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Original Article

# CYTOTOXIC STUDIES AND PHYTOCHEMICAL ANALYSIS OF *ORTHOSIPHON THYMIFLORUS* (ROTH) SLEESEN

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**ABSTRACT**

**Objective:** Plant-derived compounds with therapeutic effect are getting attention all over the world. Researchers are mainly targeting to ethnic and folk knowledge for the exploration of novel drug candidates. *Orthosiphon thymiflorus* is a medicinal, aromatic herb used in folk medicine and Ayurvedic preparations. Hence, it is important to evaluate the cytotoxic potential and chemical profile of the plant.

**Methods:** Cytotoxicity was evaluated *in vitro* in DLA and EAC cell lines and *in vivo* by *Allium* chromosome aberration assay. Phytochemical screening of the extract was done by UV/Vis spectroscopy and FT-IR.

**Results:** Plant extracts showed significant toxicity in cell lines in higher concentration (100 µg/ml and 200 µg/ml). Cytotoxicity *in vivo* was determined by the reduction of mitotic index (MI) and induction of chromosome aberration (CA) by the extracts in meristematic cells of *Allium*. Plant extracts showed a time and dose dependent effect on MI and incidence of CA. Both extracts induced a spectrum of clastogenic as well as non-clastogenic CA. Phytochemical analysis of both extracts revealed the presence of bioactive compounds.

**Conclusion:** Bioactivity of the crude extracts may be ascribed to the active compounds present in the extract as indicated by spectroscopic analysis. Further phytochemical investigations may pave a new avenue to cancer drug research. Also, the cytotoxicity of extract apprises the necessity of further studies in the mammalian system.

**Keywords:** *Orthosiphon thymiflorus,* Cytotoxicity, Mitotic Index, *Allium cepa* Chromosome aberration assay, UV-Vis spectroscopy, FT-IR.



## INTRODUCTION

Herbal medicines are attracting the world attention mainly because of its fewer side effects and low cost. Ethno-pharmacognosy is a proliferating field of science which is becoming the center of attraction of many drug developing industries because of the escalating awareness of the unlimited potential of plant and plant derived products in therapeutics. Plants having potential therapeutic effects were identified, its phytochemical constituents were profiled, and the biological activities *in vitro* as well as *in vivo* have been proved by many authors. Even though herbs are being used in medicinal preparations, its safety for uncontrolled use is not assured [1]. Misuse or/and adulterations of medicinal plants can cause even death [2]. Plant extract having cytotoxic potential have been reported by various authors. Unrefined preparations and undefined standard of dosages set major challenges to herbal medicine system [3].

Cytotoxicity in the system is a major effect of crude extracts which are being used in herbal preparations. Cytotoxic phyto-compounds itself have some therapeutic value since components like vincristine, vinblastine, methotrexate, taxol *etc*. isolated from certain plants are being used as anti-cancer drugs [4-6]. *Allium* assay is a powerful tool to detect the cytotoxic potential of a sample by observing the clastogenic as well as the non-clastogenic CA and analyzing the MI. It's a simple, easy and reliable *in vivo* assay. The similarity to the mammalian system makes the model plant highly preferred by many researchers [7].

There are different chromatographic techniques which can be used to detect the phytochemicals in the crude extract. The separation of plant constituents is generally performed using one, or a combination of chromatographic techniques like column chromatography with high-pressure liquid chromatography or high-performance thin layer chromatography. Separated components can be identified by Mass Spectrum (MS). The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated. Relatively cost effective and simple spectroscopic techniques like UV/Vis and FT-IR can be used to detect the class of compounds. Even though it is limited to a conjugate system, it can be used as a primary data for structure elucidation of organic samples to indicate whether the conjugation is present in the given sample. UV-Vis data can provide the corroboration information from the data of IR, MS or NMR.

