An important way to fight against the new borne diseases is by developing new medicines and finding medicinal sources. Hexagonia tenuis (Hook.) Fr., is a least explored and potential bracket fungus. Chemical profiling of H. tenuis revealed the presence of 37 compounds. Few of these were with anti tumor and anti oxidant properties. Because of the presence of number of bioactive components, cytotoxic studies were also carried out and it resulted in a wide spectrum of chromosomal abnormalities. This can be considered as a stepping stone for the further detailed studies in animal assays which may leads to the production of potential mycopharmaceuticals.

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a potential bracket fungus

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INTRODUCTION

"Applied mycology is one of the most stimulating and rapidly evolving areas of the biological sciences"

Plant resources are the back bone of traditional medicine, which means that more than 3.3 billion people in less developed countries utilize medicinal plants on a regular basis (Davidson, 2000). There are nearly 2000 ethnic groups in the world and almost every group has its own traditional medicinal knowledge and experience (Liu *et al.*, 2009; Kebriaee-Zadeh, 2003).

Ethnobotany deals with the study of the relationship between plants and people as well as their culture. It is recognized as an effective way to discover medicines in future. Before the introduction of chemical medicines, man relied on the healing properties of the medicinal plants. Some are giving value to these plants due to the ancient belief which says plants are for supplying man with food, medical treatment, industrial products and other effects. It is believed that, out of the 5.2 billion people of the world, 80% dwells in these less developed countries. As per the estimations of WHO, 80% of these people rely almost exclusively on traditional medicine for their primary health care needs.

According to Paracelsus, the alchemical genius of the Middle ages, "In all things there is a poison, and there is nothing without a poison. It depends upon the dose whether a compound/ substance is poison or not". There is a Chinese saying "Shi Yao San Fei Du", which means that any drugs can have toxic effects.

Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by majority of cultures around the world, especially the Chinese and Indian medicines.

Studying medicinal plants helps to understand plant toxicity and protect man and other animals from natural poisons. Cultivation and preservation of medicinal plants also promotes the protection of biological diversity. Among plant kingdom, Angiosperms contributes more in the medicinal resources. The medicinal effects of plants are due to the metabolites especially secondary compounds produced by plant species. Plant metabolites include primary metabolites and secondary metabolites. Primary metabolites include carbohydrates, amino acids, nucleotides, fatty acids, steroids and lipids whereas; secondary metabolites include

terpenoids, glycosides, alkaloids, phenolics, *etc*. serving a non specific function (Meskin and Mark, 2002).

Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification and structure of the large number of secondary metabolic compounds found in plants. Every plant produces compounds as part of their normal metabolic activities. Plants produce primary and secondary metabolites of which the latter is not much involved in metabolism. But many secondary metabolites like glycosides, alkaloids, terpenoids *etc*. are commmercially imporant and find use in a number of pharmaceutical compounds. Mixture of volatile monoterpenes and sesquiterpenes found in plants are called essential oils. Phytochemical analysis can be done by different techniques. The gas chromatographic methods are proved to be effective when unknown oil samples are subjected to this technique. The versatality of the gas chromatographic method provides information not only about the number of constituents in the sample but the constituents are quantified with high precision even when microgram levels of samples are available. This method also provides easy and effective detection of foreign constituents in the essential oil (Jose and Rajalkshmi, 2005).

A recently developed technique regarding the analysis of total essential oil is GC-MS. This technique is rapidly used for the analysis of essential oils. GC is a tool for separating the volatile components while analysis depends upon retention characteristics under standard conditions. The mass spectrometer can be used as a detector for a gas chromatograph in which the high degree of specificity of the mass spectrum is an aid to the identification of the sample. The large number of spectra obtained in a short time from GC-MS technique and the routine nature of the data obtained, makes the computer a very useful accessory to the GC-MS unit. With the help of GC-MS technique, it has now been possible to analyse directly the fragrances of natural as well as artificial material without the use of heat or solvents directly by the use of head space analysis. A GC-MS machine which has computarised library search disc can be regarded as the best single tool for essential oil analysis (Jose and Rajalkshmi, 2005).

Knowledge of chemical compounds especially essential oil composition allows a better and specially directed application. This can be achieved by means of carefully performed capillary gas chromatography (Buchbauer, 2000) and mass spectrometry experiments.

Number of plants and plant products are widely used in the modern medicine also. For example, *Rauwolfia serpentina* is used for the treatment of hypertension and mental diseases;

Podophyllum for the treatment of cancerous tumours; *Digitalis purpurea* for the treatment of heart diseases; *Aloe vera* for the radiation burns and even third degree burns; *Taxus baccata* for the treatment of breast and uterine cancer; *Catharanthus roseus* for the treatment of leukemia; *Phyllanthus amarus* for the treatment of liver ailments and jaundice; *Evolvus alsinoides*, *Centella asiatica* and *Bacopa monnieri* for improving the intelligence and memory power among children (Trivedi and Sharma, 2004). Although traditional medicine flourished in India for quite a long time; yet for a while it was subdued under the impact of modern medicine. Emergence of life threatening diseases is increasing day by day. This leads to the development of new and novel medicines especially in the field of cancer.

A tumor or neoplasm is an abnormally growing cellular mass that has no normal metabolic purpose. When tumor cells move from their place of origin to another location, take up residence and cause new tumerous growth. When this happens, the disease is known as cancer and the tumor is termed malignant.

Cancer is a genetic disease caused by mutations that arise first in a single cell. Early diagnosis is a key to successful anticancer treatment. Traditional treatments for cancer include chemotherapy, radiation therapy and surgery. Today, biotechnology and immunology offer several alternative means of treatment. One biotechnological approach is to use gene therapy. Cancer treatment is also possible by using monoclonal antibodies that are attracted specifically to the abnormal antigens of tumor cells. The monoclonal antibodies are altered to carry cytotoxins or radioactive molecules that will destroy only the cancer cells, not the adjacent normal tissue (Bourgaize *et al*., 2003).

The drawback of the conventional treatment apart from its high cost is the immune suppressive effects. Drugs that could reduce these side effects as well as stimulate immunity will be of great help in improving cancer treatment strategies (Leemol and Girija, 1998). Many plants have been evaluated in clinical studies and are currently being investigated phytochemically to understand their anti-tumour actions against various cancers. Thus the cancer patients are further burdened by drug induced toxic side effects and have now turned to seek help from the complementary and alternative medicine hoping for a better cure (Rao *et al*., 2008).

Cytotoxicity is the quality of being toxic to cells. Treating cells with the cytotoxic compound can result in a variety of cell fates. Chemotherapy as a treatment of cancer often

relies on the ability of cytotoxic agents to kill or damage the cells which are reproducing; this preferentially targets rapidly dividing cancer cells. Decrease in the mitotic index as a result of treatment with a particular substance shows its capacity to arrest cell division together with its ability to kill the actively dividing cells (Tajo and Thoppil, 1998). The degree of cytological aberration either in mitosis and meiosis is regarded as one of the dependable criteria for estimating the effect of mutagens (Reddy and Reddy, 1985).

Cytotoxicity is the degree to which something is toxic to living cells. It is also a subject of heavy pharmaceutical study, particularly in the area of cancer research. A wide variety of anti- cancer drugs exhibit 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 (Gali- Muhtasib and Bakkar, 2002). The ultimate goal of many chemotherapy drugs is the low cytotoxicity to normal cells and high cytotoxicity to cancerous cells. Cytotoxicity as an index of chromosomal aberrations is found to be caused by plant extracts and several other chemicals. These findings have promoted scientists to induce extracts as herbal cytotoxicants.

Number of fungi and bacteria are also having great significance in the treatment of diseases. Chemicals produced by fungi possess different biological properties. It may even kill or arrest the growth of other organisms. Due to the least explored nature of fungal groups, medical mycology became an important area of research. Fungi are important as decomposers, food, medicine and in the production of dye, enzymes, beverages, antibiotics *etc*. With respect to medically important compounds, the fungi have proved to be an extremely important group of organisms (Alexopoulos *et al*., 1996a). Historically a number of fungi including fruiting bodies of bracket fungi have been used in herbal medicine.

The fungi comprising the division Basidiomycota commonly are known as basidiomycetes. These are a large and diverse group and include forms commonly known as mushrooms, puffballs, earthstars, stinkhorns, bird's nest fungi, bracket or shelf fungi *etc*. The Basidiomycetes are an important group of fungi including harmful as well as useful species. Basidiomycetes are well known for their production of a wide variety of interesting secondary products especially for their scents, tastes, colours and toxic properties.

The order Aphyllophorales (Polyporales) is an important group of Basidiomycota. Many species of Aphyllophorales decay wood of trees that are already dead. In their role as wood

rotters, species of Aphyllophorales are also important to animal life. A surprisingly large number of the basidiocarps of the species of Aphyllophorales have been used medicinally. A notable example is *Ganoderma lucidum*. Cures for everything from veneral disease to cancer as well as purification of blood, lowering of cholesterol and general increased longevity have been attributed to some of these fungi (Alexopoulos *et al*., 1996b).

Polyporaceae is the largest and most diverse group of poroid Aphyllophorales. *Lentinus, Trametes, Ganoderma, Polyporus, Pycnoporus, Cryptoporus, Hexagonia etc*. comprises the important genera among the family Polyporaceae. Among these, *Hexagonia* is least explored for its biological activities.

Hexagonia is a potential bracket fungi. The genera *Hexagonia* has a wide spread distribution especially in tropical regions. It is a lignicolous fungi with thin and leathery bracket. The fruiting body is firmly attached to the dry and dead branches. Fruiting body is differentiated into dorsal and ventral surface. Dorsal surface is velvety with concentric zones in various shades of fawn or tan. Ventral surface (hymenium) is characterised by the presence of numerous minute hexagonal pores. Fruiting bodies are extremely persistent. The genera consist of 16 species. It includes *H. apiaria, H. dermatiphora, H. hirta, H. hydnoides, H. leprosa, H. niam-niamensis, H. nitida, H. pobeguinii, H. similis, H. speciosa, H. tenuis, H. umbrinella, H. variegata, H. velutina, H. vesparia and H. zambesiana.*

Hexagonia is economically important bracket fungi. Among these *H. apiaria* is used as food. *H. hydnoides* has been screened for antimicrobial activities. It could inhibit the growth of *Bacillus cereus* (Rosa *et al*., 2003). *H. tenuis* is used for the purification, characterisation and synthesis of a thermostable enzyme called laccase (Chaurasia *et al*., 2015). Organic dyes were obtained from *H. hirta*. These previous works reveal the medical significance of *Hexagonia*, for which the genera was selected for the study. Synthesis of thermostable enzyme called laccase from *H. tenuis* shows its biological importance. Because of this least explored nature, the species *H. tenuis* is selected for the present study.

Systematic Position:

Kingdom : Fungi

Division : Basidiomycota

Class : Basidiomycetes

Order : Polyporales

Family : Polyporaceae

Genus : *Hexagonia*

Species : *tenuis*

***H. tenuis* (Hook.) Fr.-Habitat, ventral and dorsal view of fruiting body**

The present investigation involves the studies on cytotoxic activity of fungal extract of *H. tenuis* (Hook.) Fr., using *Allium cepa* root tip assay as well as GC-MS analysis. *A. cepa* root tip assay was introduced by Leven (1938) and was later proposed as a standard method to study genotoxicity (Fiskesjö, 1985).

Objectives of the present work are:

- To study the cytotoxic effects of fungal extract of *H. tenuis* using *Allium cepa* root tip assay.
- GC-MS analysis of the fungal extract to reveal the chemical compounds present in it.

REVIEW OF LITERATURE

The perspective of "a strong mind in a strong body" insists that both emotional health and spiritual vigour are essential to maximum vitality. Self esteem, the ability to forge and maintain, trusting relationships, the capacity to love and to be loved and a sense of peace in the quietest of moments and in the deepest recesses of the soul are accordingly esteemed as major determinants of health. To maintain a healthy condition, the demand for plant based medicines (phytomedicines and pharmaceuticals) is increasing worldwide. Plants are also found to contain disease specific curative properties and extracts of such plants are increasingly being used to manufacture effective drugs.

From time immemorial, mankind has relied on plants and plant products for its health care requirements. By trial and error, empirical reasoning and even by experimentation, all of which had later evolved into ethno-medical traditions (Udayan and Balachandran, 2009).

Tribal medicines have often led to the discovery of valuable new drugs. The study of the plants used in their manufacture and fresh experimentations and trials using them can lead to further new effective formulations. Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are considered to play a beneficial role in health care. Despite their importance, medicinal plants are seldom handled within an organized, regulated sector; most of them are still exploited with little or no regard to the future. Thus medicinal plants are viewed as a possible bridge between sustainable economic development, affordable health care and conservation of vital diversity.

Many higher plants produce economically important organic compounds such as oils, resins, tannins, natural rubber, gums, waxes, dyes, flavors and fragrances, pharmaceuticals and pesticides. According to Balandrin *et al*. (1985), most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents and new sources of commercially valuable materials remain to be discovered. For security, people are returning to the naturals (Joy *et al*., 2001). Cytotoxic investigations and the analysis of chemical compounds can be considered as a preliminary step towards such a kind of novel discovery.

CYTOTOXIC STUDIES

Cancer is obviously the most serious disease and primary treatment rests with the practitioner of scientific medicine. Several herbs have been used to fight against cancer. The anticancerous effect is believed to be due to the plants' ability to boost the immune function (Cammarata, 2006).

Plants are the main sources of clinically used anticancer drugs such as vinblastine and vincristine (Nobel *et al.,* 1958; Svoboda, 1961) as well as taxol (Wani *et al*., 1971).

Studies on cytotoxicity have been a stepping stone to the development of anticancer drugs. For the confirmation and completion of the experiment, animals are used as the test material. In toxicology research, one of the fundamental concerns for both science and ethics is the issues regarding the animal use and care. Emphasis has been given to the use of alternatives to mammals in testing, research and education (Mukhopadhyay *et al*., 2004).

Allium cepa assay has been used to study cytotoxicity in plants (Fiskesjö, 1985). Based on the report of Fiskesjö and Levan (1993), the *Allium* test has been found to have a correlation with other test system (MIT-217 cell test with mice, rats or human *in vivo*) and could be used as an alternative to laboratory animals in toxicological research (Abu and Mba, 2011).

Allium cepa root tip assay was introduced by Levan (1938) and was later proposed as a standard method to study genotoxicity (Fiskesjö, 1985).

The cause of chromosomal abnormalities by studying the chromosome morphology, gene action, nucleic acid chemistry and metabolism was studied by Darlington (1942).

Sharma and Sharma (1960, 1962) studied the importance of spontaneous and chemically induced chromosome breaks and emphasized the importance of nucleic acids in controlling chromosome breaks induced by different compounds.

The cytological effect of water extract of *Pulicaria crispa* on *A. cepa* was studied by Shehab (1979). Spindle disturbances, stickiness, bridges and laggards are the major anomalies detected. From the study it was clear that, as the duration of treatment and concentration increases the percentage of abnormality also increased.

Chromosome breakage, bridge formation, C-mitosis, micronuclei formation, stickiness and chromosome condensation were the main abnormalities observed in studies conducted by Prasad and Das (1977) on the effect of growth substances on mitosis in *Vicia faba*.

Deena and Thoppil (2000) studied the cytotoxic properties of *Lantana camara* on *Allium cepa* root tip cells. Chromosome stickiness, chromosome clumping and diagonal orientation of chromosomes were the major abnormalities observed.

The cytotoxic potential of *Artemisia nilgirica* extract comprising both polar and non-polar fractions and plant extract having polar chemical compounds alone were evaluated on the meristematic root tip cells of *Allium* cepa (Leeja and Thoppil, 2004). Both clastogenic and non-clastogenic abnormalities were noticed. Chromosome stickiness, bridges, binucleate cells *etc*. were the major aberrations observed.

Minija *et al*. (1999) studied mitoclastic activities of *Mentha rotundifolia* and the abnormalities observed were clumped metaphase, scattered metaphase, polyploidy, bridge, ball metaphase *etc*.

The cytotoxic effects studied by Saj and Thoppil (2006) in two species of *Corymbia* include, many clastogenic abnormalities like nuclear lesions, chromosome stickiness and chromosome breakage. The non-clastogenic aberrations include C-metaphase and polyploidy.

MEDICINAL IMPORTANCE OF FUNGI

Words of the father of medicine, Hippocrates is as follows: “Let food be your medicine and medicine be your food”. This saying aptly suits mushrooms as they have tremendous medicinal, food, drug and mineral values; hence they are the most valuable asset for the welfare of humans.

Medicines of fungal origin have been used very long back in history. The invention of the so called “wonder drug” Penicillin was a land mark in this field. Since then many fungi are known for their antifungal, antibacterial, antiviral, antitumor and many other such properties of pharmacological values.

Concurrent with an increasing interest in the role of fungi in natural environment, it has been realized from laboratory studies that fungi have the capability for a whole range of

chemical transformations, leading to compounds of very considerable economic importance. The production of ethanol by yeast is a long standing example of this ability (Jennings and Lysek, 1999).

Terrestrial fungi are known to be rich sources of biologically active compounds for medicinal and agricultural applications (Zjawiony, 2004).

Mushrooms had long been used for medicinal and food purposes since decades. It is now increasingly recognized that correct diet and control modulates many functions of human body and consequently participates in the maintenance of state of good health, necessary to reduce the risk of many diseases. Modern pharmacological research confirms large parts of traditional knowledge regarding the medicinal effects of mushrooms due to their antifungal, antibacterial, antioxidant and antiviral activities, besides being used as functional food (Wani *et al*., 2010).

An antitumor substance namely, "clavacin" has been obtained from the giant puff ball, *Clavatia gigantea (*Beneke, 1963). Mushrooms were identified for their antitumor principle and include *Pleurotus* (Yoshika *et al*., 1963), *Agaricus bisporus* (Vogel *et al*., 1974), *Flamulina velutipes* (Komatsu *et al*., 1963) and *Agaricus blazei* (Kawagishi *et al*., 1989).

The pharmacological uses of mushrooms demand further intensive research for its wider application. Even though a variety of mushrooms are well known for their edibility and nutritive values and number of them are under cultivation, the possibility of their maximum exploitation for food and medicines for human health is yet to be explored (Balakrishnan and Nair, 1994).

Mushrooms have been found to be effective against cancer, reduction of cholesterol, stress, insomnia, asthma, allergies and diabetes (Bahl, 1983).

The first successful research discovered the antitumor effects of the hot water extracts from several mushrooms (Ikekawa *et al.*, 1969). The components were proved to be polysaccharides especially β-D glucans.

Chihara *et al.* (1969) isolated an antitumor polysaccharide from the Shitake fruiting bodies, which was named lentinan.

The antioxidants are an important defense of the body against free radicals and mushrooms are a rich source of these antioxidants (Mau *et al.,* 2004*)*.

Kimura *et al*. (1994) reported that, schizophyllan from *Schizophyllum commune* is effective against head and neck cancer.

Several different polysaccharide antitumor agents have been developed from the fruiting body, mycelia and culture medium of various medicinal mushrooms (*Lentinus edodes, Ganoderma lucidum, Schizophyllum commune, Trametes versicolor, Inonotus obliquus* and *Flamulina velutipes*). Both cellular components and secondary metabolites of a large number of mushrooms have been shown to affect the immune system of the host and therefore could be used to treat a variety of disease states (Wasser and Weis, 1999).

Lemieszek *et al*. (2009) reported that *Piptoporus betulinus* fraction elicited anticancer effects that were attributed to decreased tumor cell proliferation, motility and the induction of morphological changes.

Joseph *et al.* (2011) isolated polysaccharides from *Ganoderma lucidum* (Polyporaceae) and their antitumor and anti inflammatory activities were investigated using *in vivo* models.

The medicinal mushroom *Agaricus blazei* produced high amounts of laccase in a complex liquid medium. The results show that in addition to the wood colonizing white rot fungi, the typical litter decomposing Basidiomycetes can also produce high amounts of laccase in liquid media (Ullrich *et al.,* 2005).

Recently Junfeng Wang *et al.* (2012) reported two novel cytotoxic polyphenols, expansols A and B, containing both phenolic bisabolane and diphenyl ether units from the fungus *Penicillium expansum* 091006 (Trichocomaceae) endogenous with the mangrove plant *Excoecaria agallocha*.

Mushrooms are a new and emerging source of potent pharmaceutical products. *Ganoderma lucidum, Lentinus edodes, Trametes versicolor, Schizophyllum commune, Flamulina velutipes, Pleurotus ostreatus, Agaricus bisporus, Agaricus brasiliensis, Pholiota nameko, Tricholoma matsutake, Auricularia auricula* and *Grifolia frondosa* possess wide range of bioactivities like anticancerous, antibacterial, antiviral, antifungal, antidiabetic and anti inflammatory potential (Tidke and Rai, 2006).

Ajith and Janardhanan (2007) reported that mushrooms like *Ganoderma lucidum, Phellinus rimosus, Pleurotus florida* and *Pleurotus pulmonaris* possessed antioxidant and anti tumor activities. Indian medicinal mushrooms are potential sources of antioxidant and anti cancer compounds. However, intensive and extensive investigations are needed to exploit their valuable therapeutic use.

According to Lucas *et al*. (1957) the most significant medicinal effect of mushrooms and their metabolites that have attracted the attention of the public is their anti tumor property. Lucas and his collaborators demonstrated the anti tumor activity of the higher Basidiomycetes in 1957 for the first time.

Mizuno (2000) reported the comparison of hot water extract of *Phellinus* with other anticancer mushrooms. *Phellinus* extract is the strongest evidence of tumor proliferation suppression.

"Lingzhi" (*Ganoderma lucidum*) is a potential medicinal mushroom. Earlier studies had reported the anticancer activity of lingzhi which has prompted its usage by cancer patients (Yuen and Gohel, 2005).

Hong *et al.* (2004) reported that the ethanolic extract of lingzhi induced apoptosis in human colon cancer cell HT-29 by increasing caspase- 3 activity and nitric oxide (NO) in a dose- dependent manner.

