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Study on Cytotoxicity and Chemical Profiling of *Hexagonia tenuis* (Hook.) Fr., a Potential Bracket Fungus (Polyporaceae)

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Abstract
Biodiversity richness forms a stepping stone to medicinal researches leading to drug development. Number of plants, bacteria and fungi play an important role in traditional medicine. *Hexagonia tenuis* (Hook.) Fr., is a bracket fungus that belongs to the family Polyporaceae. Among the 16 species of *Hexagonia*, *H. tenuis* is mainly used for the purification, characterisation and synthesis of a thermostable enzyme called laccase. The present study aims to reveal the potential biochemical components present in *H. tenuis* using GC-MS analysis and to study the cytotoxic efficacy using *Allium cepa* root tip assay. Due to the presence of some potential chemicals such as sterols, terpenoids *etc*. that shows antitumor and antioxidant properties, *H. tenuis* has been tested for cytotoxicity using *Allium cepa* root tip assay. Cytotoxicity screening revealed the existence of different types of chromosomal aberrations which may form a key to further studies in animal assays also.

Keywords
Aberrations, Cytotoxicity, *Hexagonia tenuis,* Mitotic index.

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INTRODUCTION
The biodiversity richness, especially medicinal plant resources and the associated indigenous knowledge, innovations and traditional practices are an important asset to mankind. The traditional herbal medicine is now becoming popular for the treatment of several diseases. Generally, the use of herbal drugs is recognized by the people as being safer than the allopathic ones. Numerous plants have been used to treat various diseases in ethno medical practices in remote villages and tribal areas. Number of fungi and bacteria are also having great significance in the treatment of diseases [1]. Many fungi produce chemicals that kill or arrest the growth of other organisms. Thus the medical mycology is now becoming an important area of research due to the emergence of various capricious diseases.

Number of fungi and bacteria are reported as significant in the treatment of various diseases. Chemicals produced by fungi possess different biological properties. It may even kill or arrest the growth of other organisms. Fungi are important as decomposers, food, medicine and in the production of dye, enzymes, beverages, antibiotics *etc*. With respect to medicinally important compounds, the fungi have proved to be an extremely important group of organisms [2]. Conventionally, number of fungi including fruiting bodies of bracket fungi has been used in herbal medicine.

In 1969, Ikekawa and his colleagues [3] discovered the antitumor effects of the hot water extracts from several mushrooms. The components were proved to be polysaccharides especially β-D glucans.

From the fruiting body, mycelia and culture medium of various medicinal mushrooms such as *Lentinus edodes, Ganoderma lucidum, Schizophyllum commune, Trametus versicolor, Inonotus obliquus* and *Flamulina velutipes* different polysaccharide antitumor agents have been developed. 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 [4]. Bracket fungi belong to basidiomycetes, in which mushrooms, puffballs, earthstars, stinkhorns, Bird's nest fungi etc. are included. The Basidiomycetes are an important group of fungi with 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.

Surprisingly large number of the basidiocarps of the species of Polyporales has 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 [5].

Polyporaceae is the largest and most diverse group of Polyporales. *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 fungus with wide distribution especially in tropical regions. It is a lignicolous fungus 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. Ventral surface (hymenium) is characterised by the presence of numerous minute hexagonal pores. Fruiting bodies are extremely persistent. The genera consist of 16 species. Among this, *H. apiaria, H. hirta, H. Hydnoides and H. tenuis* are economically important. *H. apiaria* is used as food. *H. hydnoides* has been screened for antimicrobial activities. It could inhibit the growth of *Bacillus cereus* [6]. *H. tenuis* is used for the purification, characterisation and synthesis of a thermostable enzyme called Laccase (7). Organic dyes were obtained from *H. hirta*. These previous works reveal the medical significance of *Hexagonia*. Synthesis of thermostable enzyme called laccase from *H. tenuis* shows its biological importance. Because of its least explored nature, the species *H. tenuis* is selected for the present study.

The present investigation involves the studies on cytotoxic activity of fungal extract of *H. tenuis* (Hook.) Fr., using *Allium cepa* root tip assay and GC-MS analysis. *A. cepa* root tip assay was introduced by Leven [8] and was later proposed as a standard method to study genotoxicity [9].