*Orthosiphon thymiflorus* is an aromatic medicinal herb belongs to Lamiaceae. It is mentioned in Ayurveda and also used in folk medicine as a medicinal plant. It is antidiarrheal, alexiteric, vulnerary and febrifuge and also mentioned as useful in erysipelas, dermatopathy such as scabies, dermatitis, and pruritus, hemorrhoids, wounds, ulcers and fever [8]. Even though it has been widely used in medicinal preparations and in folk medicines, little literature is available about the cytotoxic potential and phytochemical profile of the plant. *Orthosiphon* contains 9 species; pharmacological properties like antidiabetic, diuretic, anti-hepatotoxic, antibacterial, hypertensive and antitumor activity of this genus have been reported [9-11].

The aim of this study was to analyse the cytotoxic effect of two solvent extracts of *Orthosiphon thymiflorus* and characterize the phytochemicals in the active crude extract by UV-Vis and FT-IR spectroscopic analysis.

## MATERIALS AND METHODS

### Preparation of the extract-Soxhlet's extraction

Plant materials from Calicut University campus were collected, identified, cataloged and preserved in the herbarium, Dept. of Botany, University of Calicut (CALI 123719). Methanol: ethanol (70:30) and hexane extract of shade dried leaves were procured using Soxhlet's apparatus and concentrated and kept at 4 °C. Later it was suspended in 2% DMSO for *in vivo* analysis.

**Cytotoxic studies**

**a) *In vitro* study**

The tumor cells aspired from the intraperitoneal cavity of tumor-bearing mice was washed thrice in PBS or normal saline. Cell viability was determined by trypan blue exclusion method [12]. Viable cell suspension ($1x10^6$ cells in 0.1 ml) was added to tubes containing various concentrations of extract and volume was made up to 1 ml using PBS. Control tubes contained only cell suspension. The mixture was incubated for 3 h at 37 °C. Further, the cell suspension was mixed with 0.1% trypan blue and kept for 2-3 min and loaded to hemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The numbers of stained and unstained cells were counted separately. Five different concentrations of the extracts (10 µg/ml, 20 µg/ml, 50 µg/ml, 100 µg/ml, and 200 µg/ml) were used in the study to analyse the toxicity and elucidation of $IC_{50}$ value.

$$\% \text{ cytotoxicity} = 100 \times \frac{(T \text{ dead})}{(T \text{ total})}$$

Where, T dead is the number of dead cells in the treated group, and T total is the number of dead and live cells in treated group.

**b) *In vivo* study-*Allium* assay**

**Selection of controls**

DMSO (2%) was taken as the negative control and Ethyl methane-sulfonate (EMS), a potential mutagenic, teratogenic and possibly carcinogenic agent, is taken as a positive control in the present study. A treatment with a sub-lethal (100 ppm) concentration of EMS at different time durations as in the extract treatment was done.

**EC 50 value**

Onion bulbs were purchased freshly from the local market. Old roots and dry scales were removed and allowed to germinate by placing in a petri dish containing distilled water When the roots reached 2-3 cm in length, the root meristem was exposed to various concentrations of the extract at its peak mitotic time for different durations (1/2 h, 3 h & 24 h) and controls. Six concentrations (600 ppm, 450 ppm, 300 ppm, 150 ppm, 100 ppm & 50 ppm in 2% DMSO) of both solvent extracts were tested for their efficacy. The length of roots was noted before and after the exposure period and compared to the negative control.

Five concentrations (300 ppm, 150 ppm, 100 ppm & 50 ppm) of both solvent extracts, which were found to be effective by EC 50 analysis, were evaluated for their cytotoxic potential by *Allium* assay. The root tips were exposed to different concentrations for different time periods (1 h & 24 h).

**Mitotic squash preparation**

Mitotic squash preparation of the root tips were done with improved techniques [13]. Each experiment was repeated five times, and, at least, five micro slides were prepared for each parameter.

**Cytological study**

The five slide replicates were randomly selected for each test concentration for cytological analysis [14]. The slides were scored for MI and percentage of CA in Leica DM 500 microscope with an attached image analyzer.