Lingzhi also inhibited the metastasis of tumor in the lungs and prolonged the life span of tumor- transplanted mice (Lee *et al.*, 1995).

In searching for new cancer treatments over the past several years, many species of Basidiomycete mushrooms have been investigated for their anticancer properties (Yang and Jong, 1989). The anticancer calvacin, a conjugated protein containing one or more carbohydrate residues was extracted from the giant puff ball *Calvatia gigantea* and tested against many experimental tumors, such as Sarcoma 180, mammary adenocarcinoma 755, leukemia L-1210 and HeLa cell lines. Many other anticancer compounds of mushroom origin, including β-D- glucans, heteropolysaccharides, glycoproteins, lectins and terpenoids have been investigated (Robles- Hernandez *et al*., 2008).

Because of the ease of cultivation and nutritional value, the popularity of the genus *Pleurotus* has mainly increased. A wide spectrum of traditional medicine systems had

attributed potential medicinal properties to *Pleurotus* sp. Scientific evidence supports their importance as producers of substances with antibiotic, antiviral, anticarcinogenic, antiinflammatory and hypocholesterolemic activities (Gunde- Cimerman, 1999).

Wasser and Weis (1999) reported that *Auricularia auricula, Tramella fuciformis, Tramella mesenterica, Ganoderma lucidum, Pleurotus ostreatus, Pleurotus pulmonarius* and *Lentinus edodes* are universally recognized and are medicinally valuable higher Basidiomycetes.

CYTOTOXICITY OF FUNGI

The presence of a cytotoxic protein phallolysin from the fruiting bodies of *Amanita phalloides* has been reported by Seeger (1975). Kawagishi *et al*. (1988) isolated steroids from the fruiting bodies of *Agaricus blazei* and were found to be cytotoxic against HeLa cells.

Lee *et al*. (2011) evaluated the effects of six mushroom extracts (*Grifolia frondosa, Pholiota nameko, Panellus serotinus, Pleurotus cornucopiae, Armillaria mellea* and *Flammulina velutipes*) on cytotoxic activity and cytokine production of lamina propria leukocytes (LPLs) isolated from small and large intestinal mucosa of rat.

Clitocybe alexandri is an edible saprophytic Basidiomycotina mushroom belonging to the family of Tricholomataceae. Vaz *et al.* (2012) reported that the ethanolic extract of *C. alexandri* has been demonstrated to possess cytotoxic and anti-proliferative activity towards a human lung tumor cell line (NCI-H460 cells).

Menikpurage *et al*. (2012) investigated the antioxidant and cytotoxic activity of *Pleurotus cystidiosus* against Hep-2 cancer cell line.

The ethanol extracts of mushroom samples of *Agaricus sp*. and *Termitomyces letestui* also showed cytotoxicity against the brine shrimp larvae (Nyigo *et al.,* 2005).

Cytotoxic lanostane triterpenoids were isolated from the fruiting bodies of the mushroom *Hebeloma versipelle* by Shao *et al*. (2005) that exhibited to possess cytotoxic activities against tumor cell lines.

Fruiting bodies of *Amanita virosa* was found to contain two highly toxic proteins - hemolytic lectin and toxovirin - that inhibited growth and decreased survival of the mammalian leukemia cells (Antonyuk *et al*., 2010).

Jiao *et al.* (2013) studied the effect of hot water extract of *Amauroderma rude* on invasive and metastatic breast cancer cell lines MT-1, MDA-MB231 and 4T1, less invasive breast cancer cell line MDA-MB468 and benign breast cancer cell line MCF7. No cancer cells could survive after treatment with 600µg/ml of *A. rude* extract. Low concentration of this extract (500 µg/ ml) also exerted a significant activity in inducing cell apoptosis as compared with the control (29 % vs 1.8 %).

Several studies have demonstrated that various *Ganoderma lucidum* extracts interfere with cell cycle progression, induce apoptosis and suppress angiogenesis in human cancer cells and thus act as anticancer agents (Hu *et al*., 2002; Stanley *et al*., 2005; Jang *et al*., 2010).

GAS CHROMATOGRAPHY-MASS SPECTROMETRY

Gas chromatography is a rapidly growing analytical tool. Gas chromatography is principally a separation process. It is often said that gas chromatography is the most widely used analytical technique.

Carlile *et al*. (2005) reported that, the fungi provide us with an enormous variety of strange and wonderful 'secondary metabolites'. Antibiotics are the secondary metabolites produced by one organism that interfere with the metabolism of another organism.

Analysis by conventional GC and GC-MS has shown at least 40 components including trace amounts of benzyl isothiocyanate which indicated the presence of benzylglucosinolate in *Agaricus bisporus* (Macleod and Panchasara, 1982).

Cronin and Ward (2006) conducted GC-MS analysis of *Agaricus bisporus*, which has shown the presence of volatile components like 3-methylbutanol, 3- octanone, oct-1-en-3-one, 3-octanol, oct-1-en-3-ol, furfural, benzaldehyde, phenylacetaldehyde and benzyl alcohol.

Chen and Wu (2006) reported about 30 volatile components which were identified by GC-MS analysis from the fruiting bodies of *Agaricus subrufecens*.

Terpenoids are the combination of two or more molecules of 2- methyl-2, 3-butadiene, also known as isoprene. Many terpenoids isolated from Polyporales and Ganodermatales mushrooms have anticancer properties. There have been reports of about 100 different types of terpenoids, which were obtained from the fruiting bodies of *Ganoderma lucidum* and *Ganoderma applanatum* (Robles-Hernandez *et al*., 2008).

PREVIOUS WORKS IN THE GENERA *HEXAGONIA*

A thermally stable laccase enzyme was purified from the culture filtrate of *H. tenuis* MTCC-1119 (Chaurasia *et al*., 2015). Laccase belongs to the group of polyphenol oxidases containing copper atoms in the catalytic centre and usually called multicopper oxidases. One of the applications of the laccase in organic synthesis is the selective oxidation of aromatic methyl group to corresponding aldehyde.

Rosa *et al*. (2003) conducted experiments in which the crude extracts of Basidiomycetes including *Hexagonia hydnoides* were noted for the significant antifungal and antibacterial activities.

Most of the species of the class Basidiomycetes (*Agrocybe perfecta, Hexagonia hydnoides, Irpex lacteus, Nothopanus hygrophanus, Pycnoporus sangineus* and *Tyromyces duracinus*) have been shown to possess antimicrobial activity (Fang and Ng, 2013).

Nineteen strains of wood inhabiting pore fungi including *Hexagonia tenuis* and *Hexagonia hydnoides* were able to decolourise partially or completely the AB 62 dye added to malt extract (Sanchez-Lopez *et al*., 2008).

Tidke and Rai (2006) reported that mushrooms like *Ganoderma applanatum, Ganoderma curtissi, Lycoperdon imbricatum, Polyporus alveolus, Hexagonia hirta* and *Hexagonia tenuis* were used for the production of dyes.

Guerrero-Vasquez *et al*. (2014) reported the total synthesis of speciosins P and G, where P is isolated from *Hexagonia speciosa*. Earlier studies have isolated a series of substituted hydroquinones, named speciosins A-T from the Chinese fungus belonging to Basidiomycetes, *Hexagonia speciosa*. Speciosins G and P are structurally similar to the biologically active siccayne, which was isolated from the fungus *Helminthosporium siccans* in 1968 and marine Basidiomycete taxa, *Halocyphina villosa*.

MATERIALS AND METHODS

FUNGAL MATERIAL

The fruiting body of *Hexagonia tenuis* (Hook.) Fr., a bracket fungi (Polyporaceae) used for the present study was collected from the University Campus, mainly from the Botanical Garden, University of Calicut. Taxonomic authentication of the fungi was done by Prof. P. Manimohan, Fungal Diversity Division, Department of Botany, University of Calicut.

Experiments of the present study were conducted mainly in the Cell and Molecular Biology Division, Department of Botany, University of Calicut. The remaining portion of the work (GC- MS analysis) was done at CEPC Laboratory and Technical Division, Cashew Bhavan, Kollam.

Objectives of study include:

- Cytotoxic assay of extracts of *H. tenuis* using *Allium cepa* root tips, as the test material
- Characterization of methanolic extracts of *H. tenuis* by Gas Chromatography - Mass Spectrometry

Fig. 1: Habitat showing the fruiting body of *Hexagonia tenuis* (Hook.) Fr.

Fig. 2: Fruiting body of *Hexagonia tenuis* (Hook.) Fr.: a. Ventral view;

b. Dorsal view

PLANT TEST MATERIAL

Allium cepa L. (2n=16) was seletced as the test material for the cytotoxic assay. Due to certain reasons, *A. cepa* is considered as the classical test material. The reasons are as follows:

- They can be easily propagated through bulbs
- They are easy to handle
- They possess fewer number of chromosomes and the chromosomes (2n=16) are large which enable easy cytological observations
- The chromosomes show less variation in size and shape
- It can produce large number of roots within a short period
- It can reveal clastogenic and non clastogenic abnormalities very easily
- Feasibilty to squash

The healthy and uniform sized bulbs of *A. cepa* were purchased from local market and stored in dry and well aerated conditions for a rational period before use, to prevent rot as well as to promote viability. Poorly preserved bulbs, mouldy and those that have started shooting green leaves were all discarded. The selected bulbs were planted in pure and sterilized sandy soil without manure to prevent other chemically induced chromosomal alterations. Roots sprouted out after 2-3 days.

CYTOTOXIC ASSAYS

Cytotoxicity of aqueous extracts of *Hexagonia tenuis* in *A. cepa* root tip meristem:

Cytotoxicity of the aqueous extracts of *H. tenuis* was analyzed. It involves the following steps.

Collection of the material:

Fruiting body of *H. tenuis* was collected from the University campus, especially from the Botanical Garden, University of Calicut. The collected materials were shade dried and powdered using a blender.

Preparation of the fungal extract:

Aqueous extracts were prepared from the powdered materials by using mortar and pestle. 0.1g of the powdered material was dissolved in 100 ml distilled water to prepare the stock solution. Varying concentrations like 0.1, 0.05, 0.01 and 0.005% were prepared.

Determination of period of peak mitotic activity:

In order to find out the time of peak mitotic activity, the untreated root tips of *A. cepa* were fixed in acetic alcohol (1:2) mixture at different times from 8.30 am to 11 am. After many trials, it was found that maximum dividing cells (peak mitotic activity) occurred between 9.00 am to 9.30 am under normal sunshine conditions.

Determination of fruiting body extracts of *H. tenuis* for cytotoxic assays:

Various concentrations were prepared with distilled water and after preliminary analysis, it was confirmed that higher concentrations were found to be extremely toxic, leading to cell death and hence they were eliminated. So the lowest concentrations, *viz*., 0.005, 0.01, 0.05 and 0.1 % were selected for the present study.

Selection of control:

For the effective comparison, both negative and positive controls were used. Distilled water was taken as the negative control and 0.01% Hydrogen Peroxide was taken as the positive control.

Mode of treatment of *H. tenuis* fruiting body extracts:

Healthy bulbs of *A. cepa* were planted in sterilized sandy soil. Germinated bulbs with roots having 1-2 cm length were collected at the peak mitotic period (9-9.30 am) and washed thoroughly with distilled water. Roots of germinated bulbs were treated with varying concentrations of the aqueous extracts taken in bottles, in such a way that only the roots were immersed in the solution. Root tips were cut from bulbs placed in the samples of each concentration at different time intervals such as ½ h, 1 h, 2 h, 3 h and 24 h. The treated root tips were washed thoroughly with distilled water and immediately fixed in modified Carnoy's fluid (1 Acetic acid**:** 2 Alcohol) for a period of 1 h.

With the help of improved techniques (Sharma and Sharma, 1990), mitotic squash preparation was done. After 1 h fixation, root tips were washed with distilled water and hydrolysed using 1N HCl for 5 min to separate the cells during squashing. Then it was washed and stained with 2% acetocarmine for 3 h. 45 % acetic acid was used for destaining. Finally it was squashed and mounted on clean glass slides.

Observation of the prepared slides:

Mounted slides were observed for aberrations under microscope (Olympus CX21 FSI, Japan) and photographs were taken using Amscope Mu1000 digital camera- FMA050 attached to the microscope.

Calculation of mitotic index and abnormality percentage:

Mitotic index were calculated using the following formula,

$$\text{Mitotic index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

Percentage of abnormality is calculated using the following formula,

$$\text{Abnormality percentage} = \frac{\text{Number of aberrant cells}}{\text{Total number of cells}} \times 100$$

CHARACTERIZATION OF METHANOLIC EXTRACTS OF *H. TENUIS* BY GC-MS

Preparation of methanolic extracts:

With the help of Soxhlet apparatus, methanolic extracts of dried powder of *H. tenuis* was prepared. 10 g of the fungal material was extracted for 6 hours in 100 ml methanol. The extract was then cooled, filtered and saturated to dryness in a vacuum evaporator. The extract was dissolved in 10 ml methanol (HPLC Grade, Merck) for the analysis. It was filtered through 0.20 mm membrane filter to ensure minimum contamination.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

GC-MS analysis of methanolic extract was performed on a Varian model CP-3800 GC interfaced with a Varian Saturn 2200 Ion Trap Mass Spectrometer (ITMS) operating at 70 eV and 250^0 C, equipped with a CP-1177 Split/ Splitless capillary injector and Combi PAL autosampler. A cross linked Factor Four capillary column, VF 5 m with 30 m x 0.25 mm ID

and 0.25 µm film thickness was utilized. Helium was used as the carrier gas at a flow rate of 1 ml/min. Injection volume was 1 µl. The split ratio was 1: 20. The temperature programme for the chromatographic analysis was set at 60^0 C for 1 min. (initial) and then heated up at a rate of 3^0 C/ min. to 280^0 C. Run time was 50 min. Quantification was performed using percentage peak area calculation and identification of individual components was done using the NIST MS Search. The relative concentration of each compound in the methanolic extract was quantified based on the peak area integrated by the analysis programme.

RESULTS

Present study revealed the cytotoxic and phytochemical potential of *Hexagonia tenuis* (Hook.) Fr. using *Allium cepa* assay and GC-MS analysis.

Cytotoxic studies

A remarkable cytotoxicity was observed in *A. cepa* root tips treated with *H. tenuis* aqueous extract with 4 different concentrations *viz.,* 0.005, 0.01, 0.05 and 0.1 % for 5 different time durations such as ½, 1, 2, 3 and 24 hours and the results are tabulated (Table 13). Present investigation revealed a wide spectrum of clastogenic and non clastogenic abnormalities (Figs. 9-193) in comparison with the control (Figs. 3-8).

Nuclear lesion, nuclear budding, nuclear erosion, chromosome stickiness, chromosome bridges, chromosome fragments, bizarre nuclei, pulverised chromatin, giant cells, abnormal condensation of chromosomes, nuclear extrusion, nuclear disintegration, cytomixis, nuclear fragmentation, nuclear enlargement, hyperchromasia, contorted chromosomes, chromosome coagulation, chromosome gap *etc*. were observed as the major clastogenic abnormalities.

The observed non clastogenic abnormalities include macro and micro cell formation, binucleate cell, chromosome clumping, C-metaphase, stellate chromosomes, misorientation of chromosomes, polyploidy, hypoploidy, diagonal arrangement of chromosomes, disturbed chromosomes, chromosome laggards, stathmo anaphase, scattering of chromosomes, shift in microtubular organizing centres (MTOC), ball shaped arrangement of chromosomes, pole to pole arrangement of chromosomes, cytostasis, tripolar anaphase, micronuclei, early movement of chromosomes, abnormal association of chromosomes, chromosome doubling and somatic pairing, vagrant chromosomes, unequal separation of chromosomes, early cell plate formation, strap cell formation, tropokinesis, chained chromosomes *etc.*

The most frequent abnormalities observed were nuclear lesions, chromatin erosion, nuclear budding, chromosome stickiness, chromosome bridges, bizarre nuclei, ball arrangement of chromosomes, diagonal orientation of chromosomes, pulverised chromatin, giant cells, abnormal condensation of chromosomes, stellate arrangement of chromosomes, misorientation of chromosomes, scattering of chromosomes, shift in MTOC, disturbed

chromosomes, C-metaphase, micronuclei, chromosome clumping, binucleate cells, stathmo anaphase *etc*.

Cytoplasmic heteropyknosis, formation of macro and micro cells, hyperchromasia, chromosome gaps, hypercondensation, cytostasis, chromosome doubling and somatic pairing, unequal separation of chromosomes, early cell plate formation, contorted chromosomes, nuclear extrusion, nuclear enlargement, nuclear disintegration, cytomixis, nuclear fragmentation *etc*. were observed rarely.

Mitotic indices of the root tip meristems of *A. cepa* treated with the aqueous extract of *H. tenuis* were found to be decreasing with increasing concentration of the extract. The mitotic indices of treated roots were lower than the roots treated with negative control (Tables 1-6) and greater than the roots treated with positive control (Tables 7-12). While the roots treated with the fungal extract showed considerable extend of abnormalities. The abnormality percentage of the treated roots was found to be higher than that of negative control. Thus it was clearly observed that the percentage of abnormalities of the treated roots lies in between the positive and negative control. From these observations, it was clear that the percentage of abnormality gradually increases with increase in the concentration of the extract.

0.005 % Extract of *H. tenuis*

Allium cepa roots treated with 0.005 % of the extract showed mitotic index, which was less than that of negative control and greater than that of positive control. Percentage of abnormality was found to be increasing with increase in the duration of time (½, 1, 2, 3 and 24 h).

Abnormalities like nuclear erosion, nuclear lesion, pulverised chromatin, nuclear peak, nuclear extrusion, abnormal condensation, nuclear expulsion, macro and micro cell formation, chromatin fragments, chromatin disintegration, coagulation of chromatin, stickiness, chromosome clumping, disturbed metaphase, contorted chromosomes, diagonal metaphase, ball metaphase, chromosome displacement, early movement of chromosomes, chromosome bridge, broken bridge, misorientation, unequal separation of chromosome, early cell plate formation, giant cells, micronucleus, abnormal association, chained chromosomes, C-metaphase, pole to pole arrangement of chromosomes, laggards, double nuclear lesion, partial-

C-metaphase, hypoploid cell, hyperploid cell, shift in MTOC, strap cells, cell fragmentation and multiple bridges were observed.

Rare abnormalities such as formation of macro and micro cell, unequal separation of chromosomes, nuclear extrusion, strap cells, contorted chromosome, nuclear disintegration, nuclear fragmentation and early cell plate formation were also obtained in roots treated with 0.005 % of *H. tenuis* extract.

Roots treated with 0.005 % of the fungal extract have shown different abnormalities which were positively correlated with the treatment duration (Table 13).

0.01% Extract of *H. tenuis*

Mitotic indices of the *A. cepa* root tips treated with 0.01 % of the fungal extract were found to be greater than that of positive control and lesser than that of negative control. Percentage of abnormality was gradually increasing with increase in the treatment duration (Table 13).

Abnormalities like pulverised chromatin, chromatin disintegration, nuclear erosion, nuclear lesion, nuclear peak, nuclear extrusion, stickiness, abnormal condensation, nuclear expulsion, macro and micro cell formation, chromatin fragments, coagulation of chromatin, chromosome clumping, disturbed metaphase, laggards, contorted chromosomes, diagonal metaphase, ball metaphase, chromosome displacement, early movement of chromosomes, chromosome bridge, broken bridge, misorientation, unequal separation of chromosome, giant cells, micronucleus, abnormal association, chained chromosomes, hypoploid cell, strap cells, C-metaphase, pole to pole arrangement of chromosomes, multiple bridges, partial-C-metaphase, shift in MTOC, early cell plate formation, double nuclear lesion, ghost cell, hypercondensation, scattering of chromosomes, tripolar movement in the cell, cytoplasmic heteropyknosis, cytoplasmic shrinkage, hyperploid cell, multiple narrow bridge, cell fragmentation and stathmo anaphase were observed.

Macro and micro cell formation, strap cells, hypercondensation, unequal separation of chromosomes, nuclear extrusion, cytoplasmic heteropyknosis, contorted chromosome, nuclear disintegration, nuclear fragmentation and early cell plate formation constitute the observed rare abnormalities.

0.05 % Extract of *H. tenuis*

Mitotic indices of *A. cepa* root tips treated with 0.05 % of the fungal extract were found to be in between positive and negative control. Percentage of abnormality was found to be increasing with increase in the time duration upto 2 h. While a gradual reduction in the abnormality percentage was also observed for 3 h treatment duration. But for 24 h treatment duration, the percentage of abnormality increased further (Table 13).

Abnormalities like abnormal condensation, pulverised chromatin, coagulation of chromatin, chromatin disintegration, nuclear erosion, nuclear lesion, stickiness, nuclear peak, nuclear extrusion, nuclear expulsion, macro and micro cell formation, chromatin fragments, chromosome clumping, disturbed metaphase, laggards, shift in MTOC, contorted chromosomes, ball metaphase, chromosome displacement, multiple bridges, early movement of chromosomes, chromosome bridge, broken bridge, unequal separation of chromosome, micronucleus, abnormal association, chained chromosomes, hyperploid cell, hypoploid cell, polyploid cell, strap cells, C-metaphase, pole to pole arrangement of chromosomes, partial-C-metaphase, early cell plate formation, double nuclear lesion, ghost cell, hypercondensation, diagonal metaphase, misorientation, scattering of chromosomes, tripolar movement in the cell, multiple narrow bridge, cell fragmentation, giant cells, stathmo anaphase, chromosome gap and diagonal stathmo anaphase, hyperchromasia and bridged binucleate cell were observed.

Chromosome gap, hypercondensation, unequal separation of chromosomes, nuclear extrusion, contorted chromosome, macro and micro cell formation strap cells, nuclear disintegration, hyperchromasia, nuclear fragmentation and early cell plate formation constitute the observed rare abnormalities.