MATERIALS AND METHODS

Fungal material

Fruiting body of *Hexagonia tenuis* (Hook.) Fr., a bracket fungus used for the present study was collected from the Botanical Garden, University of Calicut. Cytotoxic experiments of the present study were conducted mainly in the Cell and Molecular Biology Division, Department of Botany, University of Calicut and the GC-MS analysis was conducted at CEPC, Kollam, Kerala.

GC-MS Analysis

Preparation of methanolic extracts:

With the help of Soxhlet apparatus, methanolic extracts of dried powder of *H. tenuis* was prepared. Fungal material (10 g) 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 ms 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.

Plant test material

Allium cepa L. (2n=16) was seletced as the test material for the cytotoxic assay.

CYTOTOXIC ASSAYS

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 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. 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. Powdered material (0.1 g) 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 08:30 AM to 11:00 AM. After many trials, it was found that maximum dividing cells (peak mitotic activity) occurred between 09:00 AM to 09:30 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 (09-9:30 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 proposed by Sharma and Sharma [10] 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 was 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,

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

Statistical analysis

Statistical analyses were performed using the SPSS version 20 software package program. Data obtained were then subjected to one-way ANOVA and Duncan's multiple range test (DMRT) to confirm the variability of the data and validity of results. All results were expressed as mean ± SE and differences between corresponding controls and exposure treatments were considered statistically significant at $P<0.05$. (Table: 1)

RESULTS AND DISCUSSION

Cytotoxicity

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 [11]. A markable 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. The major aberrations obtained include chromatin erosion, bizarre nucleus, chromosome laggards, pulverized chromosomes, binucleate cell, nuclear lesions, chromosome bridge, chromosome rosette, hyperchromasia, coagulated chromosomes, stathmo anaphase, ghost cell, cytomixis, cytostasis, shift in microtubule organizing centre (MTOC), C-metaphase, macro and micro cell, hypoploid cell, diagonal chromosomes, stickiness, tripolar anaphase and strap cell (Fig. i and Fig. ii).

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 [12]. Studies by Pasqualini [13] 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.

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 [14].

Sharma [15] attributed nuclear erosion to the partial dissolution of nucleoproteins. The normal organization of chromatin in the nucleus and chromosome segregation is genetically controlled [16].

Chromatin degeneration occurs under the influence of stress or due to the action of environmental mutagens, which may become visible in the nucleus as degeneration or erosion zones [17].

Chromosome coagulation 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 [18].

Strap cells were noticed in the treatment of *A. cepa* root tips with the extract of *H. tenuis*. High frequency of strap nucleus in Furadan and Monosodium glutamate treated *A. cepa* root meristem was noticed early [19].

Chromosome stickiness was one of the most frequently scored abnormality and it was observed in almost all the treatments in the present study. 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 [20], partial dissolution of nucleoproteins [21] and stripping of protein covering of DNA in chromosomes [22] The sticky nature of chromosomes is probably due to the heterochromatinisation resulting in denaturation of nucleic acid and thus making the chromosome contour adhesive [23] Induction of stickiness is sometimes manifested as the cytotoxic effect of the chemical substances [24]. 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 could account for stickiness of chromosomes [12]

At the interphase stage in the cells of *A. cepa* after treating with the *H. tenuis* extract, bizarre form of nuclei was observed. Deformed nuclei became vacuolated and elongated, often curved and coiled. They can be considered as restitution nuclei formed after abnormal nuclear division [25].

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 [26].

Ghost cell is a dead cell in which the outline remains visible, but whose nucleus and cytoplasmic structures are not stainable. Celik and Aslanturk [27] 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.

Extrusion of chromatin from one cell into the cytoplasm of an adjoining cell is termed as cytomixis. Bowes [28] reported the migration of chromatin in somatic cells in his earlier works. 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 [29] as well as abnormal genetic behaviour due to treatment with a chemical mutagen [30].

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 [31], 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*.

Patil and Bhat [32] attributed macro and micro cell formation to the failure of normal organization and function of spindle apparatus.

Binucleate conditions are the peculiarities of cancer cells [33]. Delay or failure or arrest of cytokinesis would account for the occurrence of binucleate cells [34].