**Phytochemicals analysis**

**a) UV-VIS spectroscopy**

The plant extracts were examined under UV and visible light for proximate analysis. The samples were diluted to 1:10, 1:20, and 1:50 according to the peak resolution in different organic solvents (methanol, ethanol, chloroform, hexane & DMSO) and scanned in a wavelength ranging from 200-1100 nm and characteristic peaks were detected.

**b) FT-IR spectroscopy**

FT-IR spectroscopy of the extracts was done in JASCO FT/IR-4700 type A, JAPAN. The characteristic peaks were recorded, and it was analyzed for their corresponding functional groups.

**Statistical analysis**

The data of MI and percentage of CA are represented in percentage mean±SE, and their level of significance was calculated by ANOVA in one factorial analysis combined with Tukey and Duncan posthoc tests. The analysis was performed using SPSS 19.0.

**RESULTS**

The test extracts showed cytotoxicity in the *in vitro* studies in cell lines. Fig. 1 shows the effect of methanol and hexane extract in DLA cell lines. Both solvent extracts exhibited a concentration-dependent effect of toxicity on DLA cell lines indicating its cytotoxic potential. Methanol extract showed more toxicity (80%) than hexane extract (72%). All the concentrations were significantly ($p \leq 0.05$) different from the negative control (2% DMSO). Lower concentrations showed a low rate of cell death below 50% in both extracts indicating its less cytotoxicity at low concentrations.

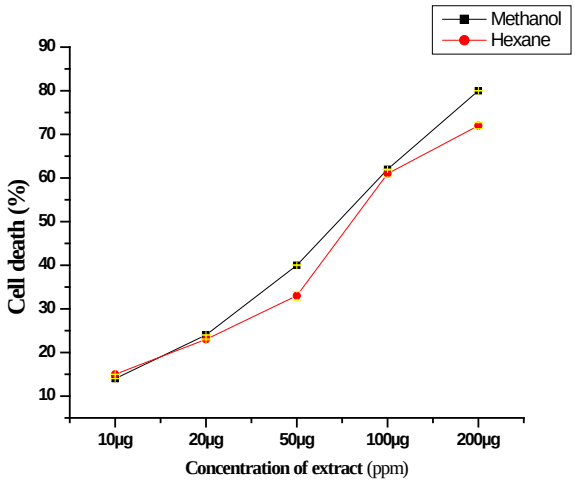


**Fig. 1: Effect of increasing concentrations *O. thymiflorus* methanol & hexane extracts in DLA cell lines**

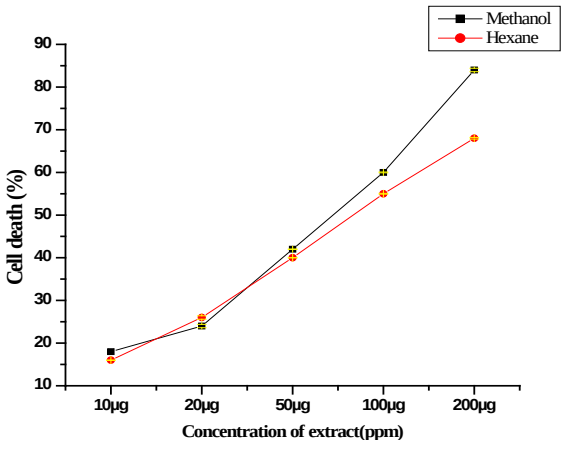


**Fig. 2: Effect of increasing concentrations *O. thymiflorus* methanol & hexane extracts in EAC cell lines**

Fig. 2 shows the effect of both solvent extracts in EAC cell lines. Both extracts showed a concentration-dependent cytotoxic potential. Concentrations of both extracts below 100 µg/ml were found to be less cytotoxic causing less than 50% of cell death but induced significant ($p \leq 0.05$) cell death compared to negative control. But highest concentration (200 µg/ml) caused 84% and 68% cell death by methanol and hexane extract respectively. Hexane extract exhibited comparatively less toxicity in tested cell lines.