0.1 % Extract of *H. tenuis*

Allium cepa root tips treated with 0.1 % fungal extract showed mitotic indices which were lesser than the negative control and greater than the positive control. Abnormality percentage was found to be decreasing at 3 h treatment duration (Table 13).

Abnormalities like stellate chromosomes, cytomictic channel formation, cytomictic channel fusion, cytostasis, stickiness, pulverised chromatin, coagulation of chromatin, chromatin disintegration, partial-C-metaphase, nuclear erosion, nuclear lesion, nuclear peak,

chromosome displacement, nuclear extrusion, nuclear expulsion, strap cells, hyperchromasia, macro and micro cell formation, chromatin fragments, chromosome clumping, abnormal condensation, disturbed metaphase, laggards, shift in MTOC, contorted chromosomes, multiple bridges, early movement of chromosomes, chromosome bridge, broken bridge, unequal separation of chromosome, micronucleus, abnormal association, chained chromosomes, hyperploid cell, hypoploid cell, polyploid cell, strap cells, C-metaphase, pole to pole arrangement of chromosomes, early cell plate formation, double nuclear lesion, ghost cell, hypercondensation, diagonal metaphase, misorientation, scattering of chromosomes, tripolar cells, multiple narrow bridge, cell fragmentation, giant cells, ball metaphase, stathmo anaphase, cytomixis, vagrant chromatin, tropokinesis, hemistellate chromosomes, chromosome gap, diagonal stathmo anaphase, nuclear enlargement, binucleate cell, bizarre nuclei, nuclear budding and chromosome doubling and somatic pairing were obtained.

The rare abnormalities observed include cytostasis, nuclear extrusion, nuclear fragmentation, chromosome gap, hypercondensation, hyperchromasia, strap cells, unequal separation of chromosomes, contorted chromosome, macro and micro cell formation, cytomixis, early cell plate formation, cytomictic channel formation, cytomictic channel fusion, nuclear enlargement, nuclear disintegration, chromosome doubling and somatic pairing.The frequency of abnormalities was found to be higher for 24 h treatment duration in all the concentration when compared to the other treatment durations such as ½, 1, 2 and 3 hours. Among this, roots treated with higher concentration (0.1 %) of the extract resulted in much higher rate of abnormality and it was comparable to the positive control.

Table No. 1

½ h treatment of *Allium cepa* root meristem in distilled water (Negative control)

Total No. of cells	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cytokinesis	Mitotic index (%)
144	33	81	15	9	4	2	77.08
160	40	91	13	8	6	2	75.00
120	28	71	8	8	3	2	76.66
139	32	74	15	11	6	1	76.97
164	40	84	17	14	6	3	75.60
157	36	93	13	10	3	2	77.07
						Average	**76.39**

Table No. 2

1h treatment of *A. cepa* root meristem in distilled water (Negative control)

Total No. of cells	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cytokinesis	Mitotic index (%)
143	27	76	18	11	7	4	81.11
145	31	77	17	12	6	2	78.62
137	30	74	16	10	5	2	78.10
157	37	81	15	14	7	3	76.43
154	34	83	17	13	6	1	77.92
126	30	68	13	9	4	2	76.19
						Average	**78.02**

Table No. 3

2 h treatment of *A. cepa* root meristem in distilled water (Negative control)

Total No. of cells	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cytokinesis	Mitotic index (%)
128	29	69	11	12	6	1	77.34
134	31	73	10	13	5	2	76.86
147	32	79	14	12	7	3	78.23
138	28	70	16	14	8	2	79.71
141	35	76	12	12	5	1	75.17
136	31	70	13	14	6	2	77.2
						Average	**77.4**

Table No. 4

3 h treatment of *A. cepa* root meristem in distilled water (Negative control)

Total No. of cells	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cytokinesis	Mitotic index (%)
161	38	79	16	17	8	3	76.39
140	33	70	15	14	6	2	76.42
129	28	69	13	11	7	1	78.29
139	31	70	17	13	6	2	77.69
147	36	74	15	12	8	2	75.51
145	34	76	13	14	5	3	76.55
						Average	**76.80**

Table No. 5

24 h treatment of *A. cepa* root meristem in distilled water (Negative control)

Total No. of cells	Interphase	Prophase	Metaphase	Anaphase	Telophase	Cytokinesis	Mitotic index (%)
156	30	90	12	14	7	3	80.76
153	31	92	13	10	5	2	79.73
149	29	89	12	11	6	2	80.53
142	30	86	12	9	4	1	78.87
146	28	90	10	11	4	3	80.82
136	27	79	11	12	5	2	80.14
Average							**80.14**

Table No. 6

Summary of treatment of *A. cepa* root meristem in distilled water (Negative control)

Treatment period	½ h	1 h	2 h	3 h	24 h
Total No. of cells	884	862	824	861	882
Interphase	209	189	186	200	175
Prophase	494	459	437	438	526
Metaphase	81	96	76	89	70
Anaphase	60	69	77	81	67
Telophase	28	35	37	40	31
Cytokinesis	12	14	11	13	13
Mitotic index (%)	**76.4**	**78.0**	**77.4**	**76.8**	**80.1**

Table No. 7

½ h treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

Total No. of cells	Interphase		Prophase		Metaphase		Anaphase		Telophase		Cytokinesis		Mitotic index (%)	Abnormality (%)
	N	A	N	A	N	A	N	A	N	A	N	A		
118	35	41	8	12	4	7	2	4	-	2	1	2	35.59	57.62
133	39	43	9	10	5	8	7	6	2	2	2	-	38.34	51.87
125	30	47	7	9	6	7	4	8	1	3	1	1	38.40	60.00
122	38	46	8	10	5	6	3	2	1	2	-	1	31.14	54.91
136	41	45	10	7	7	9	3	5	3	2	3	1	36.76	50.73
145	45	43	11	12	10	8	4	3	4	2	1	2	39.31	48.27
Average													**36.59**	**53.90**

N – Normal; A – Abnormal

Table No. 8

½ h treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

Total No. of cells	Interphase		Prophase		Metaphase		Anaphase		Telophase		Cytokinesis		Mitotic index (%)	Abnormality (%)
	N	A	N	A	N	A	N	A	N	A	N	A		
134	40	42	5	12	4	10	4	8	1	4	1	3	38.80	58.95
157	53	57	5	8	3	11	5	7	1	5	-	2	29.93	57.32
128	37	50	4	7	4	8	3	6	1	4	1	3	32.03	60.93
142	44	56	6	10	3	7	4	5	2	3	2	-	29.57	57.04
129	39	52	3	8	3	9	2	6	2	3	-	2	29.45	62.01
150	49	60	4	9	5	7	4	6	3	1	1	1	27.33	56.00
Average													**31.18**	**58.70**

N – Normal; A – Abnormal

Table No. 9

½ h treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

Total No. of cells	Interphase		Prophase		Metaphase		Anaphase		Telophase		Cytokinesis		Mitotic index (%)	Abnormality (%)
	N	A	N	A	N	A	N	A	N	A	N	A		
138	42	57	4	9	3	8	2	7	1	3	-	2	28.26	62.31
146	43	59	4	10	4	9	3	6	2	2	3	1	30.13	59.58
163	50	62	5	9	3	12	5	8	2	4	1	2	31.28	59.50
152	47	64	3	8	3	10	4	7	1	3	-	2	26.97	61.84
138	42	53	4	10	2	9	3	6	1	4	1	3	31.15	61.59
142	43	61	3	12	4	6	2	5	3	1	1	1	26.76	60.56
Average													**29.09**	**60.89**

N – Normal; A – Abnormal

Table No. 10

½ h treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

Total No. of cells	Interphase		Prophase		Metaphase		Anaphase		Telophase		Cytokinesis		Mitotic index (%)	Abnormality (%)
	N	A	N	A	N	A	N	A	N	A	N	A		
130	43	59	3	8	2	6	1	4	1	2	-	1	21.53	61.53
132	38	57	4	9	3	7	2	5	1	3	1	2	28.03	62.87
148	42	65	5	10	5	9	2	6	1	2	-	1	27.70	62.83
162	48	69	3	14	4	12	1	3	2	2	1	3	27.77	63.58
135	38	57	3	8	3	7	4	6	1	4	1	3	29.62	62.96
160	50	67	4	11	2	10	2	7	1	3	1	2	26.87	62.50
Average													**26.92**	**62.71**

N – Normal; A – Abnormal

Table No. 11

½ h treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

Total No. of cells	Interphase		Prophase		Metaphase		Anaphase		Telophase		Cytokinesis		Mitotic index (%)	Abnormality (%)
	N	A	N	A	N	A	N	A	N	A	N	A		
139	35	70	4	7	2	5	3	6	1	3	1	2	24.46	66.90
128	30	67	4	6	2	7	2	5	1	2	1	1	24.21	68.75
135	36	64	2	6	2	9	2	7	2	4	-	1	25.92	67.40
153	35	78	2	9	3	11	1	6	1	3	1	3	26.14	71.89
160	41	79	3	8	2	8	2	9	1	4	1	2	25.00	68.75
119	27	64	3	7	3	6	1	4	1	2	-	1	23.52	70.58
Average													**24.87**	**69.04**

N – Normal; A – Abnormal

Table No. 12

Summary of treatment of *A. cepa* root meristem in H_2O_2 (Positive control)

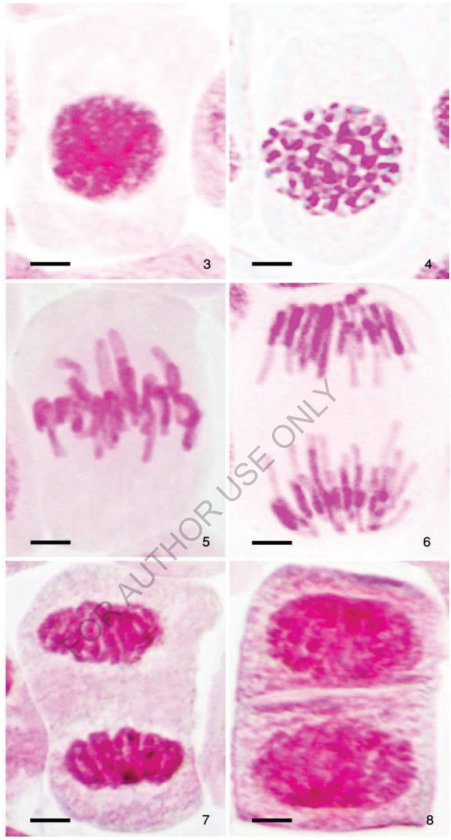
Treatment period	½ h	1 h	2 h	3 h	24 h
Total No. of cells	779	840	879	867	834
Interphase	493	579	623	633	626
Prophase	113	81	81	82	61
Metaphase	82	74	73	70	60
Anaphase	51	60	58	43	48
Telophase	24	30	27	23	25
Cytokinesis	15	16	17	16	14
Mitotic Index (%)	**36.6**	**31.1**	**29.1**	**26.9**	**24.9**
Abnormality (%)	**53.9**	**58.7**	**60.9**	**62.71**	**69.04**

Table No. 13

Summary of cytotoxicity of *Hexagonia tenuis* aqueous extract on *Allium cepa* root meristem

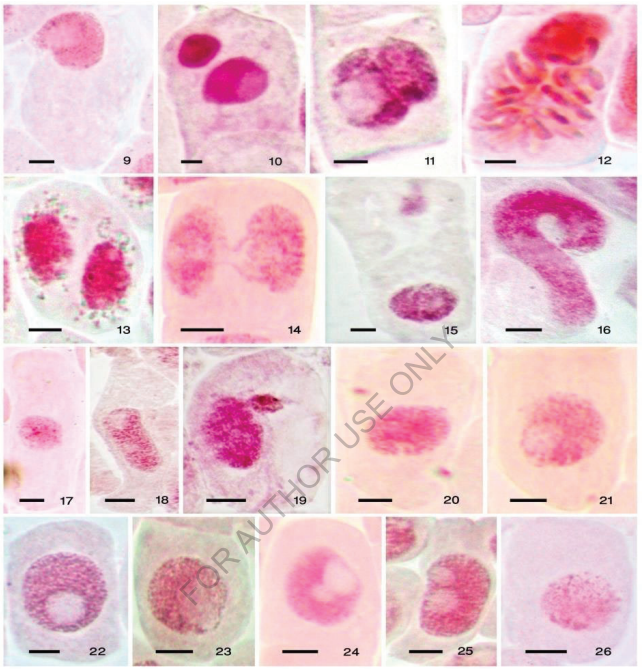
Concentration of the extract (%)	Treatment duration (hour)	Mitotic index (%)	Abnormality (%)
0.005	½	66.04	39.23
	1	64.40	42.32
	2	59.80	44.02
	3	55.83	47.57
	24	61.50	49.80
0.01	½	60.16	41.48
	1	61.14	43.49
	2	57.12	44.58
	3	51.73	50.29
	24	56.70	53.54
0.05	½	53.88	52.31
	1	55.91	54.32
	2	50.64	54.41
	3	48.12	51.93
	24	53.99	56.25
0.1	½	44.55	53.52
	1	51.57	56.13
	2	47.18	56.36
	3	46.82	55.78
	24	39.51	60.45

PLATE-2



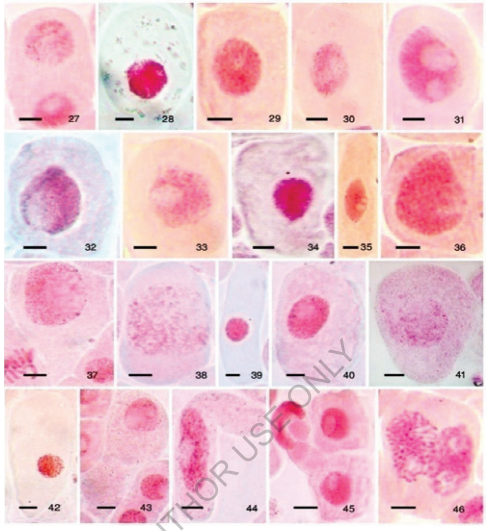
Normal mitotic stages in *Allium cepa* L. Figs 3-8: 3. Interphase, 4. Prophase, 5. Metaphase, 6. Anaphase, 7. Telophase, 8. Cytokinesis

PLATE-3



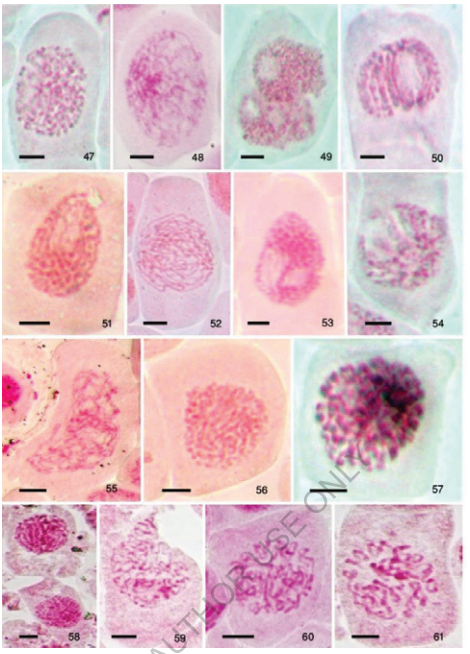
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 9-26: Interphase; 9 Nuclear expulsion, 10 Binucleate cell, 11 Binucleate cell showing lesion, 12 Binucleate cell with one at interphase and other at metaphase, 13 Nuclear disintegration in a binucleate cell showing ghost cell formation, 14 Bridged binucleate cell, 15 Bizarre binucleate cell, 16 Bizarre nucleus, 17 Abnormal condensation, 18 Cell fragmentation, 19 Micronucleus, 20 Cell showing two micronuclei, 21 Nuclear lesion and micronucleus, 22 Nuclear lesion, 23 Formation of nuclear lesion, 24 Complex nuclear lesion, 25 Double nuclear lesion, 26 Nuclear disintegration

PLATE-4



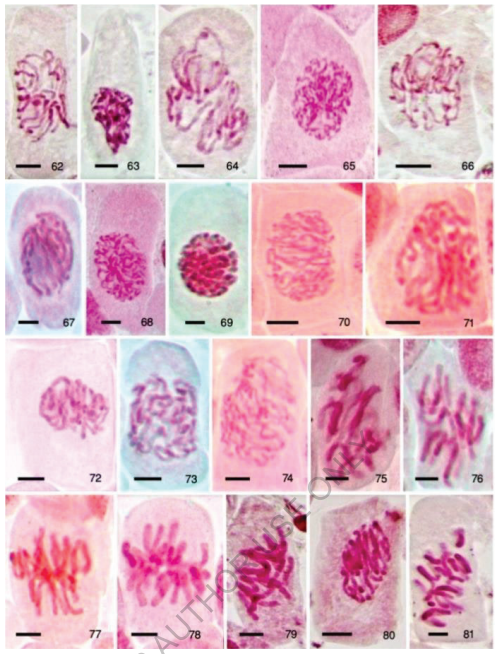
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 27-46: Interphase; 27 Nuclear disintegration in a binucleate cell, 28 Nuclear disintegration and ghost cell formation, 29 Nuclear disintegration and lesion, 30 Chromatin erosion, 31 Nuclear erosion and double lesions, 32 Nuclear erosion and formation of budding, 33 Nuclear erosion and lesion, 34 Nuclear peak, 35 Nuclear peak and lesions, 36 Nuclear enlargement, 37 Nuclear extrusion, 38 Pulverised chromatin, 39 Hyperchromasia, 40 Cytoplasmic shrinkage, 41 Ghost cell, 42 Cytoplasmic heteropyknosis, 43 Cytomictic channel, 44 Cytomictic channel formation, 45 Cytomictic channel fusion, 46 Non synchronized binucleate cell

PLATE 5



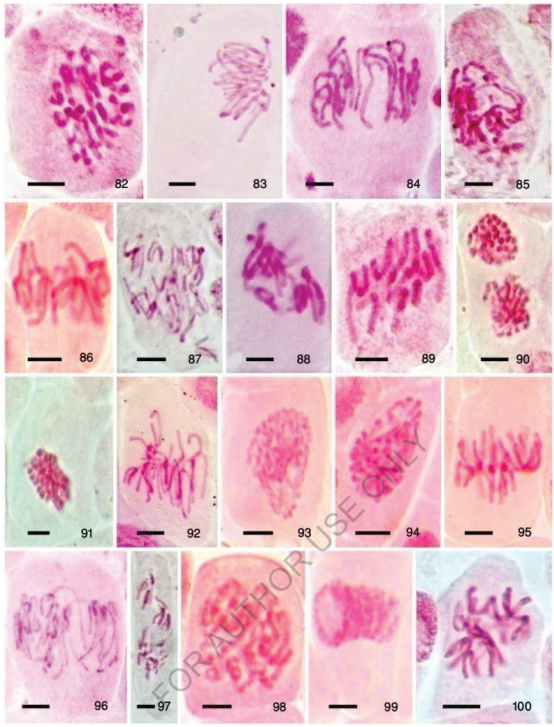
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 47-61: Prophase; 47 Pulverised chromatin, 48 Sticky chromatin, 49 Binucleate cell with single lesions, 50 Nuclear lesion, 51 Nuclear and cytoplasmic lesions, 52 Formation of lesion, 53 Double nuclear lesion, 54 Chromatin disintegration, 55 Chromatin erosion, 56 Chromatin fragmentation, 57 Chromatin gaps, 58 Cytomixis, 59 Nuclear and cellular extrusion at late prophase, 60 Abnormal association at late prophase, 61 Abnormal condensation at late prophase

PLATE 6



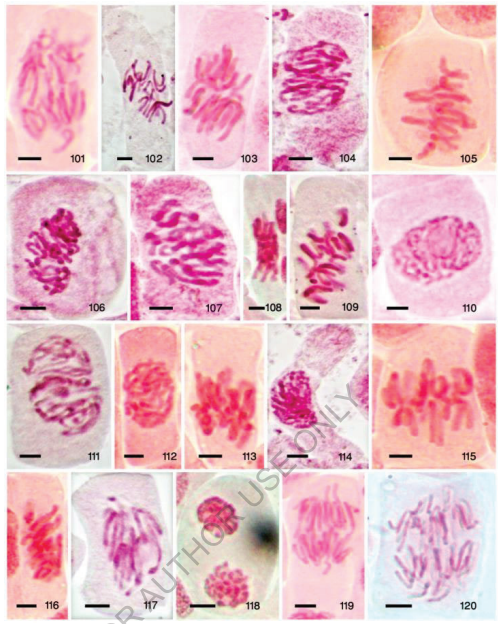
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 62-81: Metaphase; 62 Abnormal condensation at early metaphase, 63 Chromosome clumping at early metaphase, 64 Chromosome erosion at early metaphase, 65 Chromosome gaps and fragmentation at early metaphase, 66 Fragmentation at early metaphase, 67 Formation of lesion in early ball metaphase, 68 Early ball metaphase, 69 Ball metaphase, 70 Ball metaphase in a polyploid cell, 71 Chained early metaphase, 72 Chained metaphase, 73 Chained metaphase in a polyploid cell, 74 Chained scattered metaphase in a polyploid cell, 75 Contorted chromosomes, 76 Cytostasis, 77 Chromosome doubling and somatic pairing, 78 Chromosome disintegration, 79 Diagonal metaphase, 80 Diagonal displaced metaphase, 81 Diagonal metaphase showing vagrant chromosomes