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 [35]. In heterophasic cells 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.

Improper functioning or slight tilt in the spindle apparatus induced by the extract may cause diagonal orientation of chromosomes [36]. Chromosome displacement along with mitotic inhibition when Chinese hamster cells were treated with estradiol was observed in earlier works [37].

Nagpal and Grover [38] 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 [39], lagging chromosomes can be attributed to the delayed terminalisation, stickiness of chromosome ends or due to failure of chromosomal movements.

Patil and Bhat [33] in 1992 pointed out that the failure of normal organization and function of spindle apparatus may lead to formation of laggards. In 1991 Saggoo *et al*. [40] noticed that the lagging of chromosome is due to the abnormal spindle activity.

In 2000 Seoana *et al*. [41] reported that multinucleate cells with hypoploid nuclei occur in case of multipolar mitosis or due to lagging chromosomes at mitosis thereby producing two hypoploid daughter cells

In 1997 Asada and Collings [42] observed that in plants the absence of organelles such as centrosome has led to the belief that MTOCs originate on the nuclear envelope and are transported to the specific intracellular locations by microtubule proteins. 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.

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. In 1979 Shehab [43] pointed out since the extract affects the spindle fibres, they can be considered as stathmo-kinetic agents.

Levan [8] in 1938 termed the scattering of the chromosomes by spindle inhibition as C-mitosis or colchicine's mitosis. In 2000 according to Gomurgn [44] C-mitosis is one of the consequences of inactivation of spindle apparatus connected with the delay in the division of the centromere. The chromosomes were found to be scattered and chromatids become clearer in C-metaphase.

Chemical profiling by GC-MS analysis

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 esters, alcohols, acyl halides, ketones, quinolines, phenols, carboxylic acid, fatty acid esters, fatty acids, alkenes, sterols, triterpenoids and hydrocarbons. 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. Altogether 37 compounds were characterized in the present study (Table: 2).

Among the 37 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%).

Ganodermic acids are the active triterpenoids reported from Lingzhi, which has been found to show antitumor effects. GC-MS analysis of *H. tenuis* also revaled the presence of triterpenoids.

Previous works of Takaku *et al*. [45] in 2001 have shown that, ergosterol may exhibit some degree of antitumor property. According to Rajakumar *et al.* [46] 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*. [47] in 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.

GC-MS analysis of *Agrocybe aegerita* conducted by Zhang *et al*. [48] in 2003 revealed the presence of linoleic acids and ergosterol. In 2013 Al- Fatimi *et al*. [49] reported that *H. velutina* exhibited strong antioxidative effects employing the DPPH assay. Ergosterol has shown mild antioxidant activities which has already obtained in the present investigation.

Chemical investigations of few species of *Hexagonia viz., H. apiaria, H. speciosa* and *H. velutina* were only carried out previously. Isolation of a thermostable enzyme called laccase is the major work reported from *H. tenuis* by Chaurasia *et al*. [7] in 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 reported already to possess antioxidant activities, which may be exploited for the production of mycopharmaceuticals.