EC 50 value obtained by measuring the root growth in the six different concentrations (600 ppm, 450 ppm, 300 ppm, 150 ppm, 100 ppm & 50 ppm) of both extracts is shown in fig. 3. Based on the EC50 values, dosage for further cytotoxic studies was selected. The highest concentrations (600 ppm & 450 ppm) were discarded in further cytotoxic studies in *Allium* assay.

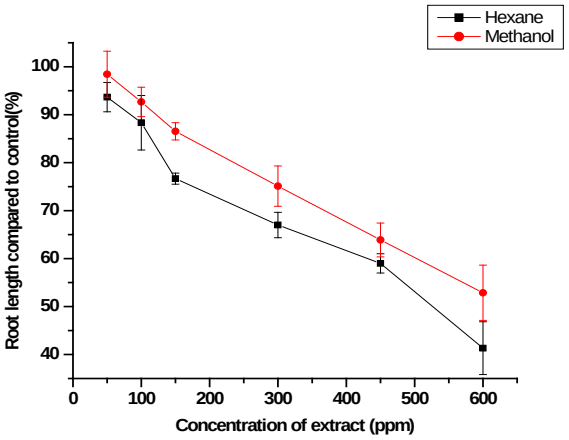


**Fig. 3: Root growth inhibition of *Allium cepa* exposed to methanol and hexane extracts of *Orthosiphon thymiflorus***

Each value (mean±S. E.) represents mean of five replicates. EC50 of methanol extract ~ 600 ppm and hexane extract ~500 ppm.

The two extracts methanol and hexane, used in the present study showed a dose and time dependent effect on MI (table 1). Different concentrations (50 ppm, 100 ppm,150 ppm & 300 ppm) of both solvent extracts reduced MI and the values were significantly ($p<0.05$) different from the negative control group. The lowest concentration of methanol extract brought down MI to 9.58% in short-term treatment (1 h) and to 14.26% in 24h treatment. The highest concentration (300 ppm) reduced MI to 28.73% and 38.97% respectively. On the other hand, the same concentrations of hexane extract reduced MI to 8.87% and 19.5% in 1h and 32.27% and 68.83 % in 24h. The result showed the high cytotoxic potential of hexane extract in the longer exposure. All the concentrations of methanol extract showed a lesser effect in short-term treatment. The mean values of MI index were not significantly different in lower concentrations showing its less toxicity, but it significantly differed from negative control. In long term treatment, higher concentrations (150 ppm, 300 ppm) of hexane extract showed high cytotoxicity.

Both extracts revealed a plethora of aneugenic, clastogenic as well as non-clastogenic aberrations in root tip cells showing its efficacy to affect the mitotic machinery of the cell in high concentrations. Fig. 4 and 5 show the frequency of various aberrations induced by different concentrations of extract. The result showed a proportional relationship between the percentage of CA and concentrations of extracts in different exposure time (fig. 6). Both solvent extracts induced CA in all the concentrations in a dose and time-dependent manner. Lower concentrations of methanol extract showed a lesser impact in mitotic apparatus but still showed a significant increase in CA compared to negative control.

**Table 1: Mitodepressive effect by increasing concentrations *O. thymiflorus* methanol & hexane extracts in *Allium cepa* root tip cells in different exposure periods**