PLATE 7



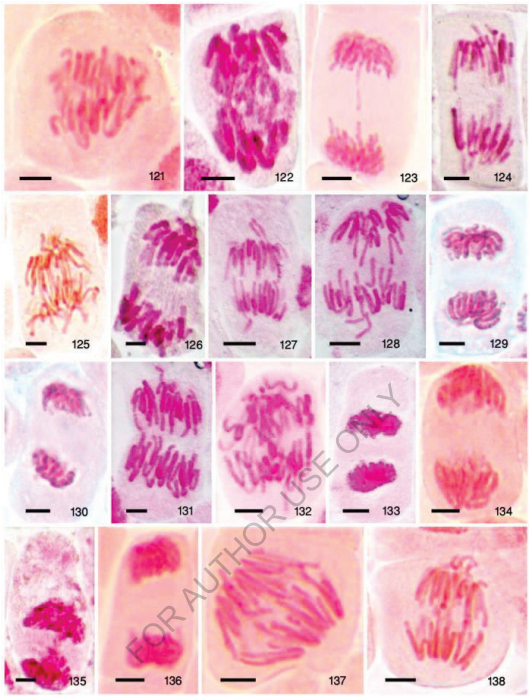
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 82-100: Metaphase; 82 Displaced early ball metaphase, 83 Displaced early metaphase, 84 Disturbed early metaphase, 85 Disturbed early metaphase in a hypoploid cell, 86 Disturbed metaphase, 87 Disturbed metaphase in a polyploid cell, 88 Hypoploid cell, 89 Hypercondensed chromosomes, 90 Hypercondensed double metaphase, 91 Hypercondensed sticky chromosomes, 92 Misorientation of chromosomes, 93 Misoriented early ball metaphase, 94 Misoriented ball metaphase, 95 Partial C-metaphase, 96 Pulverised chromosomes at early metaphase, 97 Pulverised chromosomes showing abnormal arrangement, 98 Pulverised chained chromosomes at early metaphase, 99 Pulverised chromosomes in hyperploid cell, 100 Scattered metaphase

PLATE-8



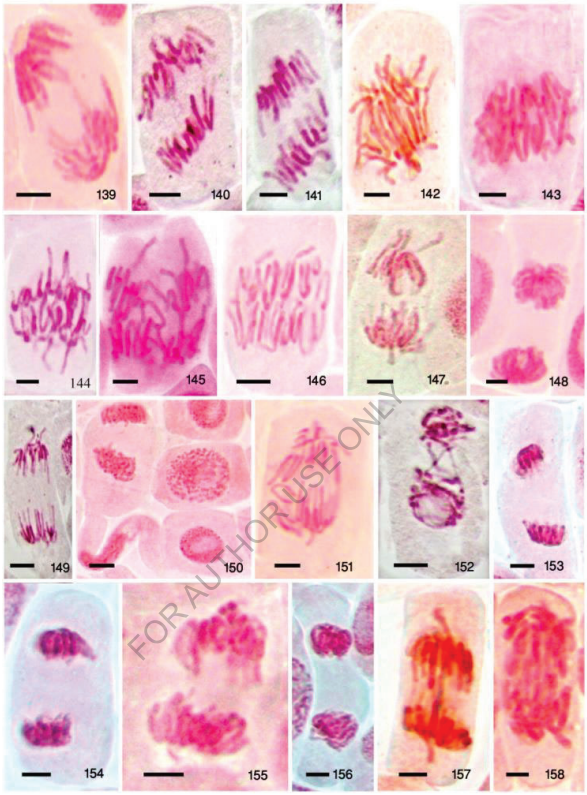
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 101-117: Metaphase; 101 Scattered metaphase in a polyploid cell, 102 Tropokinesis, 103 Stellate metaphase, 104 Pole to pole arrangement of chromosomes at early metaphase, 105 Pole to pole arrangement of chromosomes at metaphase, 106 Sticky early metaphase showing pole to pole arrangement, 107 Sticky early metaphase, 108 Sticky metaphase, 109 Sticky diagonal metaphase, 110 Sticky early ball metaphase, 111 Sticky early ball metaphase showing misorientation, 112 Sticky ball metaphase, 113 Sticky chained metaphase, 114 Sticky chained metaphase in a hyperploid cell, 115 Sticky C-metaphase, 116 Sticky disturbed pole to pole metaphase, 117 Sticky metaphase in a hypoploid cell, Figs 118-120: Anaphase; 118 Ball anaphase, 119 Chromosome bridge at early anaphase, 120 Chromosome bridges

PLATE- 9



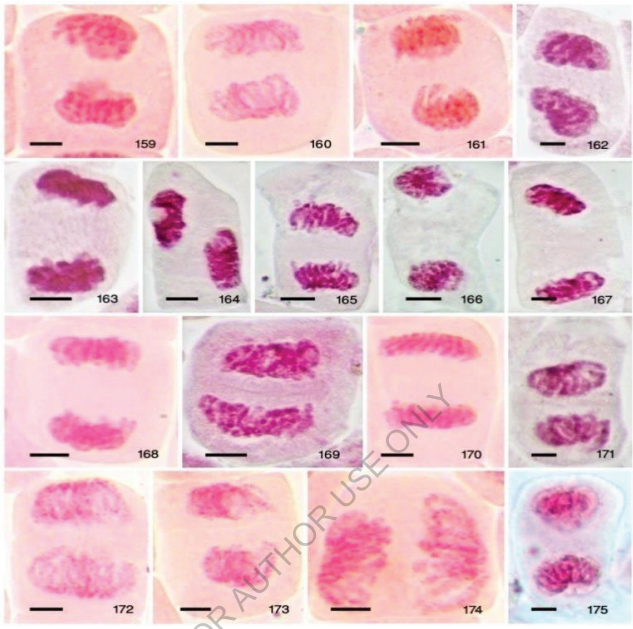
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 121-138: Anaphase; 121 Bridges showing disintegration, 122 Bridges and laggards in a sticky hypoploid cell, 123 Broken chromosome bridge, 124 Broken chromosome bridge and early movement, 125 Multiple bridges, 126 Multiple narrow bridges, 127 Early movement of chromosomes, 128 Chromosome laggards and early movement, 129 Early cell wall formation, 130 Chromosome erosion, 131 Chromosome fragments, 132 Fragmentation of chromosome with a bridge, 133 Coagulated chromosomes, 134 Coagulated late anaphase, 135 Coagulated anaphase with a bridge, 136 Coagulated diagonal anaphase, 137 Diagonal anaphase, 138 Diagonal anaphase showing early movement

PLATE- 10



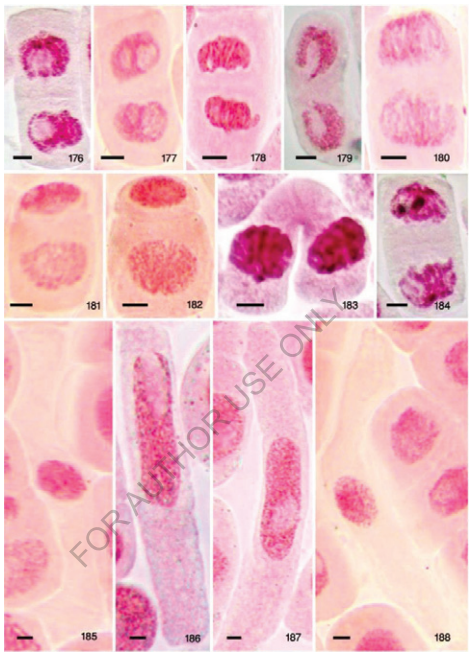
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 139-158: Anaphase; 139 Diagonal anaphase showing laggards, 140 Diagonal sticky anaphase, 141 Diagonal sticky anaphase with early movement, 142 Diagonal stathmo anaphase, 143 Stathmo anaphase, 144 Stathmo anaphase showing erosion, 145 Disturbed anaphase, 146 Sticky disturbed anaphase, 147 Hemistellate anaphase, 148 Sticky stellate anaphase, 149 Misorientation of chromosomes, 150 Cytomixis, 151 Tripolar movement, 152 Unequal separation in disturbed anaphase showing diagonal bridges, 153 Shift in MTOC at late anaphase, 154 Sticky anaphase, 155 Stickiness and fragments at early anaphase, 156 Sticky late anaphase, 157 Sticky anaphase showing chromosome bridges, 158 Sticky multiple bridges

PLATE-11



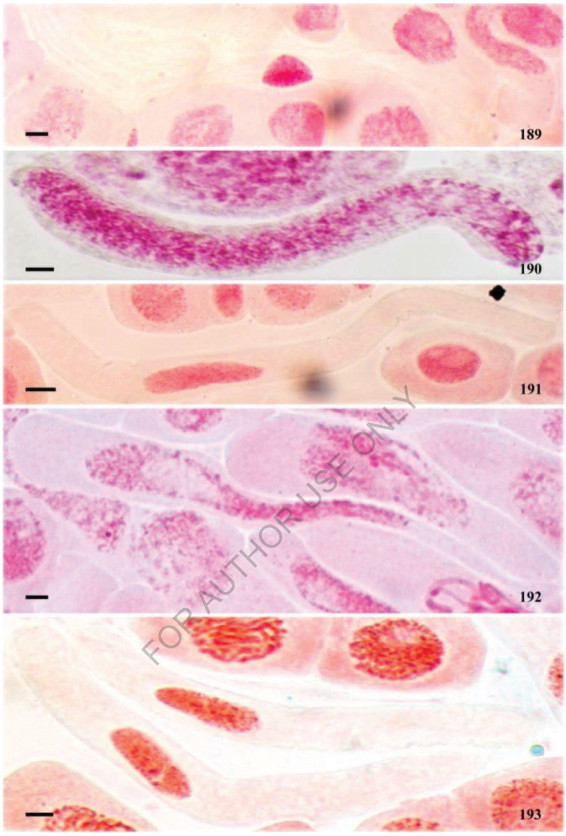
Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 159-171: Telophase; 159 Ball telophase, 160 Chromosome erosion, 161 Chromosome disintegration, 162 Coagulated telophase, 163 Coagulated telophase with brokoen bridge, 164 Disturbed telophase after equatorial separation showing chromatin erosion, 165 Pulverised fragments, 166 Pulverised telophase showing shift in MTOC, 167 Shift in MTOC at late telophase, 168 Sticky telophase, 169 Sticky telophase showing early cell plate formation, 170 Sticky telophase showing fragment, 171 Vagrant chromosome, Figs 172-175: Cytokinesis; 172 Chromatin bridge, 173 Chromatin disintegration and displacement, 174 Chromatin disintegration after equatorial separation, 175 Coagulated chromatin and micronucleus

PLATE-12



Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 176-188: Cytokinesis; 176 Lesion, 177 Double nuclear lesions, 178 Vagrant chromatin at early cytokinesis, 179 Nuclear erosion, 180 Pulverised chromatin, 181 Pulverised chromatin and formation of macro and micro cells, 182 Macro and micro cell formation, 183 Stickiness, 184 Sticky cytokinesis showing abnormal condensation, Figs 185-188: Giant cells; 185 Nuclear breakage,

PLATE- 13



Cytological aberrations induced in *Allium cepa* root meristem with the aqueous extract of *Hexagonia tenuis*. Figs 189-193: Strap cells; 189 Cone shaped nucleus, 190 Strap nucleus, 191 Cellular shrinkage and nuclear peak, 192 Strap nucleus showing lesion, 193 Nuclear breakage

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY (GC-MS) ANALYSIS

The chemical profile of the methanolic extract of *Hexagonia tenuis* was determined by using GC-MS analysis. Different types of compounds were determined and are enlisted in Table 14. The gas chromatogram obtained for *H. tenuis* is given in Fig. 194 and the mass spectrums of the identified compounds in Fig. 195-203. The total number of chemical compounds detected by GC-MS was found to be 37. The compounds were categorized into 13 classes of compounds such as ester, alcohol, acyl halide, ketone, quinoline, phenol, carboxylic acid, fatty acid ester, fatty acid, alkene, sterol, triterpenoids and hydrocarbons (Table 15).

Among the thirty seven compounds detected in the methanolic extract of *Hexagonia tenuis*, major components include 9-Methyl-10-[2-(10- methylanthracen-9-yl) ethyl] anthracene (14.15%), Linoleic acid (11.88%), Anthraergostatetraenol hexahydrobenzoate (26%), Ergosterol (16.72%), Stellasterol (6.69%), 1,54-Dibromo-Tetrapentacontane (2.9%), Methyl octadeca-8,11-dienoate (2.4%), Anthraergosta-5,7,9,14-tetra-ene (1.84%), Hexacosene (1.8%), 2-Bromo-4-fluoro 2,6-Difluorobenzoic acid (1.59%) and Anthraergostapentene (1.4%).

Among the thirteen classes of compounds obtained, hydrocarbons were found to be the leading group (50.05%). Sterols (24.44%) and fatty acids (11.88%) constitute the other major classes of compounds.

