 and 295nm (LPE), 220, 252 and 295nm (LDE) 220, 255 and 295nm (LAE) and 220, 255 and 296nm (RPE), 222, 256 and 295nm (RDE) and 220 and 299 (RAE) using acetone and at 220, 257 and 296nm (LPE), 220, 255 and 299nm (LDE) , 224, 255 and 295nm (LAE), 220, 256 and 295nm (RPE), 220, 250 and 296nm (RDE) and 221 and 253nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 236nm (LPE), 236nm

(LDE), 235nm (LAE), 237nm (RPE), 236nm (RDE) and 237nm (RAE) in acetone and 235nm (LPE), 236nm (LDE), 235nm (LAE), 235nm (RPE), 235nm (RDE) and 236nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol.

Table 45: UV- Vis and FTIR bands in the spectra of root extracts of *H. suaveolens*.

UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		Root aqueous extract		Root acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
220	220	220	220	220	224
258	255	252	255	255	255
295	294	295	299	295	296
236	235	236	236	235	235

FTIR ANALYSIS (cm^{-1})			
Functional groups	Root petroleum ether extract	Root aqueous extract	Root acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3458.48, 1563.84	3434.85, 1598.08	3448.36, 1555.64
Alkane	2928.52	2925.63	2928.52
C-H	2865.35	2859.56, 1422.55	2866.8, 1451
C=O	1734.06	-	1705.13
C-O	1219.05	1247.02	1219.53
C-H (phenyl ring)	836.17	826.53	-
C=C	-	1646.78	1641.48
Std. Taxol	1083.07 to 3458.48	1071.01 to 3477.77	1076.8 to 3489.34
NO_2	1367.09	-	1366.13
C,H (aromatic bends)	1083.07	1071.01	1076.8

The FTIR band at 3400cm^{-1} (LPE), 3431.96cm^{-1} (LDE), 3452.21, 1550.34cm^{-1} (LAE), 3458.48, 1563.84cm^{-1} (RPE), 3434.85, 1598.08cm^{-1} (RDE) and 3448.36, 1555.64cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2927.56, 2864, 1735.51, 1219.53 and 1078.24cm^{-1}(LPE), 2925.15, 2860.53, 1436.05, 1725.87, 1008.32, 1672.82 and 1071.01cm^{-1}(LDE), 2928.04, 2865.83, 1443.28, 1736.96, 1219.05, 766.25, 1639.55 and 1090.3cm^{-1} (LAE), 2928.52, 2865.35, 1734.06, 1219.05, 836.17 and 1083.07cm^{-1} (RPE), 2925.63, 2859.56, 1422.55, 1247.02, 826.53,

1646.78 and 1071.01cm^{-1} (RDE) and 2928.52, 2866.8, 1451, 1705.13, 1219.53, 1641.48 and 1076.8cm^{-1} (RAE) respectively whereas C-H (phenyl ring) were absent in leaf petroleum ether and aqueous extracts and C=C groups were absent in leaf petroleum ether extract and C=O in root aqueous extract, C-H (phenyl ring) in root aqueous extract were absent (Table 44 and 45)

Peaks at 1078.24 to 3460.41cm^{-1} (LPE), 1008.32 to3482.11cm^{-1} (LDE), 1090.3 to 3452.21cm^{-1} (LAE), 1083.07 to 3458.48cm^{-1} (RPE), 1071.01 to 3477.77cm^{-1} (RDE) and 1076.8 to 3489.34cm^{-1} (RAE) favoured the presence of standard taxol group. At 1366.61cm^{-1} indicated presence of NO_2 (nitro compounds) in leaf acetone extract and absent in leaf petroleum ether and aqueous extracts and at 1367.09cm^{-1} and 1366.13cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract.

4.7. *Antirrhinum majus:*

4.7.1. Quantification:

The data on quantification of petroleum ether, distilled water and acetone is given in Table 1. From 25gm of leaf powder 0.228gm, 14.54gm and 1.696gm of crude compounds were obtained whereas from 12.5gm of root powder 0.106gm, 2.453gm and 0.422gm of crude compounds were obtained using petroleum ether, distilled water and acetone as solvent, respectively.

4.7.2. Mitotic index:

The data on effect of leaf and root extracts of *A. majus* on mitotic index and active mitotic index is tabulated in Table 46 and 47. The cell divisions were differentiated and number of cells in each phase of cell division i.e. prophase, metaphase, anaphase and telophase were recorded (Plate no. 3). The mitotic index frequency, in control was

11.26% and active mitotic index frequency was 7.93%. The standard control i.e. methotrexate showed mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 4.80%, 5.29%, 5.41% and 6.18% respectively. Active mitotic index frequencies were 1.77%, 2.40%, 2.59% and 2.62% respectively for 3hrs of methotrexate treatment.

Table 46: Effect of leaf extracts of *Antirrhinum majus* on mitosis in *A. cepa*.

Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
LPE	50	2527	30	40	28	21	119	4.70 ± 0.16	2.69	0.36
	100	1946	35	43	22	22	122	6.26 ± 0.29	3.34	0.65
	150	1908	38	36	22	27	123	6.44 ± 0.36	3.03	0.82
	200	1815	35	30	26	36	127	6.99 ± 0.36	3.08	0.82
LDE	50	1872	24	33	19	28	104	5.55 ± 0.25	2.77	0.56
	100	1860	34	48	25	19	126	6.77 ± 0.27	3.92	0.61
	150	1867	39	54	21	31	145	7.76 ± 0.53	4.01	1.19
	200	1849	43	48	25	30	146	7.89 ± 0.55	3.94	1.23
LAE	50	2044	33	45	16	18	112	5.47 ± 0.26	2.98	0.59
	100	1919	32	29	20	32	113	5.88 ± 0.30	2.55	0.67
	150	1878	35	37	17	23	112	5.96 ± 0.18	2.87	0.41
	200	1851	39	44	26	24	133	7.18 ± 0.48	3.78	1.09

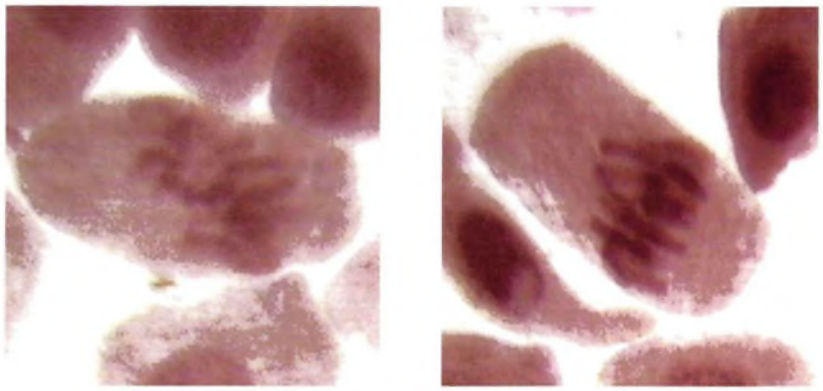
P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

Table 47: Effect of root extracts of *Antirrhinum majus* on mitosis in *A. cepa*.

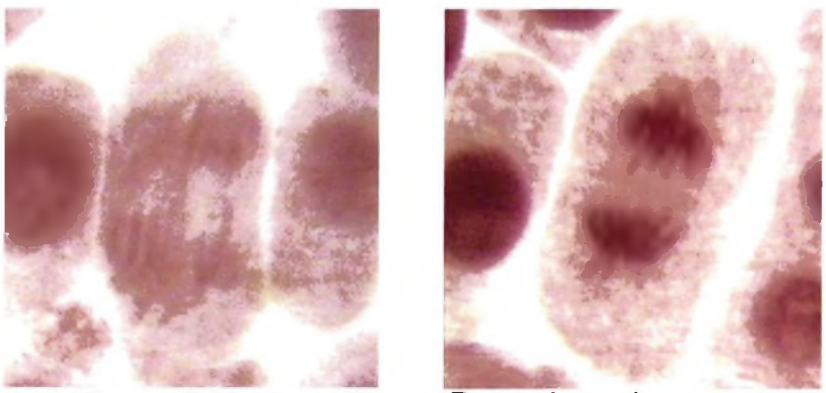
Treatments	Conc. (µg)	No. of cells observed	No. dividing cells				Total no. of dividing cells	Mitotic index frequency, S.E.	Active mitotic index freq.	S.D
			P	M	A	T				
Control	00	1714	33	78	58	24	193	11.26±0.41	7.93	0.92
MTX	50	1686	26	15	15	23	79	4.80 ± 0.46	1.77	1.03
	100	1996	36	33	15	22	106	5.29 ± 0.32	2.40	0.72
	150	1831	30	29	19	20	98	5.41 ± 0.16	2.59	0.37
	200	1810	37	22	25	27	111	6.18 ± 0.24	2.62	0.54
RPE	50	2112	37	23	12	15	87	4.13 ± 0.17	1.65	0.39
	100	2113	38	30	14	18	100	4.76 ± 0.37	2.08	0.84
	150	1857	31	34	13	15	93	4.98 ± 0.23	2.53	0.52
	200	1968	39	41	16	26	122	6.19 ± 0.13	2.89	0.31
RDE	50	1884	27	19	16	21	83	4.39 ± 0.14	1.85	0.33
	100	1767	26	23	13	22	84	4.77 ± 0.51	2.03	1.14
	150	1681	31	24	15	25	95	5.66 ± 0.30	2.32	0.68
	200	1901	38	33	18	19	108	5.70 ± 0.13	2.68	0.29
RAE	50	2022	18	20	14	19	71	3.5 ± 0.10	1.68	0.23
	100	1905	32	26	15	19	92	4.85 ± 0.25	2.15	0.57
	150	1983	36	26	17	18	97	4.89 ± 0.20	2.16	0.46
	200	1736	15	26	15	16	72	5.3 ± 0.30	2.36	0.68

P – Prophase, **M** – Metaphase, **A** – Anaphase, **T** – Telophase, **S.E.** – Standard error, **S.D.** – Standard deviation.

The treatment with petroleum ether, distilled water and acetone extracts of leaf and root showed mitotic index frequencies were 4.70%, 6.26%, 6.44% and 6.99% (LPE); 5.55%, 6.77%, 7.76% and 7.89% (LDE); 5.47%, 5.88%, 5.96% and 7.18% (LAE); 4.13%, 4.76%, 4.98% and 6.19% (RPE); 4.39%, 4.77%, 5.66% and 5.70% (RDE) and 3.5%, 4.85%, 4.89% and 5.3% (RPE) in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

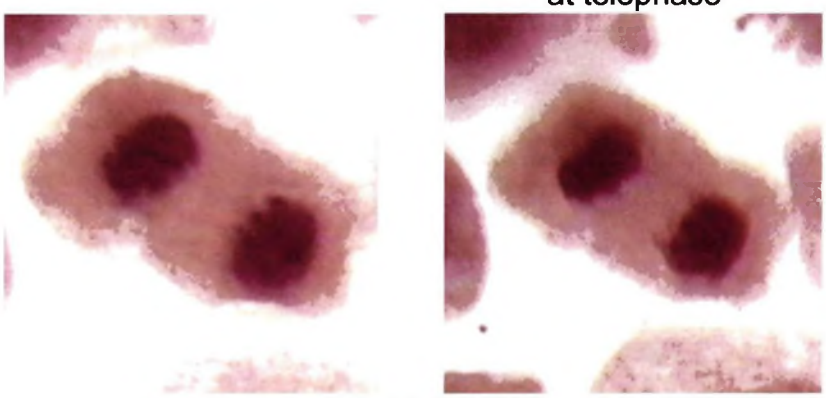


Clumped metaphases



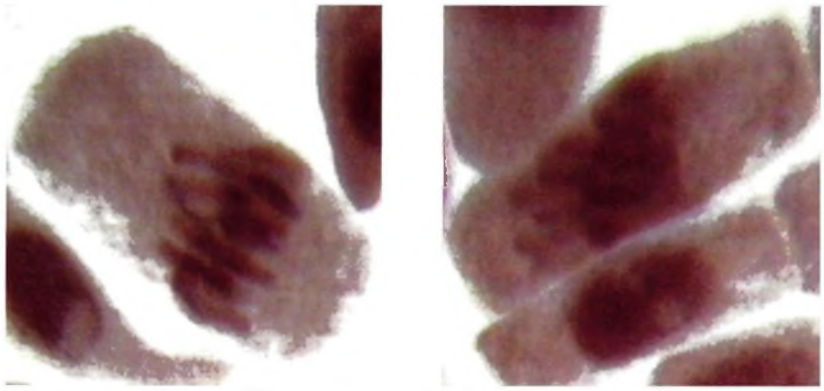
Chromatid bridge

Precocious chromosomes at telophase

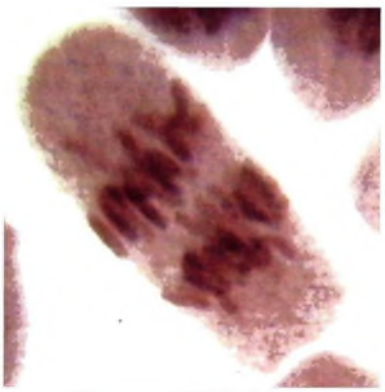


Arrested telophases

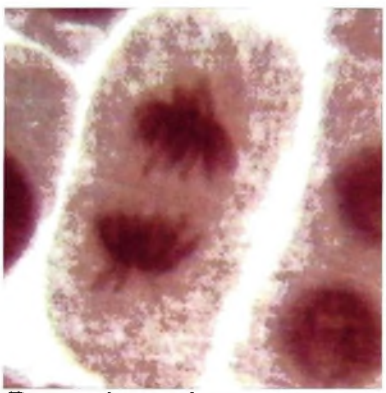
Plate No. 38: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Antirrhinum* leaf petroleum ether extract.



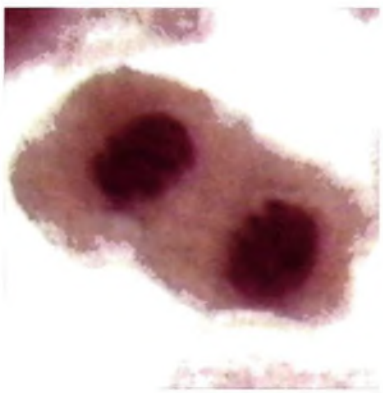
Clumped metaphases



Chromatid bridge

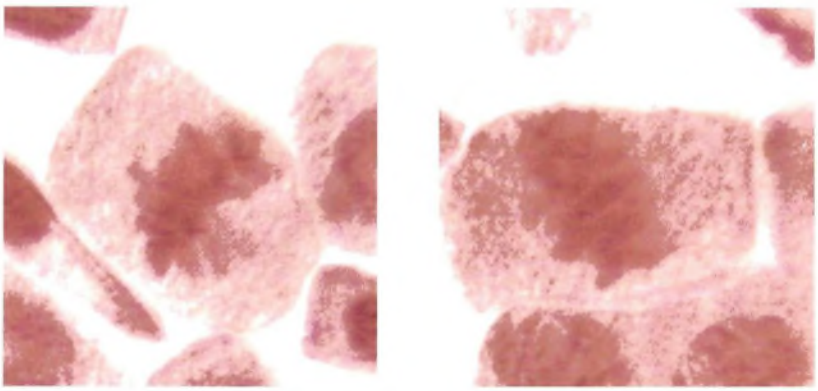


Precocious chromosomes at telophase

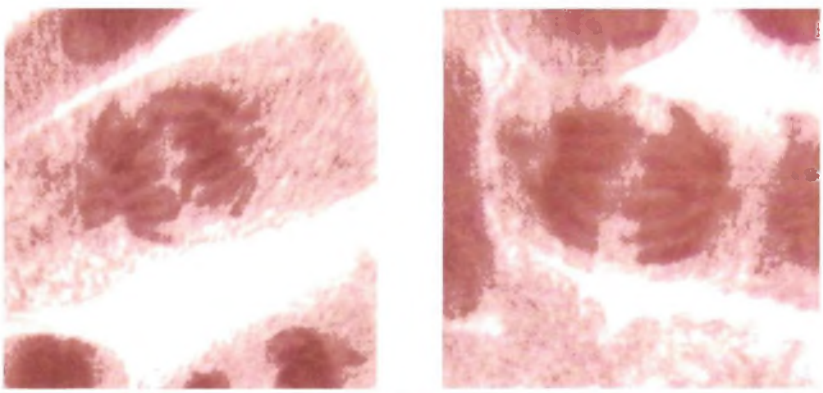


Arrested telophases

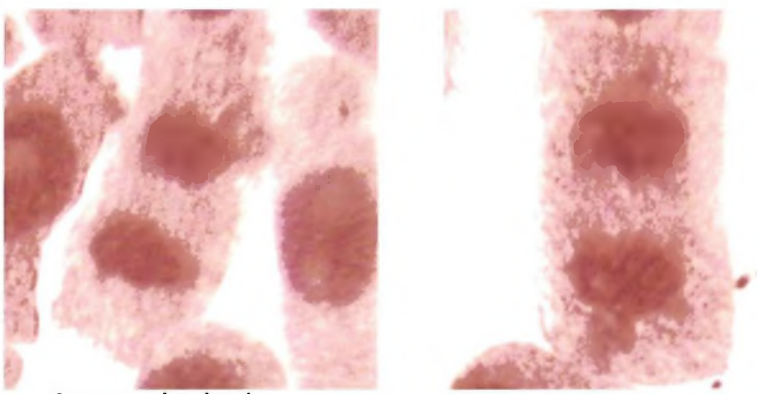
Plate no. 39: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Antirrhinum* leaf aqueous extract.



Clumped metaphases



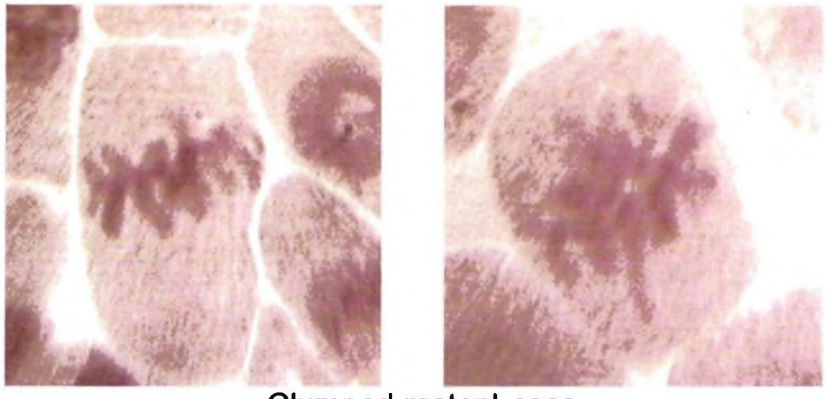
Chromatid bridges



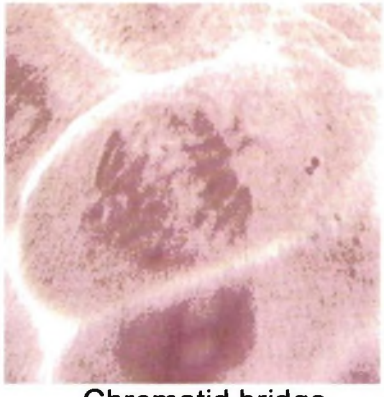
Arrested telophase

Precocious chromosomes at telophase

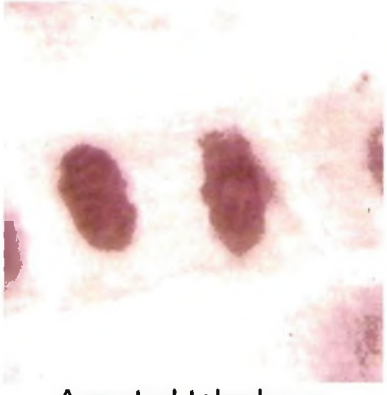
Plate No. 40: Microphotograph showing chromosomal abnormalitiesin *Allium cepa* (2n=16) after the treatment of *Antirrhinum* leaf acetone extract.



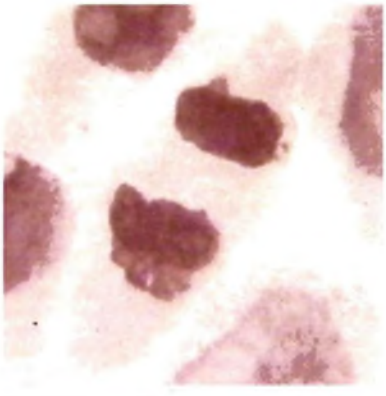
Clumped metaphases



Chromatid bridge

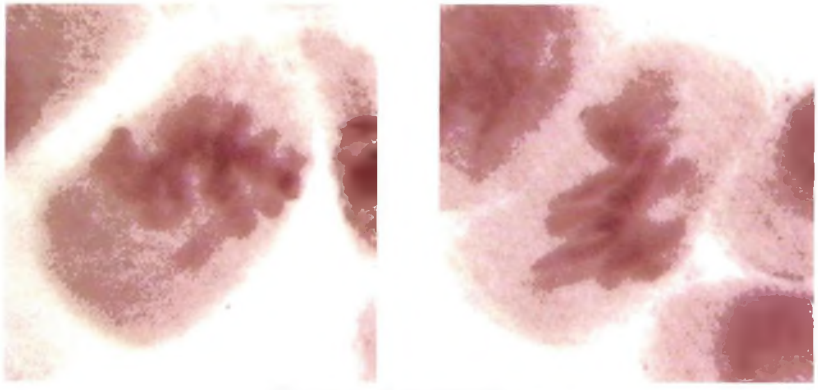


Arrested telophase

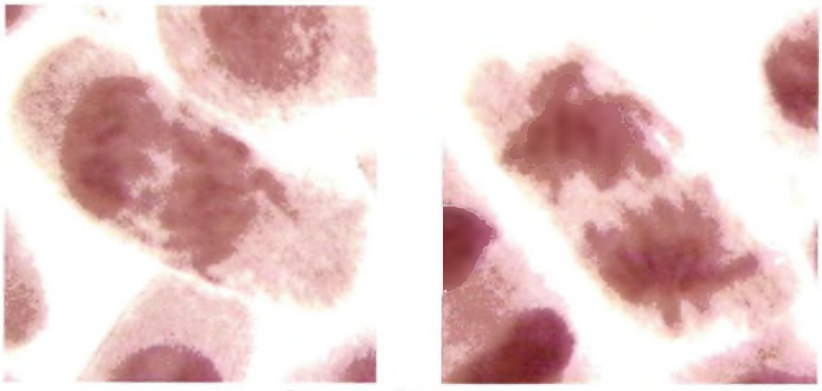


Precocious chromosomes
at telophases

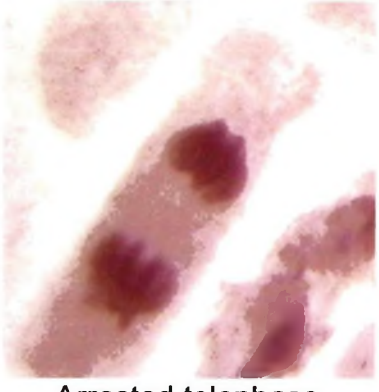
Plate No. 41: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Antirrhinum* root petroleum ether extract.



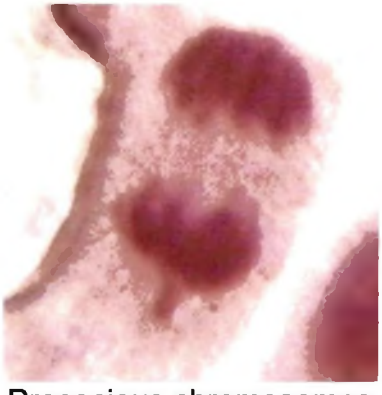
Clumped metaphases



Chromatid bridges

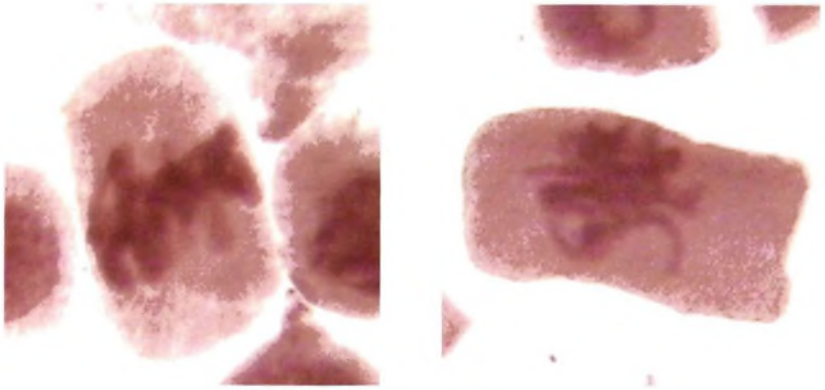


Arrested telophase

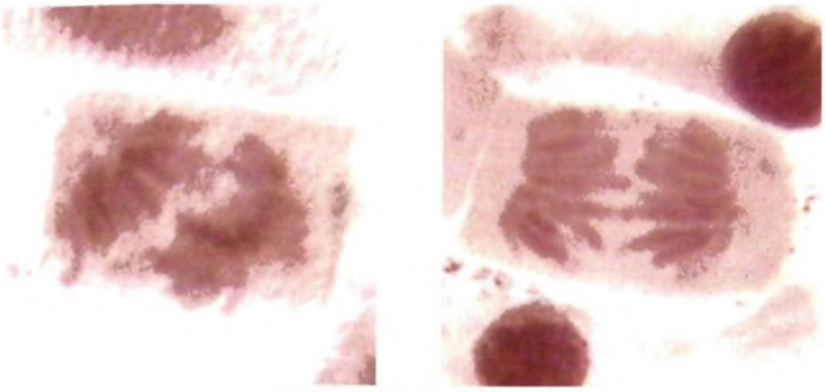


Precocious chromosomes at telophase

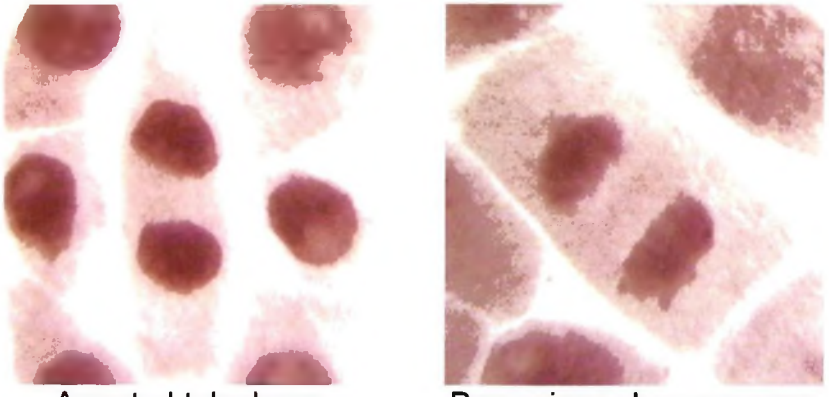
Plate No. 42: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Antirrhinum* root aqueous extract.



Clumped metaphases



Chromatid bridges



Arrested telophase

Precocious chromosomes at telophase

Plate No. 43: Microphotograph showing chromosomal abnormalities in *Allium cepa* (2n=16) after the treatment of *Antirrhinum* root acetone extract.

4.7.3. Active mitotic index:

Active mitotic index frequencies in 50µg, 100µg, 150µg and 200µg per ml concentrations were 2.69%, 3.34%, 3.03% and 3.08% (LPE); 2.77%, 3.92%, 4.01% and 3.94% (LDE); 2.98%, 2.55%, 2.87% and 3.78% (LAE); 1.65%, 2.08%, 2.53% and 2.89% (RPE); 1.85%, 2.03%, 2.32% and 2.68% (RDE) and 1.68%, 2.15%, 2.16% and 2.36% (RAE) respectively for 3hrs of treatments. At 50µg and 100µg concentration all the leaf and root extracts showed lowest mitotic index.

4.7.4. Cytological abnormalities:

Table 48: Effect of leaf extracts of *Antirrhinum majus* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	2527	14	40	12	9	75	2.96
	100	1946	16	43	10	10	79	4.05
	150	1908	18	36	11	16	81	4.24
	200	1815	19	30	19	17	85	4.68
LDE	50	1872	12	33	15	12	72	3.84
	100	1860	18	48	11	6	83	4.46
	150	1867	13	54	18	13	48	5.24
	200	1849	15	48	18	12	93	5.02
LAE	50	2044	7	45	10	8	70	3.42
	100	1919	13	29	19	14	75	3.90
	150	1878	15	37	12	11	75	3.99
	200	1851	16	44	13	11	84	4.53

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

The cytological abnormalities are scored in mitotic cells and results are shown in Table 48 and 49. The treatment with standard control (methotrexate) Plate no. 4, leaf and root extracts resulted into an observable cytological changes (Plate no. 38-43) these were chromatid bridges, clumped metaphases, arrested telophases and precocious

chromosome at telophases. The frequencies of chromosomal abnormalities after the treatment of methotrexate were 2.96%, 3.30%, 3.48% and 3.49% (Table 48, 49 and Plate no. 4) for 3hrs in 50µg, 100µg, 150µg and 200µg per ml concentrations respectively.

The frequencies of chromosomal abnormalities in leaf and root extracts treatments were 2.96%, 4.05%, 4.24% and 4.68% (LPE); 3.84%, 4.46%, 5.24% and 5.02% (LDE); 3.42%, 3.90%, 3.99% and 4.53% (LAE); 2.32%, 2.79%, 3.23% and 3.96% (RPE); 2.60%, 3.16%, 3.50% and 3.52% (RDE) and 2.42%, 2.99%, 2.92% and 3.04% (RAE) respectively at 50µg, 100µg, 150µg and 200µg per ml concentrations for 3hrs treatments. No mitotic aberrations were recorded in control. The above data indicated that the chromosomal aberrations were increased with increase in concentrations.

Table 49: Effect of root extracts of *Antirrhinum majus* on somatic chromosomes in *Allium cepa*.

Treatments	Conc. (µg)	No. of cells observed	Type of mitotic abnormalities				Total no. of abnormal cells	Frequencies of chromosomal aberrations
			CB	CM	AT	PT		
Control	00	1714	0	0	0	0	00	0.0
MTX	50	1686	12	15	8	15	50	2.96
	100	1996	11	33	8	14	66	3.30
	150	1831	15	29	8	13	63	3.48
	200	1810	14	22	10	16	64	3.49
LPE	50	2112	11	23	5	10	49	2.32
	100	2113	11	30	7	11	59	2.79
	150	1857	11	34	2	13	60	3.23
	200	1968	12	41	12	13	78	3.96
LDE	50	1884	11	19	6	13	49	2.60
	100	1767	11	23	7	15	56	3.16
	150	1681	11	24	10	14	59	3.50
	200	1901	15	33	6	13	67	3.52
LAE	50	2022	11	20	6	12	49	2.42
	100	1905	12	26	7	12	57	2.99
	150	1983	13	26	7	12	58	2.92
	200	1736	12	25	6	10	53	3.04

CB – Chromatid Bride, **CM** – Clumped metaphases, **AT** = Arrested Telophase, **PT** = Precocious chromosome at Telophase.

4.7.5. Statistical analysis:

The mitotic index, active mitotic index and somatic chromosomal aberrations were scored. The significance among the mean results of total number of aberrations, mitotic index and active mitotic index has been analysed by 't' test (Table 50 and 51). The reduction in mitotic index and number of aberrations induced by extracts of leaf and root in petroleum ether, distilled water and acetone as solvent with 50µg, 100µg, 150µg and 200µg per ml dosage which represented its mutagenic/genotoxic actions in *Allium cepa.*

Table 50: *Antirrhinum majus* leaf extracts: - Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Leaf petroleum ether extracts	10.70
Control and 100µg / ml Leaf petroleum ether extracts	7.36
Control and 150µg / ml Leaf petroleum ether extracts	6.62
Control and 200µg / ml Leaf petroleum ether extracts	5.83
Control and 50µg / ml Leaf aqueous extracts	8.72
Control and 100µg / ml Leaf aqueous extracts	6.68
Control and 150µg / ml Leaf aqueous extracts	4.04
Control and 200µg / ml Leaf aqueous extracts	3.81
Control and 50µg / ml Leaf acetone extracts	8.75
Control and 100µg / ml Leaf acetone extracts	7.87
Control and 150µg / ml Leaf acetone extracts	8.48
Control and 200µg / ml Leaf acetone extracts	4.96

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level.

Statistically calculated value $|t|$ (for independent samples) for methotrexate, petroleum ether, distilled water and acetone extracts of leaf and root compared with untreated control were 8.18, 8.60, 9.48 and 7.78 (in MTX); 10.70, 7.36, 6.62 and 5.83

(LPE); 8.72, 6.68, 4.04 and 3.81 (LDE); 8.75, 7.87, 8.48 and 4.96 (LAE); 11.59, 8.92, 9.74 and 8.32 (RPE); 11.31, 7.85, 8.15 and 9.19 (RDE) and 13.08, 8.70, 9.78 and 8.70 (RAE) respectively.

Table 51: *Antirrhinum majus* root extracts:- Calculated value of t-test (an independent sample) for the 8 degree of freedom.

Treatments	't' Value
Control and 50µg / ml Methotrexate	8.18
Control and 100µg / ml Methotrexate	8.60
Control and 150µg / ml Methotrexate	9.48
Control and 200µg / ml Methotrexate	7.78
Control and 50µg / ml Root petroleum ether extracts	11.59
Control and 100µg / ml Root petroleum ether extracts	8.92
Control and 150µg / ml Root petroleum ether extracts	9.74
Control and 200µg / ml Root petroleum ether extracts	8.32
Control and 50µg / ml Root aqueous extracts	11.31
Control and 100µg / ml Root aqueous extracts	7.85
Control and 150µg / ml Root aqueous extracts	8.15
Control and 200µg / ml Root aqueous extracts	9.19
Control and 50µg / ml Root acetone extracts	13.08
Control and 100µg / ml Root acetone extracts	8.70
Control and 150µg / ml Root acetone extracts	9.78
Control and 200µg / ml Root acetone extracts	8.70

Tabulated value is 3.335 and 2.306 for 8 degree of freedom at 1% and5% level).

These calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore null hypothesis is rejected i.e. the values of $|t|$ are significant hence samples are dependent on each other when treated with control.

4.7.6. Spectroscopic analysis:

Table 52: UV- Vis and FTIR bands in the spectra of leaf extracts of *Antirrhnium majus*.

UV- VIS ANALYSIS (nm)					
Leaf petroleum ether extract		Leaf aqueous extract		Leaf acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
224	220	221	219	224	218
255	257	253	258	250	255
296	293	297	296	-	293
235	-	236	235	236	-

FTIR ANALYSIS (cm^{-1})			
Functional groups	Leaf petroleum ether extract	Leaf aqueous extract	Leaf acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3445.46, 1559.98	3491.27, 1591.81	3435.82
Alkane	2926.11	2930.45	2925.15
C-H	2857.64, 1427.37	2858.12, 1447.62	2855.22
C=O	1733.1	1730.21	1730.21
C-O	1220.02	-	1219.53
C-H (phenyl ring)	769.14	771.55	835.69
C=C	1631.83	-	1640.51
Std. Taxol	1074.87 to 3445.46	1081.14 to 3491.27	1065.71 to 3435.82
NO_2	1366.13	-	1366.61
C,H (aromatic bends)	1074.87	1081.14	1065.71

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. . The results of UV-Vis showed that the intense absorption at 224, 255 and 296nm (LPE), 221, 253 and 297nm (LDE) 224 and 250nm (LAE) and 221, 255 and 296nm (RPE), 222, 258 and 293nm (RDE) and 220, 255 and 296 (RAE) using acetone and at 220, 257 and 293nm (LPE), 219, 258 and 296nm (LDE) , 218, 255 and 293nm (LAE), 254 and 293nm (RPE), 223, 257 and 294nm (RDE) and 218 and 258nm (RAE) using methanol which correlates to the presence of alkaloids similar to that of vincristine in *Catharanthus roseus.* At 235nm (LPE), 236nm (LDE), 236nm (LAE), 235nm (RPE), 272nm (RDE) and 236nm (RAE) in acetone and

235nm (LDE), 269nm (RPE), 235nm (RDE) and 237nm (RAE) in methanol the alkaloid peaks are resemble to that of standard taxol (Table 52 and 53).

Table 53: UV- Vis and FTIR bands in the spectra of root extracts of *Antirrhinum majus* .

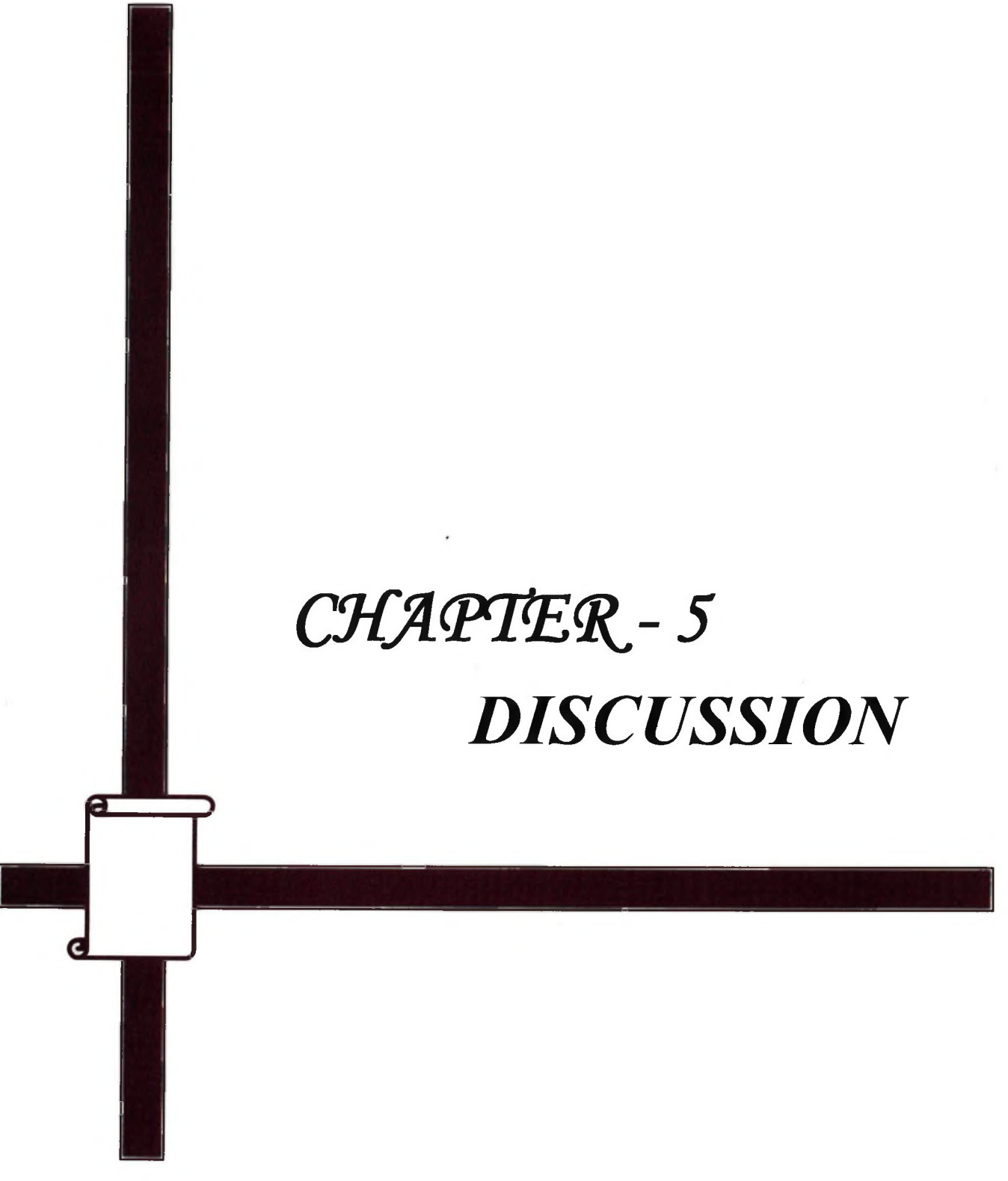
UV- VIS ANALYSIS (nm)					
Root petroleum ether extract		Root aqueous extract		Root acetone extract	
Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)	Solvent (Acetone)	Solvent (Methanol)
221	-	222	223	220	218
255	254	258	258	255	258
296	293	293	294	296	-
235	269	272	235	236	237

FTIR ANALYSIS (cm^{-1})			
Functional groups	Root petroleum ether extract	Root aqueous extract	Root acetone extract
	Solvent (Acetone)	Solvent (Distilled water)	Solvent (Acetone)
N-H	3422.8, 1597.27	3450.77, 1596.63	3459.45
Alkane	2926.59	2926.59	2927.08
C-H	2857.15, 1449.55	2860.03, 1426.89	2862.94, 1454.86
C=O	1731.65	1722.49	1728.76
C-O	1221.95	1246.57	1220.5
C-H (phenyl ring)	775.41	-	835.69
C=C	1632.8	-	-
Std. Taxol	1087.41 to 3494.17	1071.98 to 3450.77	1079.69 – 3459.45
NO_2	1366.61	-	1367.09
C,H (aromatic bends)	1087.41	1071.98	1079.69

The FTIR band at 3445.46, 1559.98cm^{-1} (LPE), 3491.27, 1591.81cm^{-1} (LDE), 3435.82cm^{-1} (LAE), 3422.8, 1597.27cm^{-1} (RPE), 3450.77, 1596.63cm^{-1} (RDE) and 3459.45cm^{-1} (RAE) indicated the presence of N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2926.11, 2857.64, 1427.37, 1733.1, 1220.02, 769.14, 1631.83 and 1074.87cm^{-1}(LPE), 2930.45, 2858.12, 1447.62, 1730.21, 771.55 and 1081.14cm^{-1}(LDE), 2925.15, 2855.22, 1730.21, 1219.53, 835.69, 1640.51 and 1065.71cm^{-1} (LAE), 2926.59, 2857.15, 1449.55, 1731.65, 1221.95, 775.41, 1632.8 and 1087.41cm^{-1} (RPE), 2926.59, 2860.03, 1426.89, 1722.49, 1246.57 and 1071.98cm^{-1} (RDE) and 2927.08, 2862.94, 1454.86, 1728.76,

1220.5, 835.69 and 1079.69cm^{-1} (RAE) respectively whereas C-O and C=C groups were absent in leaf aqueous extract. C-H (phenyl ring) in root aqueous extract and C=C in root aqueous and acetone extracts.