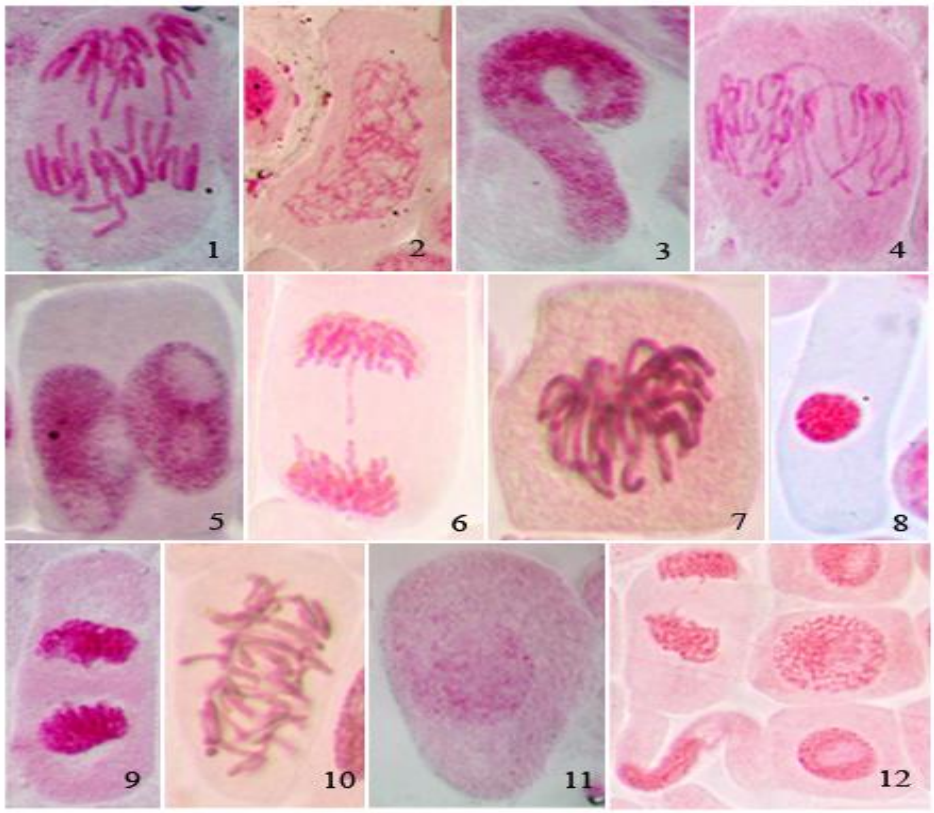

Fig.i.1.Chromosome laggards and early movement at anaphase 2. Chromatin erosion at prophase 3. Bizzare nucleus 4. Pulverize chromosomes at early metaphase 5. Binucleate cell showing double nuclear lesions 6. Broken chromosome bridge at anaphase 7. Chromosome rossete at early metaphase 8. Hyperchromasia 9. Coagulated chromosomes at anaphase 10. Pole to pole stathmo anaphase 11. Ghost cell 12. Cytomixis