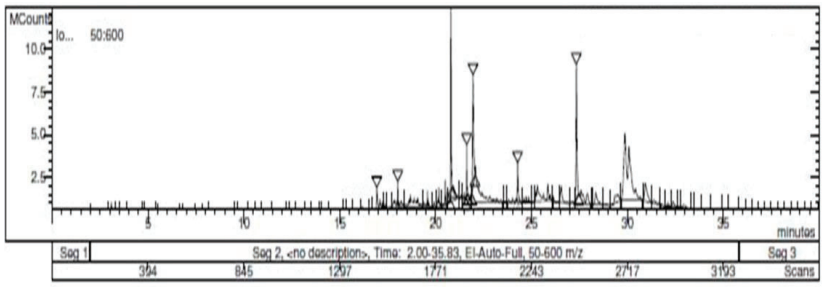


Fig. 194: Gas chromatogram of the methanolic extract of *Hexagonia tenuis*

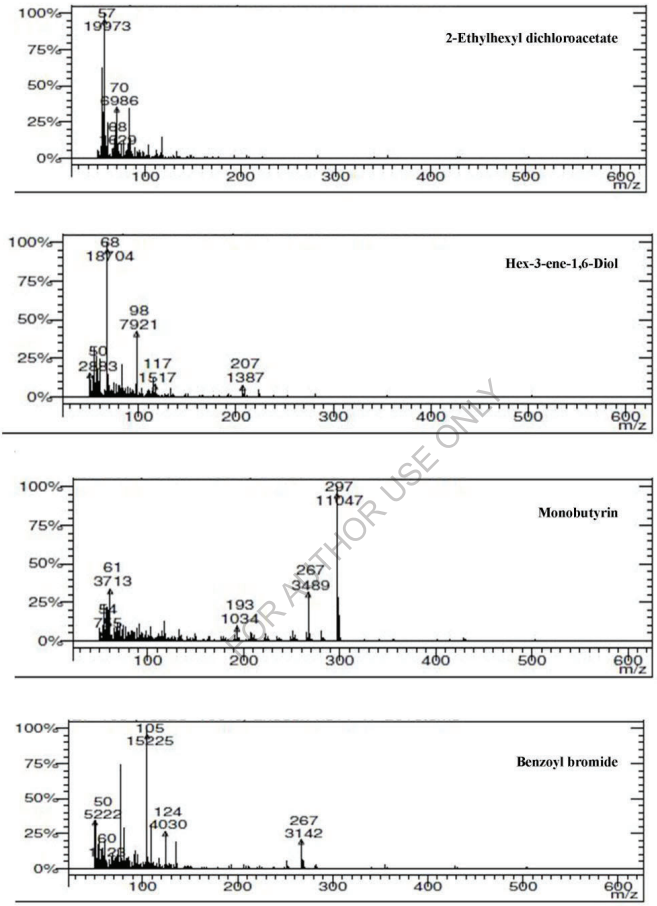


Fig. 195: (i) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

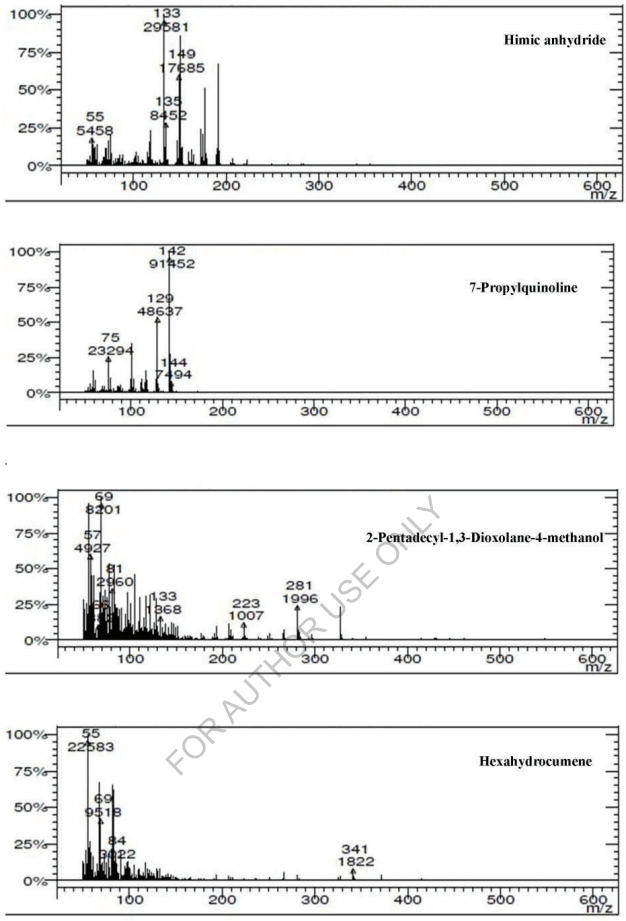


Fig. 196: (ii) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

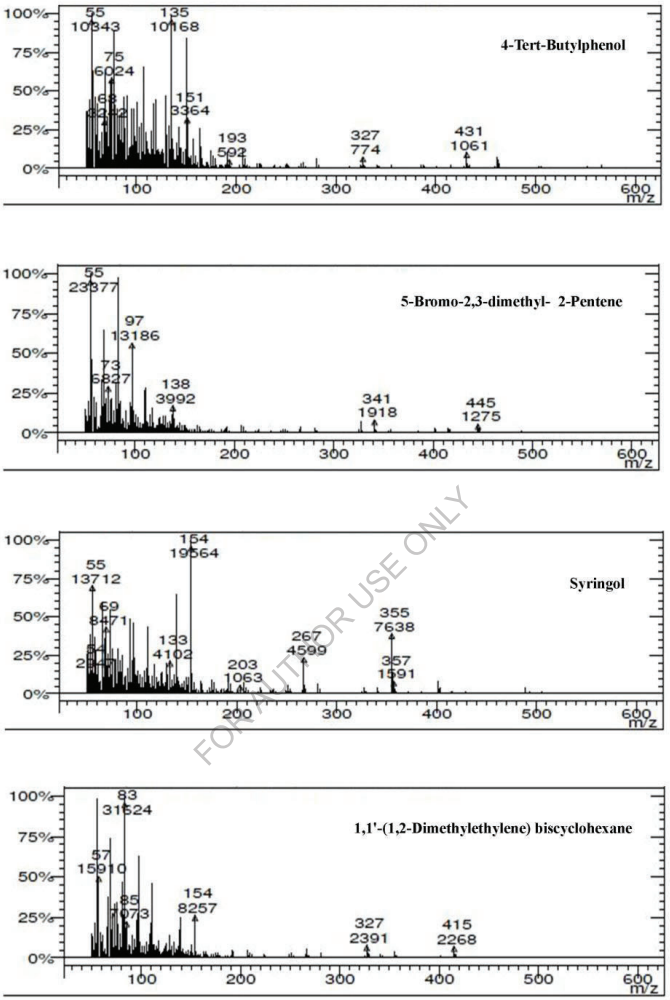


Fig. 197: (iii) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

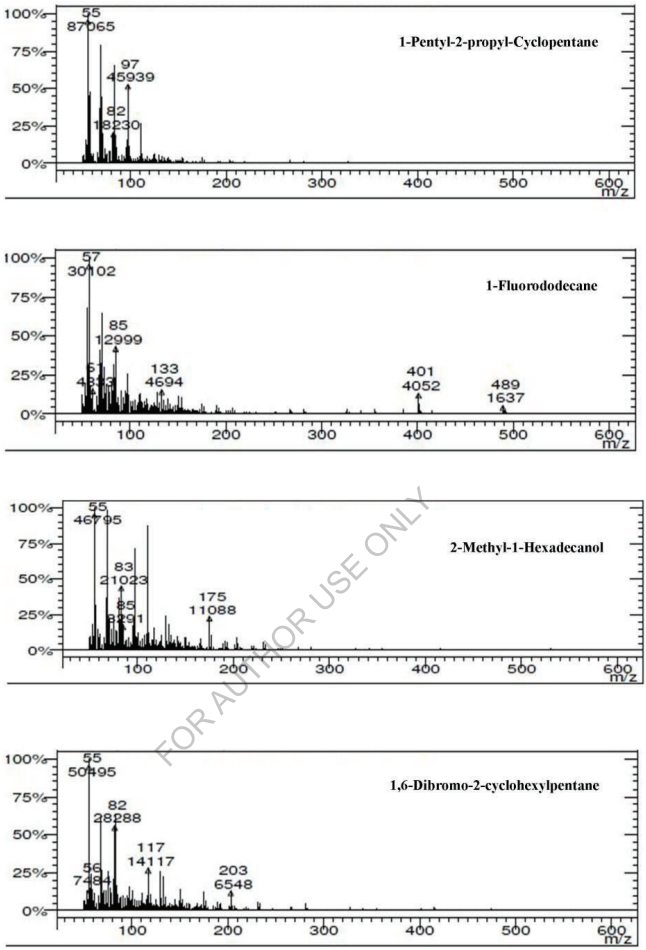


Fig. 198: (iv) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

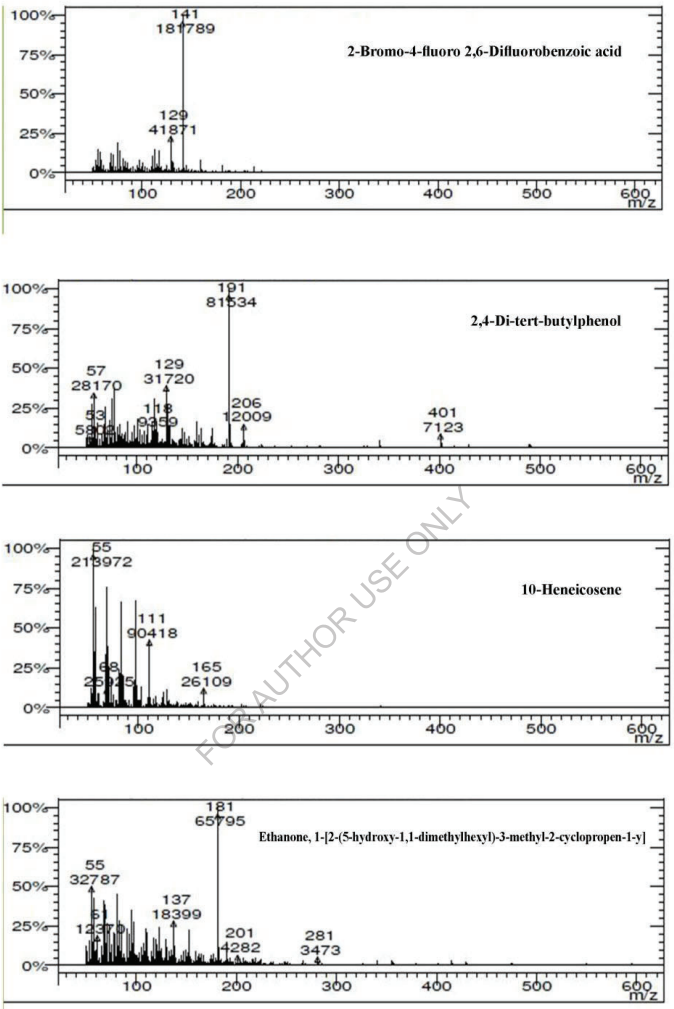


Fig. 199: (v) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

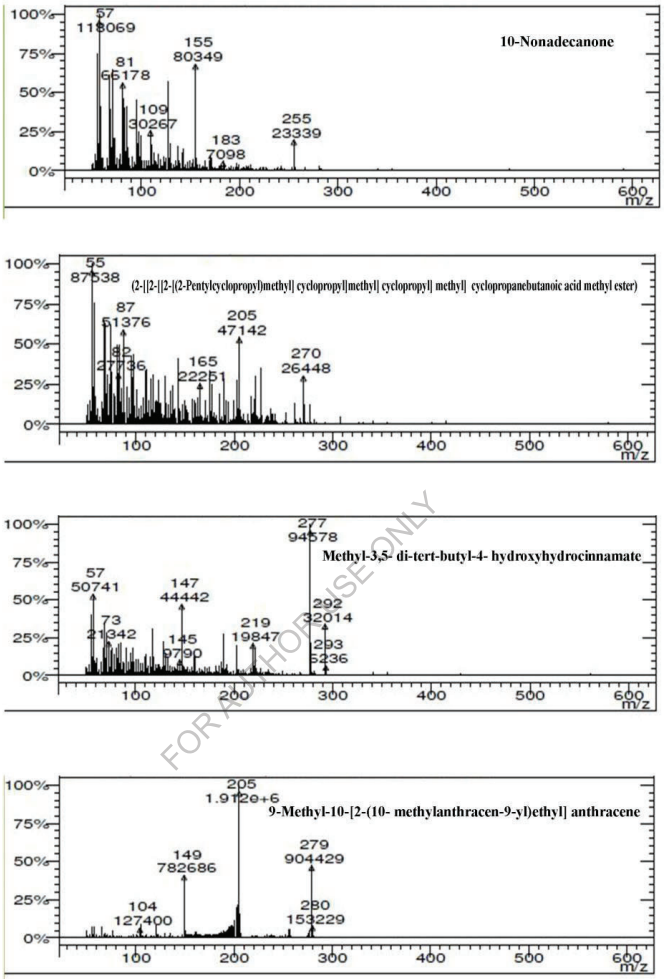


Fig. 200: (vi) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

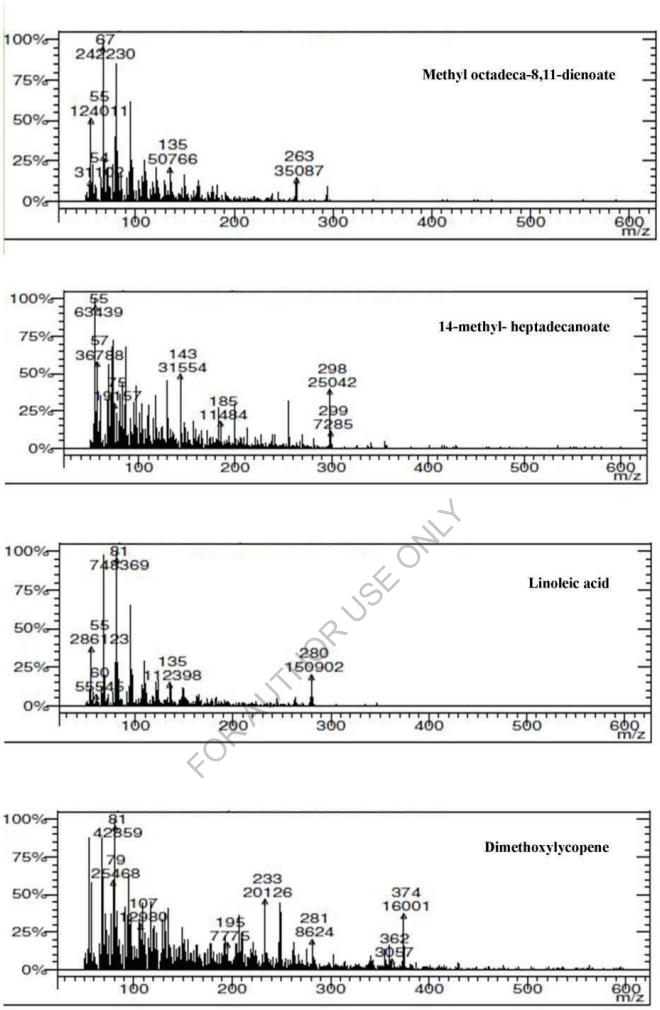


Fig. 201: (vii) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

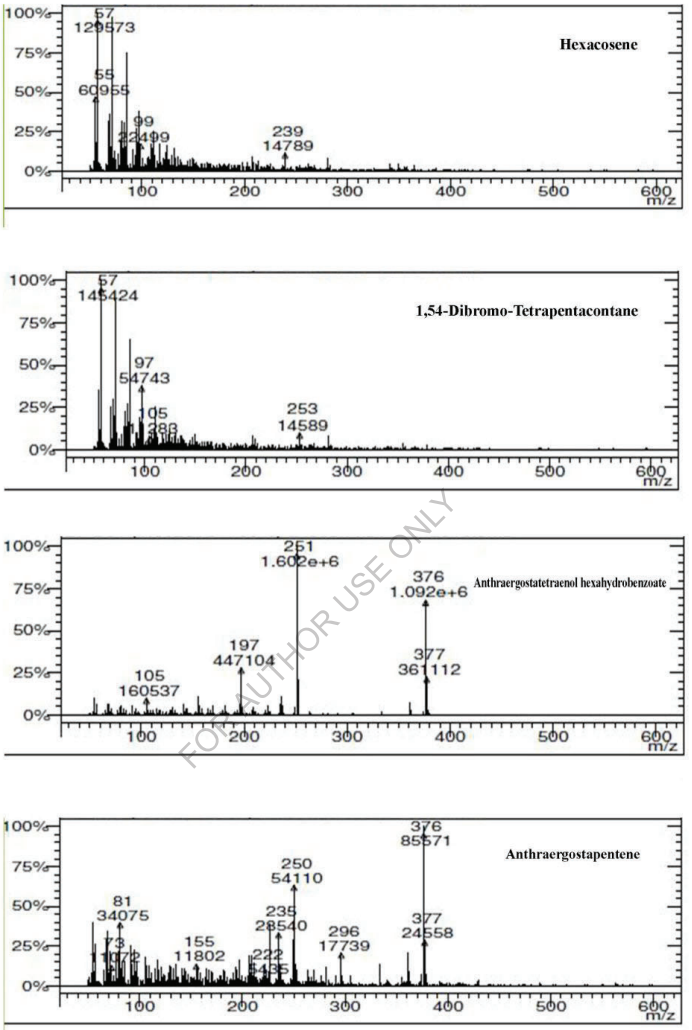


Fig. 202: (viii) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

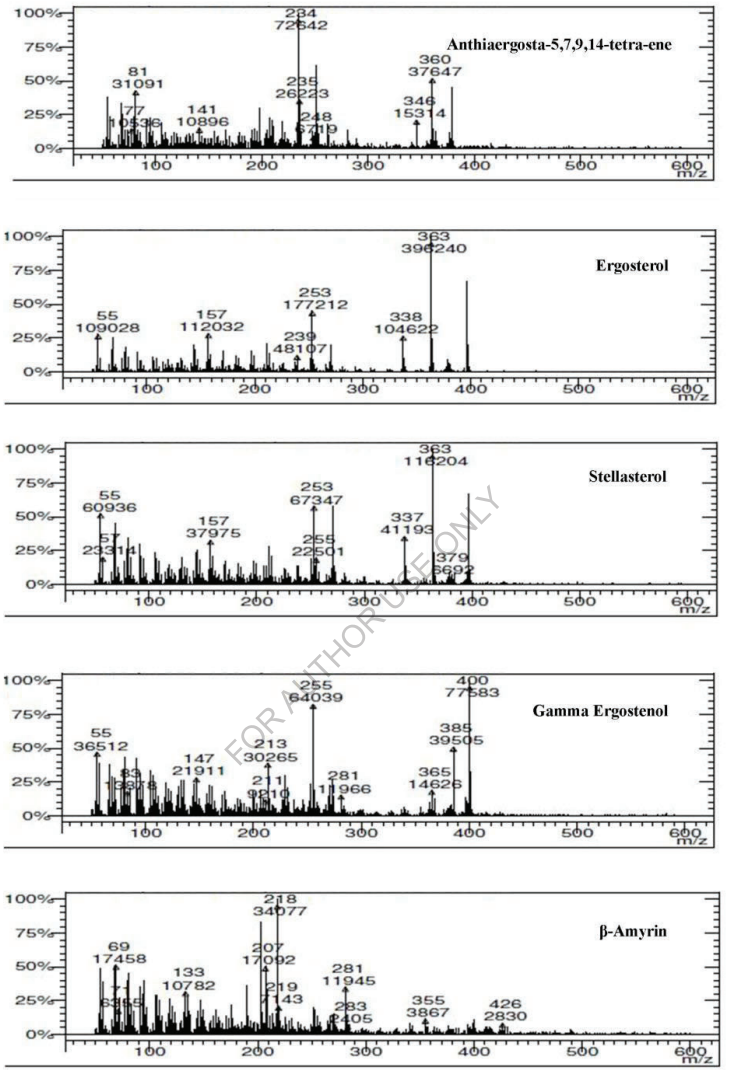


Fig. 203: (ix) Mass spectra of the components detected in the methanolic extract of *Hexagonia tenuis*

Table 14. Phytochemical constituents of methanolic extract of *Hexagonia tenuis* revealed through GC-MS analysis

Sl. No.	Retention time	Constituents	Peak area %
1	9.809	2-Ethylhexyl dichloroacetate	0.2
2	10.111	Hex-3-ene-1,6-Diol	0.3
3	11.359	Monobutyrin	0.1
4	11.758	Benzoyl bromide	0.6
5	12.773	Himic anhydride	0.4
6	13.351	7-Propylquinoline	1.1
7	13.833	2-Pentadecyl-1,3-Dioxolane-4-methanol	0.1
8	14.384	Hexahydrocumene	0.17
9	15.416	4-Tert-Butylphenol	0.15
10	15.772	5-Bromo-2,3-dimethyl- 2-Pentene	0.17
11	15.853	Syringol	0.46
12	15.98	1,1'-(1,2-Dimethylethylene) biscyclohexane	0.18
13	16.177	1-Pentyl-2-propyl-Cyclopentane	0.6
14	16.254	1-Fluorododecane	0.19
15	16.408	2-Methyl-1-Hexadecanol	0.32
16	16.809	1,6-Dibromo-2-cyclohexylpentane	0.3
17	17.22	2-Bromo-4-fluoro 2,6-Difluorobenzoic acid	1.59
18	17.355	2,4-Di-tert-butylphenol	0.8
19	18.023	10-Heneicosene	1.06
20	19.916	Ethanone, 1-[2-(5-hydroxy-1,1-dimethylhexyl)-3-methyl-2-cyclopropen-1-yl]	0.91

21	20.17	10-Nonadecanone	0.61
22	20.504	(2-[[2-[[2-[(2-Pentylcyclopropyl)methyl] cyclopropyl]methyl] cyclopropyl] methyl] cyclopropanebutanoic acid methyl ester)	0.7
23	20.602	Methyl-3,5- di-tert-butyl-4- hydroxyhydrocinnamate	1
24	20.799	9-Methyl-10-[2-(10- methylanthracen-9-yl)ethyl] anthracene	14.15
25	21.623	Methyl octadeca-8,11-dienoate	2.42
26	21.801	14-methyl- heptadecanoate	0.3
27	21.948	Linoleic acid	11.88
28	25.618	Dimethoxylycopene	0.23
29	25.849	Hexacosene	1.8
30	26.55	1,54-Dibromo-Tetrapentacontane	2.9
31	27.341	Anthraergostatetraenol hexahydrobenzoate	26
32	27.576	Anthraergostapentene	1.4
33	27.935	Anthiaergosta-5,7,9,14-tetra-ene	1.84
34	29.875	Ergosterol	16.72
35	30.084	Stellasterol	6.69
36	30.954	Gamma Ergostenol	1
37	32.109	β-Amyrin	0.66

Table 15. Percentage composition of various classes of compounds detected in the methanolic extract of *Hexagonia tenuis* revealed through GC-MS analysis

Sl. No.	Class of compounds	Peak area %
1	Ester	1
2	Alcohol	0.7
3	Acyl halide	0.58
4	Ketone	1.9
5	Quinoline (Nitrogen containing compound)	1.1
6	Phenol	1.4
7	Carboxylic acid	1.59
8	Fatty acid ester	2.7
9	Fatty acid	11.88
10	Alkene	2
11	Sterol	24.44
12	Triterpenoids	0.66
13	Hydrocarbons	50.05
	Total	100

DISCUSSION

Biological diversity forms the wealth of medicines and it act as the platform for medicinal researches. Exploration of natural resources for compounds of therapeutic potential has been an emerging field of medicine. Large scale use of medicinal plants and herbs in the preparation of drugs is increasing day by day due to the growing concern about the side effects of chemicals and synthetic substances. The medicinal plant based drugs have the added advantage of being simple, effective and offering a broad spectrum of activity with an emphasis on the preventive action of drugs. Because of these factors, the demand for plant based medicines is increasing worldwide. Thus, as a biologist our duty is to find out new resources of medicines other than from plant sources only. Because of this aspect, present study has focused on a least explored polyporous fungus, *Hexagonia tenuis*.

Many novel biologically active compounds have been reported as a result of research on medicinal mushrooms (De Silva *et al*., 2013). Bioactive compounds found in medicinal mushrooms may provide anti-cancer action with a minimum of side effects.

In the present study, cytotoxicity of the aqueous extract of *Hexagonia tenuis* by using *Allium cepa* assay was conducted to evaluate the cytotoxic potential. An attempt had also been made for the GC-MS analysis of methanolic extract of *H. tenuis*.

Normal mitotic divisions were observed in *A. cepa* root tips after treatment with distilled water (Figs 3-8; Tables 1-6). When the positive control was used, high frequency of abnormalities was observed (Tables 7-12). A wide range of abnormalities including clastogenic and non clastogenic aberrations were resulted after the treatment of *A. cepa* root meristem with the different concentrations of *H. tenuis* extract.

Nuclear lesion, nuclear budding, nuclear erosion, chromosome stickiness, chromosome bridges, chromosome fragments, bizarre nuclei, pulverised chromatin, giant cells, abnormal condensation of chromosomes, nuclear extrusion, nuclear disintegration, cytomixis, nuclear fragmentation, nuclear enlargement, hyperchromasia, contorted chromosomes, chromosome coagulation, chromosome gap *etc*. were observed as the major clastogenic abnormalities. The observed non clastogenic abnormalities include macro and micro cell formation, binucleate cell, chromosome clumping, C-metaphase, stellate arrangement of chromosomes,

misorientation of chromosomes, polyploidy, hypoploidy, diagonal arrangement of chromosomes, disturbed chromosomes, chromosome laggards, stathmo anaphase, scattering of chromosomes, shift in microtubular organizing centres (MTOC), ball shaped arrangement of chromosomes, pole to pole arrangement of chromosomes, cytostasis, tripolar anaphase, micronuclei, early movement of chromosomes, abnormal association of chromosomes, chromosome doubling and somatic pairing, vagrant chromosomes, unequal separation of chromosomes, early cell plate formation, tropokinesis, chained chromosomes *etc.*

CLASTOGENIC ABNORMALITIES

Nuclear lesion

Nuclear lesions were induced by the treatment of *A. cepa* root tips with all the concentrations of the extract of *H. tenuis* (Figs 11, 21-25, 29, 31, 33, 35, 49-53, 67, 176, 177, 187). The occurrence of nuclear lesions induced by plant derived chemicals in *A. cepa* root tips may be due to the disintegration of portion of nuclear material by the action of the plant extracts (Mercykutty and Stephen, 1980). Studies by Pasqualini *et al*. (2003) revealed that nuclear lesions are associated with programmed cell death in plants. So the wide spread and enormous occurrence of nuclear lesions in all the fungal treatments in the present investigation proclaims the acute cytotoxicity of the extract.

Pulverisation

Pulverised chromatin (Figs 38, 47, 96, 97, 98, 99, 165, 166, 180, 181) was observed in *A. cepa* root tip meristem after the treatment with *Hexagonia tenuis* extract. Sakari *et al*. (1981) suggested that the pulverisation of chromosomes is due to the premature condensation of chromosomes as a result of the action of chemical substances found in the extract.

Nuclear erosion

Nuclear erosion (Figs 30, 31, 32, 33, 55, 64, 160, 164, 179, 186) was commonly observed *A. cepa* root tips treated with the extract of *H. tenuis*. Sharma (1980) attributed nuclear erosion to the partial dissolution of nucleoproteins. The normal organization of chromatin in the nucleus and chromosome segregation is genetically controlled (Franklin and Cande, 1999). According to Caetano-Pereira *et al*. (1999), under the influence of stress or due to the action of environmental mutagens, chromatin degeneration occurs, which may become visible in the nucleus as degeneration or erosion zones.

Abnormal condensation

In the present study treatment of *A. cepa* root tips with the extract of *H. tenuis* resulted in abnormal condensation of chromosomes (Figs 17, 61, 62, 184).

Sperling and Rao (1974) reported that abnormal condensation of chromosomes may be due to the premature condensation of chromatin during prophase. Differential condensation of chromosomes may be due to the improper heteropyknosis or due to the quantitative changes in the heterochromatin content present in the cells (Verma, 1998).

Chromosome fragmentation

In the present study chromosome fragmentations (Figs 56, 65, 66, 131, 132, 155, 165, 170) were observed on the roots of *A. cepa* treated with *H. tenuis* extract. This might be due to the stretching of chromosome at metaphase followed by their breakage. Fragmentation is the result of breakage at the fragile sites of the chromosome (Chauhan and Chuahan, 1999). The reduction in the size of chromosomes is partly due to strain caused on account of tighter coiling of nucleoprotein complex in the presence of high ion concentration of calcium salt. Changes brought about in the viscosity of cytoplasm are responsible for chromosomal aberrations like fragmentation during anaphase, micronuclei formation and multinucleate cells (Sharma, 1980).

Nuclear extrusion

Chromatin extrusion from the nucleus was observed in the roots of *A. cepa* treated with *H. tenuis* extract (Figs 37, 59). This may be due to the intense activity of cytotoxicant on the nuclear membrane leading to its disruption at the site, followed by the extrusion of the nuclear material. Such an effect was noticed with gamma rays by Raghuvanshi and Singh (1976).

Nuclear disintegration

Roots of *A. cepa* treated with *H. tenuis* extract, resulted in nuclear disintegration (Figs 13, 26, 27, 28, 29, 54, 161, 173, 174). Changes in the viscosity of protoplasm brought about by stress or after exposure to toxic chemicals may lead to nuclear deformation. Nuclear DNA on the root tip meristem was found to be deformed or degraded after treatment with cytotoxicants, followed by disintegration of the nucleus (Liu *et al*., 2000). Richardson *et al*. (2001) reported similar results in *Solanum tuberosum* and it was found that nuclear damage occurred in the

root tip cells, followed by the nuclear degradation, which seems to be induced after exposure to the cytotoxicant.

Contorted chromosomes

Chromosomes were found to be twisted or contorted (Fig. 75) after the cytotoxic treatment on *A. cepa* root tip meristem with the extract of *H. tenuis*. Experimental evidences obtained from *Xerophylla villosa* reveals that nucleus with dense contorted chromatin develop at the time of destruction of the plant cells by dessication, either natural or induced (Hallam and Luff, 1980).

Certain spindle poisons not only affect the mitotic apparatus, but also cause marked proliferation of the nuclear envelope, increase in nuclear size and contortion. Contorted chromatin is a prominent nuclear anomaly detected together with cytoplasmic compartmentalization and unusual associations of several organelles after treatment with toxic chemicals (Walne, 1967). In the present study, treatment of *A. cepa* roots with *H. tenuis* extract leads to a physiological condition similar to that mentioned above, which was confirmed by the occurrence of contorted chromosomes.

Chromosome gaps

In the present study chromosome gaps (Figs 57, 65) were observed on the roots of *Allium cepa* treated with *H. tenuis* extract. *A. cepa, Hordeum vulgare* and *Secale cereale* reveal the severe chromosomal aberrations in their root tip cells after exposure to a potent mutagen, LSD.