Peaks at 1074.87 to 3445.46 cm-1(LPE), 1081.14 to 3491.27cm^{-1}(LDE), 1065.71 to 3435.82cm^{-1} (LAE), 1087.41 to 3494.17cm^{-1} (RPE), 1071.98 to 3450.77cm^{-1} (RDE) and 1079.69 – 3459.45cm^{-1} (RAE) favoured the presence of standard taxol group. At 1366.13cm^{-1} and 1366.61 indicated presence of NO_2 (nitro compounds) in leaf petroleum ether and acetone extracts and absent in leaf aqueous extract and at 1366.61cm^{-1} and 1367.09cm^{-1} in root petroleum ether and root acetone extracts showed NO_2 compounds and that was absent in root aqueous extract.



CHAPTER - 5

DISCUSSION

CHAPTER 5

DISCUSSION

Properties elicited by plant species have a full range of prospective applications in human healthcare. Herbal remedies and phytotherapy drugs containing active principles are currently being developed to protect against electrophile attack on DNA and its widespread outcomes such as aging and cancers. Even for population that use herbs traditionally, encouraging the use of species with chemo preventive actions could be helpful as a strategy for improving life expectancy; the costs are significantly lower, herbs usually have little or no toxicity during long-term oral administration and they are relatively easy to obtain on large scale. On the other hand, an evaluation of the genetic toxicity potential of these herbal remedies is essential for their safe use by humans (Andraws, et al., 2005).

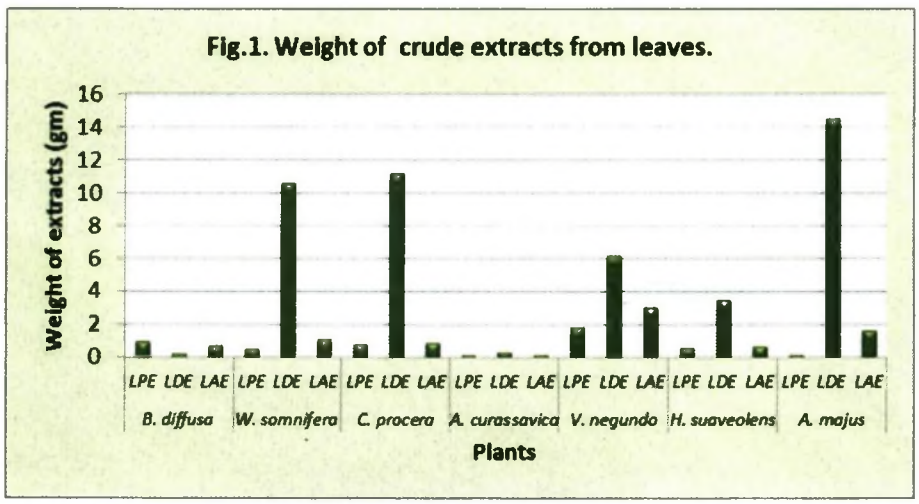
In new era of plant material used as anticancerous agents. There were the first agents to advance into clinical use for the treatment of cancer (Cragg and Newman, 2005). The induction of mutations those at the molecular level are similar to events known to be involved in carcinogenesis. In the recent year's scientist all over the world are alarmed. Man-made chemicals, antibiotics are important for the care of various diseases of plants and animals also some compounds acts as cytotoxic, genotoxic, mutagenic and anticarcinogenic, thereby affecting plants and animals. In the present study crude compounds isolated from *Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens* and *Antirrhinum majus* and its effectivity has been studied in *Allium cepa* test system to determine compound action whether these compounds acts as a cytotoxic, genotoxic, mutagenic or antimitotic property and the chemical structures of these isolated

compounds were established by spectroscopic techniques such as UV-Vis spectrophotometer and FTIR.

5.1. Quantification:

Quantification of crude compounds has been estimated from 25 gm of leaf powder and 12.5 gm of root powder of *B. diffusa, W. somnifera, C. procera, A. curassavica, V. negundo, H. suaveolens* and *A. majus* by soxhlet method using petroleum ether, distilled water and acetone as solvent system for extraction.

The extraction process is an important factor for assessing the biological activity of medicinal plant extracts (Berlin and Berlin, 2005) as it influence yield of extracts, extractive capacity of as extractant and quality parameters of herbal preparations.

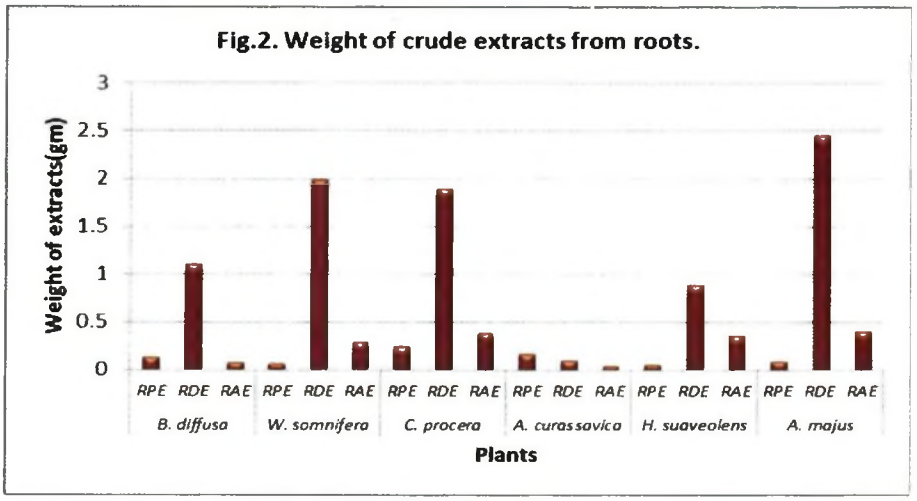


Results in the present study indicated that, in leaf extracts of petroleum ether, more amount of crude compound were obtained in *V. negundo* (1.911gm) whereas, least amount in *A. curassavica* (0.228gm) and *A. majus* (0.228gm). Phenolics with only few hydroxyl groups are soluble in ether, chloroform, ethyl acetate, methanol and ethanol (Van Sumere, 1989). Low polar solvent extractants such as hexane, petroleum ether and dichloromethane extracts non-polar compounds mainly terpenoid or highly methoxylated phenolics. In contrast, medium and high polar solvents such as ethyl

acetate, acetone, methanol, ethanol, water or mixture of these solvents extracts the polar compounds ranging from simple phenolics to complex (tannins) polymeric phenolics (Albuquerque and Hanazaki,2006).

leaf extract quantity of crude compounds showed significant increase in *A. majus* (14.545gm) as compared to other plants i. e. *C. procera* (11.240gm), *W. somnifera* (10.624gm), *V. negundo* (6.23gm), *H. suaveolens* (3.593gm), *A. curassavica* (0.411gm) and *B. diffusa* (0.340gm) respectively using distilled water as solvent as compared to that of leaf petroleum ether and acetone as a solvent. Study showed that more dissolution of chemical compounds of leaves of these plants in aqueous medium than petroleum ether and acetone as a solvent (Table 1 and Fig.1).

While in case of acetone as solvent, more quantity of crude compound was extracted from *V. negundo* leaf (3.142gm) and less quantity was obtained from the leaves of *A. curassavica* (0.256gm). Water is a good solvent for the extraction of many types of active constituents such as alkaloidal salts, colouring agents, glycosides, gums, sugars, anthraquinone derivatives and tannins. Petroleum ether soluble constituents are oils, fats, waxes, resins and alkaloidal bases. Highly inflammable produces physiologically effects (Henry, 1960; Trease and Evans, 1983; Mehta, 2002). Acetone has been adjudge to be the best extractant of plant extract of bioassay because it extracts broad spectrum of components (polar and non-polar) is miscible with all other solvents is highly volatile and exhibit low toxicity to biological organisms in various assay (Eloff, 1998).



In petroleum ether solvent (Fig. 2), the crude compounds were obtained from roots shows significant increase in *C. procera* (0.270gm) and decrease in *H. suaveolens* (0.071gm). Most often chemical speciation of organic carbon (OC) is performed on a fraction that extracts in relatively nonpolar organic solvents such as benzene, ether, hexane or dichloromethane. The nonpolar organic solvent extractable mass typically accounts for only 50-60% of the total organic aerosol (Cass, 1998).

In both aqueous and acetone root extracts (Fig. 2.) the high amount of crude compounds were obtained from *A. majus* (2.453gm and 0.422gm) whereas, low amount obtained from roots of *A. curassavica* (0.122gm and 0.066gm). Samy and Gopalakrishnakone, 2008 analysed different sovent sytems for extraction of various organic compounds viz. n-hexane extacts wax, lipid, fat, soluble oil and esters; dichloromethane extracts terpenoid; ethanol and acetone are used for ester extraction and methanol for extraction of alkaloids.

Gilani and Atta-ur- Rahaman (2005), used the plant extracts or plant-derived pure chemicals to treat diseases is a therapeutic modality which has stood the test of time. It indicated that medicinal plants contain substances like peptide, unsaturated long

chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds.

Methanol, ethanol, acetone, water, ethyl acetate and to a lesser extent, chloroform, dichloromethane and their combinations are frequently used for the extraction of Taxol (Chen and Kingston, 1994 and Gabetta et al., 1999).

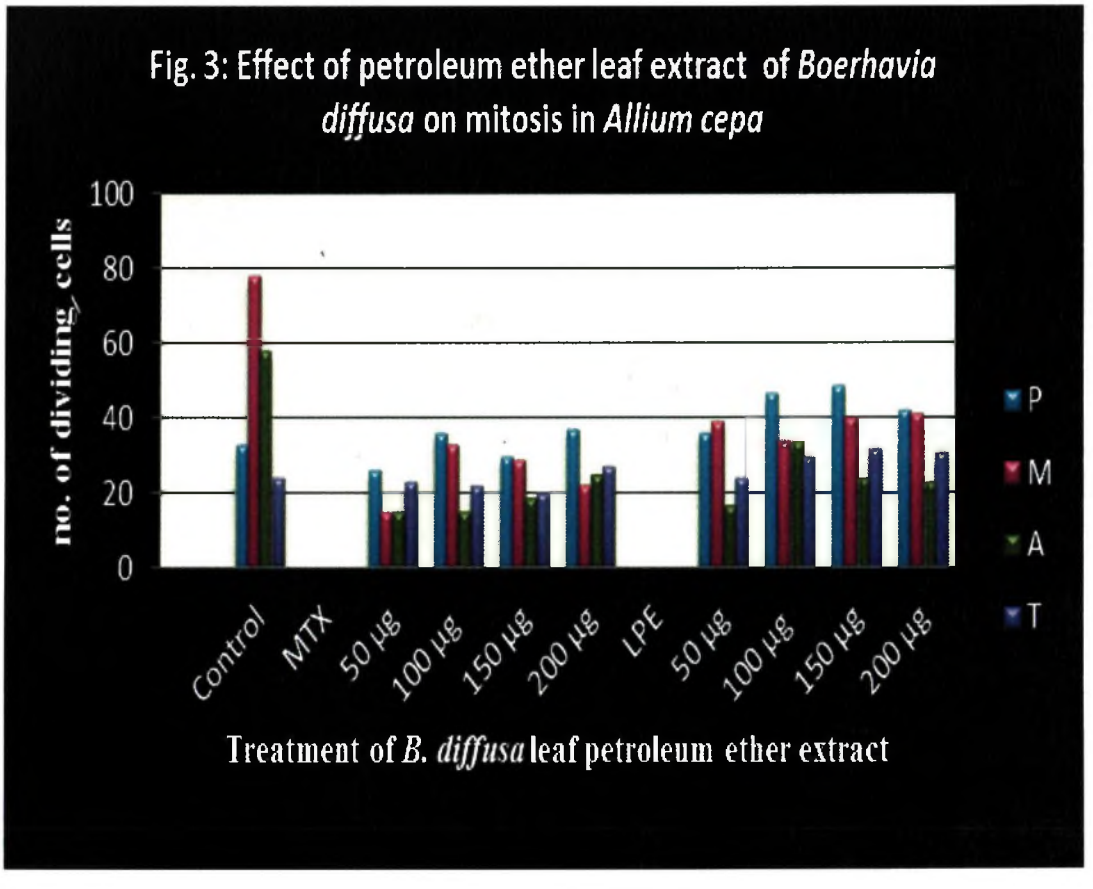
In the present study, choosing the different solvent systems extracted the crude compounds from the different parts of plants which give higher amount of the bioactive compounds. These crude extracts expresses good biological capacity of the extractant which indicates that the substance with powerful biological effects exist in these extracts and must be isolated to purified to confirm its utility in pharmacological and medicinal uses after confirming cytotoxic / genotoxic activity.

5.2. Cytotoxicity:

The normal mitosis in *Allium cepa* showed the number of dividing cells i.e. prophase, metaphase, anaphase and telophase. Prophase having chromosomes become visibly distinct as long thin threads divided into chromatids, each sister chromatid is attach to other in region of the centromere, nucleolus and nucleolar membrane are present. In metaphase, disappearance of nuclear membrane and nucleolus occurred; spindle formation takes place which results into movement of chromosome at equatorial regions. In anaphase, centromere divides, become functionally double, chromatid converted into independent chromosomes that separate and move to opposite poles and in telophase, spindle disappears and reconstruction of nuclear envelopes around the two groups of offspring chromosomes and chromosomes uncoiled to become like slender thread and nucleoli reappears.

5.2.1. *Boerhavia diffusa* leaf extracts:

The data presented in Table 2 showed the cytotoxic effect of petroleum ether, aqueous and acetone leaf extract of *B. diffusa*. In control, mitotic index frequency was 11.26% in which prophases were thirty three, metaphases seventy eight, anaphases, fifty eight and telophases were twenty four. In Methotrexate control exhibited reduction in mitotic index frequency with 50µg concentration (4.80%). In this concentration there is considerable reduction in telophases (15) and Anaphases (15) over the control. Whereas, LPE, LDE and LAE there is slight increase in mitotic index with increase in concentration of crude compound (Fig.3, 4, 5).



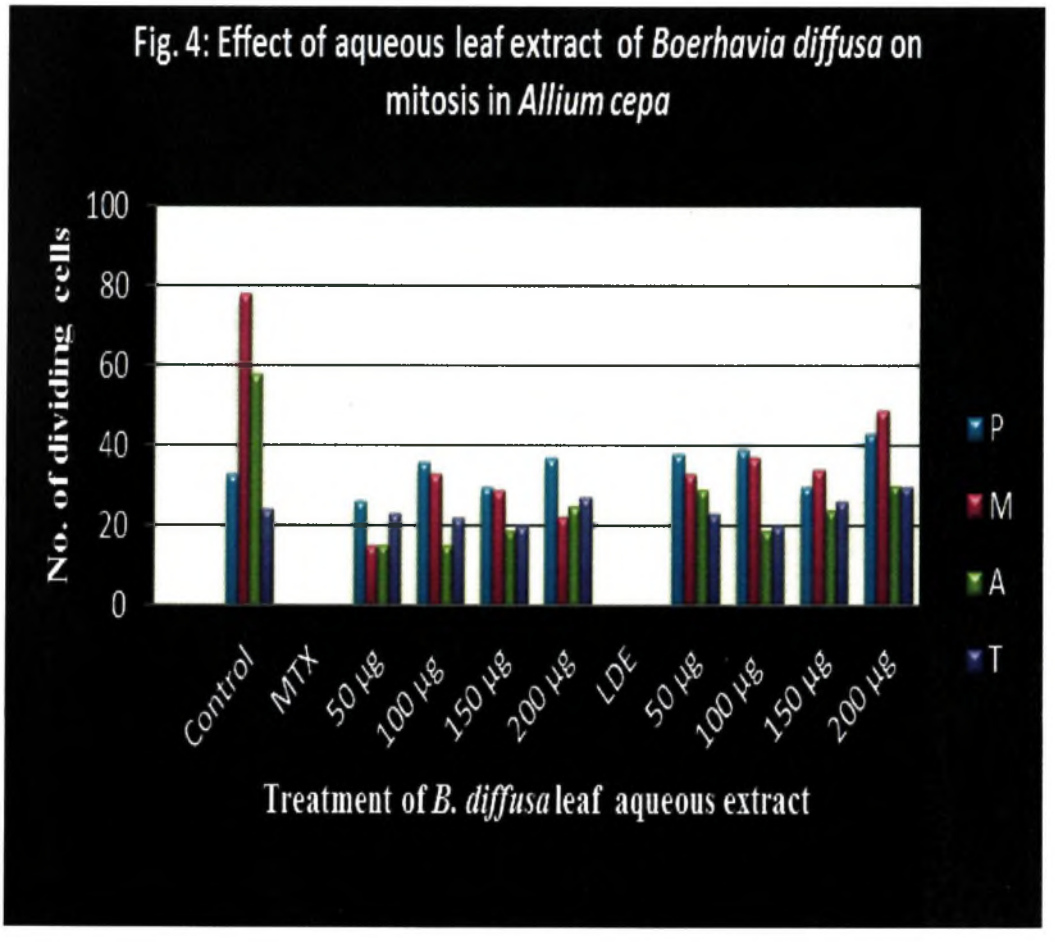


Fig. 4: Effect of aqueous leaf extract of *Boerhavia diffusa* on mitosis in *Allium cepa*
No. of dividing cells
100
80
60
40
20
0
Control
MTX
50 µg
100 µg
150 µg
200 µg
LDE
50 µg
100 µg
150 µg
200 µg
P
M
A
T
Treatment of *B. diffusa* leaf aqueous extract

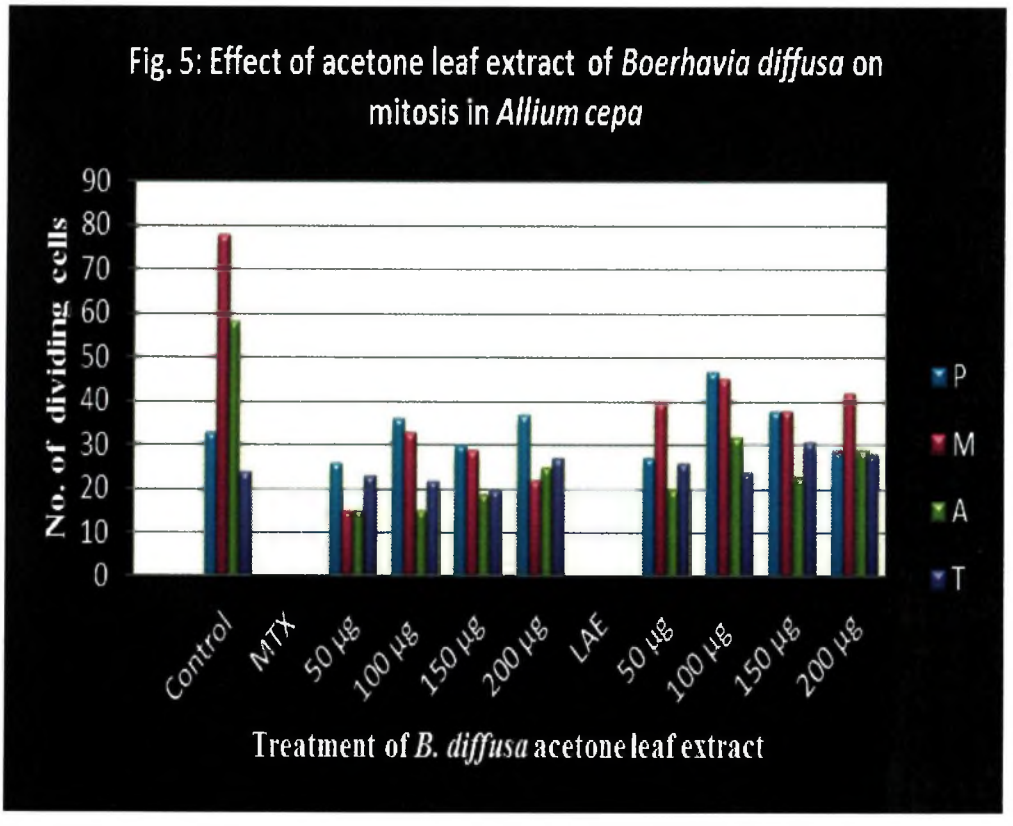
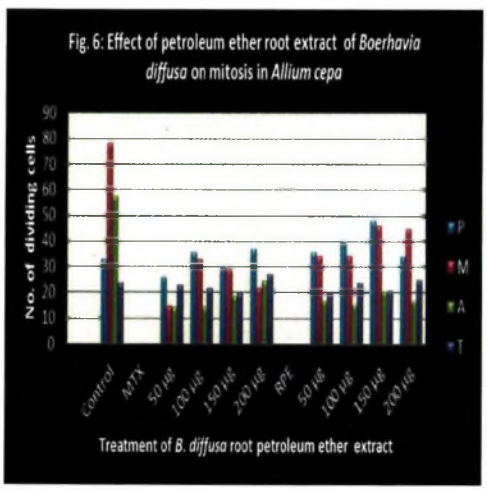


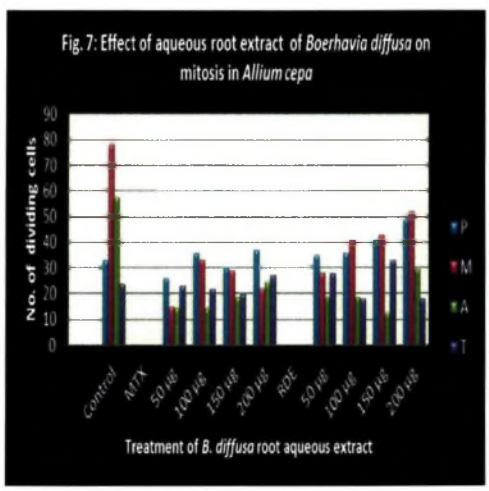
Fig. 5: Effect of acetone leaf extract of *Boerhavia diffusa* on mitosis in *Allium cepa*
No. of dividing cells
90
80
70
60
50
40
30
20
10
0
P
M
A
T
Control
MTX
50 µg
100 µg
150 µg
200 µg
LAE
50 µg
100 µg
150 µg
200 µg
Treatment of *B. diffusa* acetone leaf extract

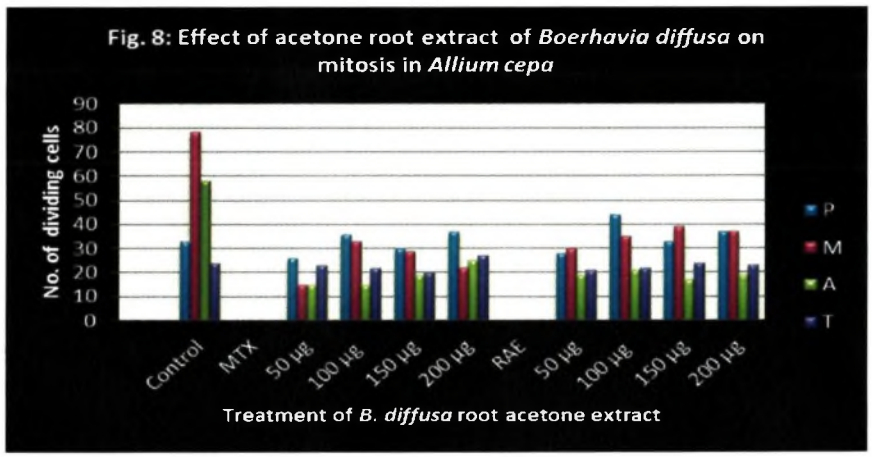
Prophases were increased in 150µg of LPE (49), 200µg of LDE (43) and 47 in 100µg of LAE as compared to that of control (33) and which is comparable to MTX (37). The significant reduction in metaphases in MTX (15) as compared to control (78), in all concentrations metaphases were lower down to half as compared to control in LPE (34 in 100µg) , LDE (33 in 50µg) and LAE (38 in 150µg) which is relatively similar to MTX (33 in 100µg) . The similar results were obtained in case of anaphases. The decrease in number of anaphases in all concentrations of LPE (34 in 100µg), LDE (30 in 200µg) and LAE (32 in 100µg) as compared to control (58). However, there are no significant variations in telophases in all leaf extracts when compared with control and MTX.

5.2.2. *Boerhavia diffusa* root extracts:

The data from Table 3 and Fig. 6, 7, 8 indicated that in all concentrations of root extracts mitotic index was increased with increase in concentrations but significantly inhibited as compared to control (11.26%) and showed similar inhibition of lowering mitotic cell division MTX (Std. Control, 4.80% to 6.18%). Active mitotic index was also increased with increase in concentrations and highly decreased (2.20% in 50µg of RDE) as compared to control (7.93%).







The numbers of prophases were higher in 150µg of RPE (48), 200µg of RDE (49) and 100µg of RAE (44) as compared to control (33) and MTX (37). In all root extracts, metaphases and anaphases showed significant inhibition as compared to control whereas, similar to that of MTX. Metaphases were reduced in 50µg and 100µg of RPE (34), 50µg of RDE (28) and 50µg of RAE (30) as compared to control (78) while anaphases showed significant decrease at 100µg of RPE (16), 150µg of RDE (13) and 150µg of RAE (17) as compared to control (58) and slightly same as in MTX (15). There was increase in telophases occurred in 150µg of RAE (33) as compared to control (24) and showed similarity in other extracts.

5.2.3. *Withania somnifera* leaf extracts:

Mitotic index of all leaf extracts was increased with increase in concentrations (Table 10 and Fig. 9, 10, 11) as compared to control (11.26%). It is somewhat similar to MTX (Std. control) and the mitotic index in LDE (9.47 % in 150µg and 10.12 in 200µg) which is comparable to control.

Studying the data of mitotic index of leaf extracts of *W. somnifera*, in all treatments rose over the prophase percentage, meanwhile metaphases and anaphases were reduced as compare to control. Prophases were higher in 150µg and 200µg of LPE

(48), 50µg and 150µg LDE (60) and 100µg LAE (43) as compared to control (33) and MTX (37). Number of metaphases were reduced in 50µg of LPE (41) and 50µg of LAE (31) and moderately lowered in remaining concentrations of leaf extracts. Anaphases were highly inhibited in 150µg of LAE (13) and as in control (58).

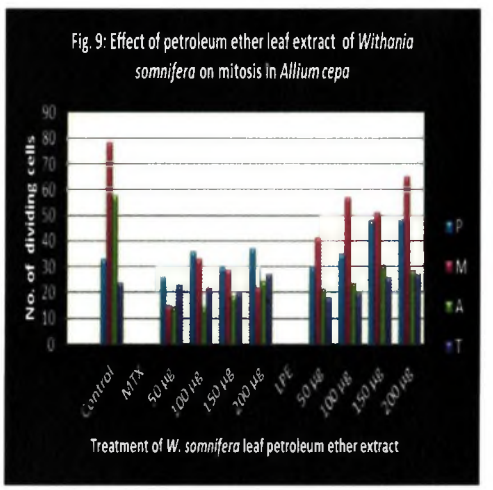


Fig. 9: Effect of petroleum ether leaf extract of *Withania somnifera* on mitosis in *Allium cepa*

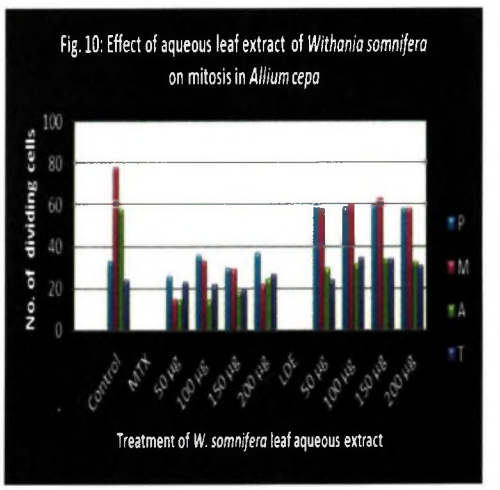


Fig. 10: Effect of aqueous leaf extract of *Withania somnifera* on mitosis in *Allium cepa*

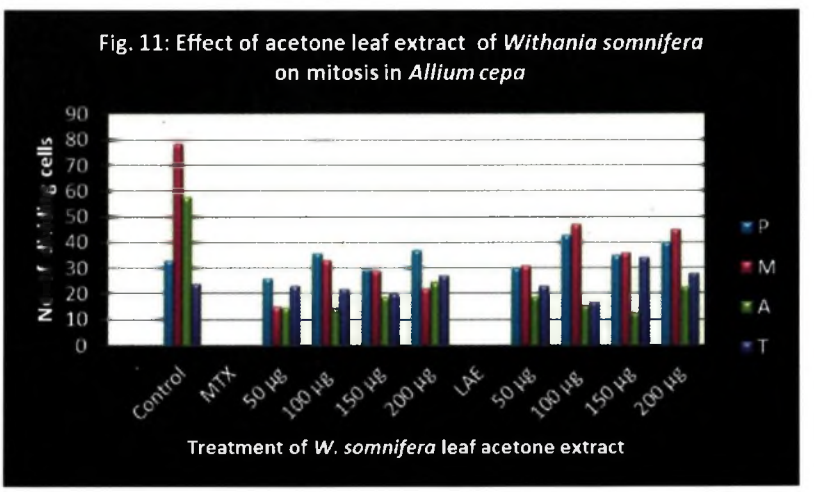
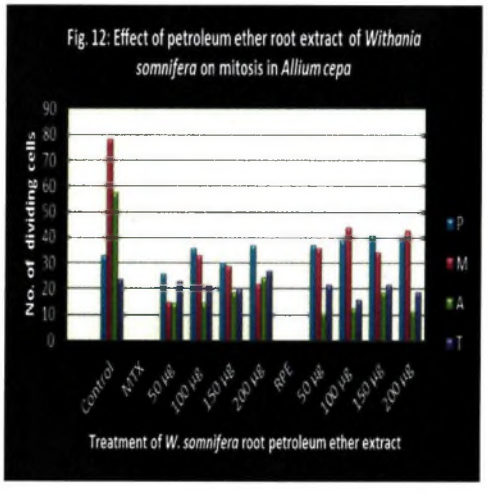


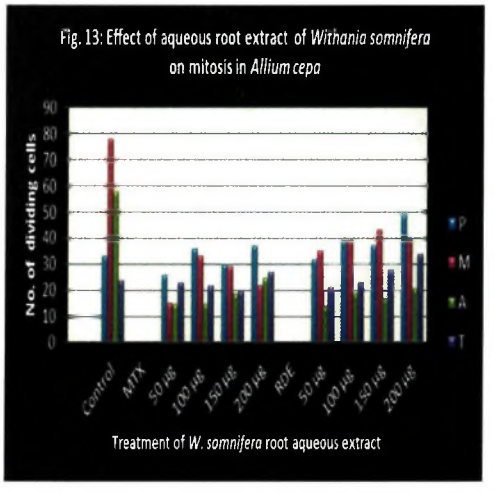
Fig. 11: Effect of acetone leaf extract of *Withania somnifera* on mitosis in *Allium cepa*

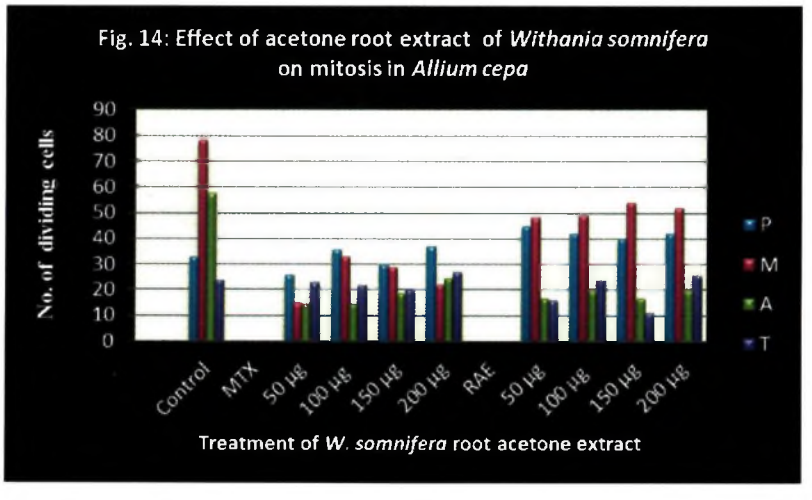
5.2.4. *Withania somnifera* root extracts:

The data from Table 11 and Fig. 12, 13, 14 showed that mitotic index and active mitotic index of root extracts of *W. somnifera* were increased with increase in concentrations and slightly lower as compared to control and somewhat same as in MTX. In all concentrations prophases were increased. In 150µg of RPE (41), 200µg of RDE (49) and 50µg of RAE (45) as compared to control (33) and MTX (37). Lowering

of metaphases occurred in 150µg of RPE (34) and 50µg of RDE (35) as compared to control (78) and anaphases also decreased in 50µg of RPE (11). 50µg of RDE (14) and 50µg and 150µg of RAE (17) as compared to control (58). Telophases were inhibited in 150µg of RAE (11) as compared to control (24) and MTX (27).



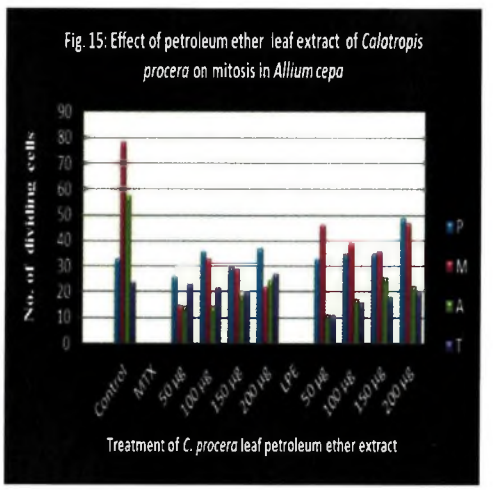


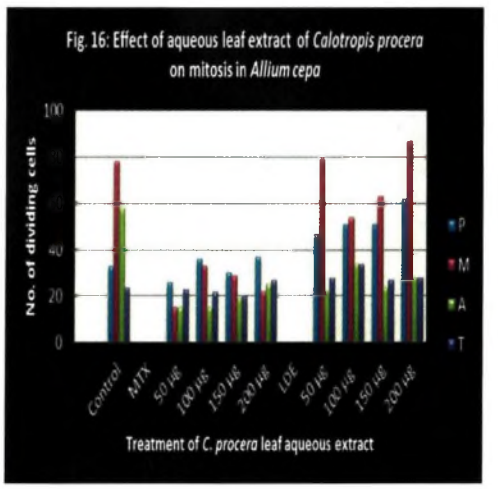


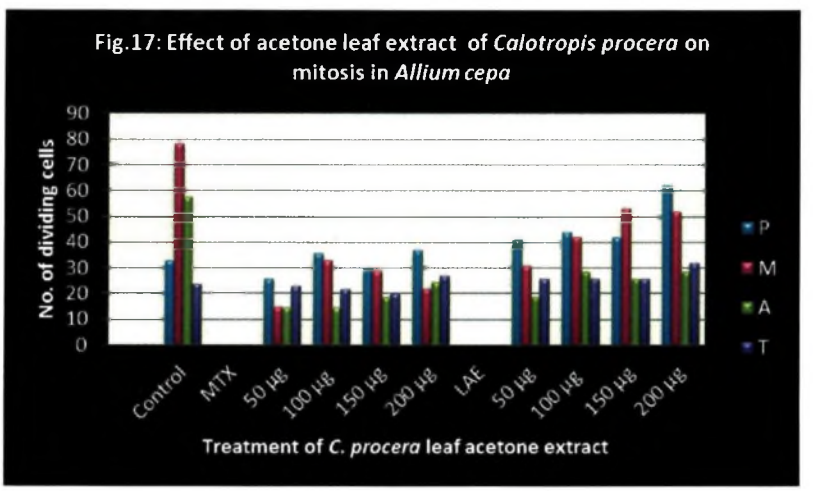
5.2.5. *Calotropis procera* leaf extracts:

Mitotic index and active mitotic index were increased with increase in concentrations and lowered down as compared to that of control (11.26% and 7.93%). Result of normal mitosis indicated that, numbers of prophases were increased in leaf extracts of *C. procera* i.e. in 200µg of LPE (49), 200µg of LDE (62) and 200µg of LAE (62) as compared to control (33) and MTX (37) while metaphases were reduced in

150µg of LPE (36) and 50µg of LAE (31) and increased in 50µg (79) and 200µg (87) of LDE as compared to control (78). Decrease in anaphases was observed in 50µg of LPE (12), LDE (22) and LAE (19). Numbers of telophases were increases in all extracts except leaf petroleum ether extracts (Table 18 and Fig.15, 16, 17).



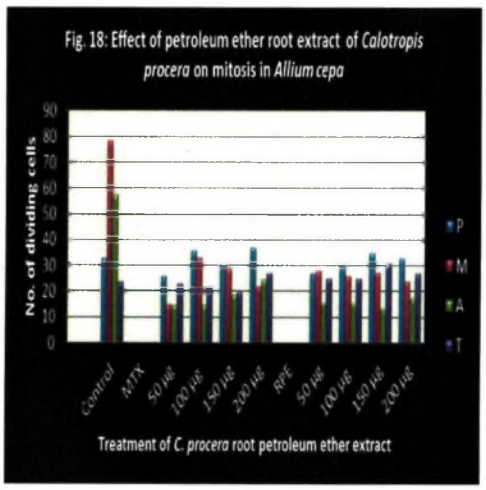


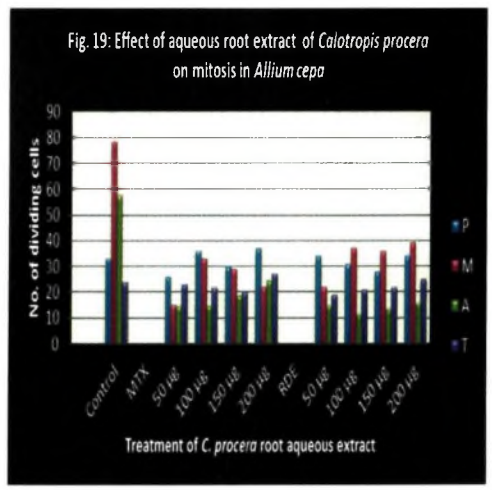


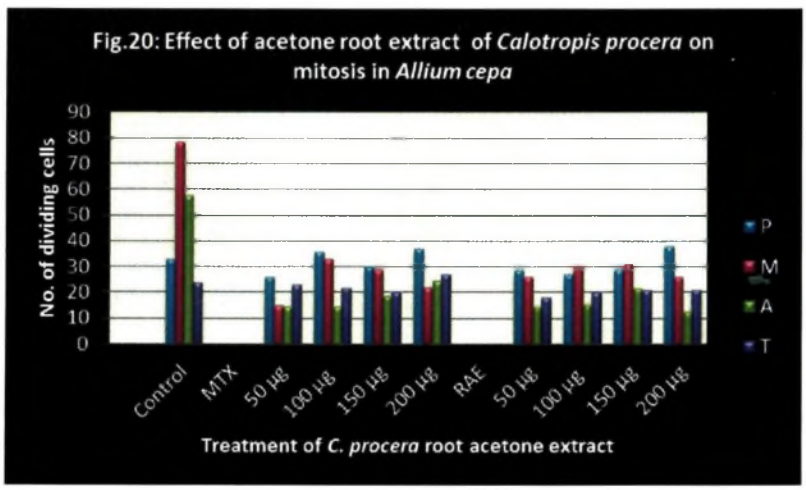
5.2.6. *Calotropis procera* root extracts:

From Table 19 and Fig. 18, 19, 20 it was observed that, mitotic index and active mitotic index frequencies were increased with increase in concentrations and reduced to half in all extract concentrations ranges from 4.17% in 50µg RPE to 5.59% in 200µg of RDE. There was no significant difference observed in number of prophases after the treatments. Metaphases were highly inhibited by the treatments i.e. in 200µg of RPE

(24), 50µg of RDE (22) and 50µg and 200µg of RAE (26) as compared to control (78). Anaphases were also decreased in 150µg of RPE (14), 100µg of RDE (12) and 200µg of RAE (13) and in control (58). In telophases moderate differences were obtained in all extracts.