| Treatment duration(h) | Concentration (ppm) | MI(mean±SE) | |
|---|---|---|---|
| | | +methanol | +hexane |
| 1 h | 50 ppm | 85.0±0$^{c}$ | 85.67±0$^{e}$ |
| | 100 ppm | 82.0±1.0$^{c}$ | 82.0±.33$^{d}$ |
| | 150 ppm | 70.67±0.3$^{b}$ | 76.67±2.0$^{c}$ |
| | 300 ppm | 67.0±2.0$^{b}$ | 63.67±1.2$^{b}$ |
| | EMS | 20.67±.6$^{a}$ | 20.67±0.6$^{a}$ |
| | negative control(DMSO) | 94.0±0$^{d}$ | 94.0±0$^{f}$ |
| 24 h | 50 ppm | 80.6±.32$^{e}$ | 75.67±.88$^{c}$ |
| | 100 ppm | 74.0±2.0$^{d}$ | 67.67±1.67$^{c}$ |
| | 150 ppm | 66.8±.92$^{c}$ | 32.3±2.4$^{b}$ |
| | 300 ppm | 57.37±.87$^{b}$ | 29.3±.67$^{b}$ |
| | EMS | 14.67±.67$^{a}$ | 14.67±67$^{a}$ |
| | negative control(DMSO) | 94.0±0$^{f}$ | 94.0±0$^{d}$ |

Each value (mean±S. E.) represents mean of five replicates. Means in a column followed by the same superscript letters are not significantly different ($P<0.05$, one-way ANOVA, Tukey and Duncan post hoc tests)

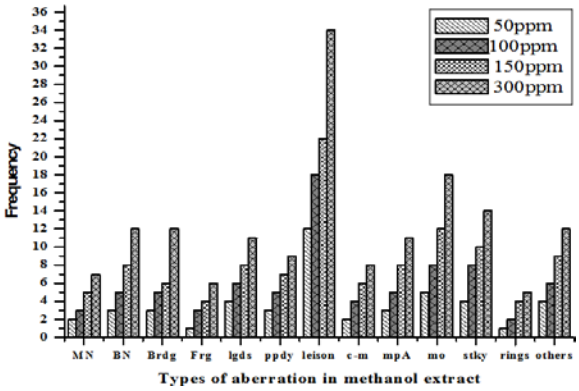


**Fig. 4: Types of aberrations induced by methanol extract in Allium root tip meristem**

MN-Micronuclei, BN-binucleated cells, Brdg-Bridges, Frg-Fragments, lgds-laggards, ppdy-polyploidy, leison-leisons in prophase, c-m-C-metaphase, mpA-multipolar anaphase, mo-misorientation in meta & anaphase, stky-stickiness in meta&anaphase, ring-ring chromosomes.

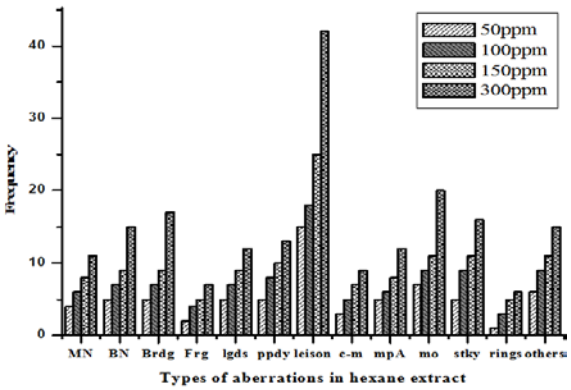


**Fig. 5: Types of aberrations induced by hexane extract in Allium root tip meristem**

MN-Micronuclei, BN-binucleated cells, Brdg-Bridges, Frg-Fragments, lgds-laggards, ppdy-polyploidy, leison-leisons in prophase, c-m-C-metaphase, mpA-multipolar anaphase, mo-misorientation in meta & anaphase, stky-stickiness in meta&anaphase, ring-ring chromosomes.

In lower concentrations of both extracts, non-clastogenic aberrations like scattering, diagonal metaphase and anaphases were observed. Aneugenic effects like nuclear erosion (fig 7a), abnormal grouping of chromosomes in a polyploid cell (fig 7b), chromosome bridge (fig 7g), chromosome fragments (fig 7i), C-metaphase (fig 7j), laggard (fig 7p), stickiness (fig 7m), pulverization(fig 7o) and micronuclei formation were observed in hexane extract even in lower concentration. In higher concentrations of both extracts, a significant increase in the frequency and diversity of aberrations were observed. Lesions (fig. 7k & 7n) in prophase were the highly frequent aberration in all treatment periods and concentrations. A proportional increase in the incidence of MN was also observed in hexane extract. The results showed that higher concentrations were able to inhibit cell division significantly. Hexane extract in its highest concentration showed ghost cells (fig 7r), cells without a nucleus and nuclear extrusion (fig 7p) obviously indicating the toxicity of the tested concentration in *Allium* root tip cells.