Aberrations occurred in the form of chromatid and isochromatid breaks/ gaps, with most of these gaps/breaks failing to rejoin. The distribution of chromosome breaks was not uniform over the length of chromosomes and a majority of the breaks were localized at the centromeric regions. These results are the outcome of the clastogenic activity of the mutagen, acting upon the fragile sites, distributed at random on the chromosomes (Sadasivaiah *et al*., 1973; Star, 1970). A tendency of chromosome breaks is that it tends to be concentrated in the heterochromatic segments of the chromosomes (Kihlman, 1957).

Chromosome coagulation

Chromosome coagulation (Figs 133, 134, 135, 136, 162, 163, 175) was observed on the roots of *A. cepa* treated with *H. tenuis* extract. This abnormality is an after effect of chromosome stickiness, where the chromosome seems to be adhering to form an intact mass of aberrant chromosome group. Coagulation of chromosomes suggests that changes might have occurred in the viscosity of their constituent materials. It has frequently been assumed that such changes in viscosity are due to depolymerisation of DNA (Hollaender, 1954).

Chromosome stickiness

Chromosome stickiness (Figs 48, 91, 106-117, 140, 141, 144, 146, 148, 154-158, 168, 169, 170, 183, 184) was one of the most frequently scored abnormality and it was observed in almost all the treatments in the present study. According to Darlington (1942), stickiness is due to the disturbances in the nucleic acid metabolism of the cell. Stickiness has been interpreted by many to be the result of depolymerisation of DNA (Darlington, 1942), partial dissolution of nucleoproteins (Kaufmann, 1956), breakage and exchange of basic folded fibre unit of chromaids (Klasterska *et al*., 1976) and stripping of protein covering of DNA in chromosomes (Stephen, 1979). It may be due to the action of the extract on the protein (El-Sadek, 1972), which forms an integral part of chromosomes.

The sticky nature of chromosomes is probably due to the heterochromatinisation resulting in denaturation of nucleic acid and thus making the chromosome contour adhesive (Grundmann, 1966). Induction of stickiness is sometimes manifested as the cytotoxic effect of the chemical substances (Panda and Sahu, 1985). Electron microscopic studies demonstrated stickiness as chromatid type aberration (Mc Gill *et al*., 1974; Klasterska *et al*., 1976). Stickiness may result from the entanglement of chromatin fibres, which fail to condense properly in preparation for mitosis (Mc Gill *et al*., 1974). There could be some substances present in the extract, which affect the DNA structure in *A. cepa* root tip meristem perhaps resulting in physical depolymerisation of DNA. This together with or without partial dissolution of nucleoprotein (Mercykutty and Stephen, 1980) could account for stickiness of chromosomes.

Chromosome bridges

Chromosome bridges (Figs 14, 119-126, 132, 135, 152, 157, 158, 163, 172) are the frequently observed clastogenic abnormality resulted from the treatment of *A. cepa* root tips with *H. tenuis* extract. According to Ahmad and Yasmin (1992) mitotic bridge may arise due to general stickiness of chromosomes at metaphase. Mitotic bridges may also arise due to the formation of dicentric chromosomes by breakage and reunion (Raj and Rao, 1972; Kaur and Grover, 1985).

Multiple bridges occur as a result of fusion between broken chromosomes (Young and Young, 1993). Bridge appears from the chromosomes stretched towards the poles. The bridges and acentric fragments arise as a result of stickiness, which hinders separation of chromosomes (Das *et al*., 1968).

Patnaik *et al*. (1984) reported that the anaphase bridges may arise due to the effect of chemicals in breaking the protein moiety of nucleoprotein backbone. Breaking up of chromosomes followed by proximal chromatid reunion, results in the formation of dicentric anaphase bridges, which can also be attributed to the general stickiness of chromosomes (Abraham and Koshy, 1979; Anis and Wani, 1997). According to Najjar and Soliman (1980) chromosomal bridges may be due to chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to the unequal translocation or inversion of chromosomal segments. Telophase bridges were also present as observed by Cabaravdic (2010) when he exposed *A. cepa* cells to Benzo(a)pyrene.

Giant cells

In the present study giant cells (Figs 185-188) were observed on the roots of *A. cepa* treated with various concentration of *H. tenuis* extract. Nuclear breakage, lesion, erosion and receeding cytoplasm were observed in giant cells after the treatment with the extract.

Either by employing chemicals or by physical means, giant cells can be induced, which are capable of affecting the cell cycle especially in the 'S' phase. Here the cell division is completely arrested and cell expansion seems to be generated and as a result the cells become large giant cells. The frequency of giant cells seems to be increased depending upon the dosage and duration of the treatment with cytotoxic agents (Verma and Van Huyste, 1971). Menzel (1988) experimentally proved that the giant cells are subjected to various kinds of stress and

normally they cannot cope with the enormous size of the protoplasm that they are liable for injury.

Nuclear budding

Roots of *A. cepa* treated with *H. tenuis* extract, resulted in the nuclear appendages or nuclear budding (Fig. 32). Naturally occurring nuclear budding, apart from the induced bud or appendage formation occurs as a result of the selective entrapment of extra chromosomal amplified DNA by the nucleus and which can probably end in micronucleation during S-phase (Shimizu *et al*., 1998). Nuclear buds and vesicles resembling protuberances were observed to originate from the outer membrane of the nuclear envelope *in situ* at certain regions of the nuclear envelope. Hellgren and Morre (1992) reported that, probably these nuclear buds arise as a result of the excessive production of nucleic acids and proteins, induced by the cytotoxicants. This may be the probable reason for scoring nuclear appendage or nuclear buds in *A. cepa* after treatment with the *H. tenuis* extract in the present investigation.

Bizarre nuclei

At the interphase sage in the cells of *A. cepa* after treating with the *H. tenuis* extract, bizarre form of nuclei was observed (Figs 15, 16). Deformed nuclei became vacuolated and elongated, often curved and coiled. They can be considered as restitution nuclei formed after abnormal nuclear division (Chauhan and Chauhan, 1999).

Hyperchromasia

After treating *A. cepa* root tips with the *H. tenuis* extract, hyperchromasia (Fig. 39) was observed at interphase. Hyperchromasia is the most distinguishable state of aberration, where the nucleus takes up intense stain than normal, probably due to heterochromatinisation. Hyperchromasia is an extremely condensed and thereby deeply staining state of nucleus observed during stress induced by the influence of toxic environmental chemicals or during incompatible conditions. Progressive heterochromatinisation seems to be responsible for this aberration (Gernand *et al*., 2005). Extremely condensed heterochromatin leading to hyperchromasia had been observed in cultured tobacco cells after treatment with toxic chemicals (Houot *et al*., 2001).

Ghost cells

Ghost cells (Figs 13, 28, 41) were observed when *A. cepa* root tips were treated with *H. tenuis* extract. Ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable (http[5]). Celik and Aslanturk (2010) have observed the presence of ghost cells when *A. cepa* root tips were treated with *H. tenuis* extract. Similarly the components in *H. tenuis* extract might have lead to the damage of nucleus and cytoplasmic structures resulting in a ghost cell.

Cytomixis

This aberrant phenomenon was observed (Figs 43, 44, 45, 58, 150) in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Extrusion of chromatin from one cell into the cytoplasm of an adjoining cell is termed as cytomixis. Migration of chromatin has also been reported in somatic cells (Bowes, 1973; George and Geethamma, 1985). The factors responsible for cytomixis are rather ambiguous. Some possible causes attributed to cytomixis are cell response as a consequence of treatment with pesticides and antibiotic dosages (Kumar and Sinha, 1991) as well as abnormal genetic behaviour due to treatment with a chemical mutagen (Kumar and Srivastava, 2001; Kumar and Sharma, 2002).

Cytomixis in the present investigation may be attributed to abnormal genetic behaviour due to treatment with toxic fungal extract. As the concentration of the extract increased, the frequency of cells that showed chromosomal stickiness and cytomixis were also increased. According to Kaul (1971), certain chemicals which cause stickiness of chromosomes, may be responsible for cytomixis. Failure to find chromosomal stickiness and cytomixis in control confirms this view. This may be the probable reason for cytomixis in *A. cepa*, after treatment with the extract of *H. tenuis*.

Nuclear enlargement

After treating *A. cepa* root tips with the *H. tenuis* extract, nuclear enlargement (Fig. 36) was observed at interphase. Kang *et al*. (2011) have reported that nuclear enlargement resulted when SW480 human colon cancer cells was treated with Arctigenin, a natural plant lignin. Changes in cell and nuclear size often correlate with the functionality of cancer treating agents. Hence nuclear enlargement can be considered as a positive action towards the curing of cancer cells.

Strap cells

Strap cells were noticed (Figs 189-193) in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Prasanth *et al*. (2013) noticed high frequency of strap nucleus in Furadan and Monosodium glutamate treated *A. cepa* root meristem.

NON CLASTOGENIC ABNORMALITIES

Micronucleus

Treatment of *A. cepa* root tips with the extract of *H. tenuis* resulted in interphase cells with micronucleus (Figs 19, 20, 21, 175). It may be formed due to the action of the extract on the spindle apparatus, leading to unequal separation of chromosomes at anaphase. The larger group of daughter chromosomes forms a larger nucleus and the smaller group forms a micronucleus.

According to Sparrow and Singleton (1953), micronuclei are a fair index of chromosome fragment production. Micronucleus may originate from a lagging chromosome at anaphase or from a chromosome fragment (Badr and Ibrahim, 1987). Micronuclei are true mutagenic aspects (Auerbach, 1962), which may lead to a loss of genetic material and have been regarded as an indication of mutagenicity of their inducers (Ruan *et al*., 1992).

Abnormal association of chromosomes

Abnormal association of chromosomes was observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis* (Fig. 60). Autotetraploid barley plants induced by the use of colchicines revealed various irregularities in chromosome configuration, including unequal grouping. The probable reason for abnormal association may be spindle poisoning (Chen *et al*., 1945). According to Smith (1943), such induced abnormal association of chromosomes, manifested as a stathmo-kinetic effect may be responsible for the detection of a wide spectrum of euploid and aneuploid variants in *Nicotiana*.

Macro and micro cells

Treatment of *A. cepa* root tips with the extract of *H. tenuis* resulted in macro and micro cells at cytokinesis (Figs 181, 182). Patil and Bhat (1992) attributed this abnormality to the failure of normal organization and function of spindle apparatus.

Binucleate cells

Binucleate cells were observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis* (Figs 10-15, 27, 46, 49). Binucleate conditions are the peculiarities of cancer cells (Oksala and Therman, 1974; Graham *et al*., 1978). Delay or failure or arrest of cytokinesis would account for the occurrence of binucleate cells (Ene Obong and Amadi, 1987).

In most binucleates, chromosome segregation movement was organized in a common spindle and the daughter nuclei at the telophase appeared to envelope each other in the newly formed nuclear membrane. Disturbances in the nuclear and microtubular cycles seem to be associated with the formation of heterophasic binucleate cells (Alberts *et al*., 1983). The heterophasic cells exhibited asynchronous nuclei at different stages of mitosis. In heterophasic cells (Fig. 46) displaying interphase-prophase and interphase-metaphase nuclei, the mitotic transition is delayed but is ultimately achieved due to the effect of the advanced nuclei, which induces a premature mitotic entry of the lagging nuclei.

Misorientaion of chromosomes

Treatment of *A. cepa* root tips with the extract of *H. tenuis* resulted in misorientation of chromosomes (Figs 92, 93, 94, 111, 149). It may be due to the disturbed functioning of the spindle apparatus. The disturbance can be due to the distortion of the spindle apparatus, a tilt in the equatorial organization of metaphase chromosomes or a change in the direction of movement of daughter chromosomes during anaphase (Saliem *et al*., 1981).

Scattering of chromosomes

Scattering of chromosomes were observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis* (Figs 74, 100, 101). It may be due to the disturbance in the mitotic spindle (Darlington, 1942). The scattered and clumped metaphases are the partial and full effects respectively of a C-mitotic agent (Hadder and Wilson, 1985). According to Mathur and Chua (2000), chromosome scattering could be attributed to the interference of the extract with tubulin or polymerization of the microtubular subunits forming the spindle apparatus.

Disturbed metaphase, anaphase and telophase

After treating *A. cepa* root tips with the *H. tenuis* extract, disturbed metaphase (Figs 84, 85, 86, 87, 116) disturbed anaphase (Figs 145, 146, 152) and disturbed telophase (Figs 164)

were observed. According to Heaps *et al*. (1982) and Saleem *et al*. (1993), disturbed chromosomes may be due to the loss of activity of microtubules in spindle fibres. Disturbed metaphase may be the result of disturbances on the spindle apparatus (Shehab *et al*., 1978) or due to inhibition of spindle formation (Amer and Farah, 1983). Shehab (1979) observed that the extract can be considered as stathmo-kinetic as it affects the spindle fibres.

Diagonal orientation of chromosomes

Diagonal orientation of chromosomes (Figs 79, 80, 81, 109, 137, 138, 139, 140, 141, 142) was found in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. This may be due to the slight tilt in the spindle apparatus induced by the extract. According to Das *et al*. (1968) the improper functioning of spindle apparatus causes the diagonal orientation of chromosome. This phenomenon has also been observed on treatment with extracts of other plants as well (Tajo and Thoppil, 1998; Deena and Thoppil, 2000; Sreeranjini and Thoppil, 2001).

Displaced chromosome

Displaced chromosome (Figs 82, 83, 173) was found in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Wheeler *et al*. (1987) have observed chromosome displacement along with mitotic inhibition when Chinese hamster cells were treated with estradiol.

Ball shaped arrangement of chromosomes

Ball metaphase (Figs 67, 68, 69, 70, 93, 941, 110, 111, 112), ball anaphase (Fig. 118) and ball telophase ((Fig. 159) were observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. It is a form of C-mitosis with characteristically clumped chromosomes. The ball metaphase is followed by either a complete degeneration of the cell or a state similar to interphase (Barber and Callan, 1942). In the present study, the formation of ball metaphase may be due to the localized activity of spindle apparatus at the centre so that the chromosomes were arranged in such a way that their centromeres remain at the equator and arms radiating in different directions and orienting in the form of a ball. Ball anaphase is the stage in mitosis in which sister chromatids separate into a hollow ball of chromosomes that result from the early cleavage divisions in some aberrant cells (Morgan, 2006).

Cytostasis

Cytostasis (Fig. 76) was observed after treating *A. cepa* root tips with the *H. tenuis* extract. Mitodepressive effect seems to be responsible for the cytostatic activity. Ahumada *et al*. (1995) have observed cytostasis and mitodepressive effect when *A. cepa* root tips were treated with aqueous extracts of *Viscum cruciatum*.

Stellate arrangement of chromosomes

Stellate metaphase (Figs 103, 147, 148) and stellate anaphase (Figs 103, 147, 148) were observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Such type of anomaly was also observed after the treatment of *A. cepa* root tips with pure "sevin" (Amer, 1965) and considered as being a fore-step of the complete disturbance of the spindle. Star shaped arrangement of anaphase may be due to the clumping of daughter chromosomes into star like structures near the polar region of the cell.

Chromosome clumping

After treating *A. cepa* root tips with the *H. tenuis* extract, chromosome clumping (Fig. 63) was observed. Clumping of chromosomes is attributed to the increased concentration of the cytotoxicants (Pritchard and Court, 1968). Scattered and clumped metaphases are considered as the partial and full effect respectively of a C-mitotic agent (Hadder and Wilson, 1958). It may be due to the action of the extract on chromosome to chromosome spindles or pole to pole spindles. Hence the chromosome to chromosome distance changed and were seen clumped together.

Chromosome doubling and Somatic pairing

Chromosome doubling was observed (Fig. 77) in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Colchicine as well as its alternative oryzalin caused chromosome doubling by mitotic inhibition in *Lilium* and *Nerine* (Tuyl *et al*., 1992). Similarly, a colchicine like compound might be present in the extract which resulted in doubling of chromosomes.

Somatic pairing was found to be a least scored and rare abnormality (Fig. 77) after treating *A. cepa* root tips with the *H. tenuis* extract. Somatic pairing in root tip cells of regenerated *Sorghum* plants were observed by Wen *et al*. (1989) and explained that it was due to the high RNA content of root tip cells induced by plant hormones in the regeneration

medium. Root meristems of *A. cepa* when treated with the extract might have had an increase in RNA content resulting in somatic pairing.

Unequal separation of chromosomes

Chromosomes were found to be separating aberrantly and thereby two unequal groups of chromosomes were found to be moving from the equatorial plane towards the poles (Fig. 152). Patil and Bhat (1992) attributed this abnormality to the failure of normal organization and function of spindle apparatus.

Laggard formation

In the present study, lagging of chromosomes were observed (Figs 122, 128, 139). Barthelmass (1957) and Nagpal and Grover (1994) noticed that lagging of chromosomes might be because of the hindrance of pro-metaphase movement of chromosomes, accompanied by adhesion of the centromere to the nuclear membrane or to the surrounding surface of the plasma membrane. According to Kaur and Grover (1985), lagging chromosomes can be attributed to the delayed terminalisation, stickiness of chromosome ends or due to failure of chromosomal movements.

The failure of normal organization and function of spindle apparatus may lead to formation of laggards (Patil and Bhat, 1992). According to Saggoo *et al*. (1991), lagging of chromosome is due to the abnormal spindle activity.

Hypoploid cell

Hypoploid cells (Figs 85, 88, 117, 122) were noticed during the treatment of *A. cepa* root tips with *H. tenuis* extract. Multinucleate cells with hypoploid nuclei occur in case of multipolar mitosis or due to lagging chromosomes at mitosis thereby producing two hypoploid daughter cells (Seoana *et al*., 2000).

Polyploid cell

In the treatment of *A. cepa* root tips with the extract of *H. tenuis,* polyploid cells were observed (Figs 70, 73, 74, 87, 101). Onfelt and Klasterska (1983) pointed out that the mitotic abnormalities are generally considered insignificant from the mutation point of view unless polyploid or aneuploid cells are induced.

Hyperploid cell

Hyperploid cells (Figs 99, 114) were noticed during the treatment of *A. cepa* root tips with *H. tenuis* extract. Hyperploidy can be induced in mammalian cells by many anti-cancer drugs, including inhibitors of the microtubule organization (Kung *et al*., 1990). It has been reported that apoptotic cell death is correlated with hyperploid formation and abrogation of the cell cycle checkpoint might have resulted both apoptosis and hyperploid formation (Hall *et al*., 1996).

Shift in microtubule organizing centre (MTOC)

Shift in MTOC (Figs 153, 166, 167) was observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. In plants the absence of organelles such as the centrosome has led to the belief that MTOCs originate on the nuclear envelope and are transported to the specific intracellular locations by microtubule proteins (Asada and Collings, 1997). Studies in both plant and animal system have suggested that stable microtubules form an integral component of MTOCs. In the present study, chemical components present in the extract might have affected the stability of microtubules thereby causing a shift in MTOC.

Multipolar anaphase

Treatment of *A. cepa* root tips with the extract of *H. tenuis* resulted in the multipolar anaphase (Fig. 151). According to Minija *et al*. (1999), abnormal movement of chromosomes may be due to severe disturbances in the spindle apparatus.

Multipolar anaphase was observed in *A. cepa* root meristematic cells by Akatrinei (1966) when treated with extracts of *Viscum album*. The number of poles in a cell depends on the position of the assemblage of RNA and polysaccharides, which remain distributed either in the form of gel (Prasad, 1974).

Stathmo anaphase

Stathmo-anaphase (Figs 142, 143, 144) was observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Here the daughter chromosomes do not separate fully, but they remain connected together by means of partial overlapping of the arms. This may also be due to the abnormal functioning of spindle fibres. Studies conducted on *Hordeum sativum, Vicia faba* and *Nigella damascena* confirms that stathmo anaphase is a radiomimetic effect caused

by the simultaneous multipolar and spindle poisoning activities induced by the spindle destructing chemicals. Since the extract affects the spindle fibres, they can be considered as stathmo-kinetic agents (Shehab, 1979).

Early cell plate formation

Early cell plate formation (Figs 129, 169) was observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Cell plate formation can start from metaphase itself (Whaley *et al*., 1966). Some substance present in the extract may be the reason for the early cell plate formation in *A. cepa* cells after the treatment.

Chained chromosomes

Chained chromosomes (Figs 71, 72, 73, 74, 98, 113, 114) were observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Similar effect was noticed by Menendez-Yuffa *et al*. (2000) when embryonic calli from coffee plants were subjected to mitotic aberration studies.

Early movement of chromosomes

Early movement of chromosomes (Figs 127, 128, 138, 141) was observed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Tajo and Thoppil (1998) pointed out that, the early movement of chromosomes may be due to the disturbance in the spindle mechanism. According to Kaur and Grover (1985), the precocious movement of the chromosomes might have been caused by the early terminalisation, resulting in the movement of the chromosome ahead of the rest during anaphase.

C-metaphase

C-metaphase (Figs 95, 115) was observed after the treatment of *A. cepa* root tips with *H. tenuis* extract. Levan (1938) termed the scattering of the chromosomes by spindle inhibition as C-mitosis or colchicine's mitosis. According to Redei (1998), in the event of C-mitosis, the mitotic anaphase gets blocked by the poisonous effect of the cytotoxic chemical and consequently the cell and its progeny may become polyploid. C-mitosis is one of the consequences of inactivation of spindle apparatus connected with the delay in the division of the centromere (Gomurgn, 2000). The chromosomes were found to be scattered and chromatids become clearer in C-metaphase.

Pole to pole arrangement of chromosomes

Pole to pole arrangement of chromosomes was observed (Figs 104, 105, 106, 116) in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Abnormal pole to pole orientation of chromosomes at metaphase leading to equatorial separation of chromosomes at anaphase is an acute aberrant condition that arises as a result of irregular pathways of spindle assembly and abnormal spindle activity (Ford and Correl, 1992).