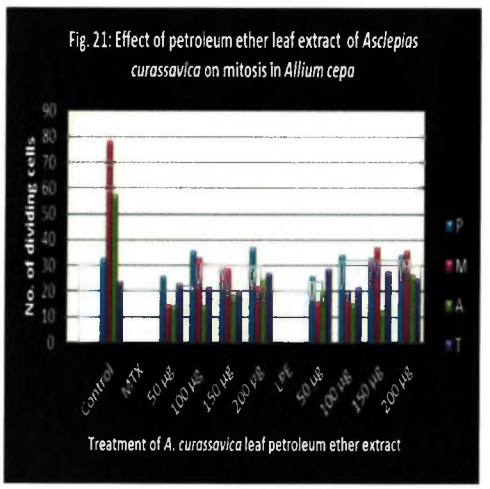


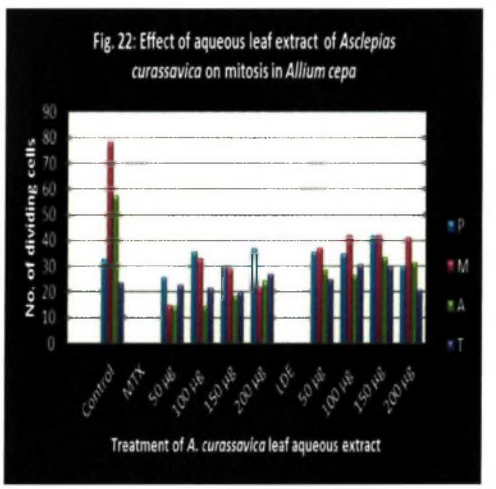


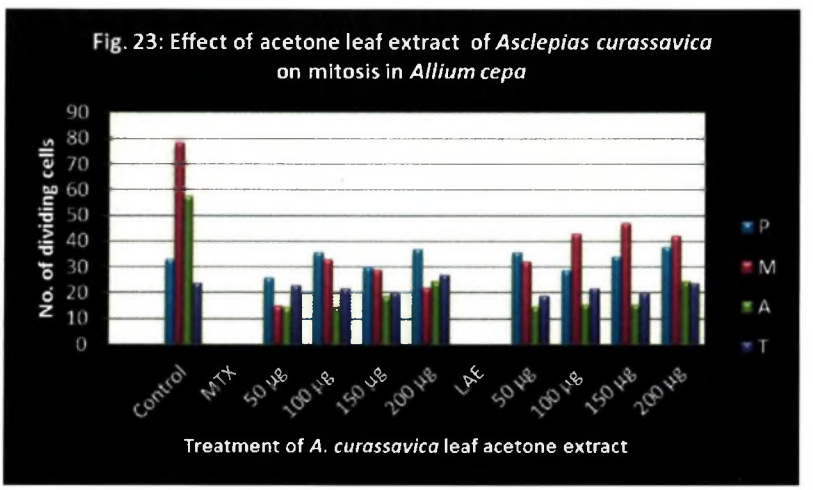
5.2.7. *Asclepias curassavica* leaf extracts:

The data from the Table 26 and Fig. 21, 22, 23 of leaf extracts of *A. curassavica* indicated that the numbers of prophases were relatively equal to that of respective control (33) and (Std. control) MTX (37) and slight increase in 150µg of LDE (42). Metaphases were highly decreased in 50µg (16) and 100µg (20) of LPE as compared to control (78) and anaphases were decreased significantly in all concentrations and very

low number is observed in 150µg of LPE (13) and 50µg of LAE (15) as compared to control (58) and telophases were same in leaf extracts . The reduction of mitotic index and active mitotic index were also found in all leaf extracts as that of control. MTX (Std. control) and leaf extracts showed significant similar property of lowering mitotic cell division.



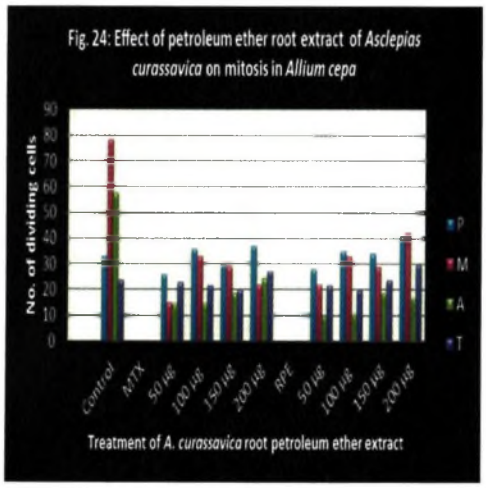


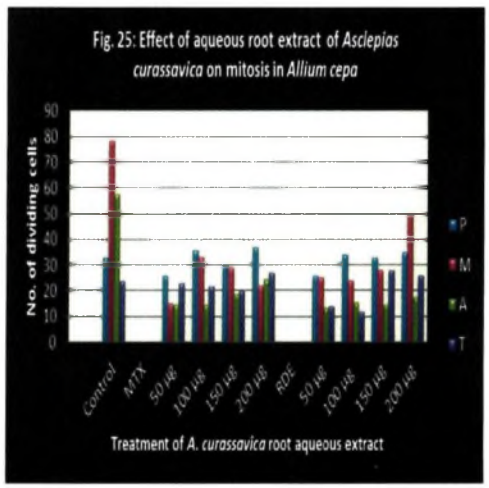


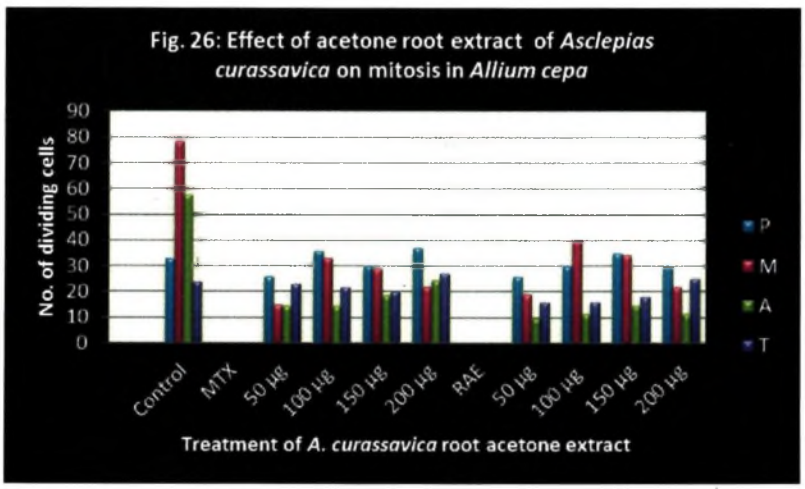
5.2.8. *Asclepias curassavica* root extracts:

In root extracts of *A. curassavica,* mitotic index 70% inhibition has been observed in 50µg of RAE (3.28%) and 66% inhibition in 50µg of RDE (3.80%). Active mitotic index frequencies is also lowered down to more than half (1.69% in 50µg of RPE, 1.87% in 50µg of RDE and 1.34% in 50µg of RAE) as compared to control

(7.93%) and also increased with increase in concentrations (Table 27). Prophases were increased in 200µg of RPE (41) and relatively same in remaining root extracts. In 50µg of RPE (22), 100µg of RDE (24) and 50µg of RAE (19) metaphases were significantly reduced as compared to control (78). Anaphases in all concentrations were highly decreased in 50µg of RPE (10), RDE (14) and RAE (10) as compared to control (58). Reductions in telophases were also observed in 100µg of RDE (10) and (24) in control (Fig. 24, 25, 26).







5.2.9. *Vitex negundo* leaf extracts:

Mitotic index in LDE and LAE extracts was significantly lowered to mitotic index of control (11.10%). The results revealed no significant changes in control as

compared to 150µg (10.62%) and 200µg (11.10%) treatments in petroleum ether leaf extracts. Normal mitosis indicated that, number of dividing cells in leaf extracts of *V. negundo* in which prophases increased in 200µg of LPE (57) and 50µg of LDE (57) as compared to control (33) and (Std. control) MTX (37) Table 34 and Fig. 27, 28 and 29. Number of metaphases in 150µg of LPE (91) and 200µg (93) were increased as compared to control (78) while reduced in other concentrations of leaf extracts. Anaphases were lowered in 50µg of LDE (18) and 100µg of LAE (14). Telophases were relatively same in all concentrations.

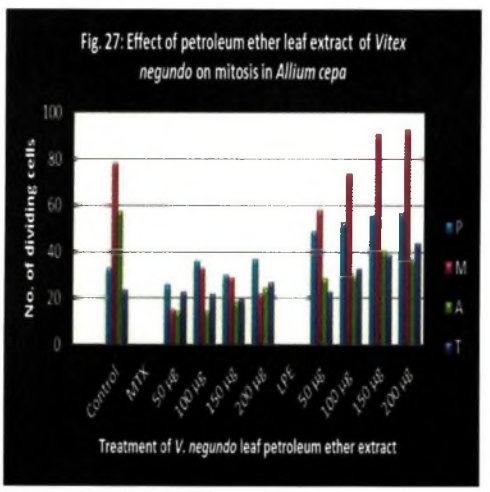


Fig. 27: Effect of petroleum ether leaf extract of *Vitex negundo* on mitosis in *Allium cepa*

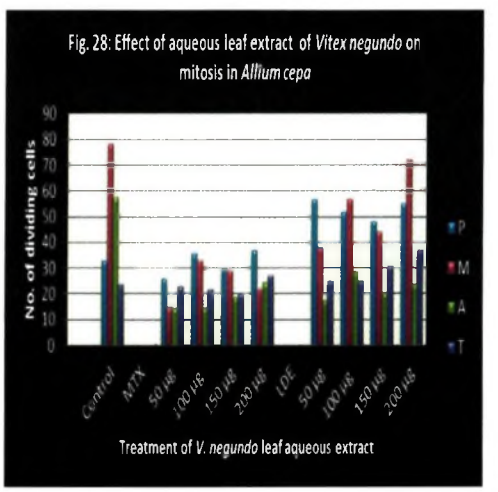


Fig. 28: Effect of aqueous leaf extract of *Vitex negundo* on mitosis in *Allium cepa*

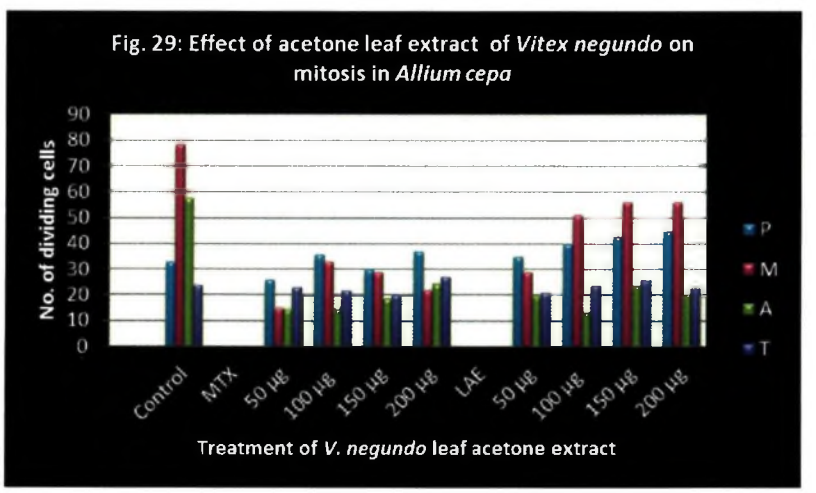
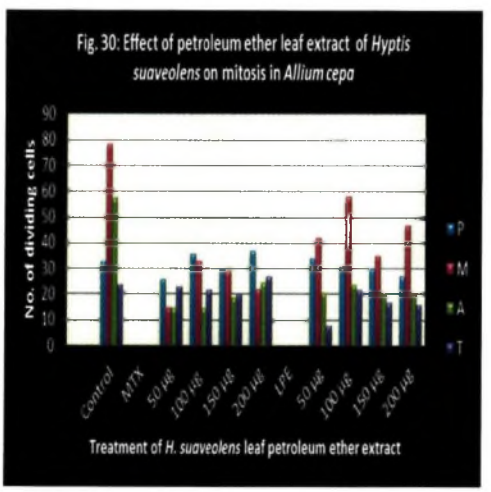
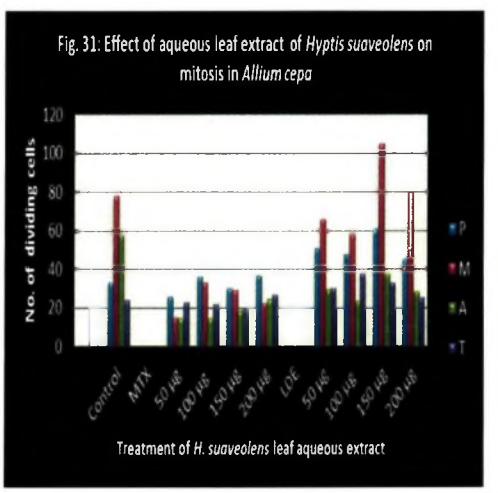


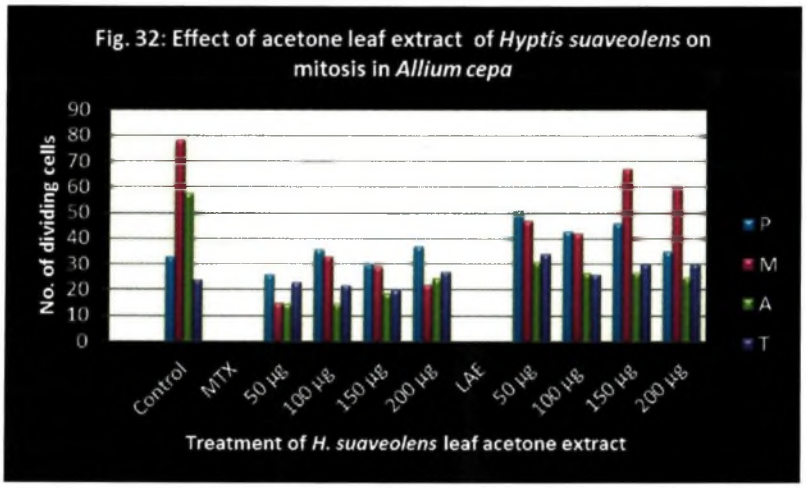
Fig. 29: Effect of acetone leaf extract of *Vitex negundo* on mitosis in *Allium cepa*

5.2.10. *Hyptis suaveolens* leaf extracts:

Mitotic index and active mitotic index frequencies increased in all extracts with increase in concentrations. Whereas, sudden increase MI (7.38%) in LPE with compare to other lower and higher LPE concentrations and decreased at 100µg concentration in leaf acetone extract (Table 38) while somewhat same as compared to that of respective (Std. control) MTX. In leaf extracts of *H. suaveolens* data of mitotic index, the prophase percentage rose over in 50µg (51) and 150µg (61) of LDE and 50µg of LAE (51) whereas, in other extract it was moderately same as in control; meanwhile metaphases were increased in 150µg (105) and 200µg of LDE (80) as compare to control (78) and reduced in remaining leaf extracts concentrations. Anaphases were decreased to half in all concentrations. Numbers of telophases were decreased in 50µg of LPE (8) as compared to control (24) while slightly increased in leaf aqueous and acetone extracts (Fig. 30, 31, 32).

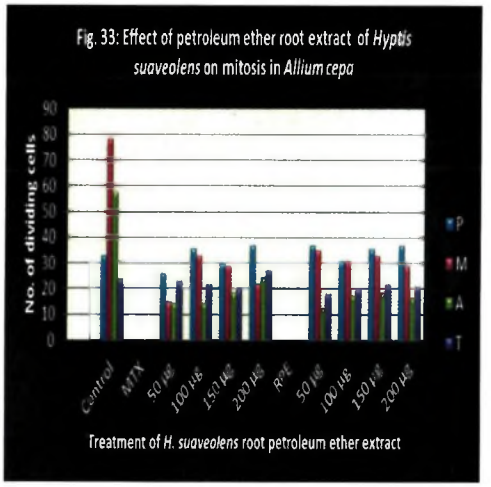


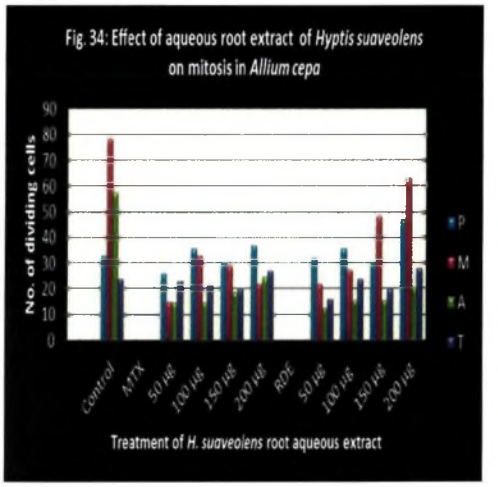


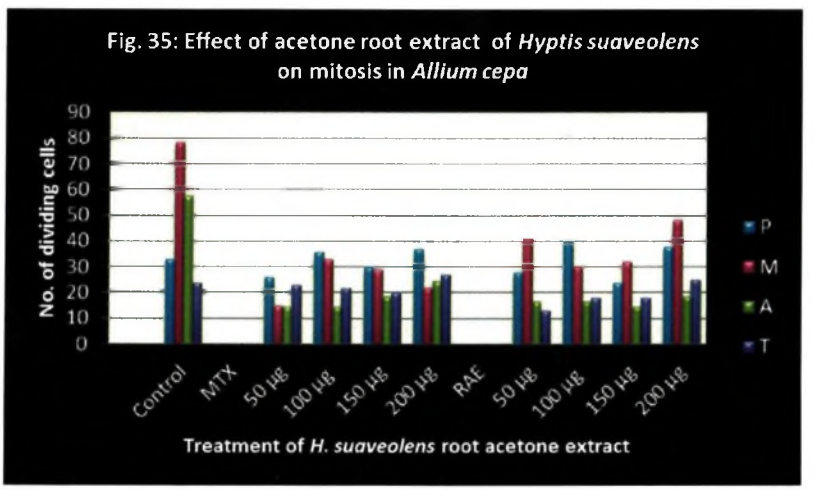


5.2.11. *Hyptis suaveolens* root extracts:

In root extracts of *H. suaveolens*, mitotic index and active mitotic index were inhibited significantly with increase in concentrations and it is lowered down as compared to control and similar to that of MTX. Prophases were moderately increased in 200µg in RDE (47) and in 100µg of RAE (40). Metaphases were enormously decreased in 220µg of RPE (29), 50µg of RDE (22) and 150µg of RAE (24) and resemblance in 200µg in RDE (63) as compared to control (78). In all concentrations anaphases were reduced more than half in 50µg of RPE (13), 50µg of RDE (13) and 150µg of RAE (15) as compared to control (58) and same as in MTX (15). Telophases in all root extracts were relatively same as in control and MTX (Table 39 and Fig. 33, 34, 35).



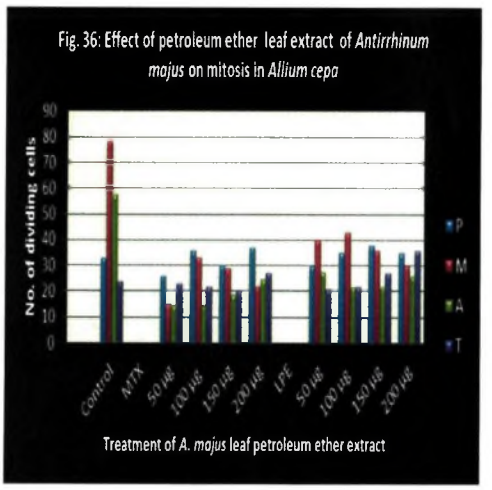


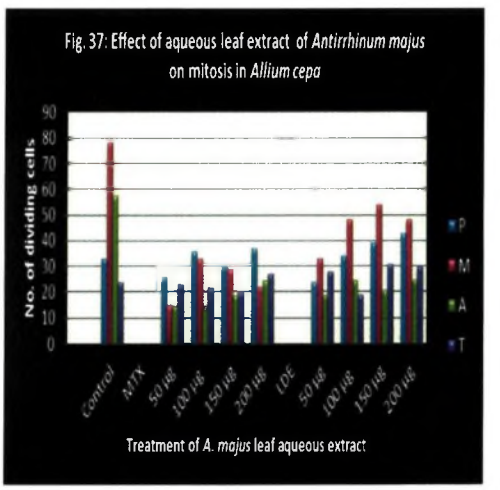


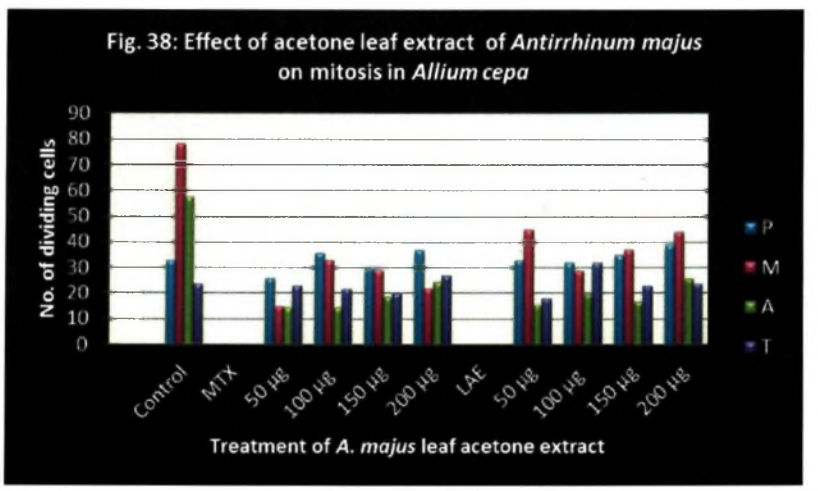
5.2.12. *Antirrhinum majus* leaf extracts:

The reduction of mitotic index and active mitotic index were also found in all extracts as compared to that of control. MTX (Std. control) showed significant similar property of lowering mitotic cell division as that of leaf extracts (Table 46). The data from the leaf extracts of *A. majus* (Fig. 36, 37, 38) indicated that the numbers of prophases in 50µg of LPE (30), 100µg of LDE (34) and 100µg of LAE (32) were relatively equal to that of respective control (33) and (Std. control) MTX (33), metaphases were significantly lesser in 200µg of LPE (30), 50µg of LDE (33) and 100µg of LAE (32) as compared to control (78) and anaphases were declined

significantly in 100µg and 150µg of LPE (22), 50µg of LDE (19) and 50µg of LAE (16) as compared to control (58) and telophases were same in leaf extracts when compared in control and MTX.





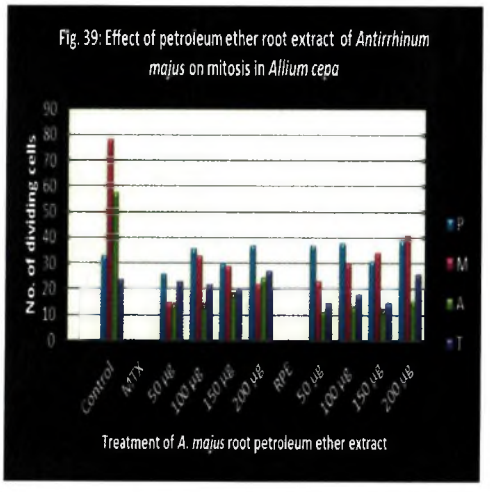


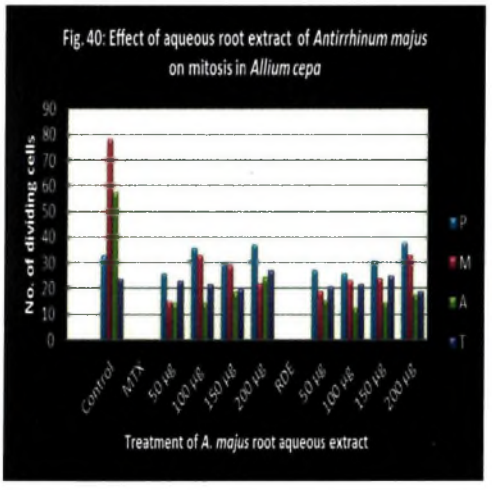
5.2.13. *Antirrhinum majus* root extracts:

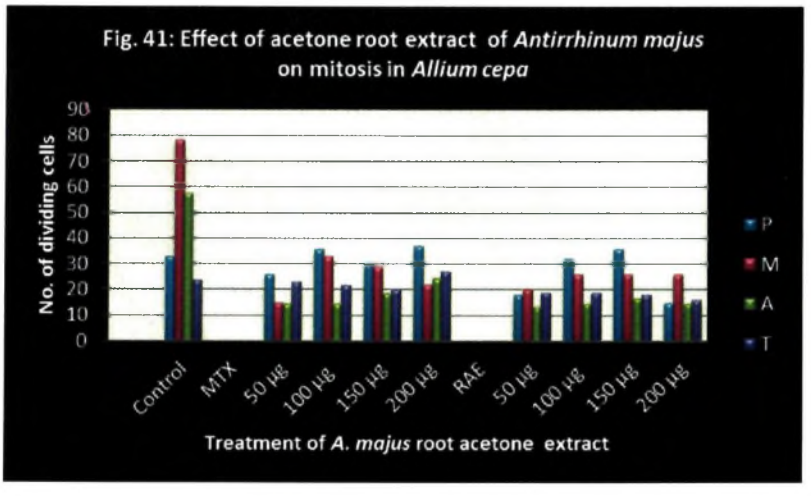
From the Table 47 and Fig. 39, 40 and 41, it is indicated that, mitotic index and active mitotic index were considerably increased with increase in concentrations and lesser as compared to control and comparatively similar to MTX.

Results of normal mitosis indicated that, number of dividing cells in root extracts of *A. majus* in which prophases comparatively same in 150µg of RPE (31), 150µg of RDE (31) and 100µg RAE of as compared to control (33) and (Std. control) MTX (37) Table 47 and Fig. 39, 40 and 41. Number of metaphase in 50µg of RPE (23),

50µg of RDE (19) and 50µg of RAE were greatly decreased as compared to control (78). Anaphases were lowered in 50µg of RPE (12), 100µg of RDE (13) and 50µg of RAE (14) as related to control (58). Telophases were relatively same in all concentrations.







Cell division is one of the most important phenomenon, which control the growth of the organism. Behaviour of the chromosomes during cell division is one of the unique features of the cell division. Effect of various drugs depends on the cell cycle of the organism. Cell treated in early interphase (G1), affects synthetic process, while latter anaphase causes unequal distribution of the chromatids. The chromosomal aberration causes changes in the gene combinations, which affects the morphological traits or metabolism of the organism. *A. cepa* root tip meristems have been widely used

for evaluation of cytotoxic and antimitotic activity of various compounds (Bhujbal et al., 2011).

The mechanism leading to the formation of daughter cells and the retention of identical chromosome numbers and other hereditary factors in the newly formed cells, following treatments with various reagents have been studied by several workers Shanthamurthy and Rangaswamy (1979), Okoli and Russom (1987), Okagbue (1990) and Umar (2004).

The general principles of the mechanism of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals which modify the normal course of mitosis (Nwakanma and Okoli, 2010). A wide variety of secondary metabolites obtained from plants are tested for their ability to treat cancer. Various anticancer drugs from plants are known to be effective against proliferating cells. They exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase. However most of the cytotoxic drugs exhibit side effects and hence, there is a need for drugs that are efficient and have less side effects (Sehgal et al., 2006).

The cell division cycle can be delineated into major phases G1, S, G2 and M phase. Cell division manifests in a periodic manner during 24h period (Ray, 1920; Winter, 1929; Solomon and Trent, 1941; Richard and Richard, 1971 and Ambrocio Melvin and Ian Kendrich, 2011). The G2 checkpoint arrests damaged cells in G2, delaying entry into mitosis until the damage gets repaired (Cuddihy and Connell, 2003).

In the present study, results obtained showed that, the mitotic index values in LPE, LDE, LAE, RPE, RDE and RAE of seven plants were increased with increase in concentrations. Mitotic index values in *B. diffusa* at 50µg of LPE (6.03%), 50µg of LDE (5.67%), 50µg of LAE (5.15%), 50µg of RPE (5.52%), 50µg and 100µg of RDE (5.15% and 5.40%) and in all concentrations of RAE; in *W. somnifera* at 50µg of LPE (5.51%), 50µg, 100µg and 150µg in LAE (4.94%, 5.69% and 6.00%), in RPE, 50µg, 100µg and 150µg (5.48%, 5.51% and 6.01%), in 50µg and 100µg of RDE (4.34% and 5.30%) and in 50µg of RAE (5.34%); in *C. procera* at 50µg, 100µg and 150µg of LPE (5.45%, 5.92% and 5.96%), in 50µg of LAE (5.45%) and in all concentrations of RPE, RDE and RAE; in *A. curassavica* in all leaf extracts of LPE, in 50µg, 100µg and 150µg of LAE (5.26%, 5.29% and 5.97%) and in all root extracts except in 200µg of RDE and RAE; in *V. negundo* 50µg of LDE and LAE (5.47% and 5.56%); in *H. suaveolens* at 50µg, 150µg and 200µg of LPE (5.16%, 5.84% and 5.76%) and in 100µg of LAE (5.95%) and in all concentrations of RPE, 50µg of RDE (4.48%) and 50µg, 100µg and 150µg of RAE (4.21%, 5.64% and 5.83%) and in *A. majus* 50µg of LPE and LDE (4.70% and 5.55%) and 50µg, 100µg and 150µg of LAE (5.47%, 5.88% and 5.96%) and all concentrations of root extract were significantly analogous to that of MTX (4.80%-6.18%) and reduced as compared to control. This suggests the supression of mitotic activities in *A. cepa* by leaf and root extracts using petroleum ether, distilled water and acetone as solvents, mitotic index is quantitative estimation of mitotic activities in an organisms. From the above findings it is suggested that all the plant extracts (LPE, LDE, LAE and RPE, RDE, RAE) inhibit MI and AMI at various concentrations because of ingredients and chemicals present in the extracts brought variations in number of dividing cells. Amongest the seven plants *Calotropis procera*;

Antirrhinum majus and *Asclepias curassavica* root extract showed significant mitotic inhibition as compared to control and similar to MTX.

The cytotoxic threshold was estimated as the concentration causing 50% mitodepression compared to the control (Sharma, 1983). This may be related to the proteins required for mitosis which were not produced at the same quantities, or the code was not reached the cell to induce it to proliferate, or the drug may cause the death of bone marrow cells (Turner et al., 1988) or due to defect occurred in the mitotic spindle composition during cell division (Shirashi, 1978). Smaka-Kincl et al. (1996) reported that the decrease in mitotic index was the result of cytotoxic effects.

Stronger concentrations of extract of immature *Solanum nigrum* L. fruits reduced the intensity of mitosis in *A. sativum* L., whereas weaker concentrations stimulated it. The presence of a cytokinin-like substance in the extract has been suggested to be responsible (Krivokapic et al., 1970). Low concentrations of tobacco leaf extract exerted a stimulating effect, whereas high concentration acted as a mitodepressant, on root-tip cells of *Allium sativum* L. (Sopova et al., 1983).

Decrease in the mitotic index was the result of the suppressive effect of some plant leaf and root extracts on DNA and nucleoprotein synthesis (Danhof and McAnally, 1983; Avila et al. 1997 and Schulze and Kirscher, 1996. El-Ghamery et al., 2000) explained the mitodepressive effect as the blockage of the G1 phase and consequently the depression of DNA synthesis. These results corroborates the findings of Bakare et al.,1999a, 1999b, 2000 on cytotoxicity of urban and rural dumps leachates.

Kabarity and Mallallah, 1980; Pandita, 1986; Badr and Ibrahim, 1987 studied effect of chromium attributed to the less proportion of interphase cells enters into prophase from which more proportion of cells become arrested at metaphase, anaphase

and telophase with the increase in the concentration of the chemical in a continuous cell cycle. Hence, chemical might have inhibited the DNA synthesis at S phase of the cell cycle and prolonged the duration of metabolic activities during interphase cells entering into prophase. At the same time, the mitodepression would have caused due to the prolonged G2 period and blockage of biosynthesis of DNA, RNA and protein.

Mitotic index (MI) measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or delay in the cell proliferation kinetics (Rojas et al., 1993).

The suppression of mitotic activity was often used in tracing cytotoxicity (Smaka-Kinel et al., 1996). It can be concluded that, together with the increase in herbicide concentrations, the mitotic index decreased due to the blocking effect of some components of extract in the G1 phase or DNA synthesis inhibition in the S phase as stated by El-Ghamery et al. (2000).

Mitotic index is the percentage of cells in culture which are in the process of division. It has proven to be a good an easy marker to evaluate and compare cell proliferation rate (Ostrosky et al., 1988). Mitotic index measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or delay in the cell proliferation kinetics (Rojas et al., 1993). Ene-Obong et al. (1991) observed a mitodepressive effect of alcoholic extracts of five molluscidal plants on root tip mitosis of *A. cepa*.

It has also been reported that extracts of some medicinal plant, *Borreria filiformis* and *Vinca rosea* had a mutagenic effect comparable to that of the alkylating agent, ethyl methane sulphonate on root tip mitosis of *A. cepa* (Ene-Obong and Osuala, 1990). The inhibition of mitotic index and reduction in percentage of

mitotic phases indicate that the treatments interfered with the normal sequence of cell division, thus preventing or reducing the number of cells entering the prophase stage at high concentrations. A lowered cell division index suggests an inhibitory effect at the interphase stage (Ene-Obong, 1995).

The higher percentage of interphase cells as compared to the control as observed in this experiment is an indication that leaf and root extracts using different solvents inhibited cell division considerably and agrees with the work of Shehab, 1979, in respect of the effect of *Teucrium pilosum* extract on the root mitosis of *A. cepa* as well as agrees with report of Tabor and Oney (2009) on their work on the effect of artificial fertilizers on mitotic index in *Vicia hybrid.* For a group of cells that rarely complete the cell cycle, a high proportion of cells to be in the resting stage of the cell cycle are expected (Darbelley et al., 1989).

Apart from the use of plant extracts for mitotic studies, there are several other reports on the use of inorganic chemicals. El-Bayoumi et al. (1979) reported high mitotic index in *A. cepa* caused by papeverine chloride. Shehab (1979) observed a depressed mitosis in *A. cepa* in the water extract of *Pulicerra crispa.* Shehab (1979) equally observed that water extract of *Teucrim pilosum* had a strong depressive effect on mitosis of *A. cepa* and toxic at (>10) high concentration. Kabarity and Mallah (1980) using Khat extract observed a mitodepressive effect in the meristematic region of *A. cepa.* Misra (1982) also reported a mitodepressive effect of calcium salt on *A. cepa*, while Okoli and Russom (1987) demonstrated the effect of high concentration of *Cassia alata* extract on the mitotic index of *A. cepa* root cells.

Many other investigations showed that the reduction in cell division activity could be due to change in the duration of the cell cycle. Van't Hoff (1968) suggested

that, the inhibition of mitotic activity by chemical compounds is due to an increase in the G2 period. This view was supported by the results of Bruneri (1971) who obtained a complete arrest of mitotic cycle at the G2.

Okagbue (1990) observed an anti-mitotic effect of three antimalarial crude plant extract - *Azadirachta indica A. jusc*, *Alstonia boonei*, *Carica papaya* and two synthetic antimalarial drugs (Fansidar and Daraprim) on the root tip mitosis of *Hippeastrum equestre*. It is possible that concentration of any chemical may have an inhibitory or stimulatory effect on the cell cycle has been shown for cytotoxicity of aqueous extracts of *Ilex paraguariensis* in *Allium* test. The extracts were prepared from commercial and laboratory and were tested at concentrations of 5-40 g/L. Both extracts significantly decreased root growth and mitotic index (Bidau et al., 2004).

5.3. Genotoxicity:

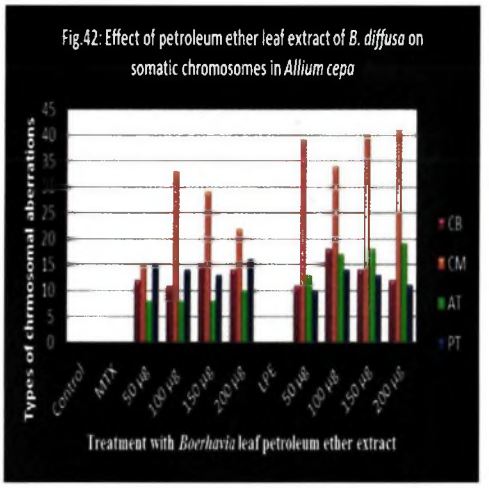
Allium cepa somatic chromosomes are as sensitive as those of human chromosome to various categories of environmental chemicals which directly or indirectly act upon genetic system and hazardous effect are depending on their cytotoxic and mutagenic potential. *A. cepa* system now-a-days is extensively used as plant test system all over the world. Since 1938 when Levan used *Allium* test for the first time, it has been the standard test for studying the effect of various chemicals, pollutants and pharmaceutical agents on the chromosome (Buiatti and Nutironchi, 1969; Fiskesjo, 1969; Kak et al., 1973; Sabharwal et al., 1975; Malode and Khandare, 2009; Malode and Khandare, 2010 and Khandare and Malode, 2012).

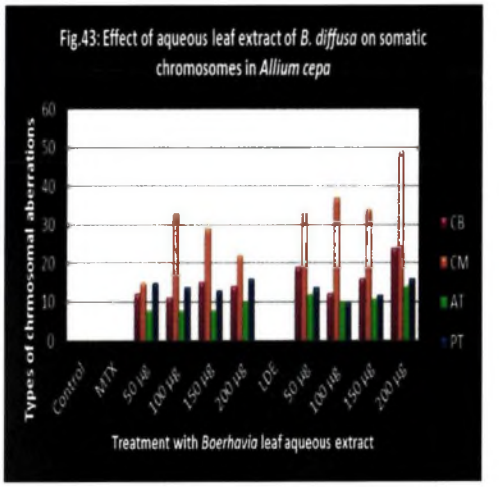
A. cepa root tip meristems have been widely used for evaluation of genototoxic and anti-mitotic activity of compounds (Shehab, 1980; Williams and Omoh, 1996 and Al-Meshal, 1987). The most frequent aberrations are clumped metaphase in which

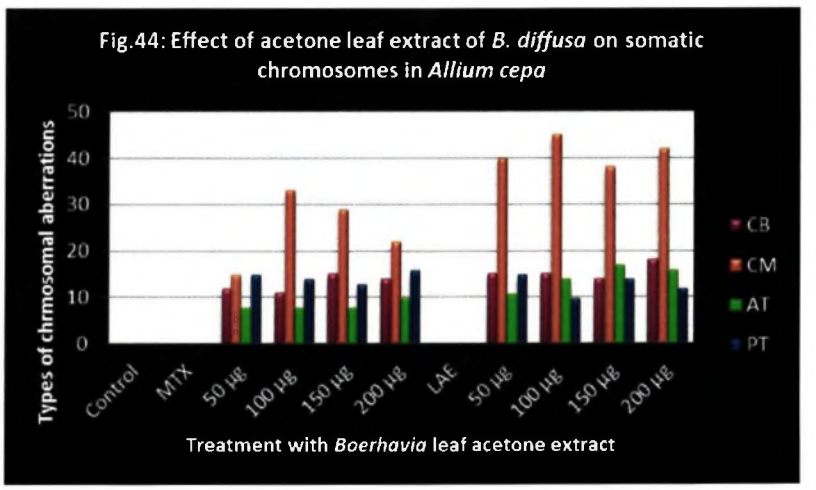
induction of cell cycle arrest at metaphase, subsequent apoptosis occur and chromosomes are intermingled with each other. In single chromatid bridge one of the chromatid become fragmented during anaphase and centromere of chromosome goes to the opposite pole and central position remains in between the two poles forming a bridge, precocious chromosome at telophase occur and reconstruction of nuclear envelop about two groups of offspring chromosomes was arrested because of arrested telophase.