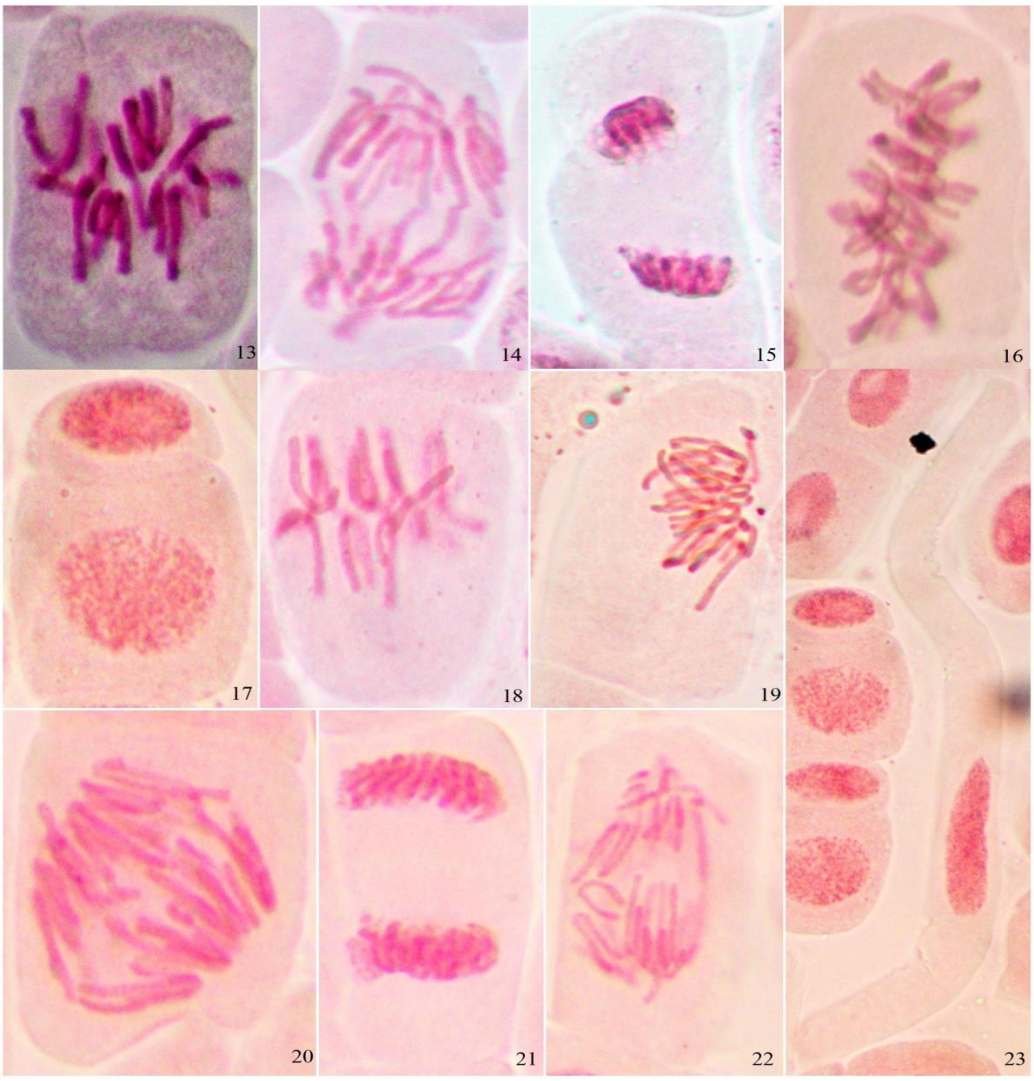

Fig. ii. 13. Cytostasis 14. Multiple bridges at anaphase 15. Shift in MTOC at late anaphase 16. Pole to pole C metaphase 17. Macro and micro cell formation 18. Hypoploid cell at metaphase 19. Displaced orientation at metaphase 20. Diagonal anaphase 21. Sticky telophase showing fragment 22. Tripolar anaphase 23. Strap cell showing nuclear peak

Table: 1. Cytotoxic potential of *Hexagonia tenuis* aqueous extract on *Allium cepa* root meristem

Treatment	Time period (hr)	No. of Dividing cells/1000 cells	No. of Aberrant cells/ 1000 cells	Mitotic index (%)	Abnormality (%)
Positive control	0.5	148.00 ± 3.61 ^{c}	696.33 ± 7.8 ^{a}	14.80 ± 0.36 ^{c}	69.63 ± 0.78 ^{a}
	1	142.67 ± 4.26 ^{c}	725.33 ± 6.39 ^{b}	14.27 ± 0.43 ^{c}	72.53 ± 0.64 ^{b}
	2	135.67 ± 6.33 ^{b,c}	805.33 ± 7.36 ^{c}	13.57 ± 0.63 ^{b,c}	80.53 ± 0.74 ^{c}
	3	122.00 ± 6.43 ^{a,b}	826.33 ± 4.81 ^{d}	12.20 ± 0.64 ^{a,b}	82.63 ± 0.48 ^{d}
	24	111.00 ± 4.35 ^{c}	863.00 ± 4.36 ^{e}	11.10 ± 0.43 ^{a}	86.30 ± 0.44 ^{e}
Negative control	0.5	743.67 ± 6.17 ^{a,b}	113.67 ± 1.45 ^{a}	74.37 ± 0.62 ^{a,b}	11.37 ± 0.14 ^{a}
	1	763.00 ± 9.30 ^{b}	79.67 ± 30.94 ^{a}	76.30 ± 0.93 ^{b}	11.30 ± 0.36 ^{a}
	2	734.67 ± 4.91 ^{a}	109.00 ± 2.31 ^{a}	73.47 ± 0.50 ^{a}	10.90 ± 0.23 ^{a}
	3	764.67 ± 11.40 ^{b}	107.33 ± 2.60 ^{a}	76.47 ± 1.14 ^{b}	10.73 ± 0.26 ^{a}
	24	805.67 ± 5.92 ^{c}	115.00 ± 1.52 ^{a}	80.57 ± 0.59 ^{c}	11.50 ± 0.15 ^{a}
0.005	0.5	666.33 ± 4.98 ^{c}	395.67 ± 3.17 ^{a}	66.63 ± 0.50 ^{c}	39.56 ± 0.31 ^{a}