Tropokinesis

After treating *A. cepa* root tips with the *H. tenuis* extract, tropokinesis (Fig. 102) were noticed. Tropokinesis is a spindle abnormality found in *A. cepa* root merisem after treatment with Furadan and Endosulphan (Ananthakrishnan *et al.*, 2013). Tropokinesis occur due to the abnormal orientation or misbehavior of spindle fibres.

Vagrant chromosomes

Roots of *A. cepa* treated with *H. tenuis* extract, resulted in the vagrant chromosomes (Figs 81, 171, 178). In vagrant chromosomes, a chromosome move ahead from its chromosomal group toward poles and leads to the unequal separation of chromosomes in the daughter cells and have been observed by many workers in different studies (Sondhi *et al*., 2008). According to Rank (2003), vagrant chromosomes are also the indicators of spindle poisoning.

Nuclear peak

Nuclear peak was observed (Figs 34, 35) in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. Nuclear abnormalities are caused by morphological alterations in the interphasic nuclei, as a result of the action of the cytotoxic agent tested (Lema and Marin-Morales, 2009).

Gas chromatography-Mass spectrometry

Gas chromatography-Mass spectrometry (GC-MS) is an instrumental technique, comprising a gas chromatograph (GC) coupled to a mass spectrometer (MS), by which complex mixtures of chemicals may be separated, identified and quantified.

GC-MS instruments have long been used for the identification of large number of components present in natural and biological systems (Binit *et al*., 2010). For the analysis of volatile components of herbal medicines, methods like GC and GC-MS are widely accepted due to its sensitivity, stability and high efficiency (Nikam *et al*., 2012).

GC-MS analysis of methanolic extract of *Hexagonia tenuis* revealed the presence of different types of chemical components (Table 14). Thirty seven compounds were identified. The chemical constituents were grouped into thirteen classes, *viz.*, ester, alcohol, acyl halide, ketone, quinoline, phenol, carboxylic acid, fatty acid ester, fatty acid, alkene, sterol, triterpenoids and hydrocarbons (Table 15). Among these, hydrocarbons (50.05%), sterols (24.44%) and fatty acids (11.88%) constitute the major classes of compounds. Previous reports on the GC-MS analysis of the extract of the *Hexagonia* were found to be rare, only few reports are available on related taxa of *H. tenuis.*

9-Methyl-10-[2-(10- methylanthracen-9-yl) ethyl] anthracene (14.15%), Linoleic acid (11.88%), Anthraergostatetraenol hexahydrobenzoate (26%), Ergosterol (16.72%), Stellasterol (6.69%), 1,54-Dibromo-Tetrapentacontane (2.9%), Methyl octadeca-8,11-dienoate (2.42%), Anthraergosta-5,7,9,14-tetra-ene (1.84%), Hexacosene (1.8%), 2-Bromo-4-fluoro 2,6-Difluorobenzoic acid (1.59%) and Anthraergostapentene (1.4%) were the major chemical constituents identified in the methanolic extract of *H. tenuis*.

In the present study fatty acid occupies 11.88% of the total components identified. Fatty acids are ubiquitous in mushrooms and may be responsible for some of the phytopharmaceutical properties attributed to many of the edible mushrooms (Zhang *et al*., 2003). Presence of fatty acids of C 12: 0 - C 24: 0 sizes were reported by Brondz *et al.* (2004) in the basidiospores of the higher fungi like *Amanita muscaria, Armillaria borealis, Agaricus sylvicola* and *Hypholoma capnoides.*

Methanolic extract of *Agrocybe aegerita* yielded a fatty acid fraction (FAF) along with palmitic acid, ergosterol, 5, 8-epidioxy ergosta-6,22-dien-3β-ol, mannitol and trehalose. Ergosterol has shown the higher inhibitory activity towards cyclooxygenase (COX) II enzyme than towards the COX I. Zhang *et al*. (2003) reported that palmitic acid and ergosterol has shown mild antioxidant activities.

GC-MS analysis of *Agrocybe aegerita* conducted by Zhang *et al*. (2003) revealed the presence of linoleic acids and ergasterol, which were already obtained in the present investigation.

Components like linoleic acid and anthraergostatetraenol were identified in the methanolic extract of *H. tenuis,* which comprises the major components of n-hexane extract of *Lentinus tuber-regium* (Fr.) Fr. (Polyporaceae) syn. *Pleurotus tuber regium* Fr. sclerotia (Afieroho and Ugoeze, 2014).

Kalac (2013) reported the presence of linoleic acid and oleic acid derivatives as the major unsaturated fatty acids which is in line with an earlier report on the mushroom.

Previous works have shown that, ergosterol may exhibit some degree of antitumor properties (Yazawa *et al*., 2000; Takaku *et al*., 2001). According to Rajakumar *et al.* (2007) ergosterols are known to act as biological precursors of vitamin D_2, hence it can be grouped as provitamins.

A chemical investigation by Thang *et al.* (2015) of *H. apiaria* resulted in the identification of 9 compounds including 5 new triterpenoids, Hexagonins A-E along with 4 known compounds – hexatenuin, ergosterol, ergosterol peroxide and urosolic acid. In the present study, GC-MS analysis of methanolic extract of *H. tenuis* yielded a compound named β- amyrin that belongs to the class triterpenoids.

Oxygenated cyclohexanoids, speciosins A-K and aporpine A were isolated from *H. speciosa* (Jiang *et al*., 2009). Al- Fatimi *et al*. (2013) reported that *H. velutina* exhibited strong antioxidative effects employing the DPPH assay.

These previous investigations give only the details of chemical constituents of various other related taxa and do not mention anything about the chemical constituents in *H. tenuis*. Chemical investigations of only few species of *Hexagonia viz*., *H. apiaria, H. speciosa* and *H. velutina* were carried out in the previous studies. Chemical investigation of *H. tenuis* involves only the isolation of a thermostable enzyme called laccase (Chaurasia *et al*., 2015). In the present study, GC-MS analysis of *H. tenuis* revealed the presence of a wide spectrum of chemical constituents, some of which were found to possess antioxidant activities, which may be exploited for the production of phytopharmaceuticals.

SUMMARY

One and only way to fight against the new borne diseases is by developing new medicines and finding medicinal resources. *Hexagonia tenuis* (Hook.) Fr., a potential bracket fungi (shelf fungi) belong to the order Polyporales in the family Polyporaceae. Polyporaceae represents the largest and most diverse group of poroid Aphyllophorales. Members of this family are highly significant in the field of medicine. The genera *Hexagonia* itself shows few biological activities.

H. tenuis is a medicinally significant bracket fungus. Cytotoxicity of *H. tenuis* was screened using *Allium cepa* root tip assay. Root tips were treated with different concentrations (0.005 %, 0.01 %, 0.05 % and 0.1 %) of the fungal extracts for different treatment durations such as ½, 1, 2, 3 and 24 hours. Cytotoxicity of *H. tenuis* on *A. cepa* root tips resulted in a wide spectrum of abnormalities.

Nuclear lesion, nuclear budding, nuclear erosion, chromosome stickiness, chromosome bridges, chromosome fragments, bizarre nuclei, pulverised chromatin, giant cells, abnormal condensation of chromosomes, nuclear extrusion, nuclear disintegration, cytomixis, nuclear fragmentation, nuclear enlargement, hyperchromasia, contorted chromosomes, chromosome coagulation, chromosome gap *etc*. were observed as the major clastogenic abnormalities.

The non clastogenic abnormalities include macro and micro cell formation, binucleate cell, chromosome clumping, C-metaphase, stellate chromosomes, misorientation of chromosomes, polyploidy, hypoploidy, diagonal chromosomes, disturbed chromosomes, chromosome laggards, stathmo anaphase, scattering of chromosomes, shift in microtubular organizing centres (MTOC), ball shaped arrangement of chromosomes, pole to pole arrangement of chromosomes, cytostasis, tripolar anaphase, micronuclei, early movement of chromosomes, abnormal association of chromosomes, chromosome doubling and somatic pairing, vagrant chromosomes, unequal separation of chromosomes, early cell plate formation, tropokinesis, chained chromosomes *etc.*

Among the abnormalities obtained, most frequent abnormalities observed were nuclear lesions, chromatin erosion, nuclear budding, chromosome stickiness, chromosome bridges, bizarre nuclei, ball arrangement of chromosomes, diagonal orientation of chromosomes, pulverised chromatin, giant cells, abnormal condensation of chromosomes, stellate arrangement of chromosomes, misorientation of chromosomes, scattering of chromosomes, shift in MTOC, disturbed chromosomes, C-metaphase, micronuclei, chromosome clumping, binucleate cells, stathmo anaphase *etc*. Rare abnormalities include cytoplasmic heteropyknosis, formation of macro and micro cells, hyperchromasia, chromosome gaps, hypercondensation, cytostasis, chromosome doubling and somatic pairing, unequal separation of chromosomes, early cell plate formation, contorted chromosomes, nuclear extrusion, nuclear enlargement, nuclear disintegration, cytomixis, nuclear fragmentation *etc*.

Lesion, erosion, stickiness, pulverisation and bridges represent the most frequently scored abnormalities. From the cytotoxic investigations on *A. cepa* root tips treated with the extract of *H. tenuis,* it was found that the percentage of abnormalities increased with the elevation in the concentration of extract. A considerable extend of abnormalities were observed in comparison with the control. Changes in the cell and nuclear size often correlate with the functionality of cancer treating agents. Thus the nuclear enlargement resulted in the present study can be considered as a positive action towards curing of cancer cells.

The chemical profiling of the methanolic extract of *Hexagonia tenuis* was determined by using GC-MS analysis. Different types of compounds were determined and were categorized into 13 classes of compounds such as ester, alcohol, acyl halide, ketone, quinoline, phenol, carboxylic acid, fatty acid ester, fatty acid, alkene, sterol, triterpenoids and hydrocarbons. Altogether 37 compounds were characterized in the present study.

Among the thirty seven compounds detected in the methanolic extract of *Hexagonia tenuis*, major components include 9-Methyl-10-[2-(10- methylanthracen-9-yl) ethyl] anthracene (14.15%), Linoleic acid (11.88%), Anthraergostatetraenol hexahydrobenzoate (26%), Ergosterol (16.72%), Stellasterol (6.69%), 1,54-Dibromo-Tetrapentacontane (2.9%), Methyl octadeca-8,11-dienoate (2.4%), Anthiaergosta-5,7,9,14-tetra-ene (1.84%), Hexacosene (1.8%), 2-Bromo-4-fluoro 2,6-Difluorobenzoic acid (1.59%) and Anthraergostapentene (1.4%).

Hydrocarbons were found to be the leading group (50.05%) among the thirteen classes of compounds obtained. Sterols (24.44%) and fatty acids (11.88%) constitute the other major classes of compounds.

Chemical investigations of only few species of *Hexagonia viz., H. apiaria, H. speciosa* and *H. velutina* were carried out previously. GC-MS analysis of *H. tenuis* revealed the presence of several chemical constituents, some of which are showing antioxidant properties. These preliminary investigations can be considered as a stepping stone for the further detailed researches in *H. tenuis*, leading to the production of potential phytopharmaceuticals.

REFERENCES

Abraham, S. and Koshy, M. P. 1979. Mutagenic potential of green chillies. Cytologia **44**: 221-225.

Abu, N. E. and Mba, K. C. 2011. Mutagenecity testing of pharmaceutical effluents on *Allium cepa* root tip meristems. J. Toxicol. Environ. Health Sci. **3**: 44-51.

Ahmad, S. and Yasmin, R. 1992. Effect of Methyl parathion and Trimiltox on mitosis of *Alium cepa*. Cytologia **57**: 155-160.

Ahumada, M. C., García, M. D., Saenz, M. T. and Aznar, J. 1995. Antimitotic and cytostatic activity of *Viscum cruciatum* Sieber parasitic on *Crataegus monogyna* Jacq. Pharm. Acta Helv. **70**: 233-236.

Ajith, T. A. and Janardhanan, K. K. 2007. Indian Medicinal Mushrooms as a Source of Antioxidant and Antitumor Agents. J. Clin. Biochem. Nutr **40**: 157–162.

Akatrinei, G. 1966. Polyploidiya I Aneuploidiya V Kornyakh Luka Pod Vityaniem Fiziologicheski Aktivnykh Veshchestv Ekstrakta Iz Omely (*Viscum album* L.). Genetika **4**: 97-104.

Alberts, B., Bray, D., Lewis, J., Raft, M., Roberts, K. and Watson, J. D. 1983. The cytoskeleton. In: Molecular Biology of Cell. Garland Press, New York. pp. 549-688.

Alexopoulos, C. J., Mims, C. W. and Blackwell, M. 1996 a. Introductory Mycology. John Wiley and Sons Inc. Publishers, Singapore. pp. 9.

Alexopoulos, C. J., Mims, C. W. and Blackwell, M. 1996 b. Introductory Mycology. John Wiley and Sons Inc. Publishers, Singapore. pp. 583-585.

Amer, S. M. 1965. Cytological effects of pesticides. I. Mitotic effects of N-methyl, 1-naphthyl carbamate “Sevin”. Cytologia **30**: 175-181.

Amer, S. M. and Farah, O. R. 1983. Cytological effects of pesticides. Effects of phosphorothionate insecticide “Dursban” on mitosis of *Vicia faba*. Cytologia **48**: 27-33.

Anis, M. and Wani, A. A. 1997. Caffein induced morpho-cytological variability in fenugreek, *Trigonella foenum graceum* L. Cytologia **62**: 343-349.

Antonyuk, V. O., Yu, K. O. and Stoika, R. S. 2010. Cytotoxic proteins of *Amanita virosa*: Purification, Characteristics and action towards mammalian cells. Toxicon. **55**: 1297-1305.

Auerbach, C. 1962. Mutation – An Introduction to Research on Mutagenesis. Part I. Methods. Oliver and Boyd, Edinburgh.

Badr, A. and Ibrahim, A. G. 1987. Effect of herbicide 'Glean' on mitosis, chromosomes and nucleic acids in *Allium cepa* and *Vicia faba* root tip meristems. Cytologia **52**: 293-302.

Bahl, N. 1983. Medicinal value of edible fungi. In: Proceeding of the International conference on science and cultivation technology of edible fungi. Indian Mushroom Science II. pp. 203-209.

Balakrishnan, B. and Nair, M. C. 1994. Medicinal Mushrooms- Advances in mushroom Biotechnology. Scientific Publishers, Jodhpur. pp. 27-29.

Balandrin, M. F., Klocke, J. A., Wurtele, E. S. and Bollinger, W. H. 1985. Natural Plant Chemicals: Sources of Industrial and Medicinal Materials. Science **228:** 1154-1160.

Barber, H. N. and Callan, H.G. 1942. The effects of cold and colchicine on mitosis in the newt. Proc. Roy. Soc. **131**: 258-271.

Barthelmass, A. 1957. Chemisch indizerte multiplare mitosekentwicklung. Amer. J. Bot. **54**: 945.

Beneke, E. A. 1963. Clavatia, Clavacin and Cancet. Mycologia **55:** 227-270.

Bourgaize, D., Jewell, T. R. and Buiser, R. G. 2003. Biotechnology- Demystifying the Concepts. Pearson Education., Singapore. pp. 333-334.

Buchbauer, G. 2000. The detailed analysis of essential oils leads to the understanding of their properties. Perfumer & Flavorist **25**: 64–67.

Cabaravdic, M. 2010. Induction of chromosome aberrations in the *Allium cepa* test system caused by the exposure of cells to benzo(a) pyrene. Med. Arh. **64**: 215-218.

Caetano-Pereira, C. M., Pagliarini, M. S. and Brasil, E. M. 1999. Cell fusion and chromatin degeneration in an inbred line of maize. Genet. Mol. Biol. **22**: 69-72.

Cammarata, J. M. D. 2006. A Guide to Herbal Remedies. Goodwill Publishing House, New Delhi. pp. 139-142.

Carlile, M. J., Watkinson, S. C. and Gooday, G. W. 2005. The Fungi. Elsevier Ltd, New Delhi.

Celik, T. A. and Aslanturk, O. S. 2010. Evaluation of cytotoxicity and genotoxicity of *Inula viscosa* leaf extracts with Allium test. J. Biomed. Biotechnol. (doi:10.1155/2010/189252).

Chauhan, N. and Chauhan, A. K. S. 1999. Genotoxicity of fluoroquinolones in *Allium* test system. J. Cytol. Genet. **34**: 153-160.

Chaurasia, P. K., Bharati, S. L., Yadava, S. and Yadav, R. S. S. 2015. Purification, Characterization and Synthetic application of a thermally stable laccase from *Hexagonia tenuis* MTCC-1119. Indian J. Biochem. Biophys. **52**: 60-67.

Chen, C. and Wu, C. 2006. Volatile Components of Mushroom (*Agaricus subrufecens*). Journal of Food Science **49**:

Chen, S. L., Shen, S. M. and Tang, P. S. 1945. Studies on colchicines-induced autotetraploid barley. I and II. Cytological and morphological observations. Am. J. Bot. **32**: 103-106.

Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T. and Fukuoka, F. 1969. Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinus edodes* (Berk.) Sing. Nature. **222**: 687-688.

Cronin, D. A. and Margaret, K. W. 2006. The characterisation of some mushroom volatiles. Journal of the Science of Food and Agriculture **22:**

Darlington, C. D. 1942. Chromosome chemistry and gene action. Nature **149**: 66-69.

Darlington, C. D. 1942. Chromosome chemistry and gene action. Nature **149**: 66-69.

Das, N. T., Raj, S. A. and Rao, R. B. V. 1968. Cytological studies in *Vicia faba* L. treated with asafetida. Cytologia **33**: 110-111.

Davidson-Hunt, I. 2000. Ecological Ethnobotany: Stumbling toward new practices and paradigms. MASA J. **16**: 1–13.

Deena, M. J. and Thoppil, J. E. 2000. Cytotoxic properties of *Lantana camara* L. J. Cytol. Genet. **1**: 111-116.

Deena, M. J. and Thoppil, J. E. 2000. Cytotoxic properties of *Lantana camara* L. J. Cytol. Genet. **1**: 111-116.

El-Sadek, L. M. 1972. Mitotic inhibition and chromosomal aberration induced by some arylasonic acids and its compounds in root tips of maize. Egypt. J. Genet. Cytol. **1**: 218-224.

Ene-Obong, E. E. and Amadi, O. C. 1987. Contribution to the cytological effects of medicinal plants. I. The mitodepressive effects of water extract of *Boerhaavea diffusa* and *Vernonia amygdalina* on *Allium cepa* root tip mitosis. Cytologia **52**: 469-474.

Fang, E. F. and Ng, T. B. 2013. Antitumor potential and the emerging medicinal properties of natural compounds. pp. 65.

Fiskesjö, G. 1985. The *Allium* test as a standard in environmental monitoring. Hereditas **102**: 99-112.

Fiskesjö, G. and Levan, A. 1993. Evaluation of the first ten MEIC chemicals in the *Allium* test. ATLA: Altern. Lab. Anim. **21**: 139-149.

Franklin, A. E. and Cande, W. Z. 1999. Nuclear organization and chromosome segregation. Plant Cell **11**: 523-534.

Gali-Muhtasib, H. and Bakkar, N. 2002. Modulating cell cycle: current applications and prospects for future drug development. Current Cancer Drug Targets **2**: 309-336.

Gernand, D., Rutten, T., Varshney, A., Rubstova, M., Prodanovic, S., Bruss, C., Kumlehn, J., Matzk, F. and Houben, A. 2005. Uniparental chromosome elimination at mitosis and interphase in wheat and pearl millet crosses involves micronucleus formation, progressive heterochromatinization and DNA fragmentation. Plant Cell **17**: 2431-2438.

Gomurgn, A. N. 2000. Cytological effect of the herbicide 2, 4-D Isocytlester 48% on root mitosis of *Allium cepa*. Cytologia **165**: 383-388.

Graham, S., Dayal, S., Swanson, M., Mittleman, A. and Wilkinson, G. 1978. Diet in the epidemiology of cancer of the colon and rectum. J. Nat. Cancer Res. Inst. **61**: 709-714.

Grundmann, E. 1966. General Cytology. Edward Arnold Ltd., London, pp. 428.

Guerrero-Vasquez, G. A., Chinchilla, N., Molinillo, J. M. G. and Macias, F. A. 2014. Synthesis of Bioactive Speciosins G and P from *Hexagonia speciosa*. Journal of Natural Products .

Gunde-Cimerman, N. 1999. Medicinal Value of the Genus *Pleurotus* (Fr.) P. Karst. (Basidiomycetes). International Journal of Medicinal Mushrooms **1**: 69-80.

Hadder, J. C. and Wilson, G. B. 1958. Cytological assay of C-mitotic and prophase poison action. Chromosoma **4**: 91-104.

Heaps, P. J. D., Timothy, J. and Spuck, P. 1982. Studies on the kinetochore function in mitosis. Effect of colchicines and cytochalasin on mitosis in the diatom (*Hantzschia amphioxys*). Eur. J. Cell Biol. **28**: 77-82.

Hellgren, L. and Morre, D. J. 1992. ATP induced budding of nuclear envelope in vitro. Protoplasma **167**: 238-242.

Hollaender, A. 1954. Radiation Biology. McGraw-Hill, Washington DC. pp. 1-694.

Hong, K. J., Dumn, D. M., Shen, C. L. and Pence, B. C. 2004. Effects of *Ganoderma lucidum* on apoptotic and anti-inflammatory function in HT-29 human colonic carcinoma cells. Phytother. Res**. 18:** 768–770.

Houot, V., Etienne, P., Petitot, A. S. Barbier, S., Blein, J.P. and Suty, L. 2001. Hydrogen peroxide induces programmed cell death features in cultured tobacco BY-2 cells, in a dose dependent manner. J. Exp. Bot.**52**: 1721-1730.

Hu, H., Ahn, N.S., Yang, X., Lee, Y. S. and Kang, K. S. 2002. *Ganoderma lucidum* extract induces cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int. J. Cancer. **102**: 250-253.

Ikekawa, T., Uehara, N., Maeda, Y., Nakanishi, M. and Fukuoka, F. 1969. Antitumor activity of aqueous extract of edible mushrooms. Cancer Res. **29**: 734-735.