5.3.1. *Boerhavia diffusa* leaf extracts:

The tabulated data (Table 4 Fig. 42, 43, 44) indicated that the chromosomal aberrations in leaf extracts of *B. diffusa* were increased with increase in concentration in all leaf extract. The frequencies of chromosomal aberrations were slightly increased in leaf extracts when compared with MTX. No mitotic aberrations were recorded in control. In leaf extracts, numbers of chromatid bridges showed similarity with that of MTX (15) while increases in 200µg of LDE (24). Clumped metaphases were significantly increased in 200µg of LPE (41), in 200µg of LDE (49) and in 100µg of LAE (45) as compared to MTX (33). In all concentrations arrested telophases were also moderately increased in 200µg of LPE (19) and 150µg of LAE (17) as compared to MTX (10). Whereas, precocious chromosome at telophases were lowered in most of the concentrations as compared to that of MTX.

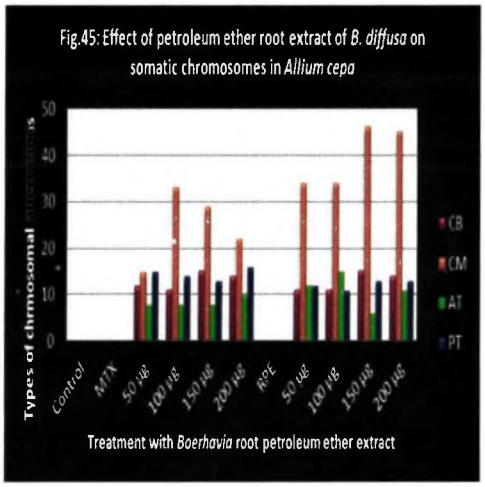


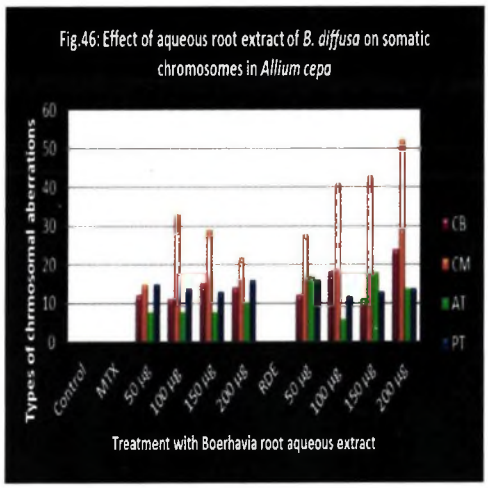


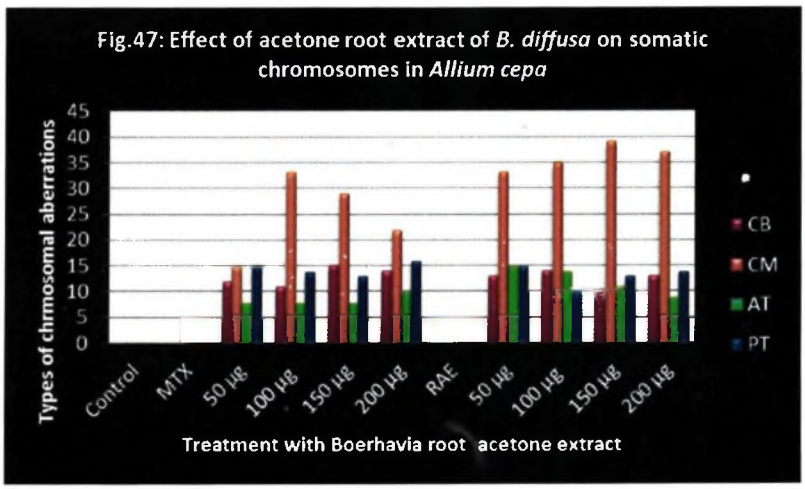


5.3.2. *Boerhavia diffusa* root extracts:

In RDE chromosomal aberrations frequencies were increased with increase in concentrations whereas, in 100µg of RPE (3.93%) it is higher than 150µg (3.84%) and in RAE 50µg shows higher frequency than other concentrations (Table 5 and Fig. 45, 46, 47). Chromatid bridges were increased in 100µg and 200µg of RPE (18 and 24) as related to MTX (15). Clumped metaphases were markedly increased in 150µg of RPE (46) and in 200µg of RPE (52) with that of MTX (33). Numbers of arrested telophases were also increased in 100µg of RPE (15), 150µg of RDE and 50µg of RAE (15) as compared to MTX (10) and there was slight difference observed in number of precocious chromosomes at telophases when compared with MTX.



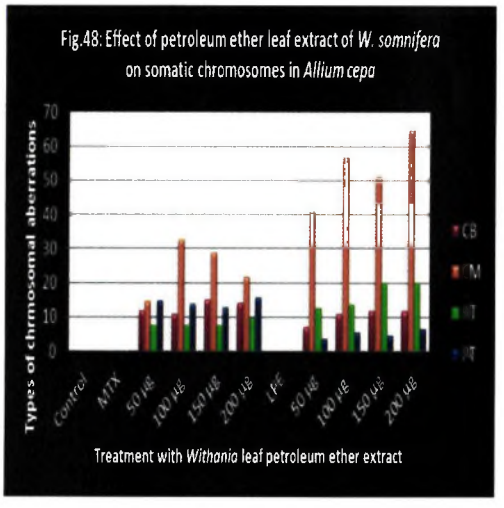


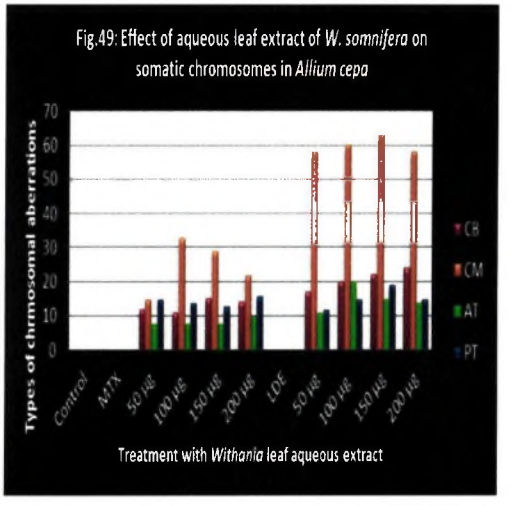


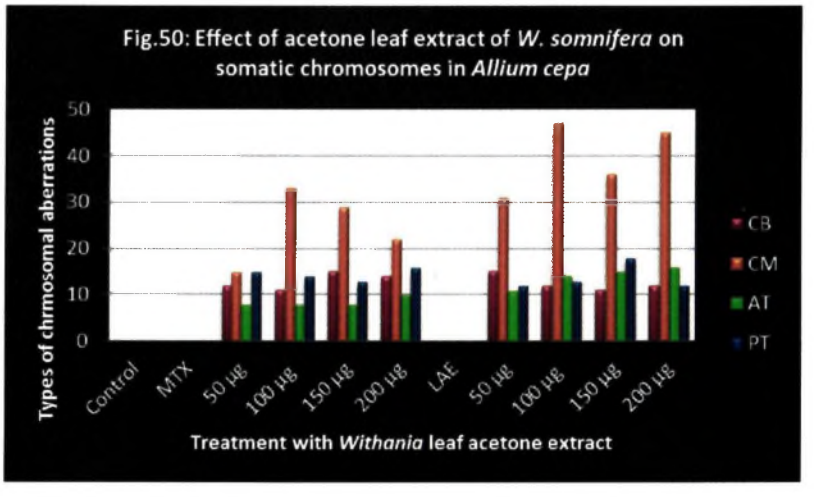
5.3.3. *Withania somnifera* leaf extracts:

Chromosomal aberrations frequencies were observed to have increased with increasing in concentrations of leaf extract of *W. somnifera* using petroleum ether, distilled water and acetone as solvent (Table 12, Fig. 48, 49, 50). Different types of chromosomal abnormalities were increased with increase in concentrations. Numbers of chromatid bridges were decreased in 50µg of LPE (7) while increased in 100µg, 150µg and 200µg of LDE (20, 22, and 24) as related to MTX (15). Clumped metaphases were highly increased in 200µg of LPE (65) and 150µg of LDE (63) to that of MTX (33). Arrested telophases were also increased in 150µg of LPE (20), 100µg of LDE (20) and 200µg of LAE (16) as compared to MTX (10) whereas, precocious chromosomes at

telophases were lower down in 50µg of LPE (4) and similar in LDE and LAE as compared to MTX(16).



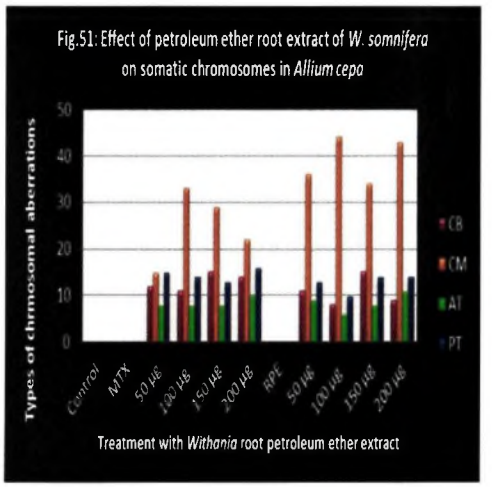


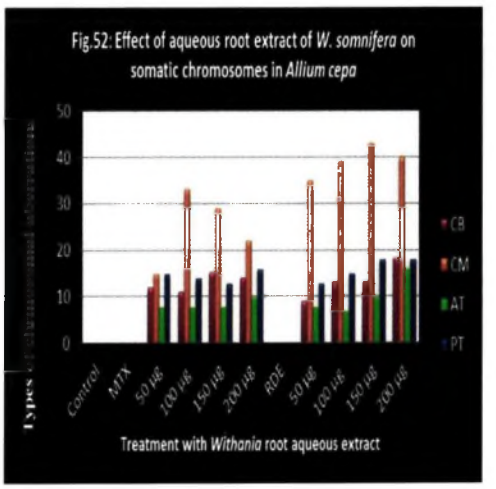


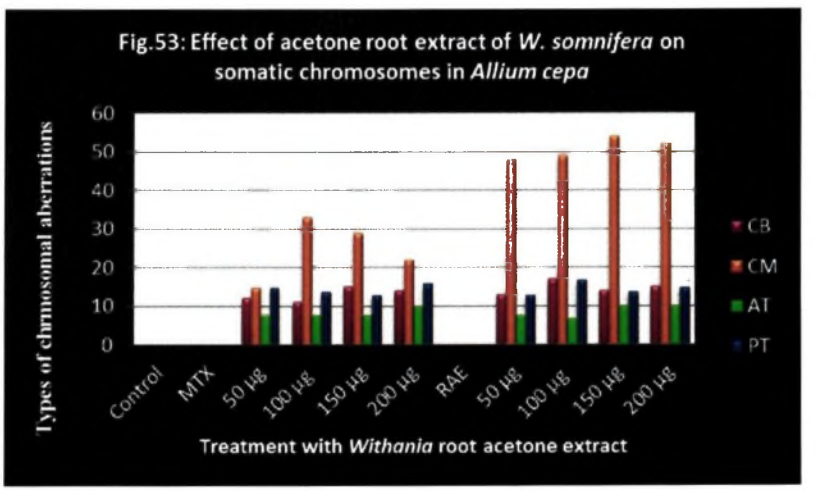
5.3.4. *Withania somnifera* root extracts:

In root extracts chromosomal aberration frequencies were increased in 50µg of RPE (3.56%) than 100µg (3.56%); in 150µg of RDE (4.69%) than 200µg (4.54) and in 150µg of RAE (5.00%) than 200µg (4.45%). Chromatid bridges were lower in 100µg of RPE (8) and 50µg of RDE (9) than MTX (15). The numbers of clumped metaphases were moderately increased in 100µg of RPE (44), 150µg of RDE (43) and 150µg of RAE (54) when compared with MTX (33). Arrested telophases and precocious

chromosomes at telophases were relatively similar to that of MTX (Table 13 and Fig. 51, 52, 53).



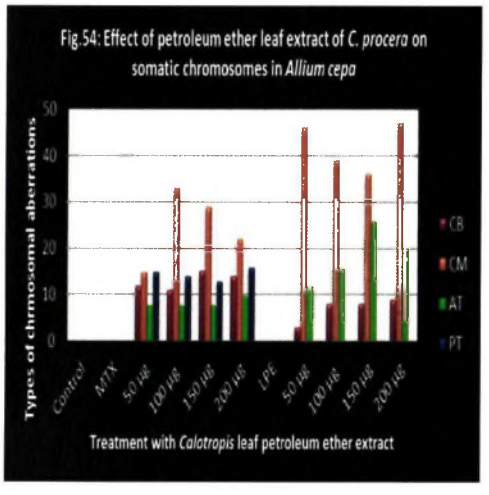


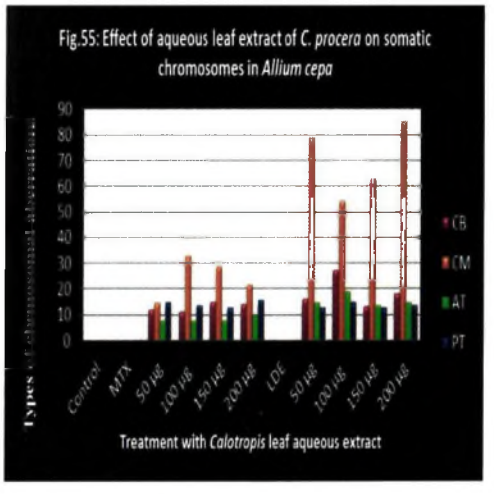


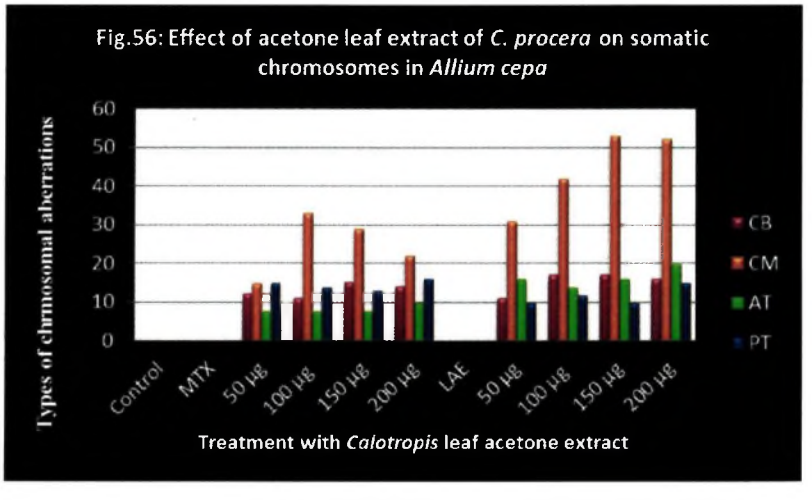
5.3.5. *Calotropis procera* leaf extracts:

Different kinds of chromosomal aberrations in leaf extracts of *C. procera* are presented in Table 20 and Fig. 54, 55, 56. In leaf petroleum ether frequencies of chromosomal abnormalities were increased with increase in concentrations whereas, in leaf distilled water extracts showed more chromosomal abnormalities in 100µg (5.38%) as compare to 150µg (5.08%) concentration and leaf acetone extracts at 150µg (4.92%) showed more chromosomal abnormalities as compared to 200µg (4.48%).

Chromatid bridges highly reduced in 50µg of LPE (3) as compared to MTX (15) while relatively equal in other concentrations. Numbers of clumped metaphases were extremely higher in 50µg and 200µg of LDE (79 and 85). Arrested telophases were increased in 150µg of LPE (26), 100µg of LDE (19) and 200µg of LAE (20) as compared to MTX (10). No precocious chromosome at telophases observed in LPE whereas, that were similar in LDE and LAE when compared with MTX.



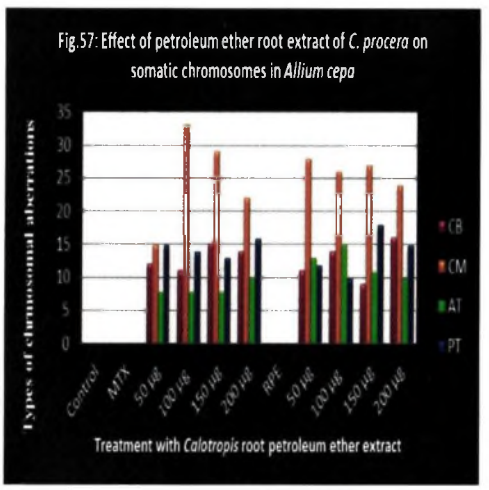


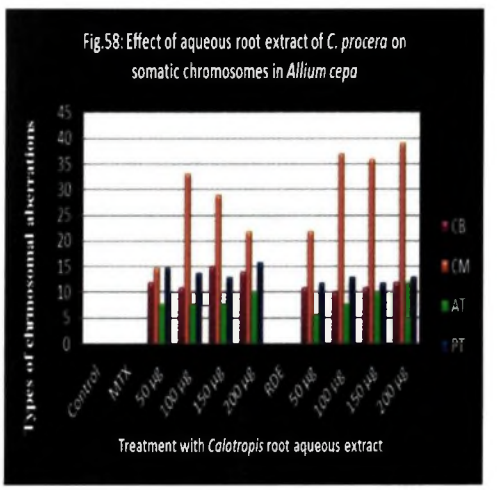


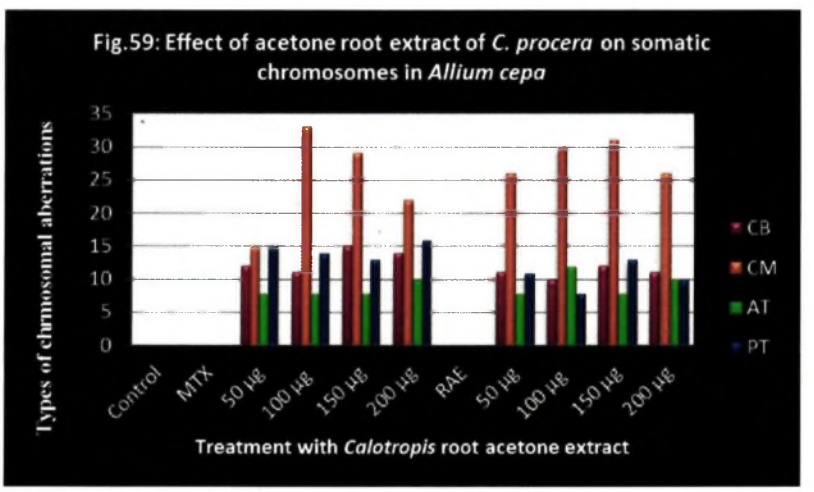
5.3.6. *Calotropis procera* root extracts:

From the Table 21 and fig. 57, 58, 59 it shows that the chromosomal aberrations in RDE were increased with increase in concentrations while in RPE frequencies were increase in 100µg (3.35%) than 150µg (3.23%). Chromatid bridges and clumped

metaphases were relatively equal in all concentrations of root extracts. Arrested telophases were increased in 100µg of RPE (15) whereas, lower down in 50µg of RDE (6) and 50µg of RAE (8) as compared to MTX (10). Precocious chromosomes at telophases were decreased in 100µg of RAE (8) while moderately similar in other concentrations as compared to MTX (16).



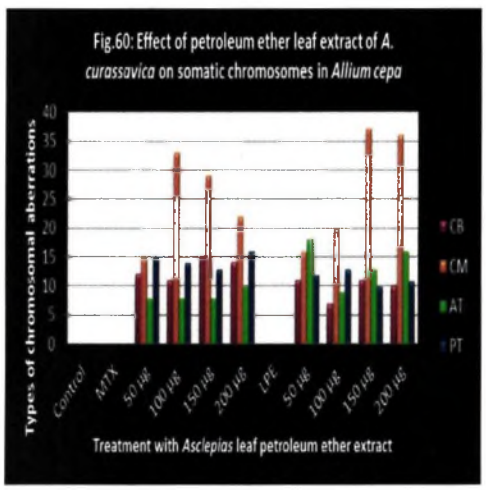


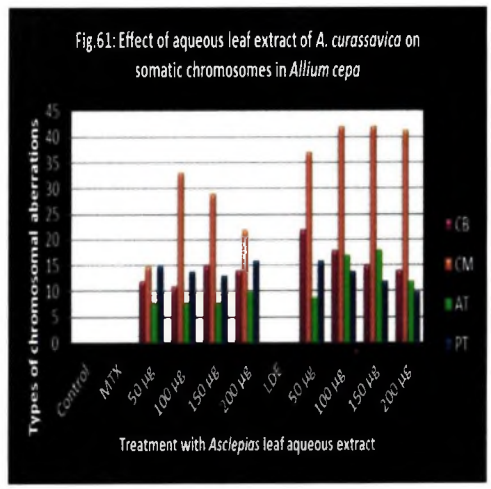


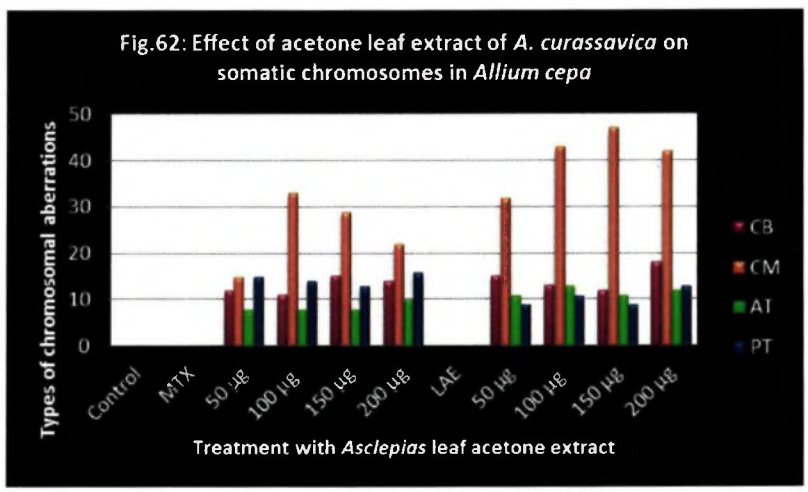
5.3.7. *Asclepias curassavica* leaf extracts:

The data presented in Table 28 and Fig. 60, 61, 62 shows the frequencies of cytological abnormalities in leaf extracts of *A. curassavica* were increased with increase in LAE concentrations whereas, increase in 150µg LPE (3.75%) than in 200µg (3.69%), and decrease in 150µg (3.95%) and 200µg (4.16%) than in 50µg (4.35%) and 100µg

(4.48%) of LDE. Chromatid bridges were decrease in 100µg of LPE (7) while increase in 50µg of LDE (22) as compared to MTX (15). Numbers of clumped metaphases were higher in 100µg and 150µg of LDE (42) and in 150µg of LAE (47) to that of MTX (33). Arrested telophases showed higher number in 50µg of LPE and 150µg of LDE (18) than MTX (10). Precocious chromosomes at telophases were relatively similar in all concentrations.

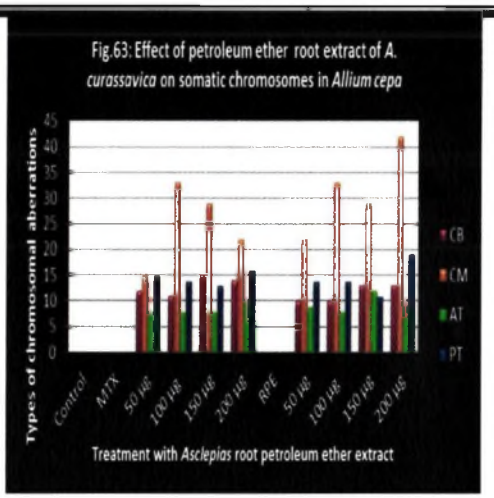


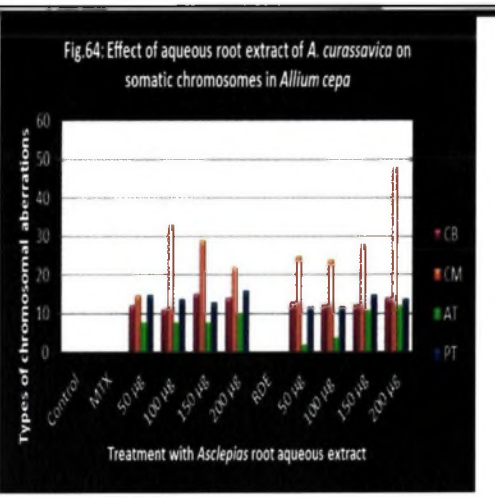


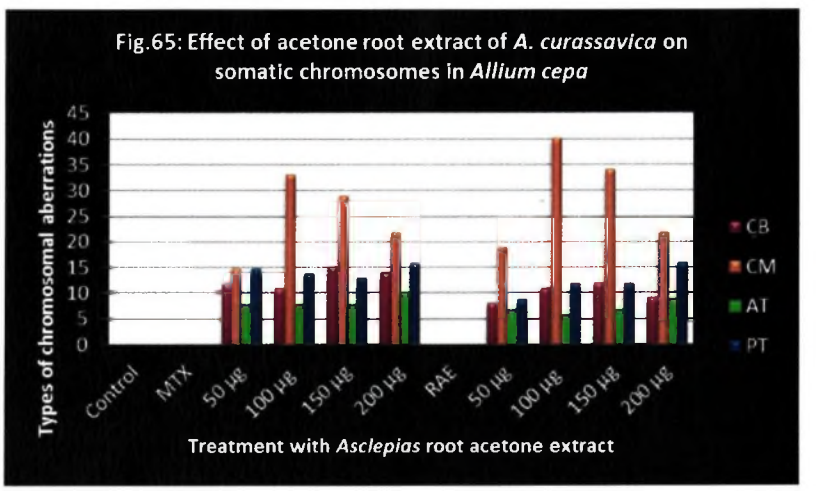


5.3.8. *Asclepias curassavica* root extracts:

In RPE and RDE frequencies of chromosomal aberrations were increased with increase in concentrations whereas, in RAE frequency was increase in 100µg (3.66%) as compared to remaining concentrations (Table 29 and Fig. 63, 64, 65).





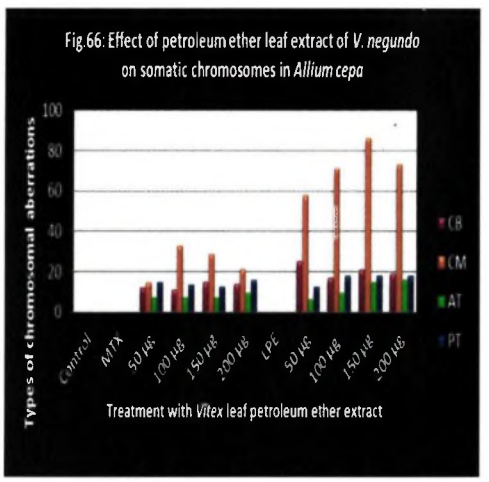


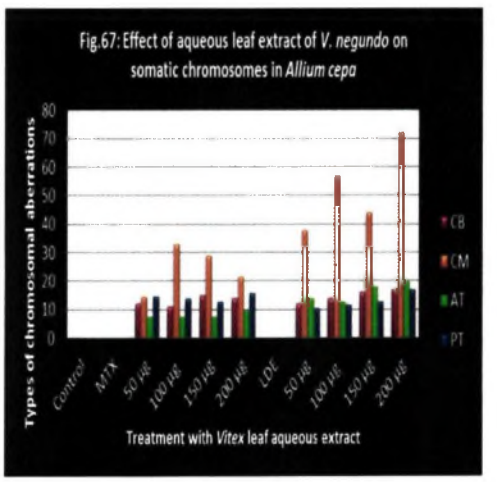
Numbers of chromatid bridges, arrested telophases and precocious chromosomes at telophases were relatively similar in all root extracts when compared with MTX. Clumped metaphases were significantly higher in 200µg of RPE (42), 200µg of RDE (48) and 100µg of RAE (40) as compared to MTX (33) .

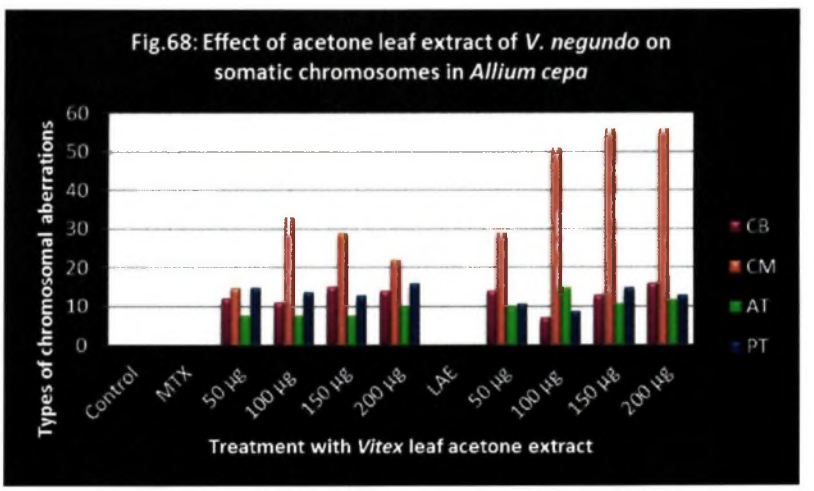
5.3.9. *Vitex negundo* leaf extracts:

Results are shown in Table 35 and Fig. 66, 67, 68. The data indicated that the chromosomal aberrations were increased with increase in concentrations in leaf extracts of *V. negundo* using petroleum ether, distilled water and acetone as solvent. Chromatid bridges were increase in 50µg of LPE (25) while decrease in 100µg of LAE. Clumped metaphases were significantly increase in 150µg of LPE (86), 200µg of LDE and 200µg of LAE (56) as compared to MTX (33). Arrested telophases were increased in 200µg of

LPE (16) and LDE (20) with that of MTX (10). Numbers of precocious chromosome at telophases were considerably analogous to MTX.





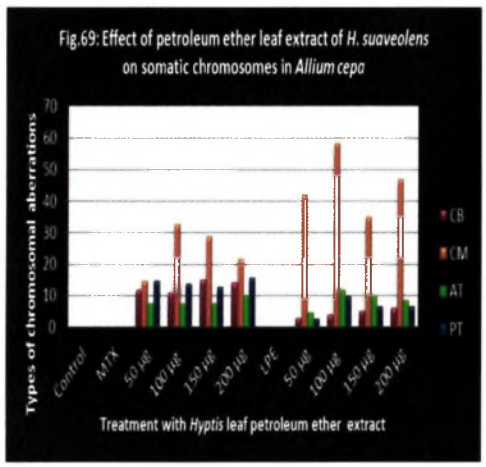


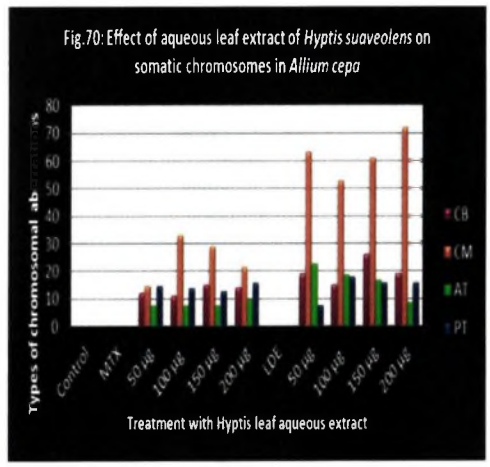
5.3.10. *Hyptis suaveolens* leaf extracts:

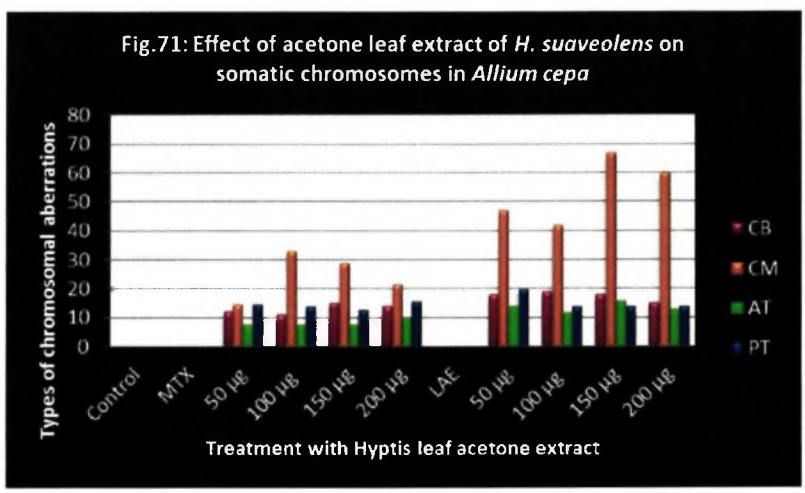
In *H. suaveolens*, leaf petroleum ether extract frequencies of chromosomal abnormalities were higher in 100µg (4.59%) as that of 150µg (3.23%) whereas, lower in 100µg LDE (4.87%) and LAE (3.72%) as compared to 50µg of LDE (5.28%) and LAE (4.79%), Table 40 and Fig. 69, 70, 71.

Chromatid bridges were significantly decreased in 50µg of LPE (3) however, increased in 150µg of LDE (26) and 100µg of LAE (19) as related to MTX (15). Numbers of clumped metaphases were significantly higher in 100µg of LPE (58),

200µg of LDE (72) and 150µg of LAE (67) as compared to MTX (33). Arrested telophases were declined in 50µg of LPE (5) but rise in 50µg of LDE (23) and 150µg of LAE (16) as to that of MTX (10). Precocious chromosomes at telophases were decrease in 50µg of LPE (3) whereas, increase in 50µg of LAE (20) compared to MTX (16).

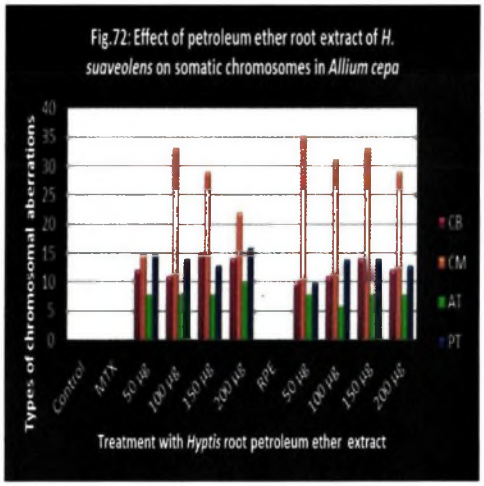


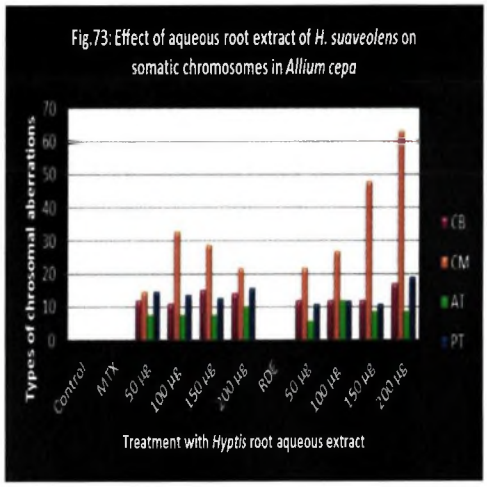


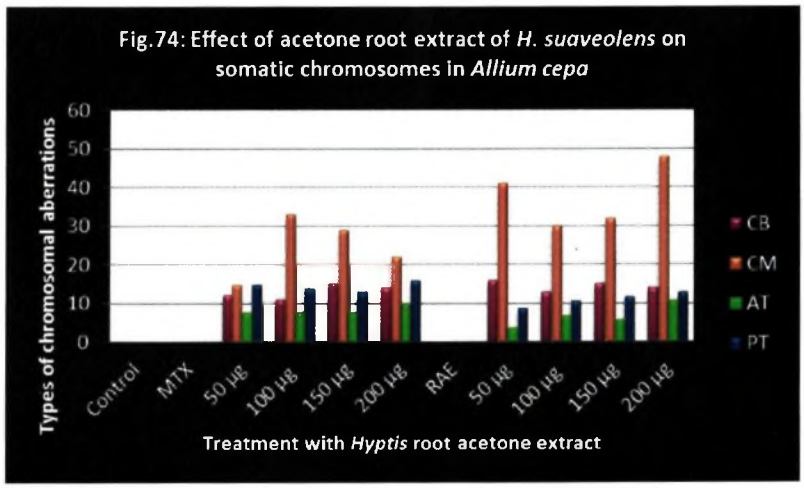


5.3.11. *Hyptis suaveolens* root extracts:

Data presented in Table 40 and Fig. 72, 73, 74, indicated that the frequencies of chromosomal aberrations RDE and RAE increased with increase in concentrations whereas, in RPE it has been increased at 150µg (3.77%) than 200µg (3.57%).





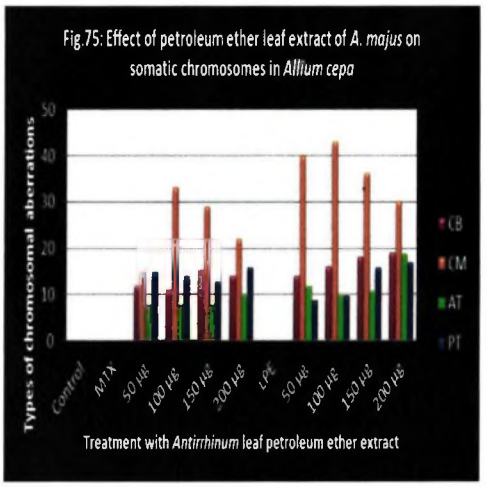


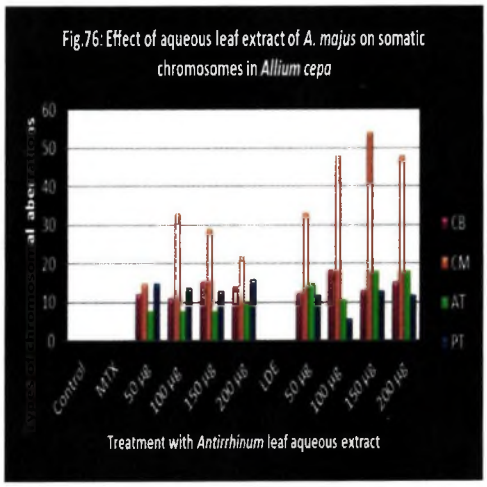
Numbers of chromatid bridges were considarably equal in all concentration when compared with MTX. In RPE clumped metaphases were relatively same while significantly higher in 200µg of RDE (63) and RAE (48) compared to MTX (33). Numbers of arrested telophases and precocious chromosomes at telophases were showed similarity with MTX.