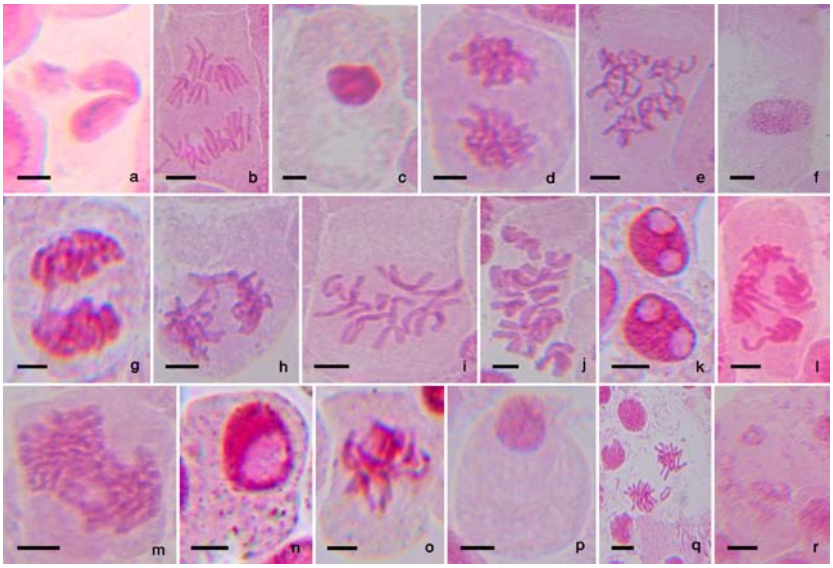


**Fig. 7: Different types of aberrations induced by methanol and hexane extract of *O. thymiflorus* in root tip meristem of *Allium cepa***

Fig. legend-a) Aberrant nucleus showing Nuclear Erosion, b) Abnormal Grouping of Chromosomes in a Polyploid Cell, c) Cellular Vacuolation, d) Chained Anaphase showing Pulverization, e) Chained Metaphase, f) Giant Cell, g) Chromosome Bridge at Telophase, h) Chromosome Displacement at Anaphase, i) Chromosome Fragments at Metaphase, j) C-Metaphase, k) Double Nuclear Lesions in a Binucleate Cell, l) Equatorial Separation at Anaphase, m) Sticky Chained Metaphase in a Polyploid Cell, n) Nuclear Lesion, o) Pulverized Metaphase, p) Nuclear Extrusion at Interphase, q) laggard, r) Ghost cell.

**Bar represents 5µm**

Spectroscopic analysis of the extracts showed the presence of active phytochemicals. Fig. 8 show FT-IR spectrum of methanol and hexane extract.

The possible bioactive compounds can be identified by tracing out the functional groups corresponding to the peaks in the spectrum (table 2). It revealed a spectrum of potential components that explicit the bioactivity of the extracts.