	1	646.33 ± 4.80 [b]	438.00 ± 9.53 [b]	64.63 ± 0.48 [b]	43.80 ± 1.06 [b]
	2	633.00 ± 4.04 [a,b]	450.67 ± 8.51 [b]	63.30 ± 0.40 [a,b]	45.83 ± 0.85 [b]
	3	626.00 ± 3.21 [a]	484.67 ± 5.48 [b]	62.60 ± 0.32 [a]	48.46 ± 0.54 [b]
	24	635.00 ± 3.60 [a,b]	445.47 ± 3.17 [c]	63.50 ± 0.36 [a,b]	44.54 ± 0.31 [c]
	0.5	603.67 ± 5.23 [c]	475.00 ± 12.66 [a]	60.37 ± 0.52 [c]	47.50 ± 1.27 [a]
	1	589.00 ± 4.72 [b]	487.33 ± 25.06 [a]	58.90 ± 0.47 [b]	48.73 ± 2.50 [a]
0.01	2	591.00 ± 2.51 [b]	540.33 ± 2.02 [b]	59.10 ± 0.25 [b]	54.03 ± 0.20 [b]
	3	559.33 ± 2.02 [a]	487.33 ± 6.69 [a]	55.93 ± 0.20 [a]	48.73 ± 0.66 [b]
	24	560.33 ± 4.17 [a]	548.67 ±10.39 [b]	56.03 ± 0.41 [a]	54.86 ± 1.03 [c]
	0.5	563.33 ± 4.37 [c]	551.67 ± 11.05 [a]	56.33 ± 0.44 [c]	55.17 ± 1.10 [a]
	1	555.67 ± 5.36 [c]	570.33 ± 14.33 [a]	55.57 ± 0.53 [c]	57.03 ± 1.43 [a]
0.05	2	539.00 ± 3.78 [c]	614.00 ± 7.21 [b]	53.90 ± 0 .40 [c]	61.40 ± 0.72 [b]
	3	514.67 ± 10.17 [b]	618.00 ± 1.52 [b]	51.47 ± 1.01 [b]	61.80 ± 0.15 [b]
	24	484.67 ± 11.40 [a]	633.00 ± 2.08 [b]	48.46 ± 1.14 [a]	63.30 ± 0.21 [b]
	0.5	411.00 ± 6.70 [e]	635.67 ± 3.75 [a]	41.10 ± 0.66 [e]	63.56 ± 0.37 [a]
	1	367.00 ± 7.63 [d]	707.33 ± 6.43 [b]	36.70 ± 0.76 [d]	70.73 ± 0.64 [b]
0.1	2	323.67 ± 7.21 [c]	742.67 ± 3.75 [c]	32.36 ± 0.72 [c]	74.26 ± 0.37 [c]
	3	285.33 ± 11.09 [b]	756.67 ± 5.92 [c]	28.53 ± 1.11 [b]	75.66 ± 0.59 [c]
	24	229.67 ± 7.00 [a]	755.67 ± 5.23 [c]	22.96 ± 0.69 [a]	75.56 ± 0.52 [c]

Table: 2. 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

Sl. No.	Retention time	Constituents	Peak area %
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	Anthraergosta-5,7,9,14-tetra-ene	1.84
34	29.875	Ergosterol	16.72
35	30.084	Stellasterol	6.69
36	30.954	Gamma Ergosterol	1
37	32.109	β-Amyrin	0.66

CONCLUSION

Cytotoxic impetus of *Hexagonia tenuis* in *Allium cepa* root tip revealed a number of chromosomal aberrations. This kind of potential in inducing different types of aberrations will be a key towards the antitumor activities of the extract. Percentage of mitotic index decreased (from 66.63 ± 0.36$^{a, b}$ to 22.96 ± 0.69^{a}) with increase in concentration of the extract. That is dose dependent mitotic index was observed. Percentage of aberrations (75.56 ± 0.52^{c}) by the extract is comparable to that of the positive control. Percentage of the aberrations was directly proportional to the concentration of the extract. 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 will form a stepping stone for the production of biopharmaceuticals. Presence of biologically active components like linoleic acid, ergosterol, stellasterol *etc.* is the highlight of *Hexagonia tenuis* extract.

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