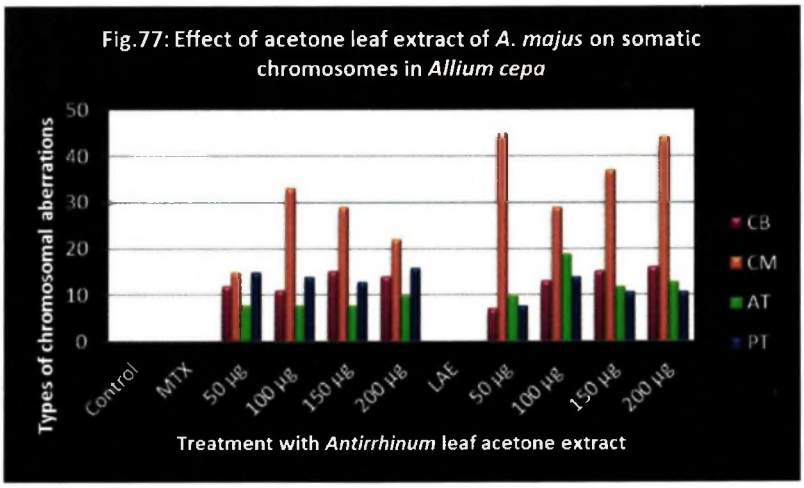
5.3.12. *Antirrhinum majus* leaf extracts:

In the LPE and LAE, frequencies of chromosomal aberrations were increased with increase in concentrations however in LDE it increases in 150µg (5.24%) than 200µg (5.02%), Table 48 and Fig.75, 76, 77. Chromatid bridges were higher in 200µg of LPE (19), 100µg of LDE (18) and lower in 50µg of LAE (7) as compared to MTX

(15). Clumped metaphases were slightly increase in 100µg of LPE (43), 100µg of LDE (48) and 50µg of LAE (45) to that of MTX (33). Arrested telophases were also increased in 200µg of LPE (19), 150µg of LDE (10) and 100µg of LAE (19) as related to MTX (10). Precocious chromosomes at telophases showed lower numbers in 50µg of LPE (9), 100µg of LDE (6) and 50µg of LAE (8) than MTX (16).

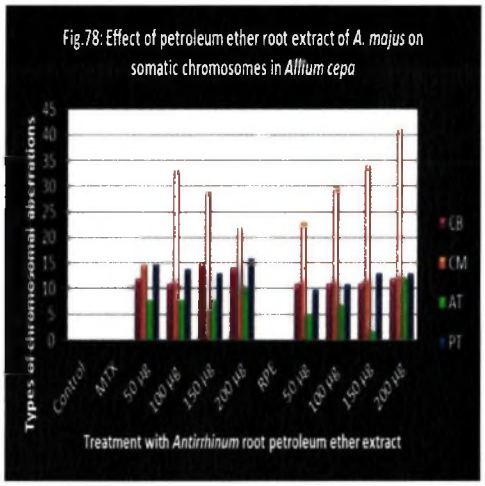


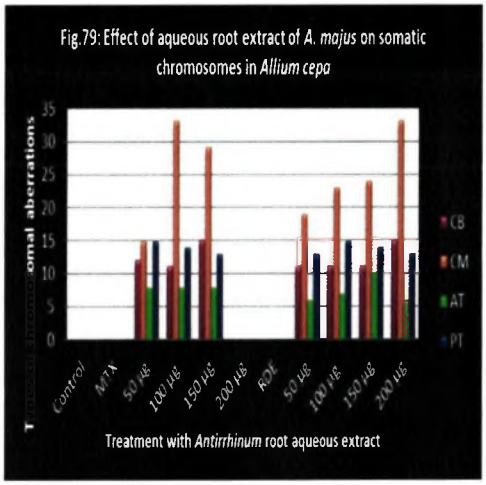


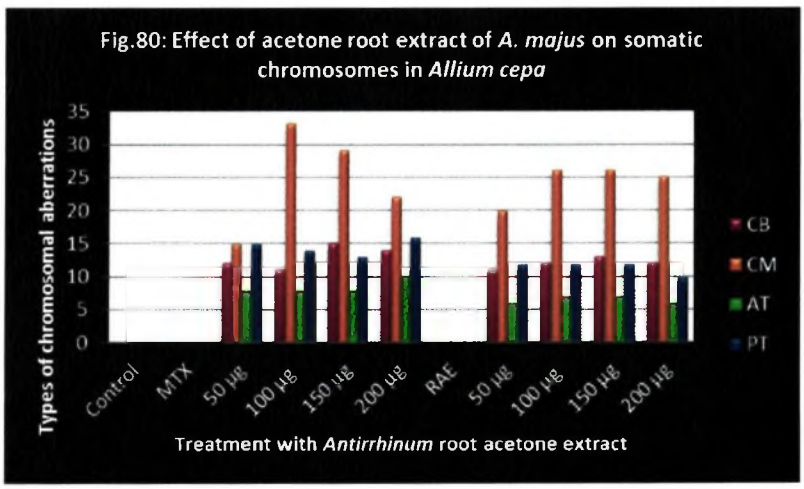


5.3.13. *Antirrhinum majus* root extracts:

Data presented in Table 49 and Fig. 78, 79, and 80 showed that in all concentrations chromosomal aberrations were increased with increase in concentrations







Numbers of chromatid bridges and clumped metaphases showed similarity to that of MTX. Arrested telophases were highly lower in 150µg of LPE (2) than in MTX (10) and in other concentrations showed equivalence. There were no variations observed in number of precocious chromosome at telophases when compared with MTX.

The use of plant tissue primarily root tip for studying the induction of chromosomal aberration is one of the oldest, simplest, most reliable and inexpensive method (Auti et al.2010). The higher plants *Allium cepa, Tradescantia paludosa* and *Vicia faba* have relatively large monocentric chromosomes in reduced numbers and are accepted as suitable test organisms for the study of environmental mutagenesis (Rank

and Nielsen, 1998; Grover and Kaur, 1999; Kong and Ma, 1999 ; Moraes and Jordao, 2001: Patra and Sharma, 2002).Chromosome aberrations provided important information and may be considered an efficient test to investigate the genotoxic potential of the treatments analyzed (Carita and Marin-Morales, 2008). Chromosomal aberrations are changes in chromosome structure resulting from a break or exchange of chromosomal material. Results showed other aberrations, induction of bridges and disturbance of spindle fibres at different stages of mitotic division in the onion root cells. In *A. cepa*, whenever chromosome aberrations occurred, there were almost always certain growth restrictions (Fiskesjo, 1993). The specific aberration induced depends on the time at which the interphase nucleus is exposed to a clastogen (Evans, 1977).

In the light of the results obtained in the present study, it is exhibited that the frequencies of chromosomal aberrations in most of the leaf and root extracts of seven plants were increased with increase in concentrations. Chromosomal aberrations frequencies in *B. diffusa* at 50µg of RDE (3.41%) and 100µg of RAE (3.48%); in *W. somnifera* at 50µg of LPE (3.27%), 50µg of RAE (3.31%), 100µg of RPE (3.35%), 50µg and 100µg of RDE (2.77% and 3.27%) and 50µg of RAE (3.48%); in *C. procera* at 50µg and 100µg of LPE (3.26% and 3.45%), 50µg of LAE (3.17%), all concentration of root extracts except at 150µg and 200µg of RDE; in *A. curassavica* at 50µg and 100µg of LPE (2.78% and 2.95%), 50µg, 100µg and 200µg of RPE (2.91%, 3.48% and 3.47%), 50µg and 100µg of RDE (2.50% and 2.80%) and 50µg of RAE (1.99%); in 50µg of *V. negundo* LDE (2.97%); in *H. suaveolens* at 50µg and 150µg of LPE (2.60% and 3.23%), 50µg and 100µg of RPE (3.10% and 3.15%), in 50µg of RDE (2.74%) and 50µg and 100µg of LAE (2.97% and 3.26%), in *A. majus* at 50µg of LPE and LAE (2.96% and 3.42%) and most of the root extracts apart from

200µg of RPE, 150µg and 200µg of RDE were conforming with that of MTX frequencies ranges from 2.96% to 3.49% respectively, these observations may be due to the action of the extracts on the disturbance of the formation of spindle fibres during cell division which leads to chromosomal aberrations. Bridges and clumping of the chromosomes were some of the most common effects of these extracts on the treated root tips. Stickiness usually leads to the formation of anaphase bridges and this end up inhibiting post metaphase, telophase and cytokinesis respectively. Stickiness might be due to the ability of the extracts to cause DNA depolymerization and partial dissolution of nucleoproteins, breakage and exchanges of the basic folded units of chromatids and the stripling of the protein covering of DNA in chromosomes as also observed by Onyenwe (1983).

These abnormalities have also been reported for several extracts and chemicals already investigated (Badr and Elkington 1982, Misra 1982, Nwakanma and Okoli, 2010). Amongest the seven plants *Calotropis procera*; *Antirrhinum majus* and *Asclepias curassavica* root extracts showed significant chromosomal aberrations inhibition as parallel to MTX.

The chromosome aberration and micronucleus assays have been shown to be highly reliable in genotoxicity testing (Grant, 1982). Thus a common effect of medicinal plant extracts on root tip mitosis of *A. cepa* is an inhibition of spindle mechanism leading to the scattering of the chromosomes, stickiness of chromosomes, anaphase bridges and diverse kinds of abnormalities (Shehab, 1979, 1980; Ene-Obong and Amadi, 1987ab; Ene-Obong and Osuala 1990; Ene-Obong et al., 1991).

According to Kabarity et al., 1974, chromosome bridges may be caused by stickiness of chromosomes which made their separation and free movements complete

and thus they remained connected by bridges. Chromosome bridges were commonly observed during anaphase. The bridges noticed in the cells were probably formed by breakage and fusion of chromatids or sub chromatids (Shehab and Adam, 1983).

Similar observations have been reported by Badr, 1983, Da et al., 1994 and Minija et al., 1999. It is possible that concentration of any chemical may have an inhibitory or stimulatory effect on the cell cycle. In recent studies suggested that *Lavandula stoechas*, aqueous extracts (Askin Celik and Aslanturk, 2007) and ethanolic extracts of *Citrus lemon* and *Citrus sinensis* (Ozmen and AskinCelik, 2007), petroleum ether and aqueous extracts of *Achyranthes aspera* (Malode and Khandare, 2009), petroleum ether leaf extracts of *Calotropis procera* (Malode and Khandare, 2010) and petroleum ether leaf extracts of *Vitex negundo* (Khandare and Malode, 2012) have low cytotoxic and genotoxic effects.

5.4. Statistical analysis:

During the statistical comparison it was found that the calculated values of 't' are greater than tabulated values at 1% and 5% level of significance for 8 degree of freedom. Therefore null hypothesis is rejected i.e. the values of $|t|$ are significant hence samples are dependent on each other when treated with untreated control. Whereas, in *W. somnifera* at 150µg (1.64) and 200µg (1.34), in *V. negundo* at150µg (0.80) and 200µg (0.016) leaf petroleum ether extracts and in *H. suaveolens* at 200µg (0.44) leaf distilled water extract the calculated values i.e. 't' are less than tabulated value (2.3) therefore null hypothesis is accepted and samples are not significant when compared with control.

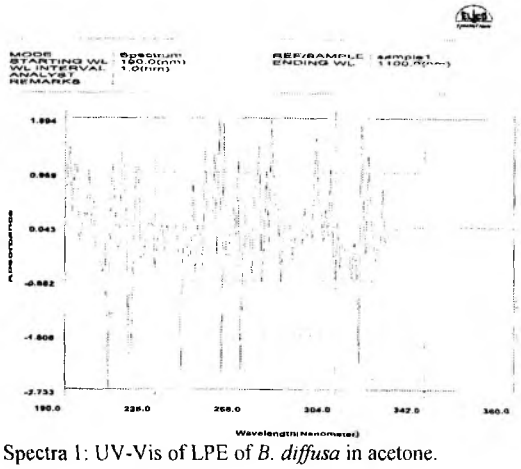
Statistical comparison and interpretation of the data of 't' test, it is showed that these seven plants contains a bioactive compounds might have anticancerous property

similar to that of MTX. Besides, this these crude show low cytotoxic / genotoxic in nature which act on protein components of the cytoplasm, the cell and chromosomes / DNA brings changes in the cell division especially lower down the metaphases and anaphases which reduced the active mitotic index frequencies in *C. procera*, *A. majus* and *A. curassavica* root extracts.

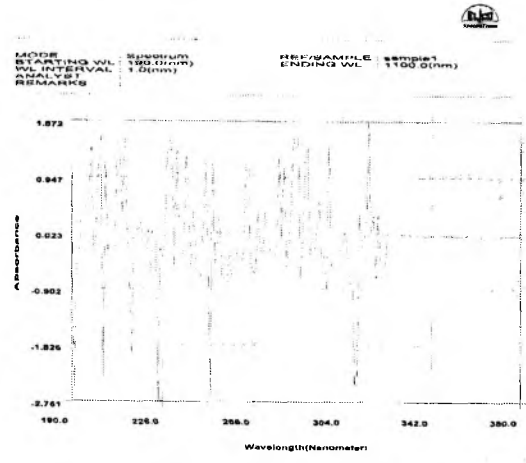
5.5. Spectroscopic analysis:

The isolated compounds were then subjected to spectral studies such as UV-Vis spectrophotometer and FTIR. TLC isolated compounds by aqueous and acetone medium were subjected to spectral analysis to study the presence of different organic group to know the preliminary bioactive compounds present in the extracts. The results of UV-Vis showed that, intense absorption in acetone and methanol as a solvent in *B. diffusa* most of the peaks were observed in LPE, LDE, RDE and RAE at 220nm, 255nm, 296, and 235nm , except in LAE (255nm in methanol) and in RPE (220 and 296nm in methanol) (Table 8, 9 & Spectra. 1- 12). *W. somnifera* showed various peaks in LPE (255nm in acetone and 296nm in methanol), LDE (220nm in methanol), LAE (296nm in methanol), RDE (296nm in acetone) and RAE (296nm in methanol) were absent while remaining are present in leaf and root extracts (Table 16, 17 & Spectra 13-24). *C. procera* all the peaks were present in leaf and root extracts (Table 24, 25 & Spectra 25-36). *A. curassavica* showed absence of certain peaks in LPE (296nm in acetone and 255nm in methanol); in LAE (255nm in acetone and methanol); in root extracts all the peaks were present in acetone solvent whereas, absent in RPE (296nm), RDE (220nm) and RAE (255nm) in methanol (Table 32, 33 & Spectra 37-48). In *V. negundo* using acetone as solvent certain peaks were absent in LPE (255nm), LDE (255 and 296nm), LAE (255nm) however, in methanol at LPE (296nm), LDE (220nm)

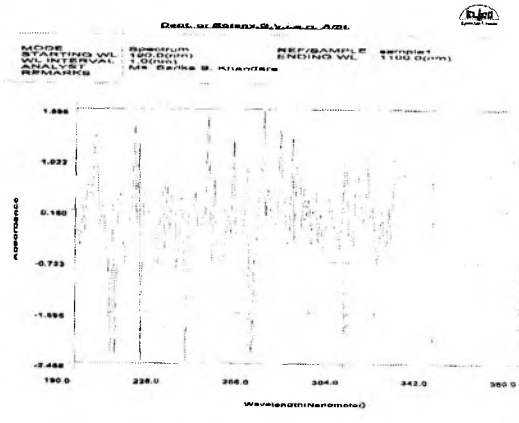
and 255nm in LAE and others are present in leaf and root extracts (Table 37 & Spectra 47-52). In case of *H. suaveolens* all the peaks were present except in RAE (296 in methanol) (Table 44, 45 & Spectra 53-66). While in case of *A. majus*, leaf extracts showed occurrence of most of the peaks apart from in methanol, 235nm in LPE, 296nm and 235 nm in LDE, 235nm in LAE, 220nm in RPE and 296 nm in RAE (Table 52, 53 & Spectra 67-78) .The presence of peaks at 220nm, 255nm, and 296nm corresponds to alkaloids vincristine, while peaks at the range of 235nm- 275nm correlates to the taxol. The intense UV absorption at 200 nm was attributable to the n-n transition of the carbonyl group and other values at 210, 230 and 250 nm correlated to the presence of benzene ring (Silverstein et al., 1981).



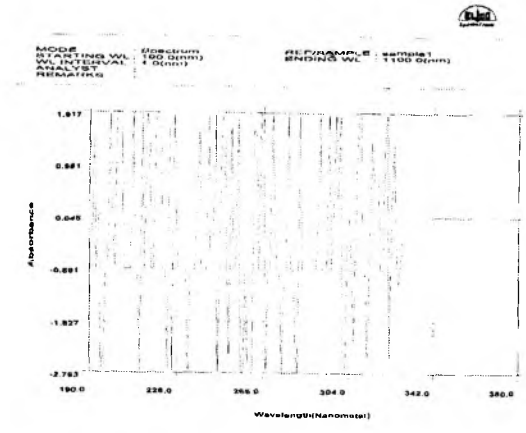
Spectra 1: UV-Vis of LPE of *B. diffusa* in acetone.



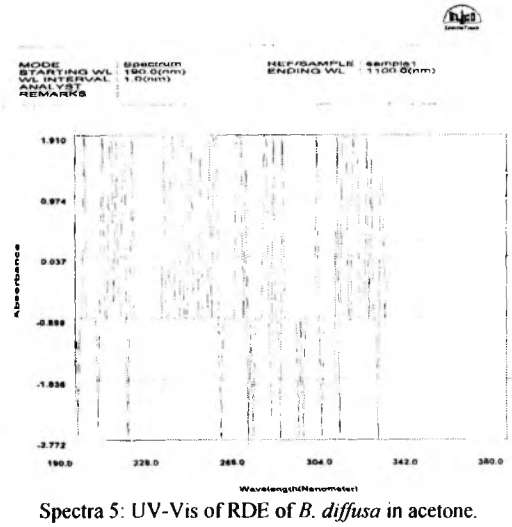
Spectra 2: UV-Vis of LDE of *B. diffusa* in acetone.



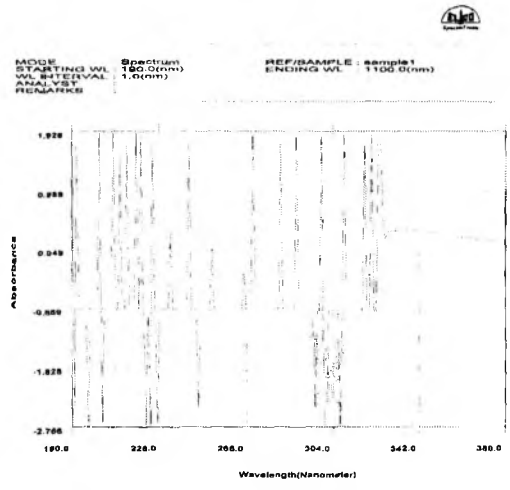
Spectra 3: UV-Vis of LAE of *B. diffusa* in acetone.



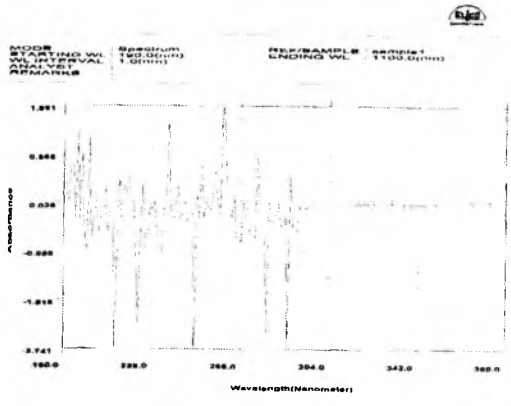
Spectra 4: UV-Vis of RPE of *B. diffusa* in acetone.



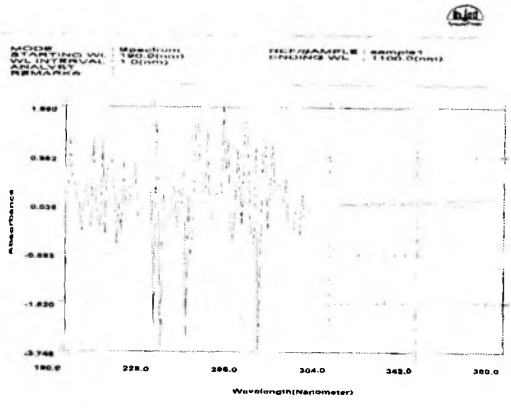
Spectra 5: UV-Vis of RDE of *B. diffusa* in acetone.



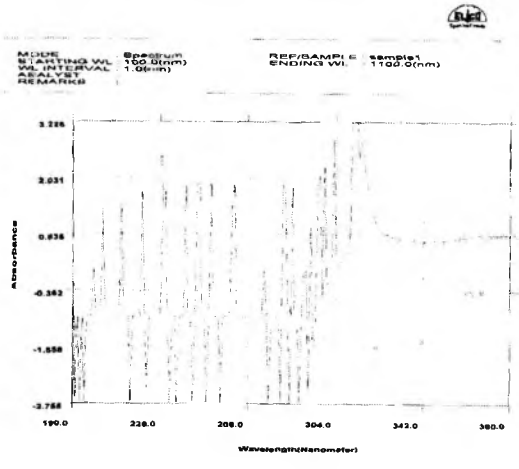
Spectra 6: UV-Vis of RAE of *B. diffusa* in acetone.



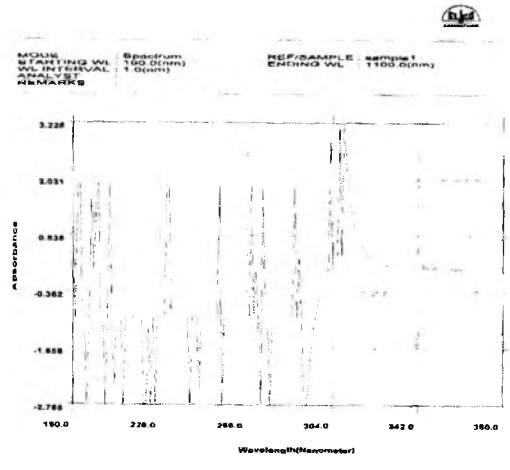
Spectra 7: UV-Vis of LPE of *B. diffusa* in methanol.



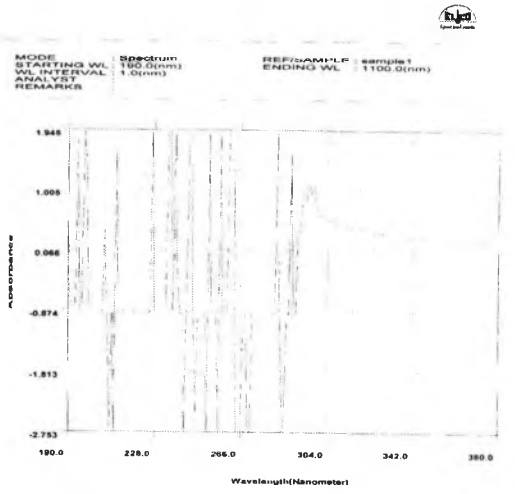
Spectra 8: UV-Vis of LDE of *B.diffusa* in methanol.



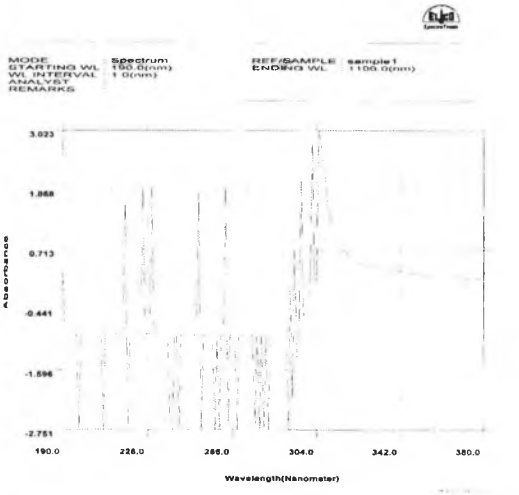
Spectra 9: UV-Vis of LAE of *B. diffusa* in methanol.



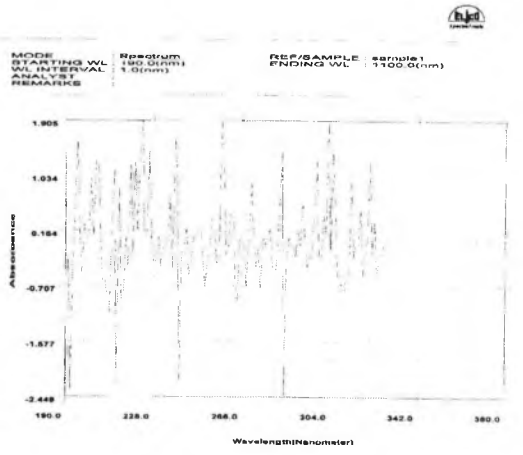
Spectra 10: UV-Vis of RPE of *B. diffusa* in methanol.



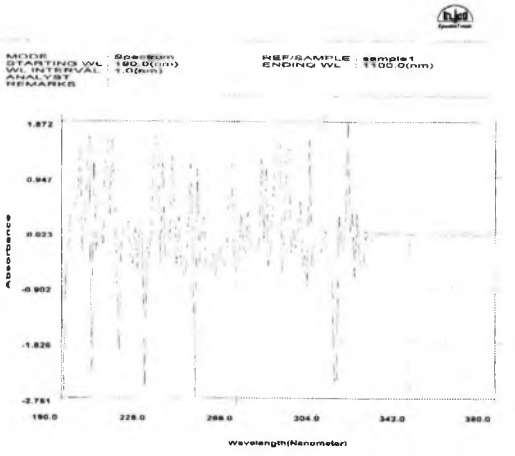
Spectra 11: UV-Vis of RDE of *B. diffusa* in methanol.



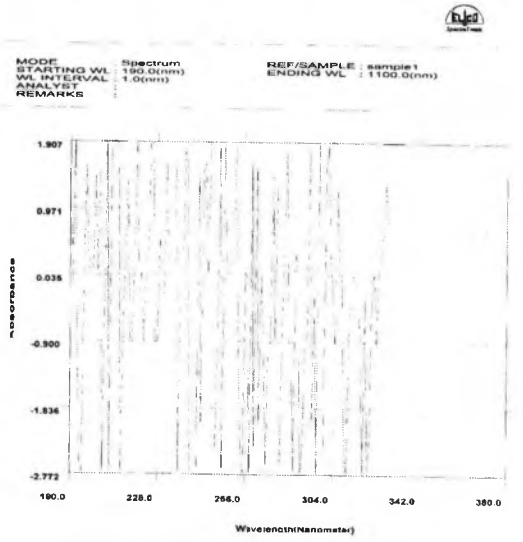
Spectra 12: UV-Vis of RAE of *B. diffusa* in methanol.



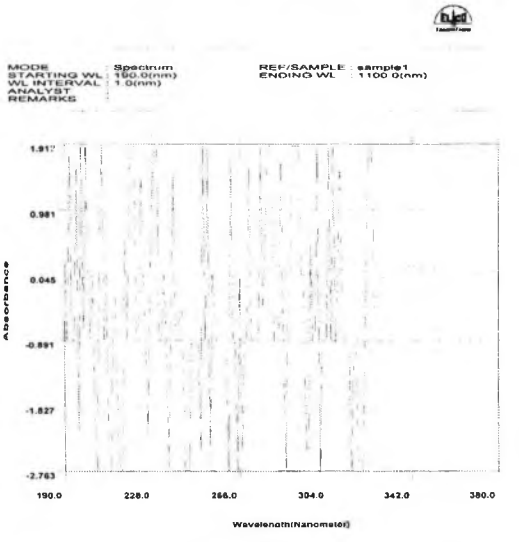
Spectra13: UV-Vis of LPE of *W. somnifera* in acetone.



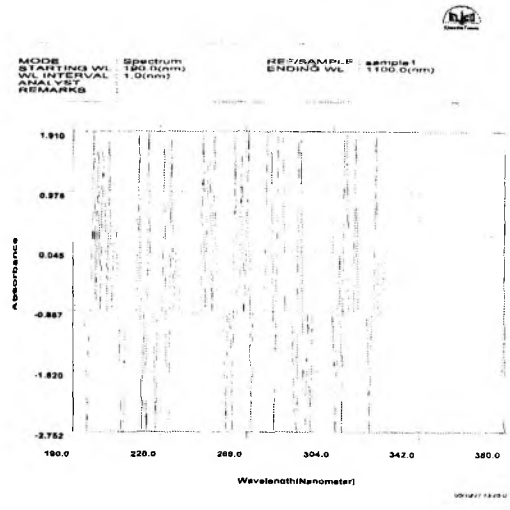
Spectra14: UV-Vis of LDE of *W. somnifera* in acetone.



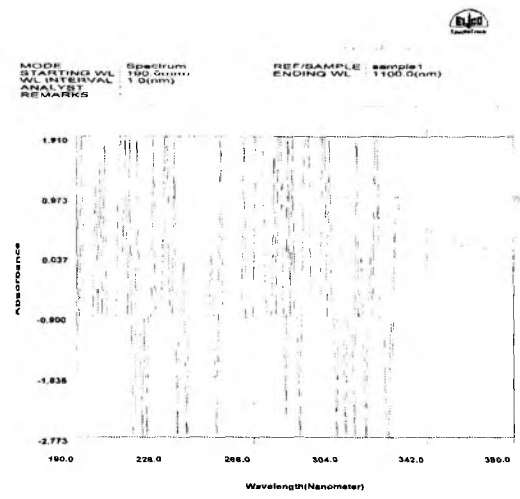
Spectra15: UV-Vis of LAE of *W. somnifera* in acetone.



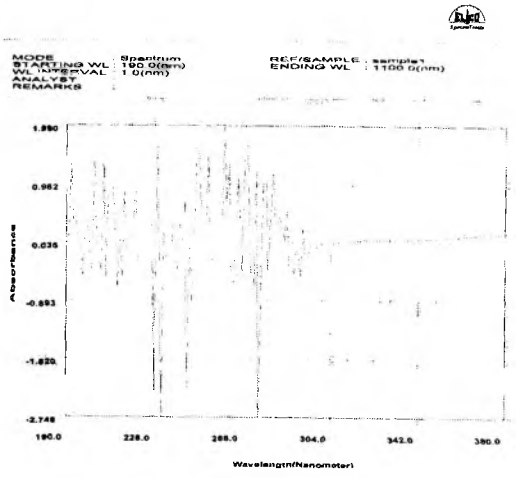
Spectra16: UV-Vis of RPE of *W. somnifera* in acetone.



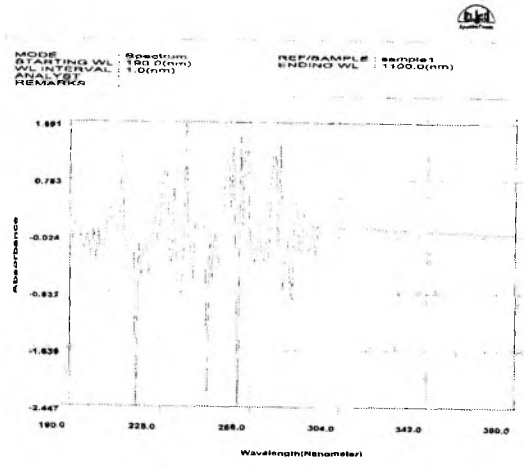
Spectra17: UV-Vis of RDE of *W. somnifera* in acetone.



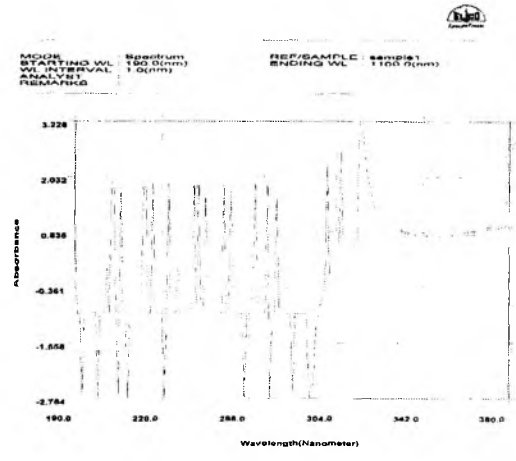
Spectra18: UV-Vis of RAE of *W. somnifera* in acetone.



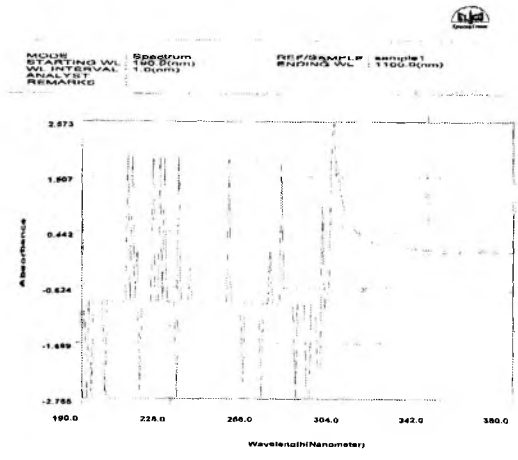
Spectra19: UV-Vis of LPE of *W. somnifera* in methanol.



Spectra20: UV-Vis of LDE of *W. somnifera* in methanol.

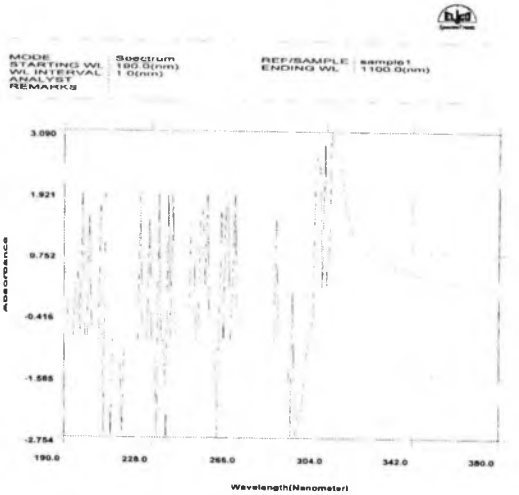


Spectra21: UV-Vis of LAE of *W. somnifera* in methanol.

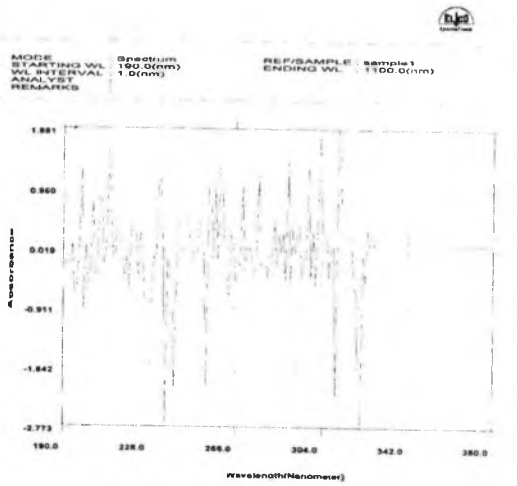


Spectra22: UV-Vis of RPE of *W. somnifera* in methanol.

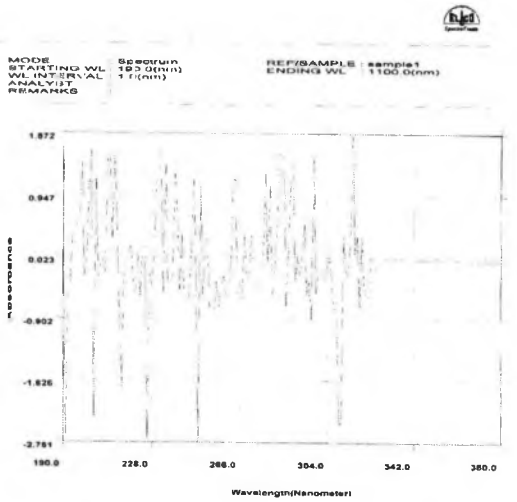
Spectra23: UV-Vis of RDE of *W. somnifera* in methanol.



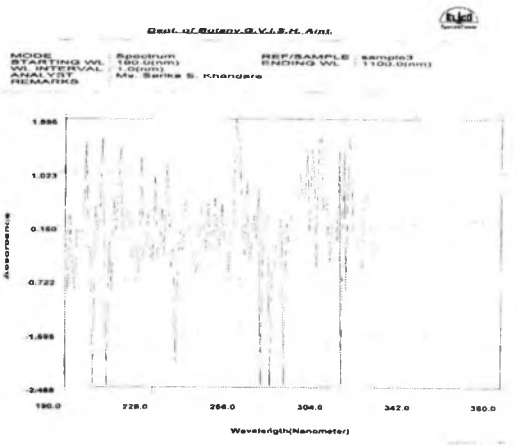
Spectra24: UV-Vis of RAE of *W. somnifera* in methanol.



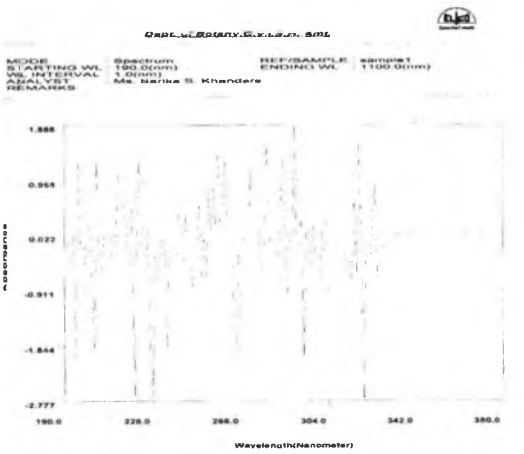
Spectra25: UV-Vis of LPE of *C. procera* in acetone.



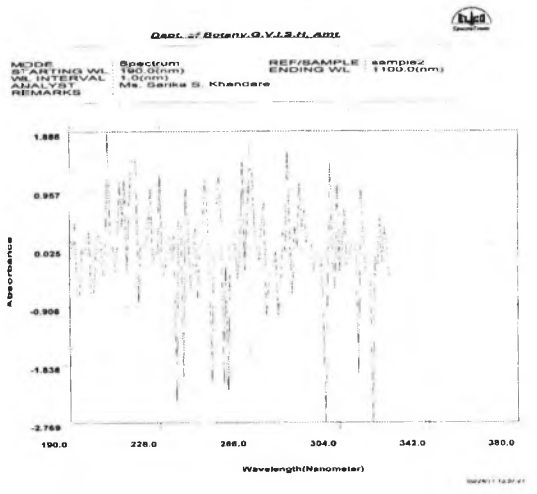
Spectra26: UV-Vis of LDE of *C. procera* in acetone.



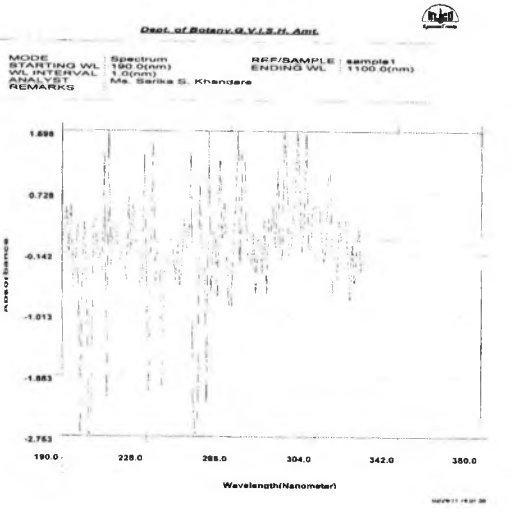
Spectra27: UV-Vis of LAE of *C. procera* in acetone.



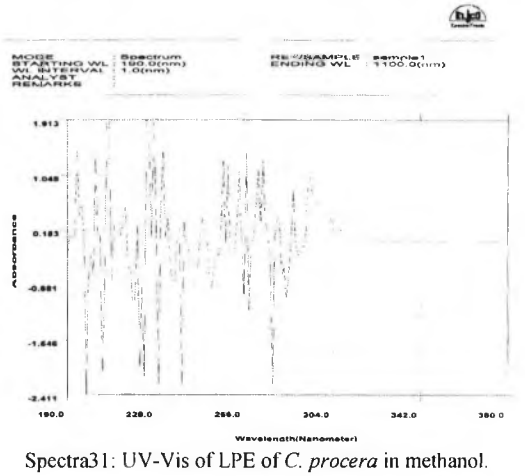
Spectra28: UV-Vis of RPE of *C. procera* in acetone.



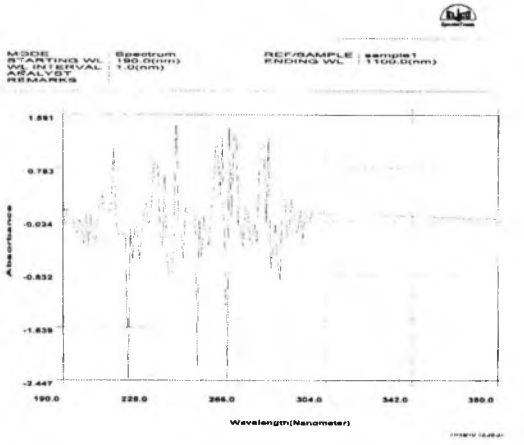
Spectra29: UV-Vis of RDE of *C. procera* in acetone.



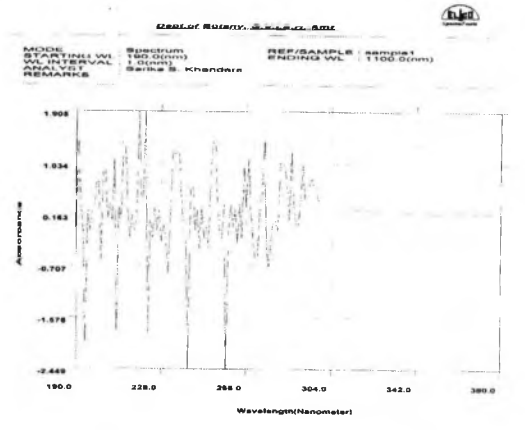
Spectra30: UV-Vis of RAE of *C. procera* in acetone.