Jang, K. J., Han, M. H., Lee, B. H., Kim, B. W., Kim, C. H., Yoon, H. M. and Choi, Y. H. 2010. Induction of apoptosis by ethanol extracts of *Ganoderma lucidum* in human gastric carcinoma cells. J. Acupunct. Meridian Std. **3**: 24-31.

Jennings, D. H. and Lysek, G. 1999. Fungal Biology- Understanding the fungal lifestyle. Bios Scientific Publishers, UK. pp. 135-140.

Jiao, C., Xie, Y. Z., Yang, X., Li, H., Li, X. M., Pan, H. H., Cai, M. H., Zhong, H. M. and Yang, B. B. 2013. Anticancer activity of *Amauroderma rude.* Plos One **8**: 1-13.

Jose, J. and Rajalakshmi, R. 2005. Medicinal and Aromatic Plants- Essential Oils and Pharmaceutical Uses. Discovery Publishing House, New Delhi. pp. 8-9.

Joseph, S., Jabula, B., George, V., Antony, K. and Janardhanan, K. 2011. Antitumor and anti-inflammatory activities of polysaccharides isolated from *Ganoderma lucidum*. Acta Pharmaceutica. **61**: 335-342.

Joy, P. P., Thomas, J., Mathew, S. and Skaria, B. P. 2001. Medicinal Plants. ed. Bose, T. K., Kabir, J., Das, P. and Joy, P. P. Calcutta pp. 449-632.

Kang, K., Lee, H. J., Yoo, J. H., Jho, E. H., Kim, C. Y., Kim, M. and Nho, C. W. 2011. Cell and nuclear enlargement of SW480 cells induced by a plant lignin, arctigenin: evaluation of cellular DNA content using fluorescence microscopy and flow cytometry. DNA Cell Biol. **30**: 623-629.

Kaufmann, B. P. 1956. Cytochemical studies of changes induced in cellular materials of ionizing radiations. Ann. N. Y. Acad. Sci. **59**: 553.

Kaur, P. and Grover, I. S. 1985. Cytological effects of some organophosphorous pesticides. I. Mitotic effects. Cytologia **50**: 187-197.

Kawagishi, H., Inagaki, R., Kanao, T. and Nakamura, T. 1989. Carbohydrate research. Brazilian *Journal* of Microbiology **186**: 267-273.

Kawagishi, H., Katsumi, R., Sazawa, T., Mizuno, T., Hagiwara, T. and Nakamura, T. 1988. Cytotoxic steroids from the mushroom *Agaricus blazei*. Phytochemistry **21**: 2111-2119.

Kebriaee-zadeh, A. 2003. Overview of national drug policy of Iran. Iranian J. Pharm. Res. **2**: 1–2.

Kimura, Y., Tojima, H. and Fukase, S. 1994. Clinical Evaluation of Sizofilan as assistant immunotherapy in treatment of head and neck cancer. Acta Otolaryngol. **511**: 192-195.

Klastreska, I., Natarajan, A. T. and Ramel, C. 1976. An interpretation of origin of sub-chromatid aberrations and chromosome stickiness as a category of chromatid aberration. Heridiatas **83**: 153-169.

Komatsu, J., Terekawa, H., Nakamishi, K. and Watanabe, Y. 1963. *Flammulina velutipes* and antitumor activities. J. Antibiot. Ser. A. **16**: 139-143.

Lee, J., Oka, K., Watanabe, O., Hara, H. and Ishizuka, S. 2011. Immunomodulatory effect of mushrooms on cytotoxic activity and cytokine production of intestinal lamina propia leukocytes does not depend on b-glucan contents. Food chem. **126**: 1521-1526.

Lee, S. S., Wei, Y.H., Chen, C.F., Wang, S.Y. and Chen, K. Y. 1995. Antitumor effects of *Ganoderma lucidum*. J. Chin. Med. **6:** 1–12.

Leeja, L. and Thoppil, J. E. 2004. Cytotoxic potential of extracts of *Artemisia nilgirica* (C. B. Clarke) Pampan. Cell Chromosome Res. **24**: 21-26

Leemol, D. and Girija, K. 1998. Suppressive effects of cyclophosphamide induced toxicity by *Withania somnifera* extract in mice. J. Ethnopharmacol. **82**: 209-214.

Lemieszek, M. K., Langer, E., Kanderfer-Szerszen, M., Sanecka, B., Mazurkiewcz, W. and Rzeski, W. 2009. Anticancer effect of fraction isolated from medicinal polypore mushroom, *Pitoporus betlinus* (Bull. Fr.) P. Karst. (Aphyllophoromycetideae): *in vitro* studies. Int. J. Med. Mushr. **11**: 351-364.

Levan, A. 1938. The effect of colchicine on root mitoses in *Allium*. Hereditas **24**: 471-486.

Levan, A. 1938. The effect of colchicine on root mitoses in *Allium*. Hereditas 24: 471-486. Mathur, J. and Chua, N. H. 2000. Microtubule stabilization leads to growth reorientation in *Arabidopsis* trichomes. Plant Cell **12**: 465-478.

Liu, T., Van Staden, J. and Cress, W. A. 2000. Salinity induced nuclear and DNA degradation in meristematic cells of soyabean (*Glycine max* L.) roots. Plant Growth Regul. **30**: 49-54.

Liu, Y., Dao, Z., Liu, Y. and Long, C. 2009. Medicinal plants used by the Tibetan in Shangri-la, Yannan, China. J. Ethnobiol. Ethnomed. **5:** 5-15.

Lucas, E. H., Montesono, R., Pepper, M. S., Hafner, M. and Sablon, E. 1957. Tumor inhibitors in *Boletus edulis* and other holobasidiomycetes. Antibiot. Chemother. **7**: 1–4.

Ma, C. N., Huang, S. J. and Chen, C. C. 2004. Antioxidant properties of methanolic extract from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food Chem. **87**: 111-118.

Macleod, A. J. and Panchasara, S. D. 1983. Volatile aroma components, particularly glucosinolate products, of cooked edible mushroom (*Agaricus bisporus*). Phyrochemistry **22**: pp. 705-709.

Mc Gill, M., Pathak, S. and Hsu, T. C. 1974. Effect of ethidium bromide on mitosis and chromosomes. Chromosoma **47**: 157-167.

Menikpurage, I. P., Soysa, S. S. and Abeytunga, D. T. U. 2012. Antioxidant activity and cytotoxicity of the edible mushroom, *Pleurotus cystidiosus* against Hep-2 carcinoma cells. J. Natn. Sci. Foundation **40**: 107-114.

Menzel, D. 1988. How do giant plant cells cope with injury? The wound response in siphonous green algae. Protoplasma **144**: 79-91.

Mercykutty, V. C. and Stephen, J. 1980. Adriamycin induced genetic toxicity as demonstrated by *Allium* test. Cytologia **45:** 769-777.

Mercykutty, V. C. and Stephen, J. 1980. Adriyamycin induced genetic toxicity as demonstrated by *Allium* test. Cytologia **45**: 769-777.

Meskin and Mark, S. 2002. Phytochemicals in Nutrition and Health. C R C Press. pp. 123. ISBN 9781587160837.

Minija, J., Tajo, A. and Thoppil, J. E. 1999. Mitoclastic properties of *Mentha rotundifolia*. L. J. Cytol. Genet. **34**: 169-171.

Minija, J., Tajo, A. and Thoppil, J. E. 1999. Mitoclastic properties of *Mentha rotundifolia*. L. J. Cytol. Genet. **34**: 169-171.

Mizuno, T. 2000. Development of an antitumor biological response modifier from *Phellinus linteus* (Berk.Et.Curt) Teng (Aphillophoromycetideae). Int. J. Med. Mushr. **2**: 21–33.

Morgan, D. O. 2006. The Cell Cycle: Principles of control. New Science Press Ltd., London pp. 1-269.

Mukhopadhyay, I., Chowdhuri, D. K., Bajpayee, M. and Dhawan, A. 2004. Evaluation of *in vivo* genotoxicity of Cypermethrin in *Drosophila melanogaster* using the alkaline commet assay. Mutagenesis **19**: 85-90.

Nagpal, A. and Grover, I. S. 1994. Genotoxic evaluation of systemic pesticides in *Allium cepa.* I. Mitotic effects. Nucleus **37**: 99-105.

Najjar, N. R. and Soliman, A. S. 1980. Cytological effects of fungicides. I. Mitotic effects of Vitavax 200 and Dithane-s-60 on wheat and related species. Cytologia **45**: 163-168.

Nobel, R. L., Beer, C. T. and Cutts, J. H. 1958. Role of chance observations in chemotherapy:*Vinca rosea*. Ann NY Acad Sci. **76**: 882–894.

Nyigo, V., Baraza, L. D., Nkunya, M. H. H., Mdachi, S. J. M., Joseph, C. C. and Waziri, A. 2005. Chemical constituents and cytotoxicity of some tanzanian wild mushrooms. Tanz. J. Sci. 31: 1-3.

Oksala, T. and Therman, E. 1974. Mitotic abnormalities and cancer. In: Chromosomes and Cancer. German, J. (ed.) John Wiley and Sons, New York.

Onfelt, A. and Klasterska, I. 1983. Spindle disturbances in mammalian cells and multiple chromatid exchanges after treatment on V 79 Chineese Hamster cells with carbaryl. Mutat. Res. **119**: 319-330.

Panda, B. B. and Sahu, V. K. 1985. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphate insecticide 'Fensulphothion'. Cytobios **42**: 147-155.

Patil, B. C. and Bhat, G. I. 1992. A comparative study on M. H. and E. M. S. in the induction of chromosome aberration on root meristem of *Clitoria ternate* L. Cytologia **57**: 259-264.

Patnaik, S., Saran, B. L. and Patnaik, S. N. 1984. Effect of zarda (processed tobacco leaf) extract on the chromosomes of *Allium cepa* L. Cytologia **49**: 807-814.

Prasad, A. B. 1974. Production of multipolar spindles in canary grass following irradiation of dry seeds. J. Cytol. Genet. **788**: 184-187.

Prasad, G. and Das, K. 1977. Effects of some growth substances on mitosis. Cytologia **42**: 323-329.

Pritchard, A. J. and Court, R. D. 1968. The cytological effects of mimosine. Cytologia **33**: 73-77.

Raj, S. A. and Rao, R. 1972. Cytolocal studies in *Vicia faba* L. treated with lathyrogens. Cytologia **37**: 245-246.

Rao, G. V., Kumar, S., Islam, M. and Mansour, S. E. 2008. Folk medicines for anticancer therapy-a current status. Cancer Ther. **6**: 913-922.

Reddy, T. V. V. S. and Reddy, V. R. 1985. Cytological effects of chemical mutagens in rice. Cytologia **50**: 499-505.

Redei, G. P. 1998. Genetics Manual Current Theory, Concepts and Terms. World Scientific Publishing Company, Florida.

Richardson, K. V. A., Wetten, A. C. and Caligari, .D. S. 2001. Cell and nuclear degradation in root meristems following exposure of potatoes (*Solanum tuberosum* L.) to salinity. Potato Res. **44**: 389-399.

Robles-Hernandez, L., Cecilia-Gonzalez- Franco, A., Soto- Parra, J. M. and Montes-Dominguez, F. 2008. Review of agricultural and medicinal applications of basidiomycete mushrooms. Technociencia Chihuaha **2:** 96-107.

Rosa, H. L., Machado, K. M. G., Jacob, C. C., Capelari, M., Rosa, C. A. and Zan, C. L. 2003. Screening of Brazilian Basidiomycetes for Antimicrobial Activity. Mem. Inst. Oswaldo Cruz. pp. 967-974.

Ruan, C., Lian, Y. and Linn, J. 1992. Application of micronucleus test in *Vicia faba* root tips in the rapid detection of mutagenic environmental pollutants. Chinese J. Environ. Sci. **4**: 56-58.

Sadasivaiah, R. S., Collins, G. B. and Davis, D. L. 1973. Effect of LSD on mitotic and meiotic plant chromosomes. Chromosoma **44**: 309-318.

Saggoo, M. I. S., Kumari, S. and Bindu, R. 1991. Cytological effects of Indian medicinal plants. I. Mitotic effects of leaf homogenate of *Tylophora indica* L. on *Allium cepa*. Cytologia **56**: 633-637.

Saj, O. P. and Thoppil, J. E. 2006. Cytotoxic studies and exploration of essential oil in two species of *Corymbia*. J. Cytol. Genet. **7**: 131-139.

Sakari, K., Martti, S. and Pekka, V. 1981. Chromosome pulverization in blood diseases. Hereditas **95**: 15-24.

Saleem, A. ., Hassan, H. Z., Budawof-Fatma, M. I., Naby, A. and Waffa, M. 1993. The mutagenic potentialities of three pesticides on three biological systems. Egypt. J. Genet. Cytol. **22**: 109-128.

Saliem, A. R., Hussein, M. M., Allam, H. Z. and Farah, A. R. 1981. The effect of three synthetic organic insecticides (Nuvacron) on cytological characters in cotton. Bull. Fac. Sci. Cairo. Univ. **32**: 52-66.

Sanchez-Lopez, M. I., Vanhulle, S. F., Mertens, V., Guerra, G., Figueroa, S. H., Decock, C., Corbisier, A. and Penninckx, M. J. 2008. Autochthonous white rot fungi from the tropical forest: Potential of Cuban strains for dyes and textile industrial effluents decolourisation. African Journal of Biotechnology **7**: pp. 1983-1990.

Seeger, R. 1975. Demonstration and isolation of Phallosyn, a haemolytic toxin from *Amanita phalloides*. Nanyn Schmiedebergus Arch. Pharmacol. **287**: 277-287.

Seoana, A. I., Giierci, A. M. and Dulout, F. N. 2000. Mechanisms involved in the induction of aneuploidy: the significance of chromosome loss. Genet. Mol. Biol. **23**: 4.

Shao, H., Qing, C., Wang, F., Zhang, Y., Lo, D. and Liu, J. A. 2005. New cytotoxic lanostane triterpenoid from the Basidiomycete *Hebeloma versipelle*. J. Antibiot. **58**: 828-831.

Sharma, A. K. and Sharma, A. 1960. Spontaneous and chemically induced chromosome breaks. Int. Rev. Cytol. **10**: 101-123.

Sharma, A. K. and Sharma, A. 1962. A study of importance of nucleic acids in controlling chromosome breaks induced by different compounds. The nucleus **5**: 127-136.

Sharma, A. K. and Sharma, A. 1990. Chromosome Techniques- Theory and Practices, Third edition, Butterworths, London.

Sharma, M. 1980. Effect of the presence of methyl, methoxy and hydroxyl groups in chemical compounds on chromosomes. Indian J. Expt. Biol. **18**: 787-790.

Shehab, A. S. 1979. Cytological effects of medicinal plants in Qatar-I. Mitotic effect of water extract of *Pulicaria crispa* on *Allium cepa.* Cytologia **44**: 607-613.

Shehab, A. S. 1979. Cytological effects of medicinal plants in Qatar-I. Mitotic effect of water extract of *Pulicaria crispa* on *Allium cepa.* Cytologia **44**: 607-613.

Shehab, A. S., Hakeem, H. A. and Abu- El- Keir, Z. 1978. Cytological effect of *Achillea fragantissima* on *Allium cepa* and *Vicia faba.* Cytologia **43**: 623-629.

Shimizu, N., Itoh, N., Utiyama, H. and Nahl, G. M. 1998. Selective entrapment of extrachromosomally amplified DNA by nuclear budding and micronucleation during S phase. J. Cell Biol. **140**: 1307-1320.

Smith, H. H. 1943. Studies on inducd heteroploids of *Nicotiana.*Am. J. Bot. **30**: 121-130.

Sperling, K. and Rao, P. N. 1974. The phenomenon of premature chromosome condensation: its relevance to basic and applied research. Hum. Genet. **23**: 235-258.

Sperling, K. and Rao, P. N. 1974. The Phenomenon of premature chromosome condensation: its relevance to basic and applied research. Hum. Genet. **23**: 235-258.

Sreeranjini, K. and Thoppil, J. E. 2001. Cytotoxic effect of *Spilanthes ciliata* on cells of onion. J. Cytol. Genet. **2**: 71-75.

Stanley, G., Harvey, K., Slivova, V., Jiang, J. and Sliva, D. 2005. Ganoderma lucidum suppresses angiogenesis through the inhibition of secretion of VEGF and TGF-1 from prostate cancer cells. Biochem. Biophy. Res. Comm. **330**: 46-52.

Star, A. E. 1970. Spontaneous and induced chromosome breakage in *Claytonia virginica.* Am. J. Bot. Bot. **57:** 1145-1149.

Stephen, J. 1979. Cytological causes of spontaneous fruit abortion in *Haemanthus katherinae* Baker. Cytologia **44**: 805-812.

Svoboda, G. H. 1961. Alkaloids of *Vinca rosea* (*Catharanthus roseus*). Lloydia **24**: 173–178.

Tajo, A. and Thoppil, J. E. 1998. Cytotoxic properties of *Ocimum americanum* L. Bull. Pure and Appl. Sci. **17**: 49-53.

Tajo, A. and Thoppil, J. E. 1998. Cytotoxic properties of *Ocimum americanum* L. Bull. Pure and Appl. Sci. **17**: 49-53.

Tidke, G. and Rai, M. K. 2006. Biotechnological Potential of Mushrooms: Drugs and Dye Production. International Journal of Medicinal Mushrooms. **8**: 351-360.

Trivedi, P. C. and Sharma, N. K. 2004. Ethnomedicinal Plants. Pointer Publishers, Jaipur.

Udayan, P. S. and Balachandran, I. 2009. Medicinal Plants of Arya Vaidyasala-Herb Garden. Geethanjali Offset Prints, Kozhikode. pp. 13-16.

Ullrich, R., Huong, L., Dung, N. L. and Hofrichter, M. 2005. Laccase from the medicinal mushroom, *Agaricus blazei*: Production, Purification and Characterisation. Applied Microbiology and Biotechnology **67**: 357-363

Van Tuyl, J. M., Meijer, B. and Van Dien, M. P. 1992. The use of oryalin as an alternative of colchicines in *in vitro* chromosome doubling of *Lilium* and *Nerine.* Acta Hort. **325**: 625-630.

Vaz, J. A., Almaida, G. M., Ferreira, I. C. F. R., Martins, A. and Vasconcelos, M. H. 2012. *Clitocybe alexandri* extract induces cell cycle arrest and apoptosis in a lung cancer cell line: identification of phenolic acids with cytotoxic potential. Food Chem. **132:** 482-486.

Verma, D. P. S. and van Huyste, R. B. 1971. Induction of giant cells in the suspension cultures of *Arachis hypogea* L. by massive irradiation. Radiat. Res. **48**: 518-530.

Verma, R. S. 1998. Heterochromatin: Molecular and Structural Aspects. Cambridge Univeristy Press, U. K.

Vogel, F. C., Garry, S. J., Kemper, L. A. K. and Graham, D. C. 1974. Bacteriocidal properties of a class of quinoid compounds related to sporulation in the mushroom, *Agaricus bisporus*. Am. J. Pathol. **76**: 165-174.

Walne, P. L. 1967. The effects of colchicines on cellular organization in *Chlamydomonas*. II. Ultrastructure. Am. J. Bot. **54**: 564-577.

Wang, J., Lu, Z., Liu, P., Wang, Y., Li, J., Hong, K. and Zhu, W. Cytotoxic Polyphenols from the Fungus *Penicillium expansum* 091006 Endogenous with the Mangrove Plant *Excoecaria agallocha.* Planta Med. **78**: 1861-1866.

Wani, B. A., Bodha, R. H. and Wani, A. H. 2010. Nutritional and medicinal importance of mushrooms. Journal of Medicinal Plants Research. **4**: pp. 2598-2604.

Wani, M. C., Taylor, H. L., Wall, M. E., Coggon, P. and Mcphail, A. T. 1971. Plant antitumor agents. VI. The isolation and structure of Taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. J. Am. Chem. Soc. **93**: 2325–2327.

Wasser, S. P. and Weis, A. L. 1999. Medicinal properties of substances occurring in higher Basidiomycete mushroom: current perspective (review). Int. J. Med. Mushr. **1**: 31-62.

Wasser, S. P. and Weis, A. L. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms; current perpespective (review). Int. J. Med. Mushr. **1:** 31-62.

Wen, F., Barnett, F. L. and Liang, G. H. 1989. Somatic pairing and separation of chromosomes in root-tip cells of regenerated Sorghum plants. J. Hered. **80**: 159-160.

Whaley, W. G., Dauwalder, M. and Kephart, J. E. 1996. The golgi apparatus and an early stage in cell plate formation. J. Ultrastruct. Res. **15**: 169- 180.

Wheeler, W. J., Hsu, T. C., Tousson, A. and Brinkley, B. R. 1987. Mitotic inhibition and chromosome displacement induced by estradiol in Chinese hamster cells. Cell. Motil. Cytoskeleton **7**: 235-247.

Yang, Q. Y. and Jong, S. C. 1989. Medicinal mushrooms in China. Mushr. Sci. **9**: 631-643.

Yoshika, P., Ikekawa, T., Noda, M. and Fuknoka, I. 1963. Studies on antitmor activities of some fractions from Basidiomycetes; An antitmour fraction from *P. ostreatus* (Fr.) Quel. Chem. Pharm. Bull. **20**: 1175-1180.

Young, S. W. and Young, P. W. 1993. Effect of plant growth regulators on mitotic chromosomes. The Nucleus **36**: 109-113.

Yuen, J. W. M. and Gohel, M. D. I. 2005. Anticancer Effects of *Ganoderma lucidum*: A Review of Scientific Evidence. Nutrition and Cancer **53**: 11-17.

Zjawiony, J. K. 2004. Biologically active compounds from Aphyllophorales (polypore) fungi. J. Nat. Prod. **67**: 300–310.