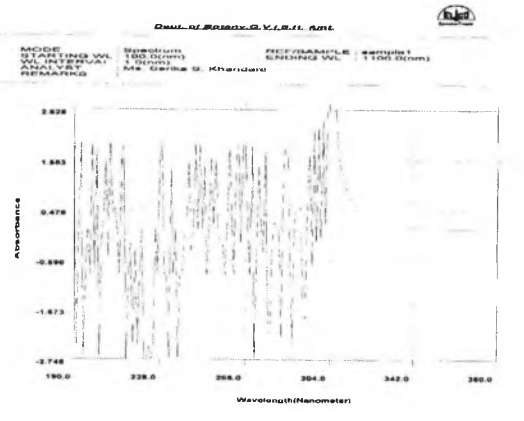
Spectra31: UV-Vis of LPE of *C. procera* in methanol.



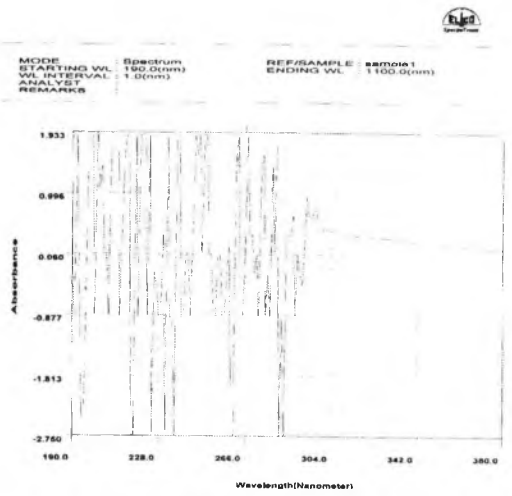
Spectra32: UV-Vis of LDE of *C. procera* in methanol.



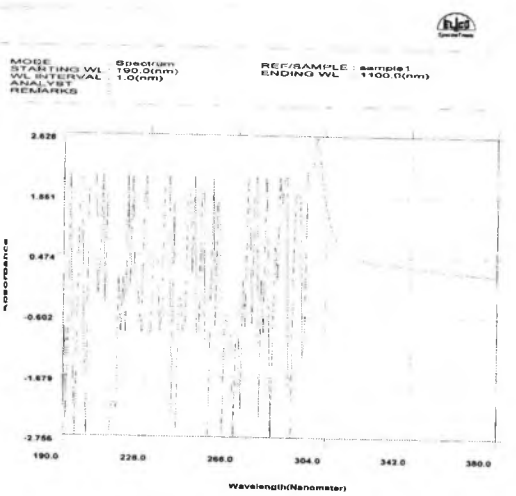
Spectra33: UV-Vis of LAE of *C. procera* in methanol.



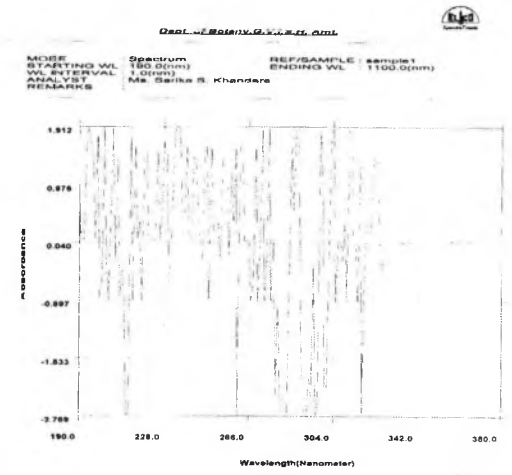
Spectra34: UV-Vis of RPE of *C. procera* in methanol.



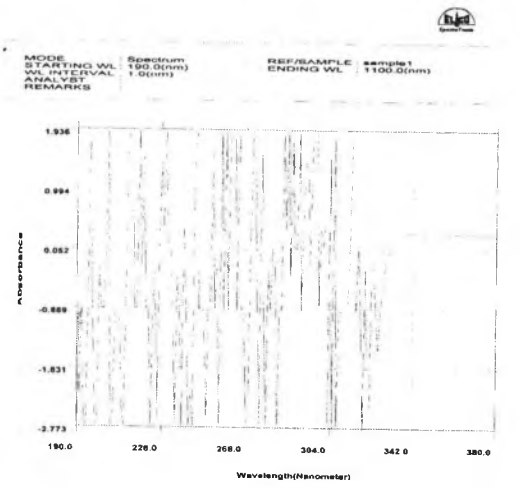
Spectra35: UV-Vis of RDE of *C. procera* in methanol.



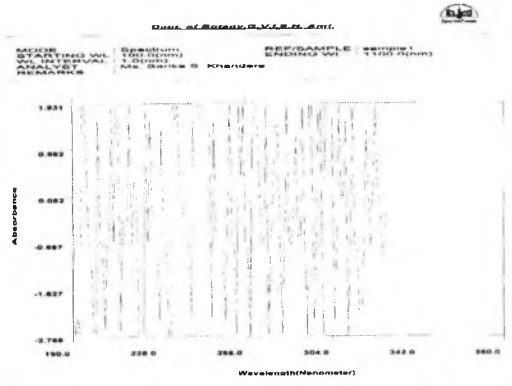
Spectra36: UV-Vis of RAE of *C. procera* in methanol.



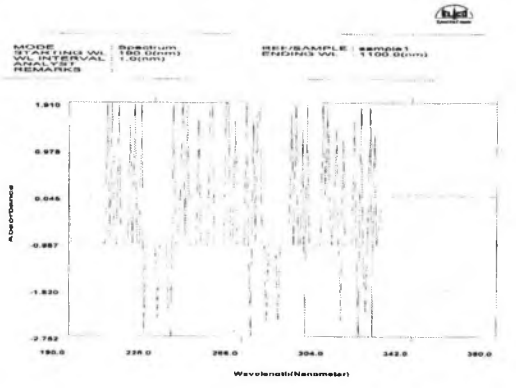
Spectra37: UV-Vis of LPE of *A. curassavica* in acetone.



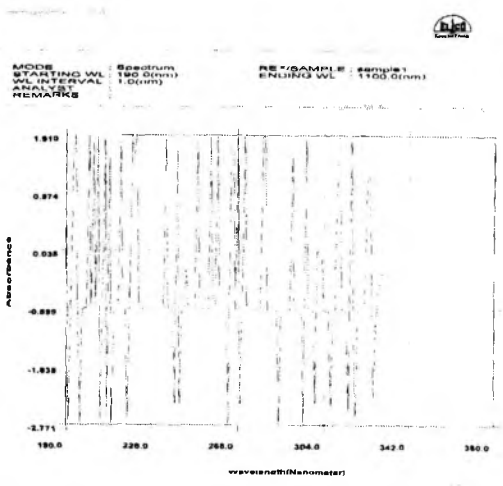
Spectra38: UV-Vis of LDE of *A. curassavica* in acetone.



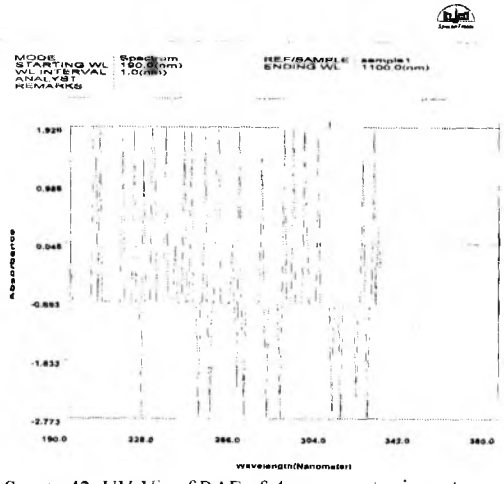
Spectra39: UV-Vis of LAE of *A. curassavica* in acetone.



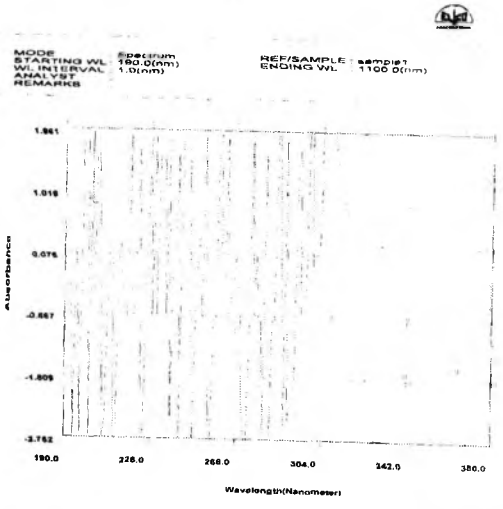
Spectra40: UV-Vis of RPE of *A. curassavica* in acetone.



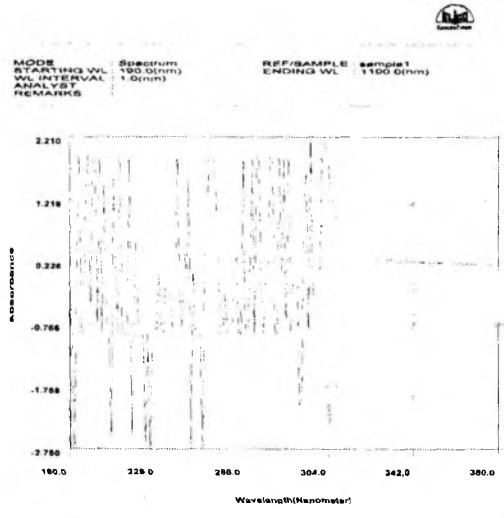
Spectra41: UV-Vis of RDE of *A. curassavica* in acetone.



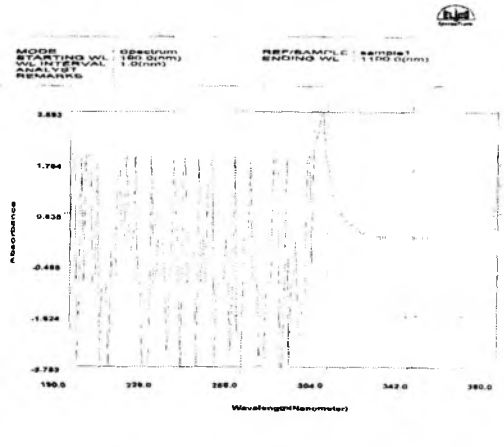
Spectra42: UV-Vis of RAE of *A. curassavica* in acetone.



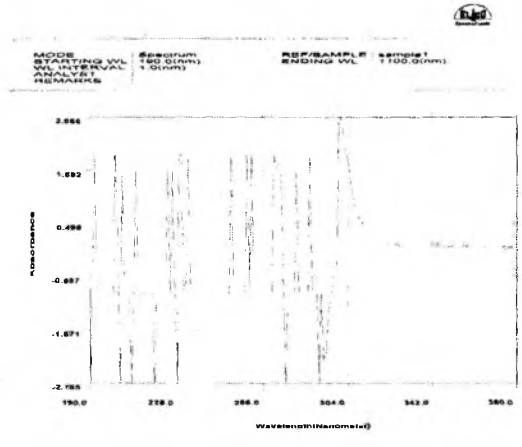
Spectra43: UV-Vis of LPE of *A. curassavica* in methanol.



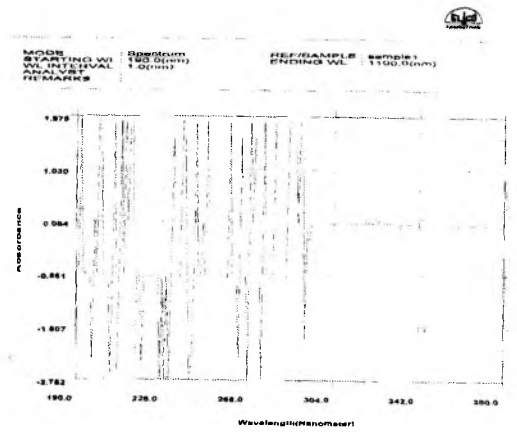
Spectra44: UV-Vis of LDE of *A. curassavica* in methanol.



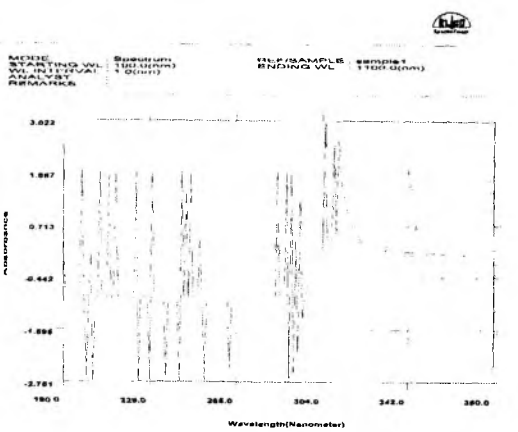
Spectra45: UV-Vis of LAE of *A. curassavica* in methanol.



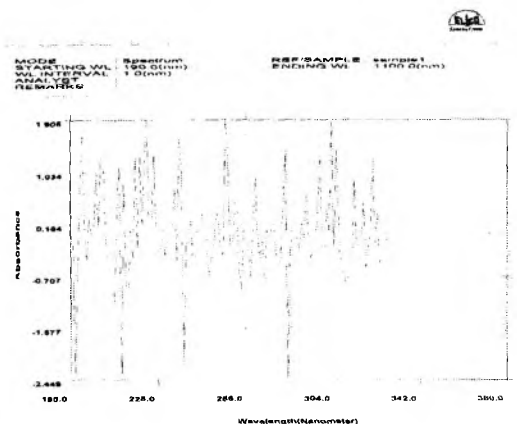
Spectra46: UV-Vis of RPE of *A. curassavica* in methanol.



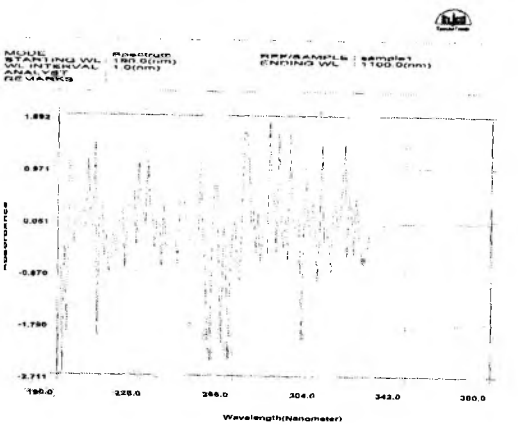
Spectra47: UV-Vis of RDE of *A. curassavica* in methanol.



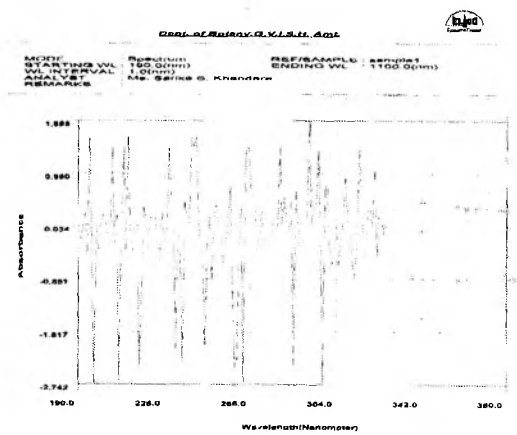
Spectra48: UV-Vis of RAE of *A. curassavica* in methanol.



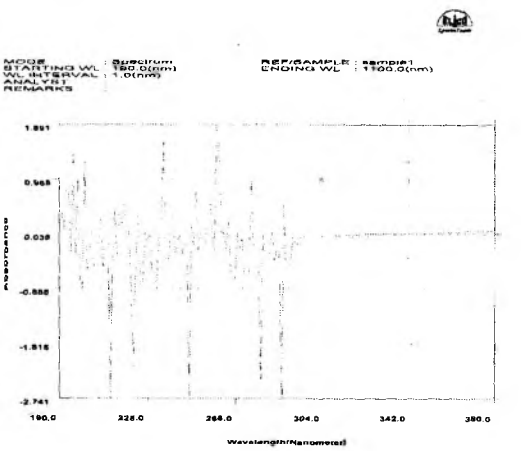
Spectra49: UV-Vis of LPE of *V. negundo* in acetone.



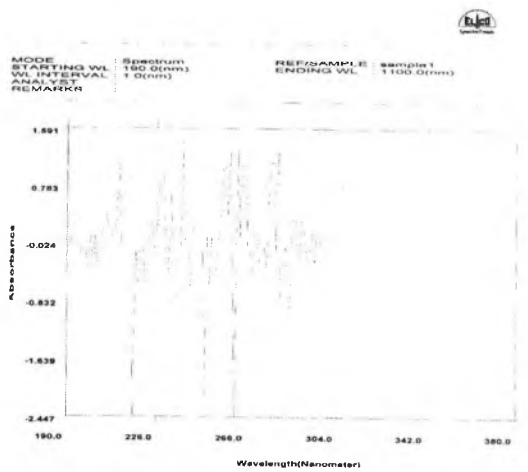
Spectra50: UV-Vis of LDE of *V. negundo* in acetone.



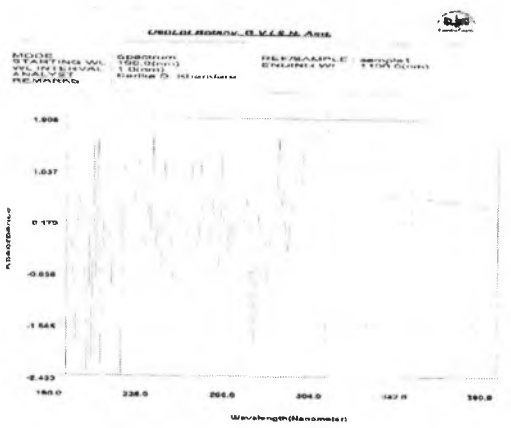
Spectra51: UV-Vis of LAE of *V. negundo* in acetone.



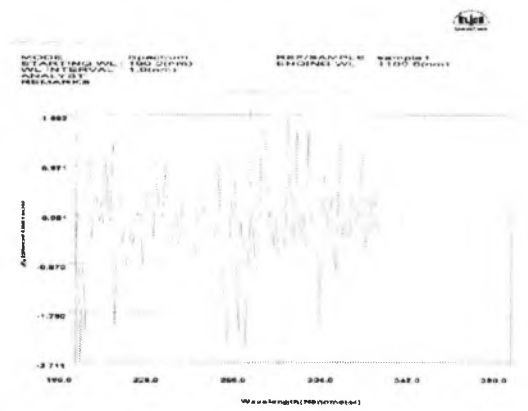
Spectra52: UV-Vis of LPE of *V. negundo* in methanol.



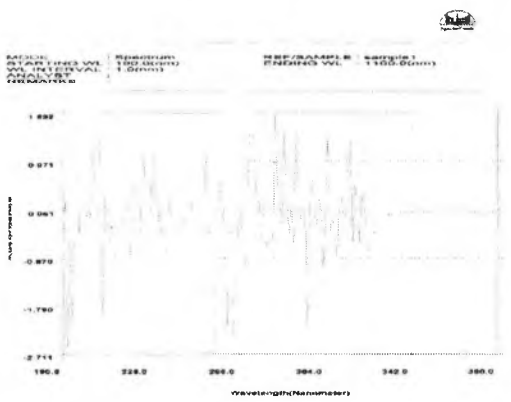
Spectra53: UV-Vis of LDE of *V. negundo* in methanol.



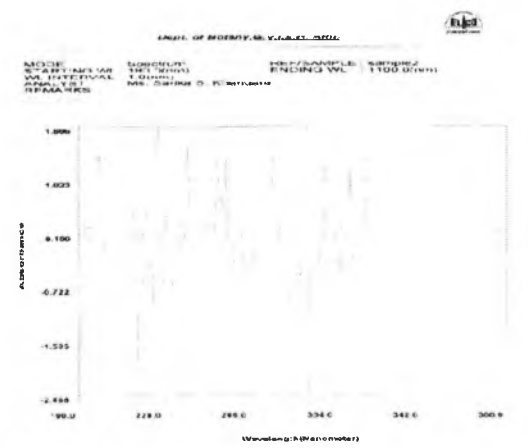
Spectra54: UV-Vis of LAE of *V. negundo* in methanol.



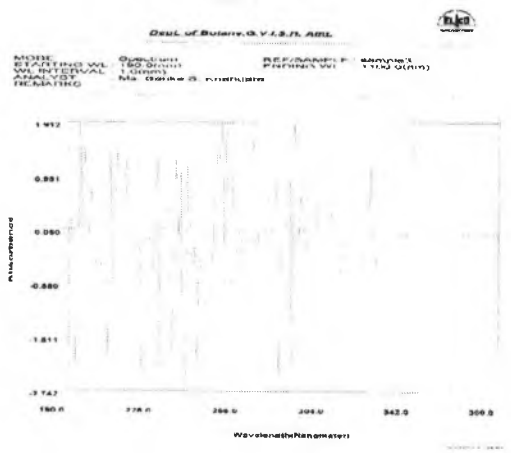
Spectra55: UV-Vis of LPE of *H. suaveolens* in acetone.



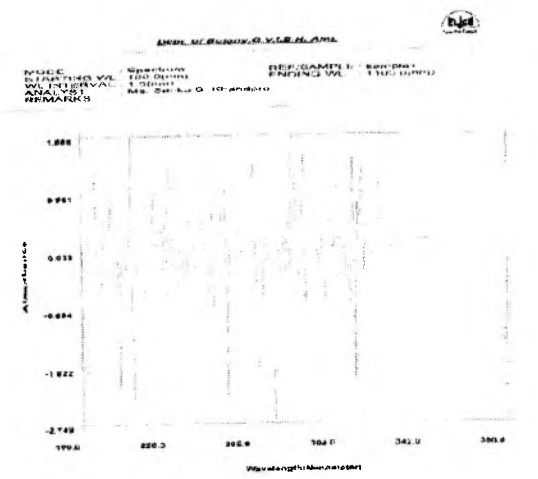
Spectra56: UV-Vis of LDE of *H. suaveolens* in acetone.



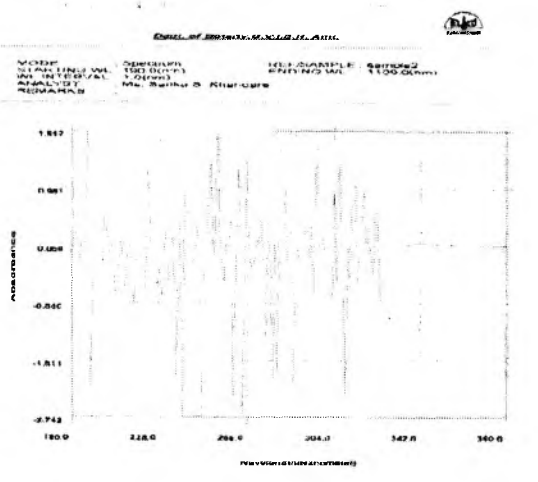
Spectra57: UV-Vis of LAE of *H. suaveolens* in acetone.



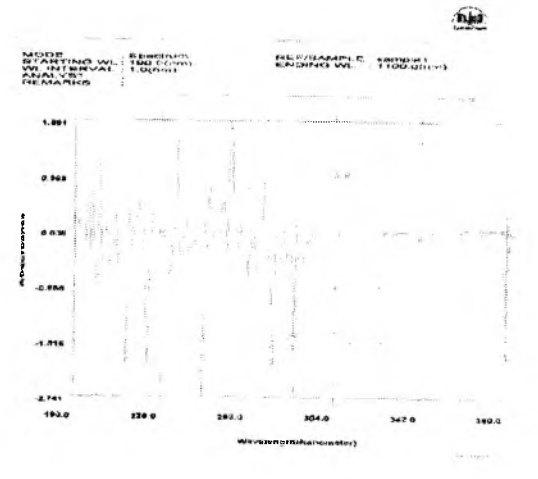
Spectra58: UV-Vis of RPE of *H. suaveolens* in acetone.



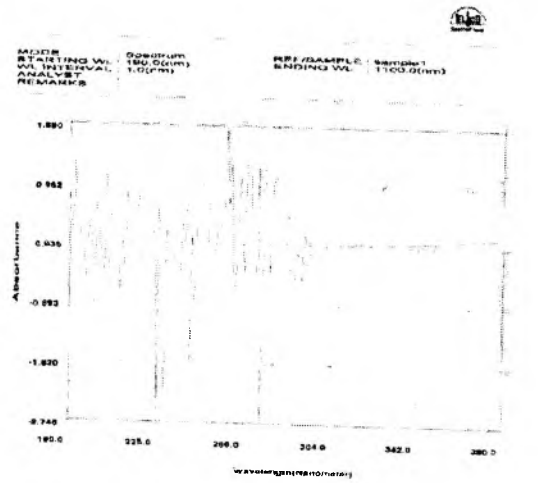
Spectra59: UV-Vis of RDE of *H. suaveolens* in acetone.



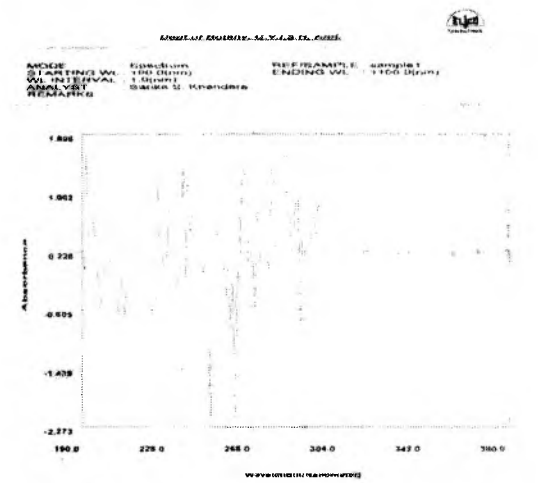
Spectra60: UV-Vis of RAE of *H. suaveolens* in acetone.



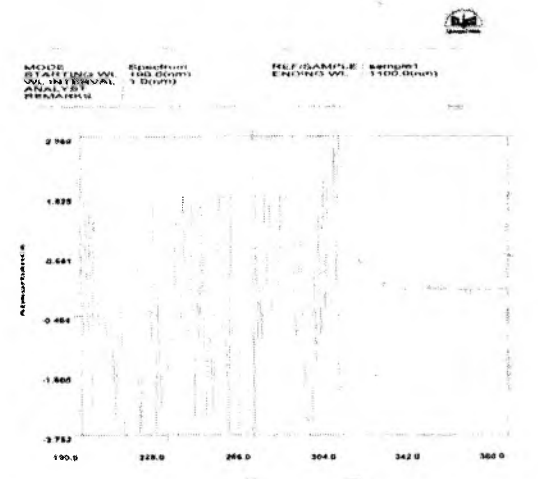
Spectra61: UV-Vis of LPE of *H. suaveolens* in methanol.



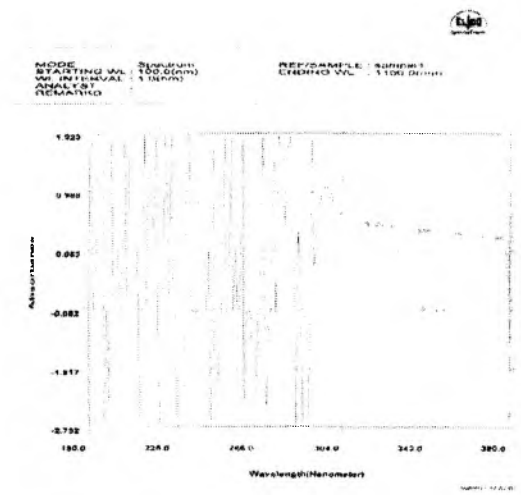
Spectra62: UV-Vis of LDE of *H. suaveolens* in methanol.



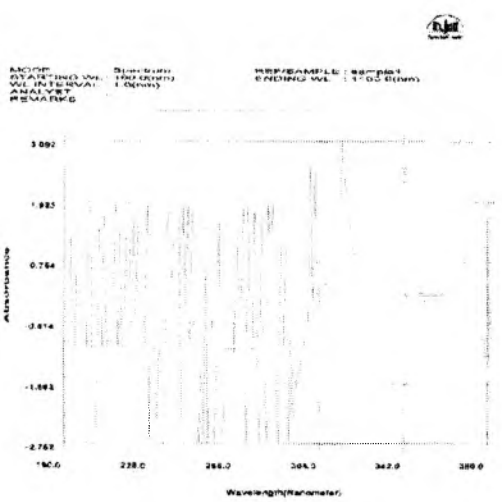
Spectra63: UV-Vis of LAE of *H. suaveolens* in methanol.



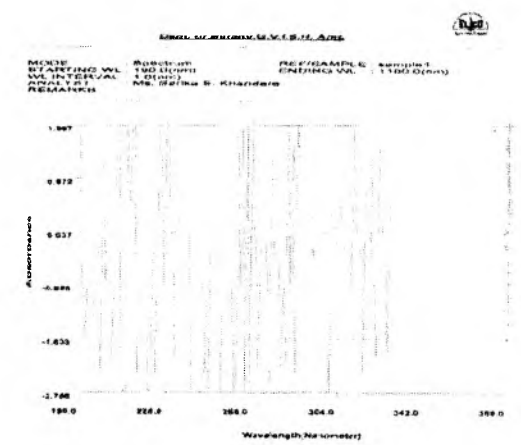
Spectra64: UV-Vis of RPE of *H. suaveolens* in methanol.



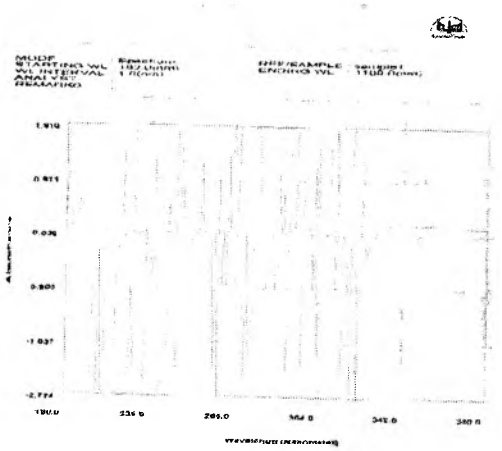
Spectra65: UV-Vis of RDE of *H. suaveolens* in methanol.



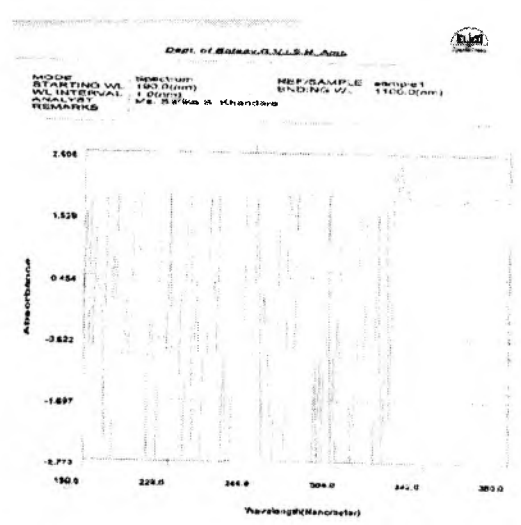
Spectra66: UV-Vis of RAE of *H. suaveolens* in methanol.



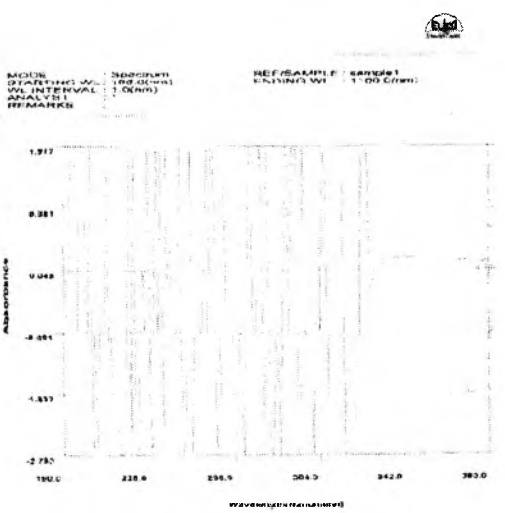
Spectra67: UV-Vis of LPE of *A. majus* in acetone.



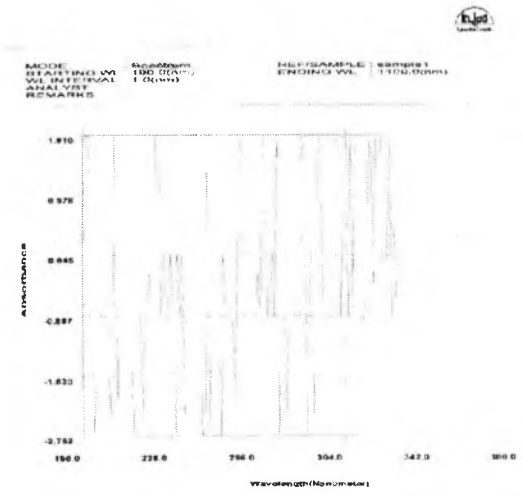
Spectra68: UV-Vis of LDE of *A. majus* in acetone.



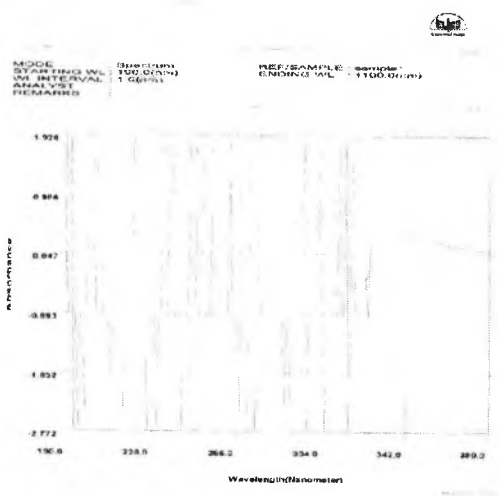
Spectra69: UV-Vis of LAE of *A. majus* in acetone.



Spectra70: UV-Vis of RPE of *A. majus* in acetone.



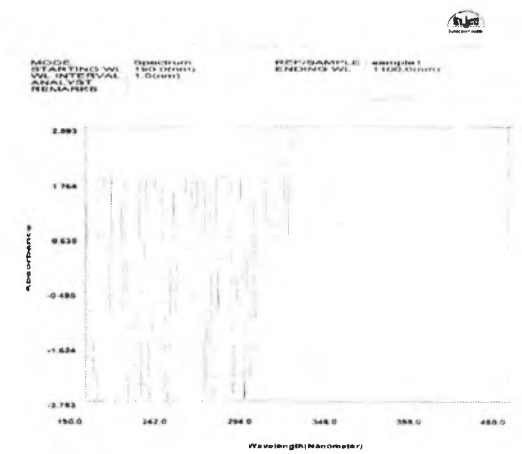
Spectra71: UV-Vis of RDE of *A. majus* in acetone.



Spectra72: UV-Vis of RAE of *A. majus* in acetone.

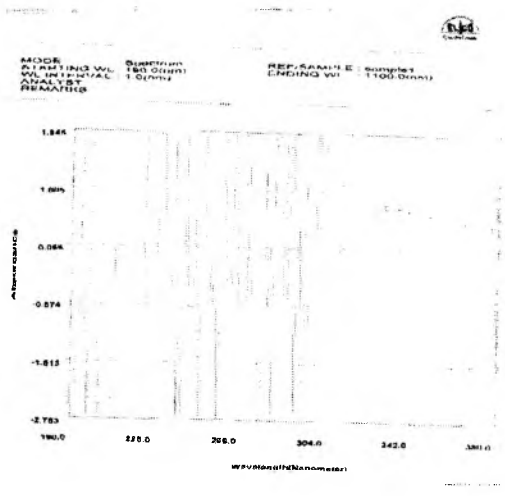
Spectra73: UV-Vis of LPE of *A. majus* in methanol.

Spectra74: UV-Vis of LDE of *A. majus* in methanol.

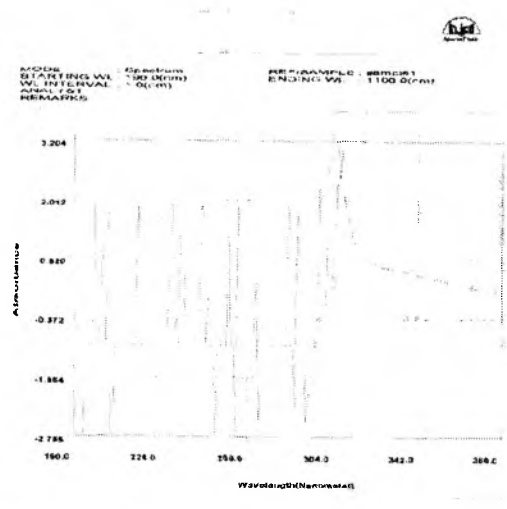


Spectra75: UV-Vis of LAE of *A. majus* in methanol.

Spectra76: UV-Vis of RPE of *A. majus* in methanol.



Spectra77: UV-Vis of RDE of *A. majus* in methanol.

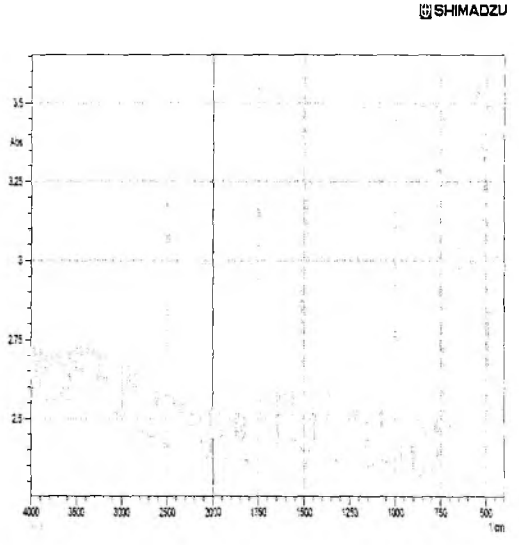


Spectra78: UV-Vis of RAE of *A. majus* in methanol.

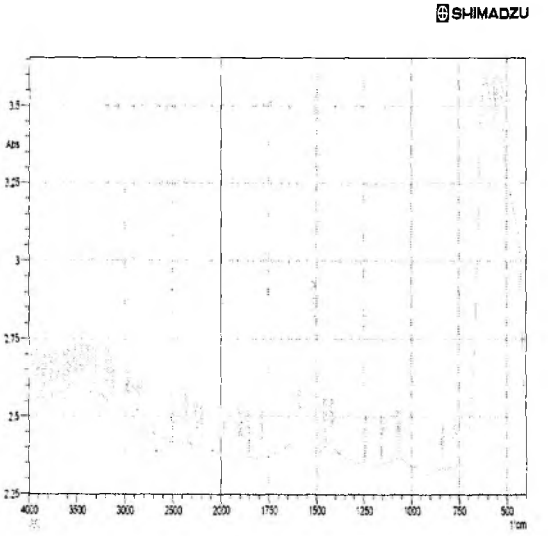
FTIR also used in the natural product chemistry for the identification of new compounds isolated from various plant species (Sati and Pant, 1985; Rawat et al., 1989; Purohit et al., 1991; Joshi et al., 1996 and Prasad et al., 1997). The FTIR band at 3500-3000 and 1580- 1650 cm^{-1} indicated the presence of -N-H group which is detected in all leaf and root extracts of seven plants.

The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2960 - 2850cm^{-1}; 3080 - 3020cm^{-1}, 2960 - 2850cm^{-1} and 1470 – 1350cm^{-1}; 1670 - 1760cm^{-1}; 1000 - 1260cm^{-1}; 1680 - 1640cm^{-1} and 675 - 870cm^{-1} from which in *B. diffusa* above functional groups were observed except in LDE (C=O and C=C) (Spectra 79-84); in *W. somnifera* leaf extracts showed absence of C=O and C=C in LDE and LAE while in root extracts, alkanes absent in RPE and in RDE (alkanes and C=C) while detected in remaining extracts (Spectra 85-90), in *C. procera,* most of the functional groups were found in leaf and root extracts whereas, showed absence in LPE (C-H phenyl ring), LDE (C-H phenyl ring and C=C), LAE (C=C), RDE (C=O, C-O and C=C) and in RAE (C=C) (91-96); in *A. curassavica*, LPE

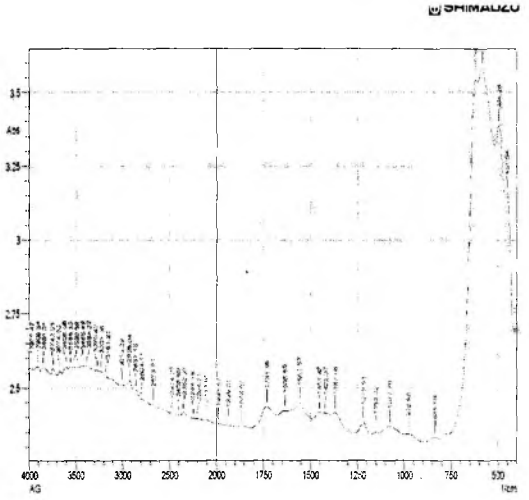
and RPE (C-H phenyl ring and C=C), LDE (C=O and C=C), LAE and RAE (C=C) and RDE (C=C) were absent however, showed presence in other extracts (Spectra 97-102); in *V. negundo* , all the functional groups present in LPE while in LDE C=O, C-O, C-H phenyl ring and C=C and C-H phenyl ring were absent (103- 105); in *H. suaveolens*, leaf and root extracts showed presence of most of the functional groups whereas, absence in LPE (C-H phenyl ring and C=C), LDE (C-H), RPE (C=C), RDE (C=O) and RAE (C-H phenyl ring) (Spectra 106- 111) and in *A. majus*, LPE, LAE and RPE exhibited all the functional groups although C-O and C=C in LDE, C-H phenyl ring and C=C in RDE and C=C in RAE were absent (112- 117). Peaks at 1000 - 3500cm^{-1} favoured the presence of standard taxol group which are noticed in all leaf and root extracts of seven plants. At 1260 – 1390cm^{-1} indicated presence of NO_2 (nitro compounds) these compound was found in most of the extracts of seven plants except in *B. diffusa* of LPE, LDE and RDE; in LDE and RDE of *W. somnifera*, *C. procera, A. curassavica* and *V. negundo*; in *H. suaveolens*, LPE, LDE and RDE of *A. majus*, LDE and RDE.



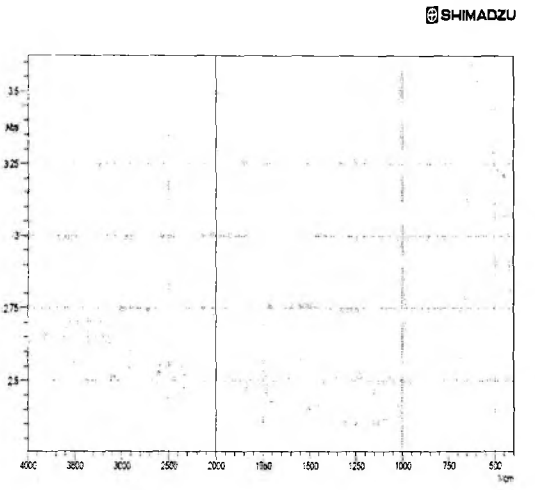
Spectra.79: FTIR Analysis: LPE of *B. diffusa.*



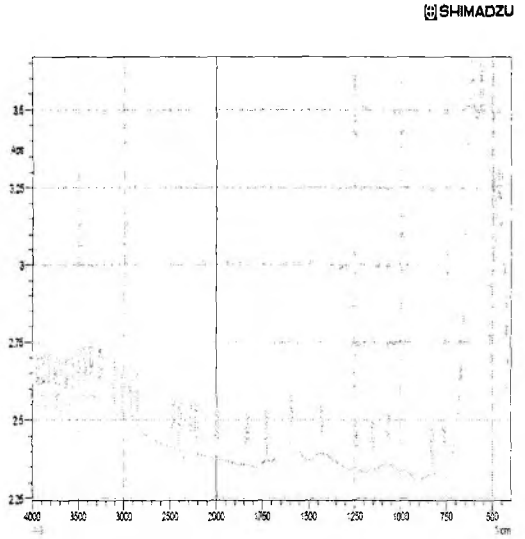
Spectra.80: FTIR Analysis: LDE of *B. diffusa*



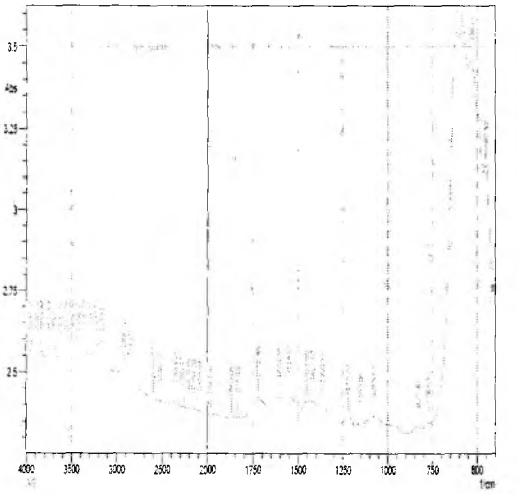
Spectra.81: FTIR Analysis of LAE of *B. diffusa.*



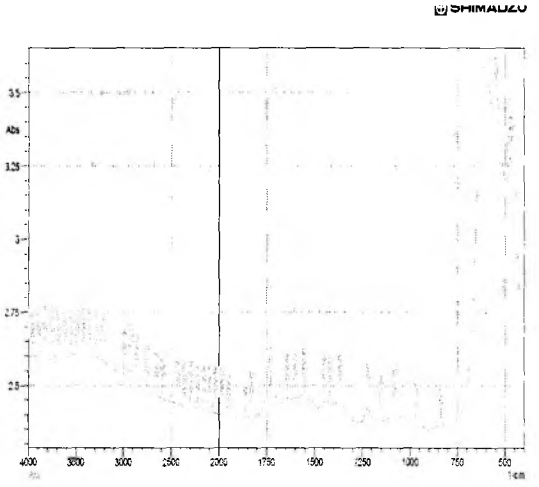
Spectra.82: FTIR Analysis of RPE of *B. diffusa.*



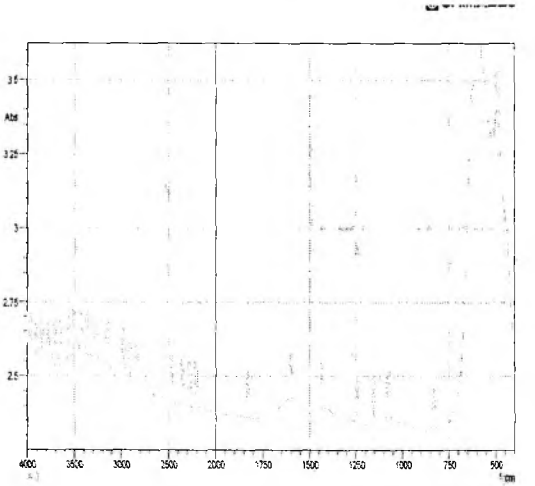
Spectra.83: FTIR Analysis of RDE of *B. diffusa.*



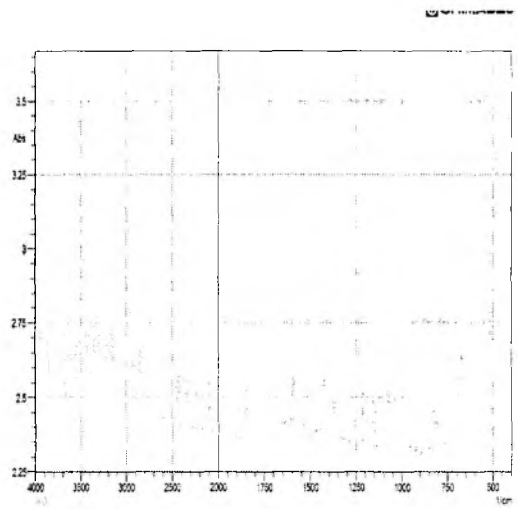
Spectra.84: FTIR Analysis of RAE of *B. diffusa*



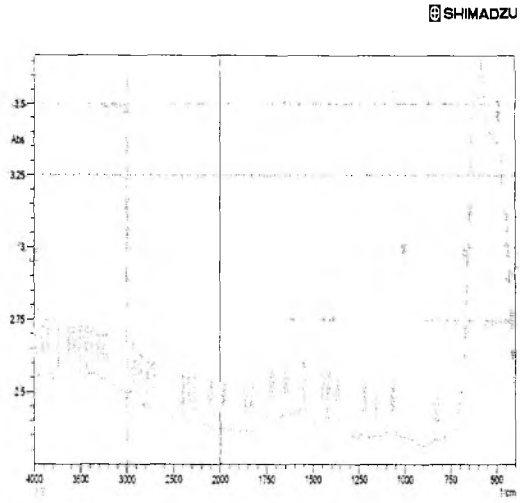
Spectra.85: FTIR Analysis: LPE of *W. somnifera*



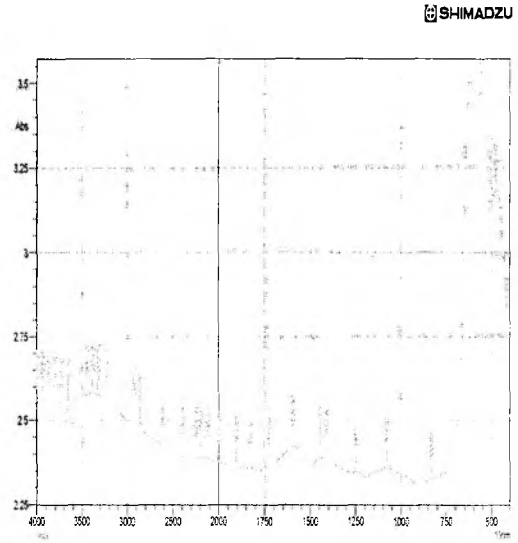
Spectra.86: FTIR Analysis: LDE of *W. somnifera.*



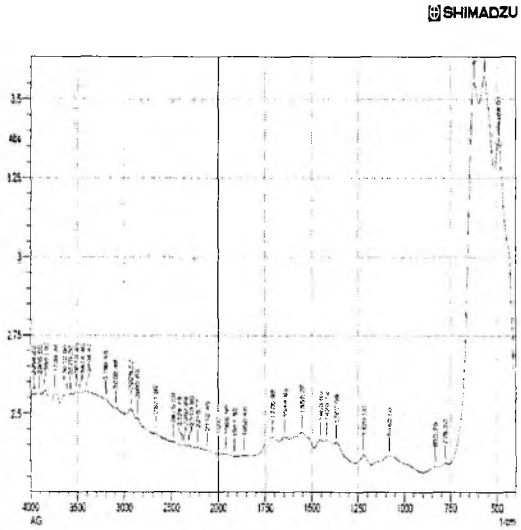
Spectra.87: FTIR Analysis: LAE of *W. somnifera.*



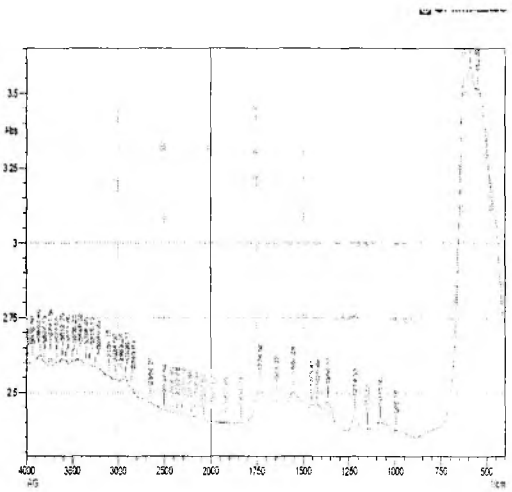
Spectra.88: FTIR Analysis: RPE of *W. somnifera.*



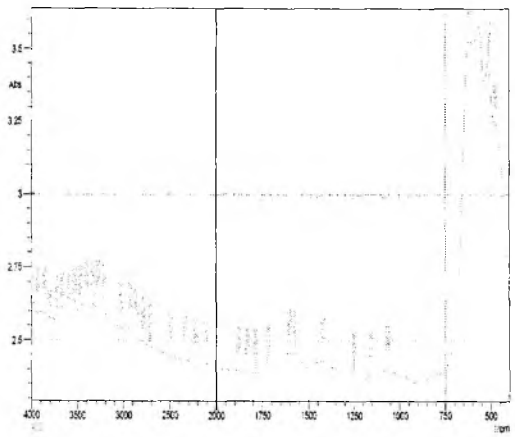
Spectra.89: FTIR Analysis: RDE of *W. somnifera.*



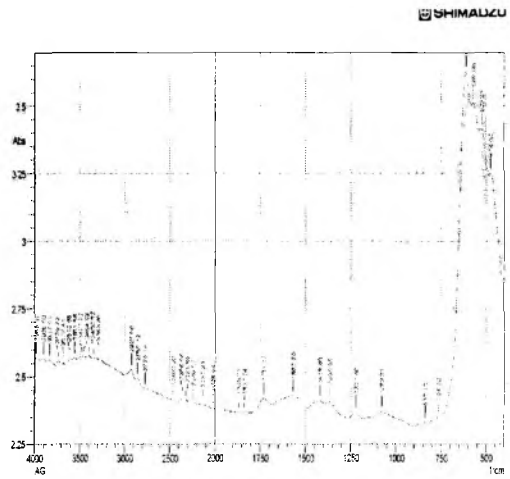
Spectra.90: FTIR Analysis: RAE of *W. somnifera.*



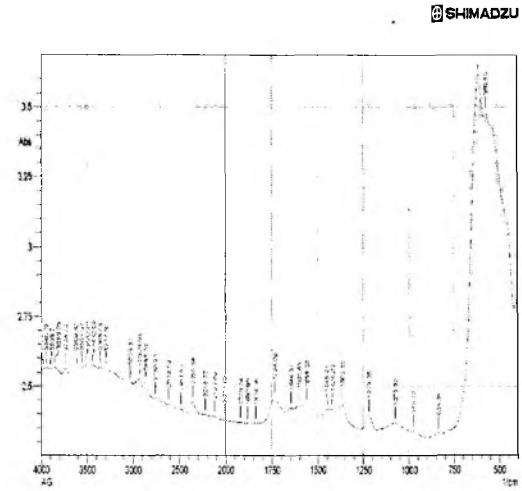
Spectra.91: FTIR Analysis: LPE of *C. procera.*



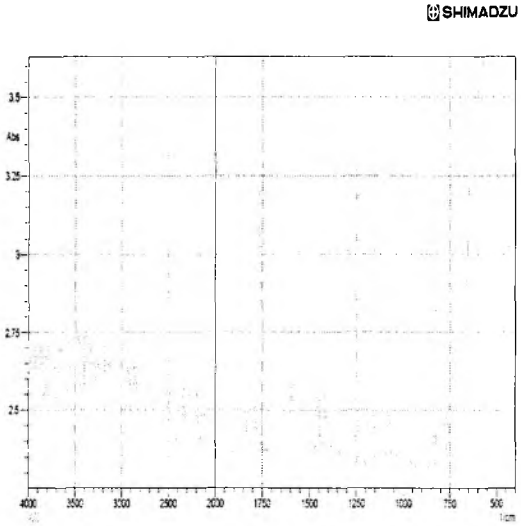
Spectra.92: FTIR Analysis: LDE of *C. procera.*



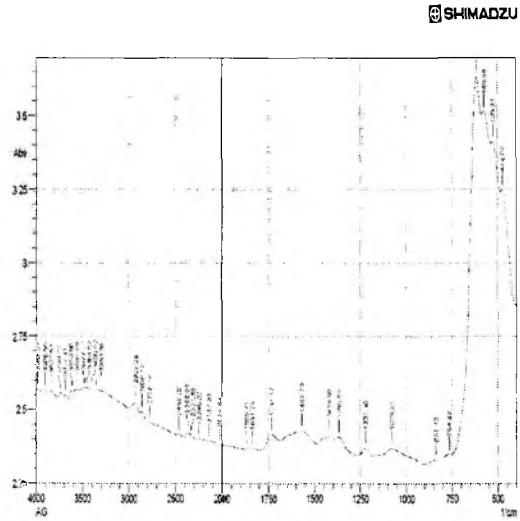
Spectra.93: FTIR Analysis: LAE of *C. procera.*



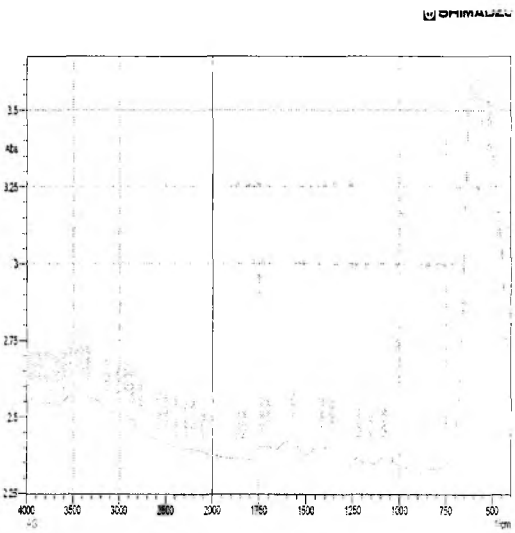
Spectra.94: FTIR Analysis: RPE of *C. procera.*



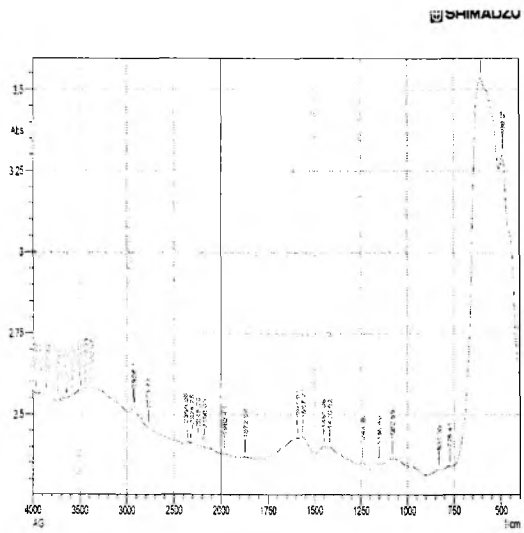
Spectra.95: FTIR Analysis: RDE of *C. procera.*



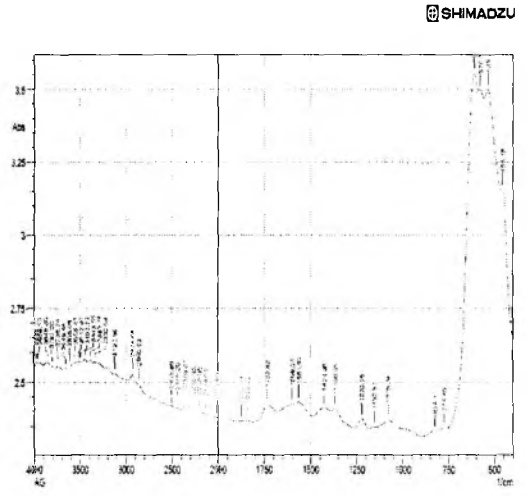
Spectra.96: FTIR Analysis: RAE of *C. procera.*



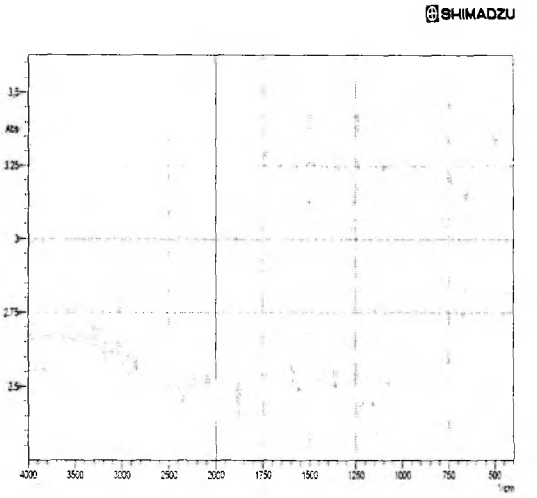
Spectra.97: FTIR Analysis: LPE of *A. curassavica*



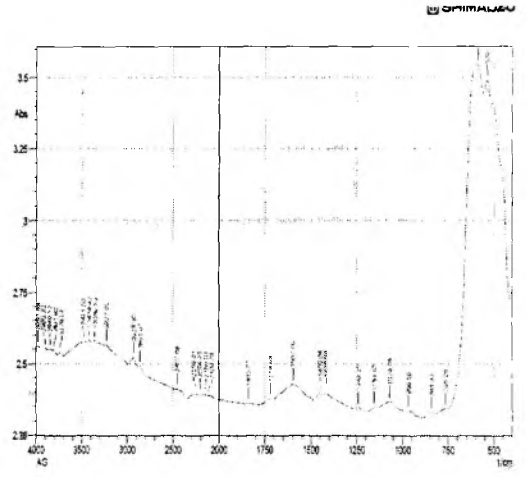
Spectra.98: FTIR Analysis: LDE of *A. curassavica*



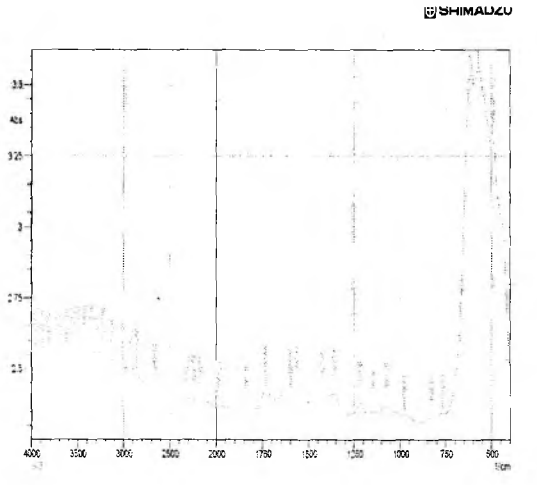
Spectra.99: FTIR Analysis: LAE of *A. curassavica.*



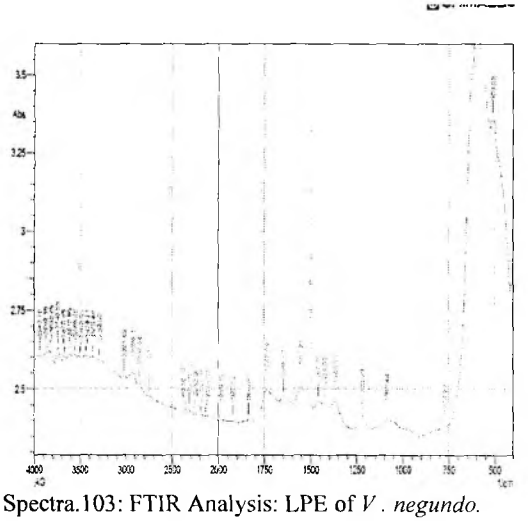
Spectra.100: FTIR Analysis: RPE of *A.curassavica.*



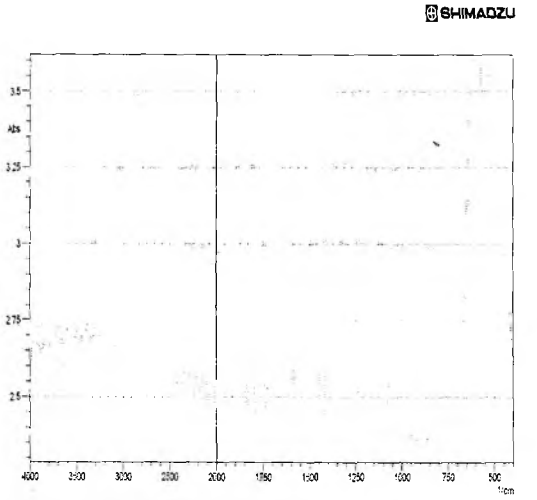
Spectra.101: FTIR Analysis: RDE of *A.curassavica.*



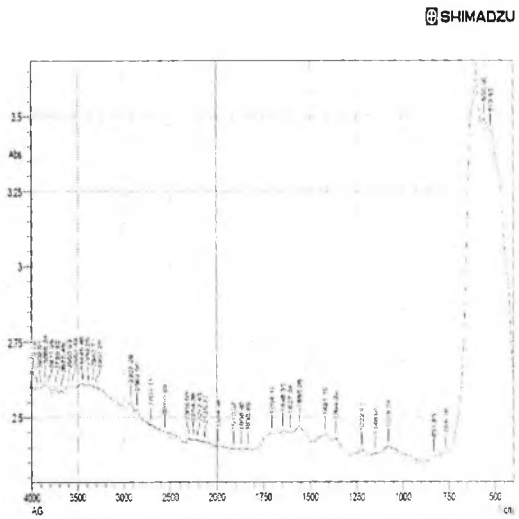
Spectra.102: FTIR Analysis: RDE of *A.curassavica.*



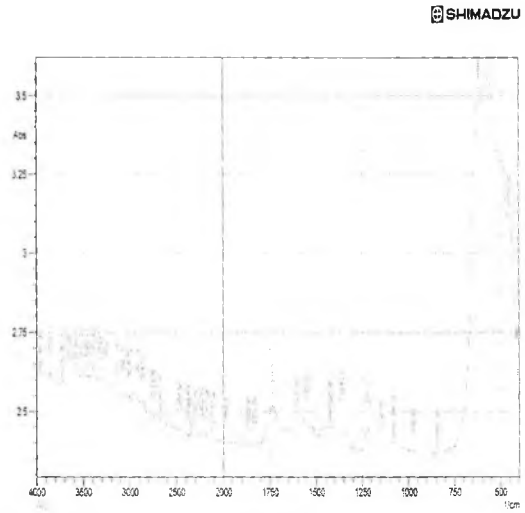
Spectra.103: FTIR Analysis: LPE of *V . negundo.*



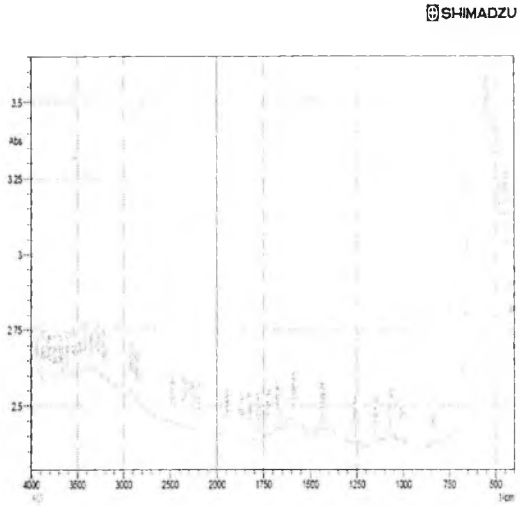
.Spectra.104: FTIR Analysis: LDE of *V. negundo.*



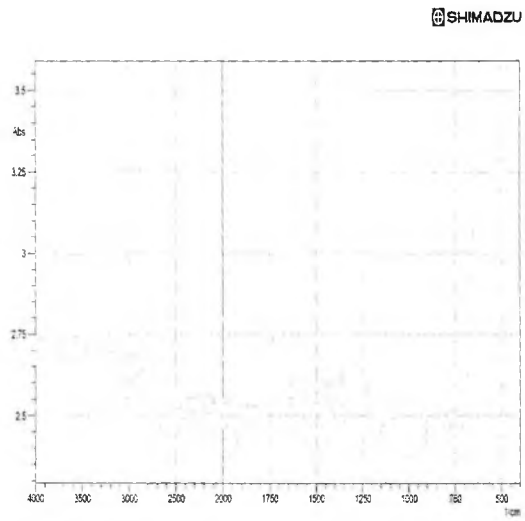
Spectra105:FTIR Analysis:LAE of *V negundo.*



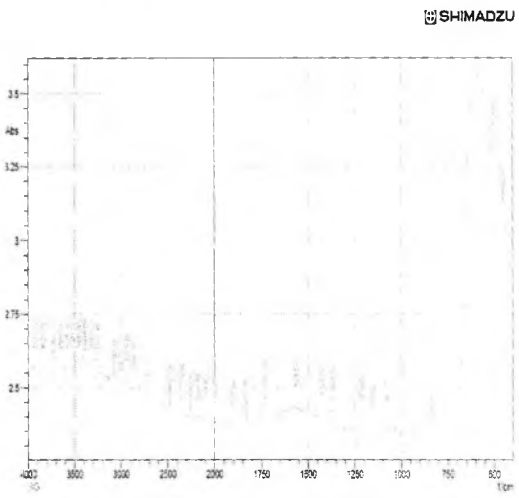
Spectra106:FTIRAnalysis:LPE of *H.suaveolens.*



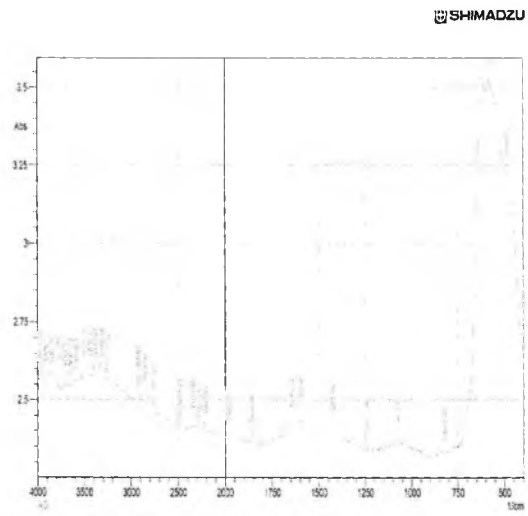
Spectra107:FTIRAnalysis:LDEof *H.suaveolens.*



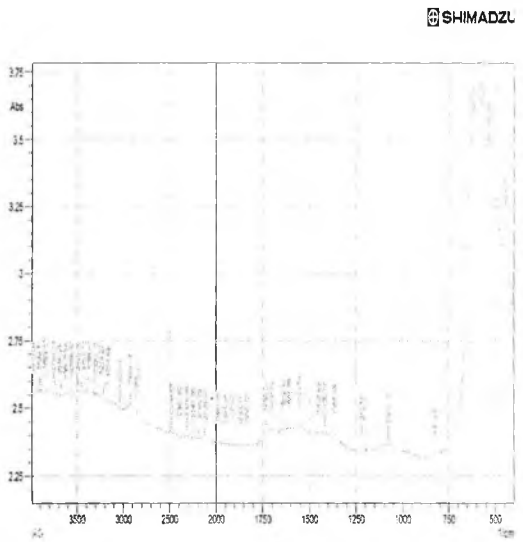
Spectra108:FTIRAnalysis:LAEof *H.suaveolens.*



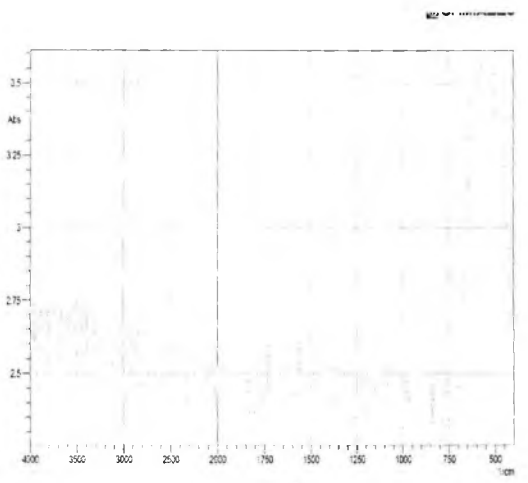
Spectra109:FTIRAnalysis:RPEof *H.suaveolens.*



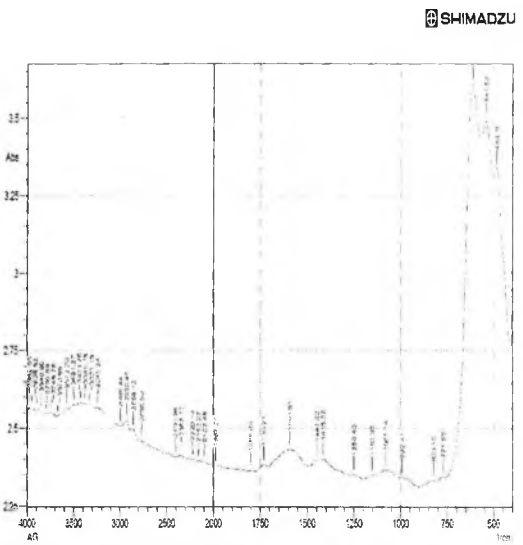
Spectra110:FTIRAnalysis:RDEof *H.suaveole*



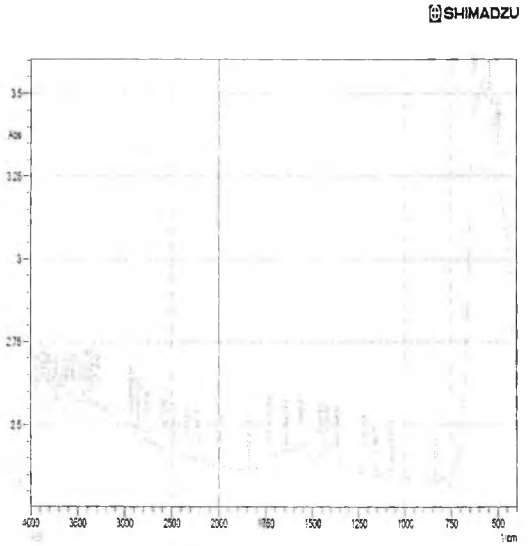
Spectra111:FTIR Analysis:RAE of *H.suaveolens.*



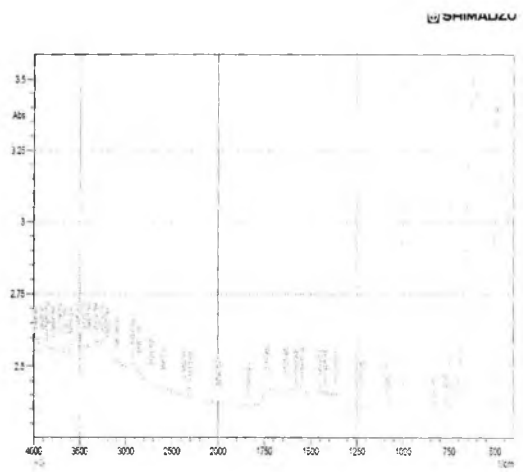
Spectra112: FTIR Analysis:LPE of *A. majus.*



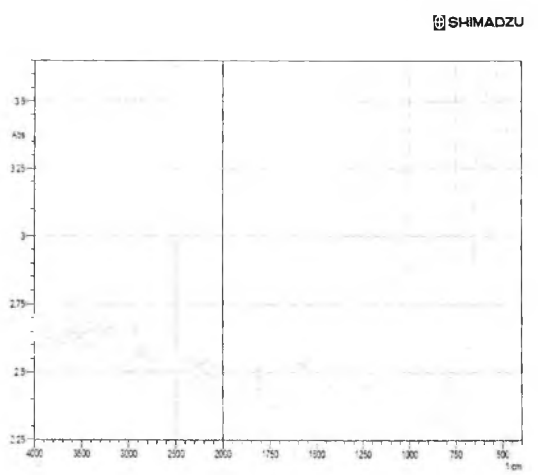
Spectra113:FTIR Analysis:LDE of *A. majus.*



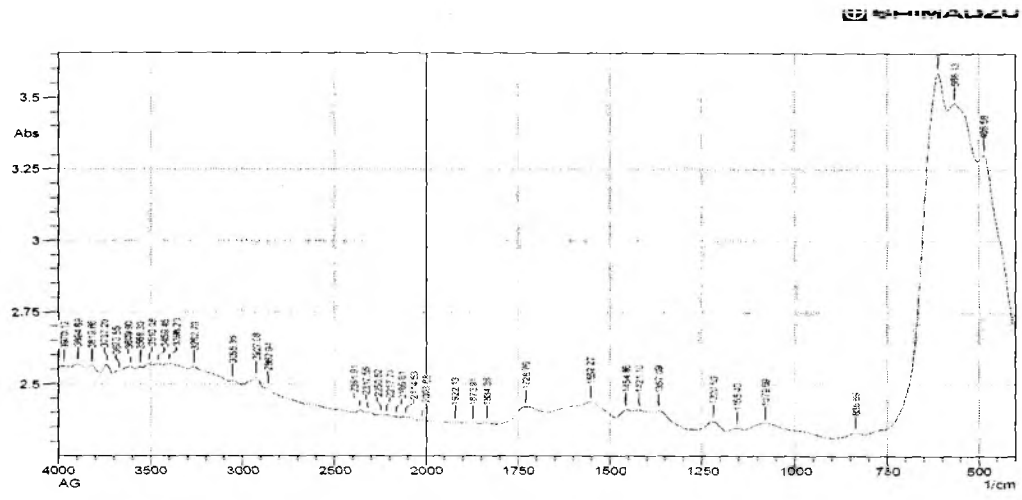
Spectra114:FTIR Analysis:LAE of *A. majus.*



Spectra115:FTIR Analysis:RPE of *A. majus.*



Spectra116:FTIR Analysis:RDE of *A. majus.*



Spectra117: FTIR Analysis:RAE of *A. majus.*

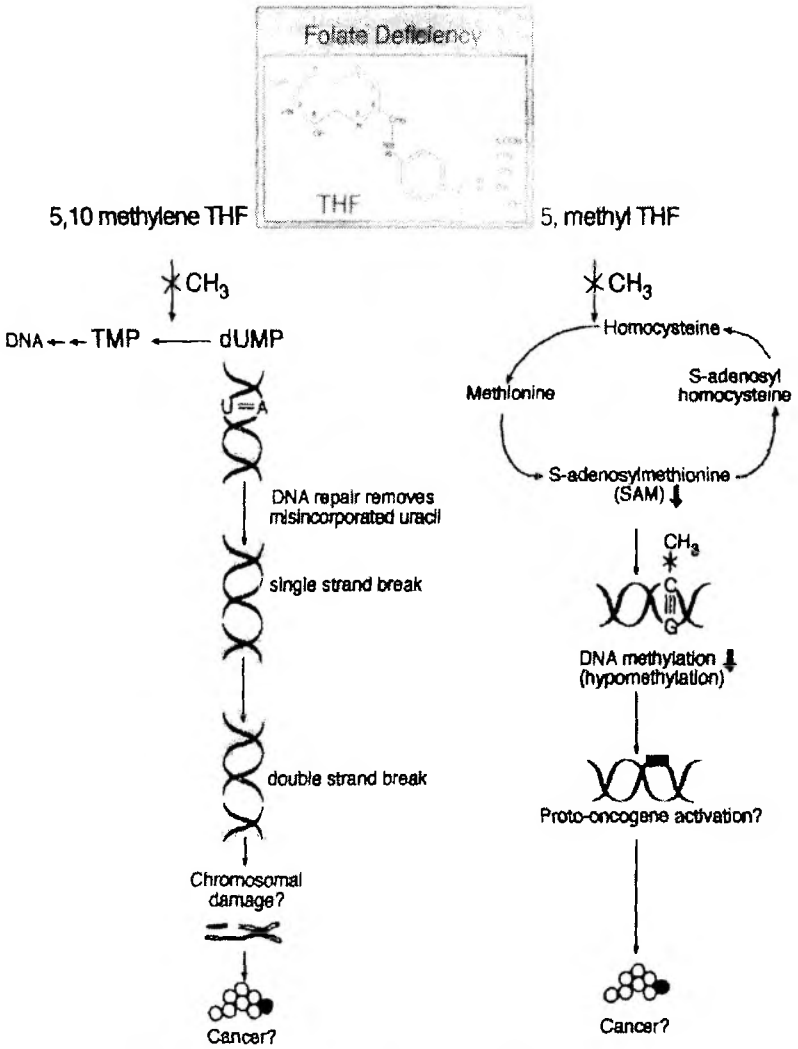
FTIR analysis revealed that all the seven plants exhibit functional groups which is correlated to alkaloids i.e. vincristine and taxol. The FTIR bond at 3369cm^{-1} indicated the presence of N-H group and the two other prominent peaks at 1719 and 1650cm^{-1} confirmed the presence of two carbonyl groups (Belleamy, 1957). The C-O-C and C-N vibrations were observed at 1026, 1111 and 1276cm^{-1} respectively. The three bands at 1451, 1489 and 1537cm^{-1} favoured the presence of benzene ring (Raphael, 1969).

Quantitative Structural Activity Relationships have been generated for a number of individual chemical classes of mutagens and carcinogens, including aromatic amines, nitroarenes, quinolines, triazenes, polycyclic aromatic hydrocarbons, lactones, aldehydes (Benigni 2005). The aromatic amines have to be metabolized to reactive electrophiles to exert their carcinogenic potential. For aromatic amines and amides, this typically involves an initial N-oxidation to *N*-hydroxyaryl amines and *N*-hydroxyaryl amides, which in rat liver is mediated primarily by cytochrome P-450 isozyme *c* (BNF-B) and *d* (ISF-G). Moreover, hydroxyl amino, nitro, and nitroso groups are able to generate amine groups (due to metabolic interconversion). The initial activation of nitro aromatic hydrocarbons is likewise through the formation of an *N*-

hydroxyarylamine, a reduction catalyzed by both microsomal and cytosolic enzymes. Microsomal nitroreduction too appears to depend on cytochrome P-450 complex, in particular rat liver isozymes *c*, *d*, *h* (PB-B) and *e* (PB-D). Cytosolic nitro reductase activity is associated with a number of enzymes, including DT-diaphorase, xanthine oxidase, aldehyde oxidase and alcohol dehydrogenase. In addition to the reactions of nitrogen oxidation and reduction (main activation pathways), certain aromatic amines and nitro aromatic hydrocarbons are converted into electrophilic derivatives through ring oxidation pathways. *N*-Hydroxyaryl amines, iminoquinones, and epoxide derivatives are directly electrophilic metabolites, while *N*-hydroxy acrylamides require esterification before becoming capable of reacting with DNA (Benigni, 2005).

The present alkaloids structural groups identified by UV-Vis and FTIR analysis similar to vincristine, which produce mitotic arrest in *Allium cepa* root tips cells as a direct consequence or due to other effect in the cell cycle. Vincristine enters in the cells during mitosis and blocks formation of microtubules of the mitotic spindle during metaphase (Anonymous overview, 2007 and Lurie et al., 1973). Drugs that act on enzymes or protein receptors that operate via specific mechanisms or essential groups, there are no principle mechanisms by which DNA-based drug can act. Cancer cells are different from normal cells in their ability to undergo uncontrolled cell division this feature requires constant mitosis and the need for a steady supply of DNA and DNA precursors. Cytotoxic substances therefore have targeted the DNA. Cancer DNA drugs are quite useful against rapidly diving cell lines (Blackburn and Gait, 1990). From the point of view of mechanism of action, carcinogens are classified into genotoxic carcinogens, which cause damage directly to DNA. Many known mutagens are in this category, and often mutation is one of the first steps in the development of cancer

(Arcos and Argus, 1995) and carcinogens that do not bind covalently to DNA, do not directly cause DNA damage and are usually negative in the standard mutagenicity assays (Woo, 2003).



Dia. 1: The role of folate in DNA methylation

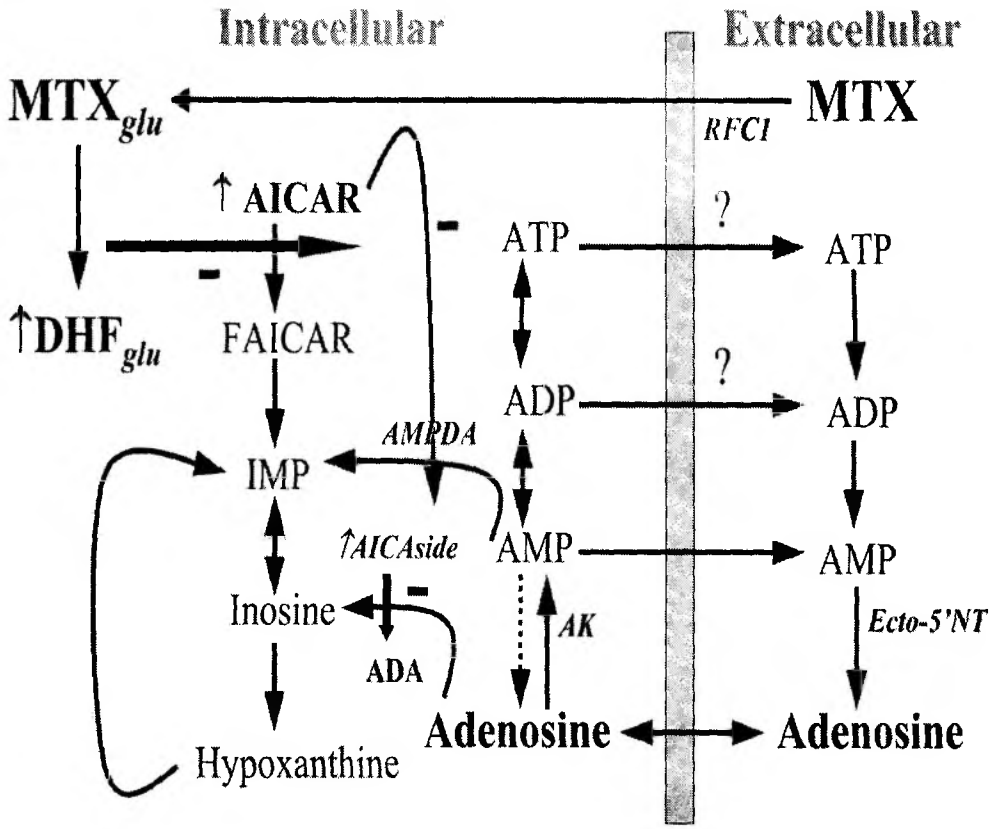
The requirement for methyl groups to be used in cellular metabolism exceeds the normal dietary supply. Insufficiency is prevented by *de novo* methyl synthesis via one carbon-donation from the folate pool. Folic acid, in the form 5'-methyltetrahydrofolate (5'-methyl-THF), is essential in the conversion of methionine to its activated form, S-adenosylmethionine (SAM), via its ability to methylate

homocysteine. SAM is the principal methyl donor in the majority of biochemical reactions, including the methylation of cytosine in DNA. Genes that are methylated at specific locations in the DNA molecule are either not transcribed or are transcribed at a reduced rate. In this way, site-specific DNA methylation controls gene expression. Alterations or disruption to DNA methylation may increase malignant transformation. Several studies have shown that specific human genes (e.g. growth hormone and proto-oncogenes) from tumor tissue (lung and colon) are substantially less methylated (hypomethylated) than genes from adjacent normal tissue (Feinberg and Vogelstein, 1983 and Fang, et al., 1996). If the level of 5'-methyl-THF, the main circulating form of folate is lowered under conditions of folate deficiency, SAM quickly becomes depleted leading to a reduction in the methylation of cytosine in DNA (Giovannucci et al., 1993). This hypomethylation may result in upregulation of proto-oncogene expression and induction of cancer (Dia.1). Indeed, methyl deficiency (lipotrope deficiency) has long been known to promote liver tumor development experimentally (Ghoshal and Farber, 1984).

Methotrexate was first developed in the 1940s as specific antagonist of folic acid. This drug inhibits the proliferation of malignant cells, primarily by inhibiting the *de novo* synthesis of purines and pyrimidines. Secondly, the administration of high doses of reduced folic acid (Folinic acid) or even folic acid itself can reverse the antiproliferative effects of methotrexate, it is clear that methotrexate does acts as an antifolate agent. Earlier it appears to be a pro-drug, i.e. a compound that is converted to the active agent after uptake. Methotrexate is taken up by cells via the reduced folate carrier and then is converted within the cells to polyglutamates (Chabner et al. 1985). Methotrexate polyglutamate are long lived metabolite that retains some of the antifolate

activities of the parent compound, although the potency for inhibition of various folate-dependent enzymes is shifted (Chabner et al. 1985; Chabner and Myers, 1989).

Mechanism of action Methotrexate:

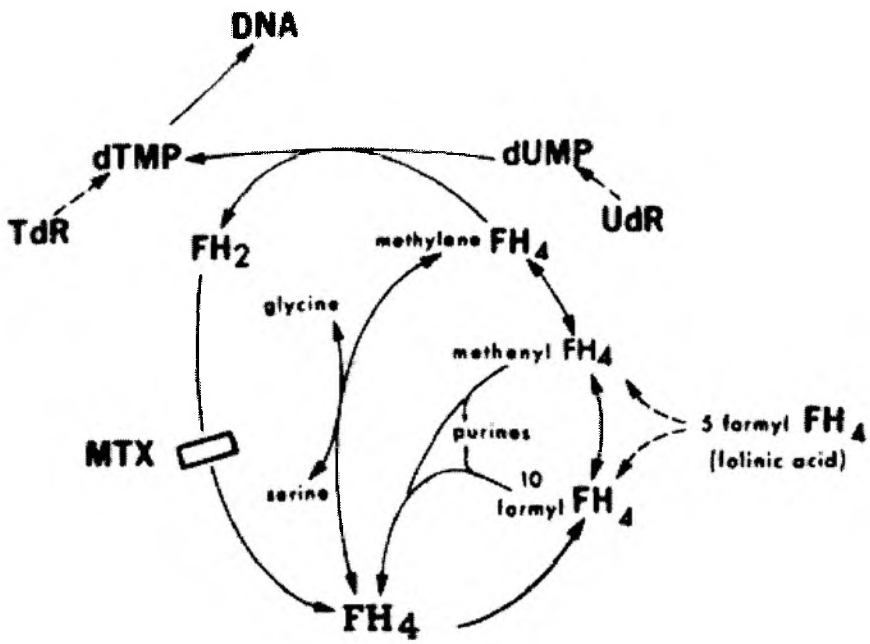


Dia. 2: Methotrexate-induced metabolic changes lead to increased extracellular adenosine.

ADA = adenosinedeaminase; **AICAR**- aminoimidazole carboxamido ribonucleotide; **AICAside** = aminoimidazole carboxamid oribonucleoside; **AK** = adenosine kinase; **AMPDA** = AMP deaminase; **DHF** = dihydrofolate; **DHFglu** = dihydrofolate polyglutamate; **ecto-5′NT** = ecto-5′nucleotidase; **FAICAR** = formyl- AICAR; **IMP** = inosine monophosphate; **MTX** = methotrexate; **MTXglu** = methotrexate polyglutamate; **RFC1** = reduced folate carrier 1.

Capacity of high doses of folinic acid to reverse the therapeutic effects: first, folinic acid may bypass the effects of methotrexate on reduction of folic acid and thereby bypass the therapeutic effects of the drug; alternatively, folinic acid but not folic acid may compete with methotrexate for a single transport site into the cell (Dia.2) and may thus interfere with cellular uptake of methotrexate (Matherly et al., 1991).

Moreover, the expected inhibition of cellular proliferation is manifested as bone marrow suppression and oral and gastrointestinal ulcers, and may require lowering the dose of the drug and, usually, the efficacy of the therapy, suggesting that inhibition of cellular proliferation alone is not responsible for the anti-inflammatory effects of methotrexate. Thus, folate antagonism appears to play, at most, a minimal role in the anti-inflammatory mechanism of methotrexate.



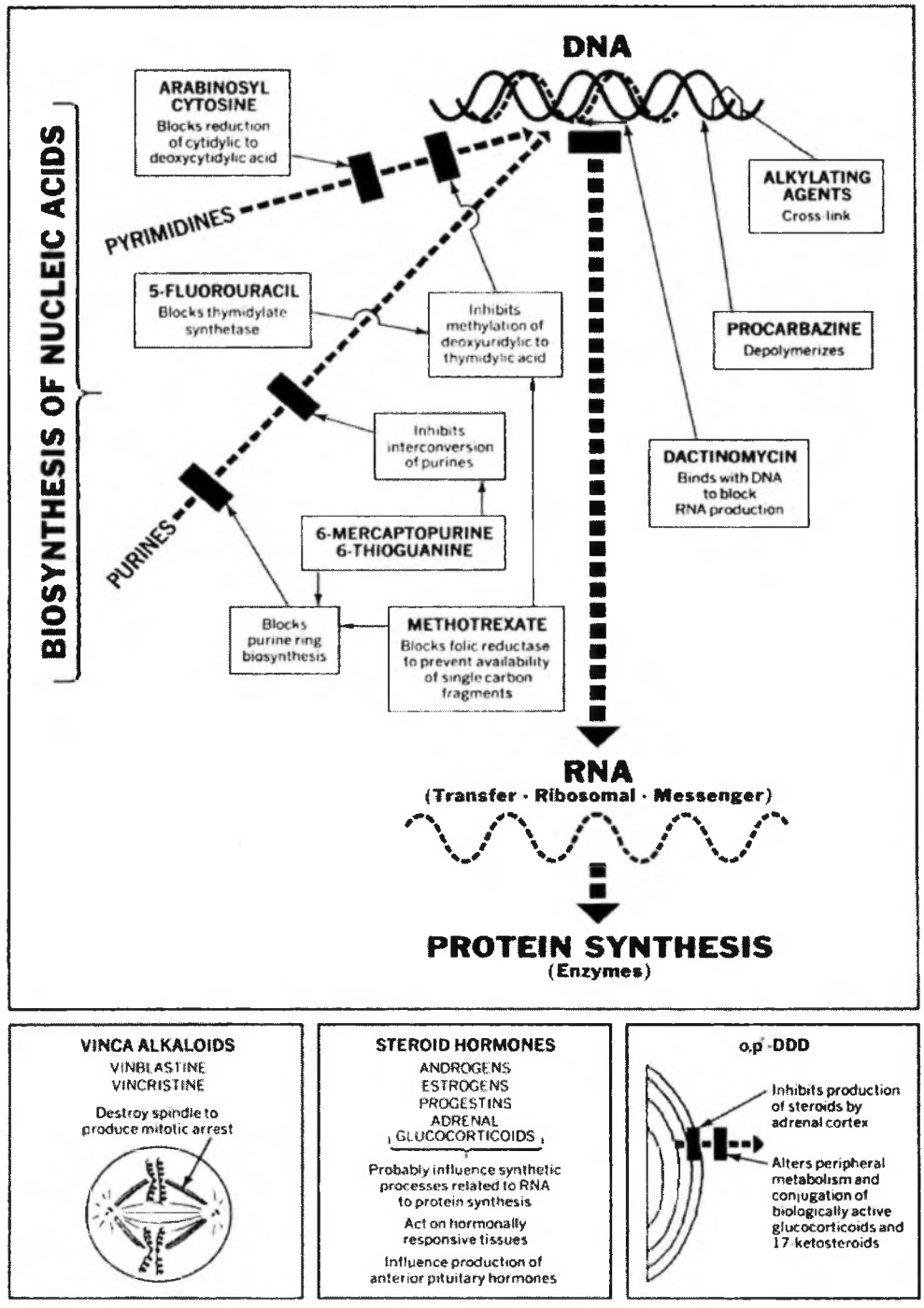
Dia. 3. Mechanism of action of MTX.
MTX inhibition of DHFR depletes reduced folate , inhibiting dUMP conversionto dTMP and in some cells, purine synthesis. Folinic acid should bypass these effects.
FH_2 - dihydrofolate; FH_4 - tetrahydrofolate.

In cancer, methotrexate allosterically inhibits dihydrofolate reductase (DHFR) an enzyme that participates in the tetrahydrofolate synthesis. The affinity of methotrexate for DHFR is about one thousand-fold that of folate. DHFR catalyses the conversion of dihydrofolate to the active tetrafolate (Dia. 3). Folic acid is needed for the de novo synthesis of the nucleoside thymidine, required for DNA synthesis. Also, folate is needed for purine base synthesis will be inhibited. Methotrexate therefore, inhibits the

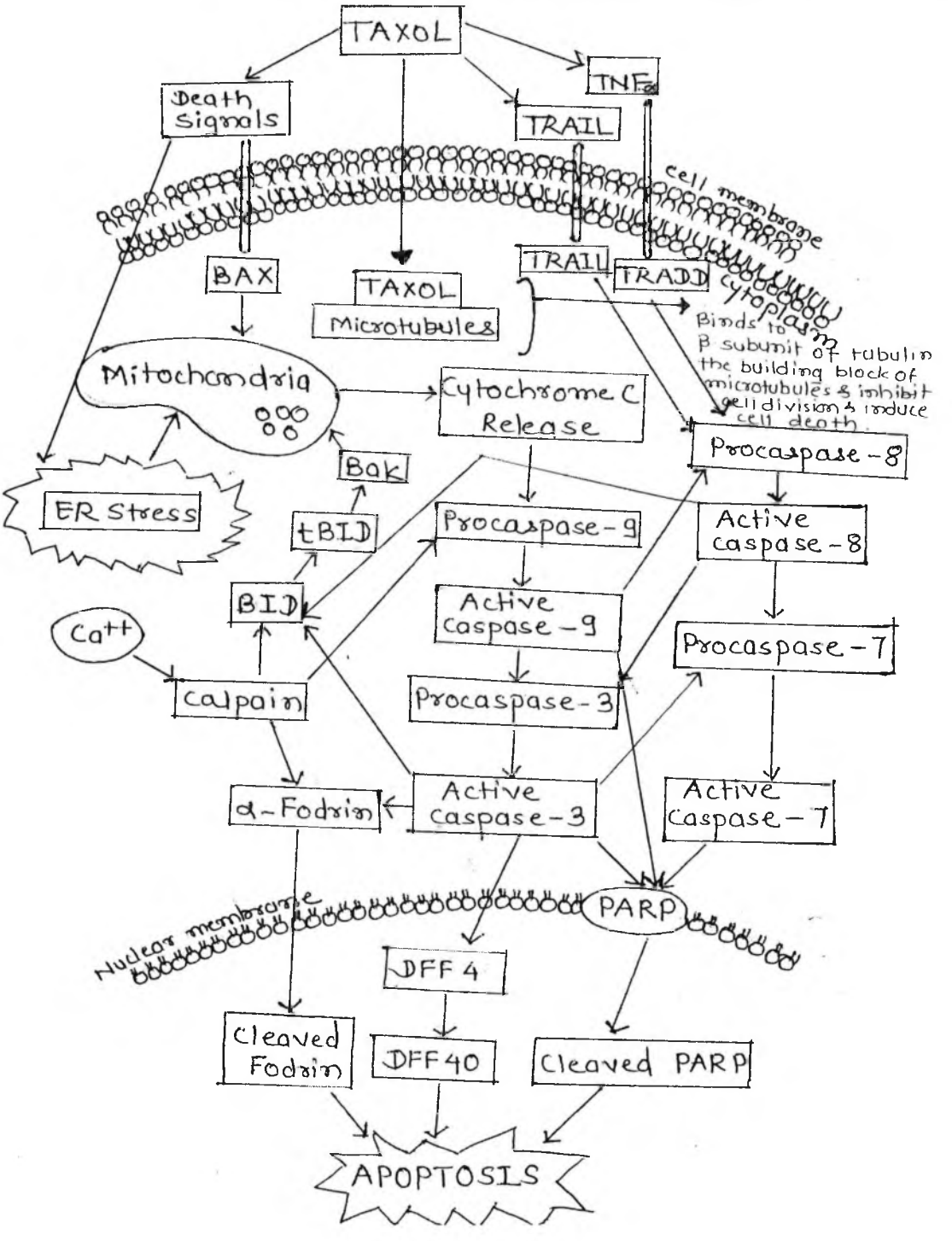
synthesis of DNA, RNA, thymidylates and proteins. Methotrexate acts specifically during DNA and RNA synthesis and thus it is cytotoxic during the S-phase of the cell cycle. It therefore has a greater toxic effect on rapidly dividing cells (such as malignant, myeloid cells, gastrointestinal and oral mucosa which replicate their DNA more frequently and thus inhibits the growth and proliferation of these noncancerous cells as well as causing the listed side effects. Facing a scarcity of dTMP, rapidly dividing cancerous cells undergo cell death via thymine less death (Rajagopalan, 2002).

The interaction of reactive chemicals with spindle fibres or the interference with spindle checkpoint proteins is a potential cause of aneuploidy. It is accepted that spindle function is inhibited by an interaction with multiple binding sites, resulting in a dose–response curve with an initiation (Parry et al., 1994). Topoisomerase I and II are enzymes that control changes in DNA structure by catalysing the breaking and re-joining of the phosphodiester backbone of DNA strands during the normal cell cycle. Topoisomerase inhibitors block the ligation step necessary for the re-joining, generating single and double strand breaks that harm the integrity of the genome. It is accepted that genotoxic effects arising via such mechanisms show an initiation (ECETOC, 1997).

The plant alkaloids vincristine and vinblastine (from the Madagascar periwinkle plant) bind to tubulin and prevent microtubule assembly in contrast to the drug paclitaxel (taxol) which binds to the β-tubulin subunit in polymers and stabilizes the microtubules against depolymerisation. Thus two opposing strategies can be used to disrupt the mitotic spindle (Dia. 4 and 5). The greatest recent impact of plant derived drug was probably felt in this area, where taxol, vinbrastine and vincristine have dramatically improved the effectiveness of chemotherapy against some of deadliest cancers (Mans et al., 2000 and Schwarstmann et al., 2002).



Dia. 4: Mechanism action of Vincristine.

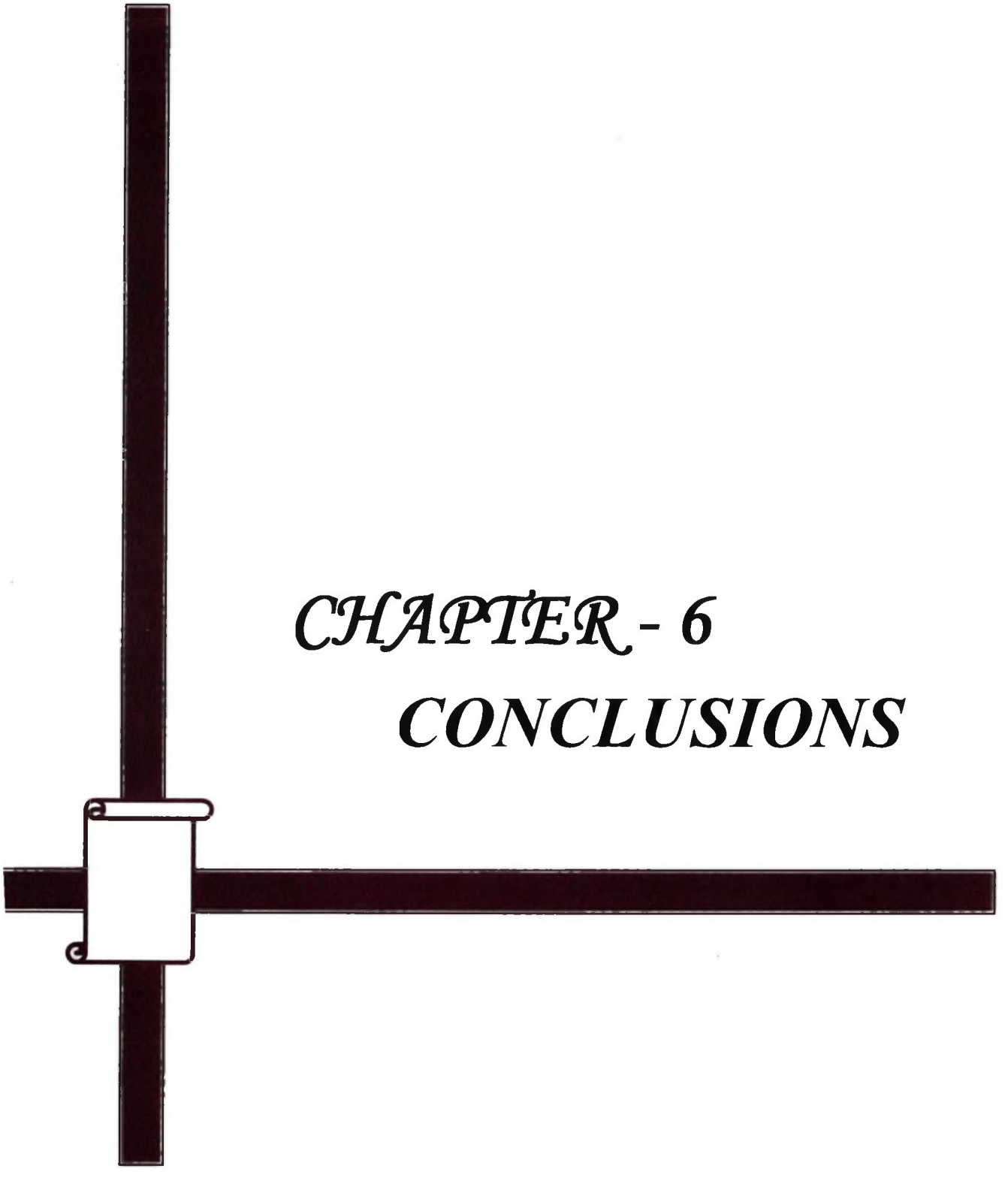


Dia. 5: Mechanism of action of Taxol.

The data from the mitotic index, active mitotic index and chromosomal aberration frequency in *Allium cepa* test system with the crude compound isolated from seven plant species and UV-Vis and FTIR analysis of certain bioactive compounds/groups which are related to taxol and vincristine for detecting cytotoxic and

genotoxic assay for detecting non-genotoxic carcinogens/mutagens is the possibility that chemicals exhibit a threshed dose. The data from the assay are possibly used for regulatory purposes, to set exposure limits below which genotoxic/mutagenic risk is considered to be much reduced in animal experimentation and cell line study. The present data showed that *Calotropis procera* and *Antirrhinum majus* root extracts all twelve concentration exhibits moderate cytotoxicity but comparable with methotrexate. While in *Asclepias curassavica* root extract showed cytotoxicity and genotoxicity assay slightly lower than *C. procera* and *A. majus*. In all seven plants root extracts *C. procera* > *A. majus* > *A. curassavica* > *H. suaveolens* > *B. diffusa* > *W. somifera* exhibits potent cytotoxicity and genotoxicity but comparable with Methotrexate. Whereas, in case of leaf extracts lower cytotoxicity is recorded in order *V. negundo* (50µg LDE and LAE) < *B. diffusa* (50µg, LPE, LDE and LAE) < *W. somnifera* (50µg of LPE, 50µg, 100µg, 150µg of LAE) < *C. procera* (50µg, 100µg, 150µg of LPE and 50µg of LAE) < *H. suaveolens* (50µg, 150µg, 200µg of LPE and 100µg of LAE) < *A. majus* (50µg of LPE and LDE, 50µg, 100µg, 150µg of LAE) < *A. curassavica* (50µg, 100µg, 150µg and 200µg of LPE ; 50µg, 100µg and 150µg of LAE).

However, no dose dependency has been recorded at higher concentrations with respect to cytotoxicity and genotoxicity assay. This may be due to these crude compounds at higher concentrations directly acted on gene/ at chromosome level instead of arresting spindle fibres. Possibly action of bioactive compounds on protein and DNA interaction and its mechanism need further experimentation.



CHAPTER - 6

CONCLUSIONS

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CONCLUSIONS

The aim of the present investigation entitled "Evaluation of bioactive compounds from some medicinal plants and its activity in *Allium cepa* with special reference to genotoxicity and antimutagenicity". Plant system is generally accepted as first tier assay system for the detection of the possible chromosomal damage, resulting from the treatment of plant extracts. *Allium* test has been widely used for screening various plant extract to reveal their genotoxic potential. Genotoxicity of chemical agents is an intrinsic chemical character based on the agent's electrophilic potential to bind with such as nucleophilic sites in the cellular macromolecules i.e. DNA, RNA and protein. Genotoxicity is thus toxicity manifested in the genetic material of cells includes both direct and indirect effects particularly in DNA and other cellular macromolecules.

Today, safer use of natural plant based chemicals; crude compounds are gaining importance in medicine and pharmaceutical industry all over the world. The isolated biologically active compounds or crude compounds from plant origin have a great therapeutic potential providing the molecular basis for most of drugs currently in clinical use, especially in cancer and infectious diseases. Studying with crude extracts is appropriate because traditional medicinal herbs are generally used as crude extracts. However, working with crude extracts also means working with complex mixtures of biologically active compounds.

The use of antimutagens and anticarcinogens in everyday life is the most effective procedure for preventing human cancer and genetic diseases. Secondly, these compounds are considered as safer to use and eco-friendly in nature physiological action of these crude compounds are multidirectional in nature but its action is still not clear. Many of the antibiotics and anticancer drugs of the future will come from soil,

plants and herbs around us. The plants were found to contain chemicals with strong anticancer activity. Some compounds act as cytotoxic, genotoxic, mutagenic and anticarcinogenic there by affecting plants and animals. Hence, it is necessary to evaluate effectively concentration of crude compound/ bioactive components present in different parts of the plants for its activity in cellular system especially in plant system before conducting animal experimentations.

Plants were selected from different families (*Boerhavia diffusa, Withania somnifera, Calotropis procera, Asclepias curassavica, Vitex negundo, Hyptis suaveolens* and *Antirrhinum majus*) on the basis of preliminary characteristics features like medicinal, therapeutic importance and easy accessibility. Experimental results obtained in the present study are briefly summarized in the following paragraphs.

6.1. Isolation and Quantification of crude compound:

Crude plant extracts were obtained using soxhlet apparatus using distilled water, petroleum ether and acetone solvent by scientific method to find out dissolution of chemicals compound present in roots (12.5gm) and leaves (25gm). Study showed that more dissolution chemical compounds of leaves in these plants in aqueous medium than acetone and petroleum ether as a solvent. Higher amount of dissolution of chemical compounds in different solvents as follows

a) In distilled water solvent, 14.54gm and 2.453gm crude compound was obtained from leaves and roots of *Antirrhinum majus.*

b) In Petroleum ether, 1.911gm crude compound was obtained from leaves of *Vitex negundo.* Whereas, 0.270gm crude compound was obtained from roots of *Calotropis procera* .

c) In Acetone, 3.142gm crude compound was obtained from leaves of *Vitex negundo* 0.422gm was obtained from roots of *Antirrhinum majus*.

6.2. Cytotoxicity and Genotoxicity:

Cytotoxicity and Genotoxicity is determined on basis of results of mitotic index and Chromosomal aberrations obtained after the treatments (3hrs at 8°C) of various plants extracts on *Allium cepa* test sytem and compare with control (Distilled water) and standared control (Methotrexate). Our findings show that amongst the seven plants, *Calotropis procera* 4 doses of RPE [50µg (4.17%), 100µg (5.00%), 150µg (5.32%) & 200µg (5.44%)], 4 doses RDE [50µg (4.52%), 100µg (4.98%), 150µg (5.25%) & 200µg (5.59%)] and 4 doses of RAE [50µg (4.36%), 100µg (4.50%), 150µg (4.77%) & 200µg (4.97%)]; *Antirrhinum majus* 4 doses of RPE [50µg (4.13%), 100µg (4.76%), 150µg (4.98%) & 200µg (6.19 %)], 4 doses RDE [50µg (4.39%), 100µg (4.77%), 150µg (5.25%) & 200µg (5.70%)] and 4 doses of RAE [50µg (3.5%,), 100µg (4.85%), 150µg (4.89%) & 200µg (5.3%)] and *Asclepias curassavica* 3 doses of RPE [50µg (3.5%,), 100µg (5.34%) & 150µg (5.67%)], 3 doses of RDE [50µg (3.80%,), 100µg (4.63%) & 150µg (6.00%)] and 4 doses of RAE [50µg (3.28%), 100µg (5.20%), 150µg (5.55%) & 200µg (5.56%)], root extracts showed significant inhibition of mitotic index as compared to control (11.26%) and similar to MTX (4.80% - 6.18%).

Present investigation study on *Allium cepa* assay on genotoxicity was determined by comparing the chromosomal aberrations of treated cells with that of control (00.00%) and MTX chromosomal aberrations ranges from 2.96% - 3.49%. Chromosomal aberrations (chromatid bridges, clumped metaphases, arrested telophases and precocious chromosome at telophases) frequencies in *Calotropis procera* 4 doses of

RPE [50µg (2.78%), 100µg (3.35%), 150µg (3.23%) & 200µg (3.46%)], 2 doses of RDE [50µg (2.56%) &100µg (3.35%)] and 4 doses of RAE [50µg (2.77%,), 100µg (2.90%), 150µg (2.96%) & 200µg (2.89%)]; *Antirrhinum majus* 3 doses of RPE [50µg (2.32%), 100µg (2.79%) & 150µg (3.23%)], 2 doses RDE [50µg (2.60%) & 100µg (3.16%)] and 4 doses of RAE [50µg (2.42%,), 100µg (2.99%), 150µg (2.92%) & 200µg (3.04%)] and *Asclepias curassavica* 3 doses of RPE [50µg (2.91%,), 100µg (3.48%) & 150µg (3.47%)], 2 doses of RDE [50µg (2.50%) and 100µg (2.80%)] and single dose of RAE [50µg (1.99%)] as similar to MTX (2.96% - 3.49%). The frequencies of chromosomal aberrations in most of the leaf and root extracts of seven plants were increased with increase in concentrations at different doses and adjudged low cytotoxic and low genotoxic consequence. Other plant extracts exhibits slighly lower cytotoxic and moderately genotoxic effects when compared with that of control.

Stastistical analysis by 't' test showed that six extracts of 150µg and 200µg of *Withania somnifera* leaf distilled water extracts, 200µg of *Calotropis procera* leaf distilled water extract, 150µg and 200µg of *Vitex negundo* leaf petroleum ether extracts and 200µg of *Hyptis suaveolens* leaf distilled water extract were not genotoxic compare to untreated control.

6.3. Evaluation of bioactive compounds/bioactive groups by UV-Vis and FTIR spectrophotometer:

UV–Vis spectral analysis revealed presence of peaks at 220nm, 255nm, and 296nm corresponds to alkaloids vincristine, while peaks at the range of 235nm - 275 nm correlates to the taxol and FTIR analysis data showed that all the seven plants exhibit functional groups, bands at 3500 - 3000 cm^{-1} and 1580 - 1650 cm^{-1} indicated the

presence of -N-H group. The alkane, C-H, C=O, C-O, C-H (phenyl ring), C=C and C, H (aromatic bends) vibrations were observed at 2960 - 2850cm^{-1}; 3080 - 3020cm^{-1}, 2960 - 2850cm^{-1} and 1470 - 1350cm^{-1}; 1670 - 1760cm^{-1}; 1000 - 1260; 1680 - 1640 and 675 - 870cm^{-1} and 1000 - 3500cm^{-1} favoured the presence of standard taxol group. At 1260 - 1390cm^{-1} indicated presence of NO_2 (nitro compounds) which are related to alkaloids i.e. vincristine and taxol and Alkaloids structural groups identified by UV-Vis and FTIR analysis similar to vincristine, which produce mitotic arrest in *Allium cepa* root tips cells as a direct consequence or due to other effect in the cell cycle. However, no dose dependency has been recorded at higher concentrations with respect to mitotic index. This may be due to these crude compounds at higher concentrations directly acted on gene/at chromosome level instead of arresting spindle fibres.

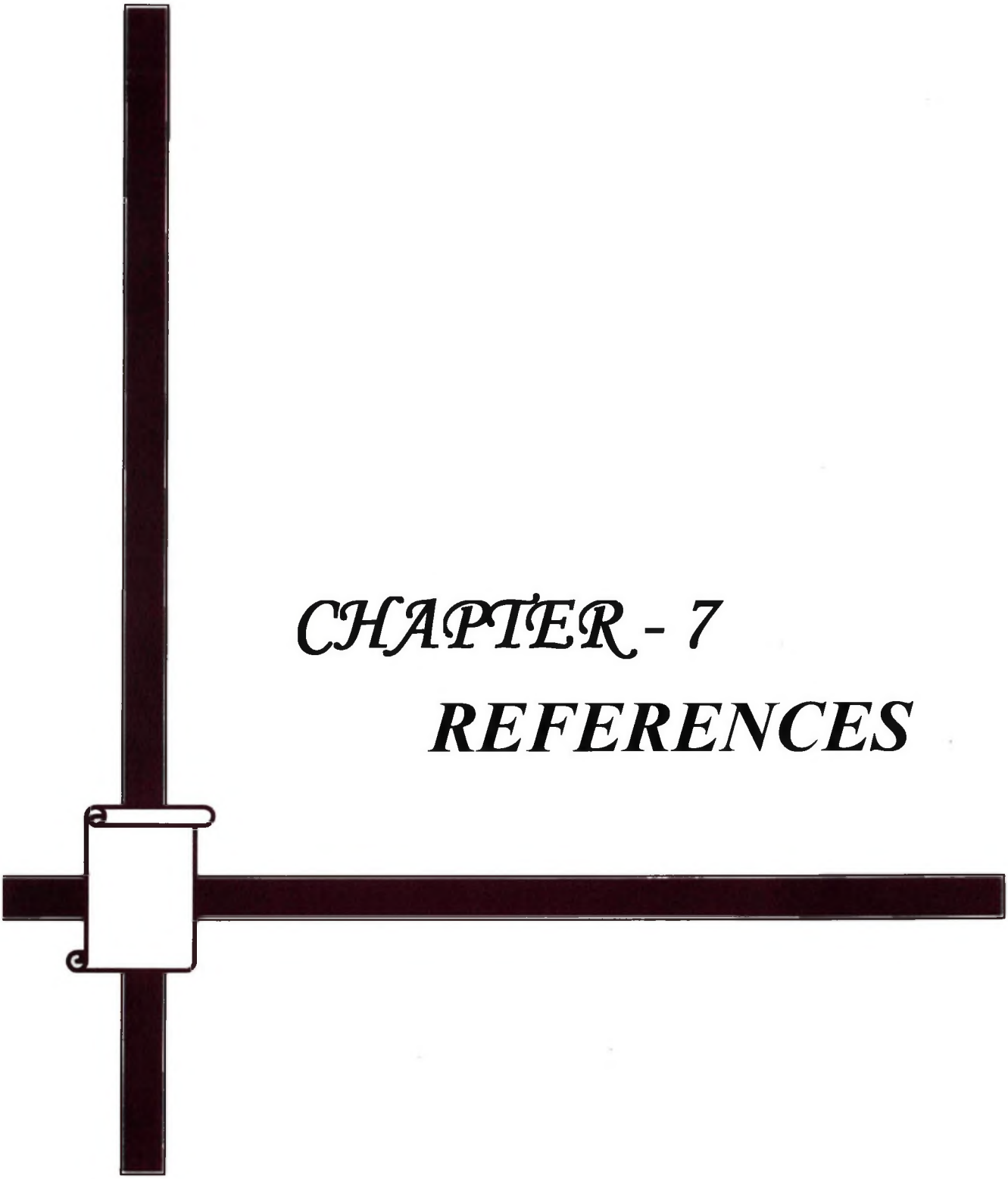
Present study resulted useful effectivity of root extracts of all the seven plant selected over other medicinal plants for the use of anticerous agent. Considering the future need *Calotropis procera*, *Antirrhinum majus* and *Asclepias curassavica* holds promise. Since such study generated low cytotoxic effect as compared to methotrexate in future for theraupeutic uses.

This study has also resulted in standardization of protocol in *Allium cepa* test assay for first hand determination of cytotoxicity and genotoxicity before directly conducting animal experimentations. *Allium cepa* test is an excellent bioindicator of chromosomal alterations that serve as an alert for the population that uses medicinal decoctions universally and that its constant use in the analysis of the treatment of plant extracts is extremely acceptable. Currently, due to major concern with therapeutic purpose, the *Allium cepa* test has occupied an important place for the prevention and

prediction of eco-friendly impact that will be caused by the use and disposal of substances including remedies and herbicides. Although, the test is merely a first assessment of cytotoxicity/genotoxicity, it always shows important scientific discoveries and new adaptations of the test might reveal in numerous possibilities of its use, avoiding the use of animals for testing. More rises and analysis, as the sophistication of the method progresses, will lead us to get the most use for the benefit of the world. Due to this methodology the future investigations on cytotoxicity and genotoxicity on cell lines and animals have been easier and accessible. However, to study the mechanism of action of bioactive compounds at molecular level, structural determination by Mass spectroscopy, proton nuclear magnetic resonance (NMR) spectroscopy is used for determining action of group of compounds of a sample and its structure and animal experimentation needs further evaluation.

6.4. Future implications:

1. Genotoxicity testing is an important part of preclinical safety assessment of new drugs.
2. It is designed to detect genetic damage such as gene mutations and chromosomal aberration, which may be reflected in tumorigenic or heritable mutation potential of the drug.
3. The importance of genotoxicity information and may have prioritized its acquisition in their strategic drug development programs.



CHAPTER - 7

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LIST OF PUBLICATIONS

1. S. N. Malode and **Sarika S. Khandare**, 2009. Effect of root and leaf extracts of *Achyranthes aspera* in *Allium cepa* with special reference to genotoxicity and antimutagenicity. Bionano frontier. 2 (2):51-55.

2. S. N. Malode and **Sarika S. Khandare**, 2010. Effect of leaf extract of *Calotropis procera* in *Allium cepa* with special reference to Genotoxicity and antimutagenicity. Int. J. Pharmacol. Biol. Sci. 4 (3): 39-46.

3. **Sarika S. Khandare** and S. N. Malode, 2012. Genotoxic potential assessment, UV-Vis spectroscopic and FTIR analysis of leaf extract of *Vitex negundo* L. International Journal of Science Innovations and Discoveries. 2 (1), 244-252