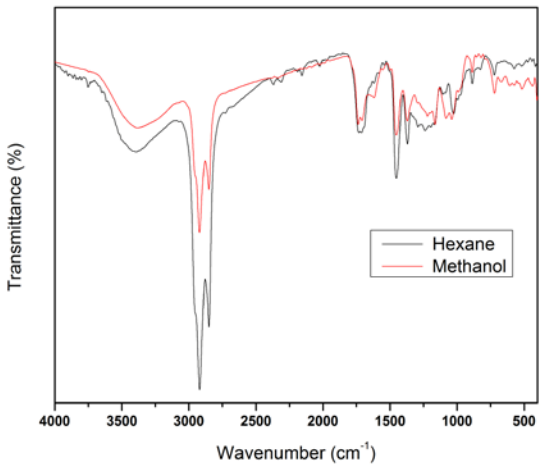


**Fig. 8: FT-IR spectrum of methanol extract of *O. thymiflorus* observed in the region of 400-4000 cm$^{-1}$**

**Table 2: FT-IR peak values and corresponding functional groups in methanol and hexane extract**

| Extract | Frequency($cm^{-1}$) | Band | Functional group |
|---|---|---|---|
| Methanol | 3392.17 | O–H stretch, H–bonded | Alcohols, phenols |
| | 2916.81 | C–H stretch | Alkanes |
| | 2846.42 | C–H stretch | Alkanes |
| | 1735.62 | C=O stretch | Esters saturated aliphatic |
| | 1709.59 | C=O stretch | α,β-unsaturated aldehydes, ketones |
| | 1618.95 | (N-H bends) | Amines |
| | 1453.1 | C–H bend | Alkanes |
| | 1366.32 | C–H rock | Alkanes |
| | 1106.94 | C–N stretch | aliphatic amines |
| Hexane | 3395.07 | O–H stretch, H–bonded | Alcohols, phenols |
| | 2917.77 | C–H stretch | Alkenes |
| | 2849.31 | C–H stretch | Alkanes |
| | 1713.44 | C=O Stretching | Aliphatic acid str |
| | 1454.06 | C–H bend | Alkanes |
| | 1366.32 | C–H rock | Alkanes |
| | 1163.83 | C–H($-CH_2X$) | alkyl halides |
| | 1107.9 | C–N stretch | aliphatic amines |
| | 1023.05 | C–N stretch | aliphatic amines |
| | 886.13 | C–H"loop" | Aromatics |
| | 719.318 | -C≡ C-H: C-H | Alkynes |

## DISCUSSION

Plant-derived compounds are being used in anticancer studies. Apoptosis is recognized as programmed cell death, which follows in several pathological situations in multicultural organisms, and it is a form of common mechanism for cell replacement, tissue remodeling, and removal of damaged cell [15, 16]. *In vitro* studies in DLA and EAC cell lines showed potential toxicity of the plant extracts. The cytotoxic effects of the extracts were estimated in terms of growth inhibition percentage and expressed as $IC_{50}$ which is the concentration of extract which inhibit cell growth of treated cells by 50% with reference to the control (untreated cells). Methanol extract showed a comparatively more inhibitory effect on both cell lines. $IC_{50}$ values of both solvent extracts found to be above 50 µg (fig. 1 & fig. 2). Percentage of cell death was above 75% in high concentration (200 µg) used in the study suggestive of its high cytotoxic potential at high concentrations. The result of the present investigation is in compromise with the earlier literature about the *in vitro* cytotoxic studies of methanolic extract of *O. thymiflorus* in DLA cells by MTT assay [17].

Cytotoxic studies in *Allium* root tip cells showed a wide range of aneugenic, clastogenic and non-clastogenic events. The result showed a significant (one-way ANOVA, $P<0.05$) decline in the MI in both extracts which is a clear indication of the Mito-depressive effect of the crude extract. A low rate of mitotic inhibition was noticed in lower concentrations (50 ppm, 100 ppm) of methanol extract. Significant ($P<0.05$) inhibition in cell cycle was observed in higher concentrations in 24h treatment. The highest concentration (300 ppm) of hexane extract in 24h decreased MI up to 50.2% compared to the positive control EMS, a potential mutagen. The results point to the potential phytochemicals possibly present in the extracts which can influence the cell cycle mechanism.

The panorama of aberrations manifested by the solvent extracts revealed its potential to affect spindle formation, spindle assembly, and DNA synthesis by affecting all the phases of cell cycle. Clastogenic effects like chromosome stickiness, pulverization, ring chromosomes, fragments, binucleated cells (BN), polyploidy, *etc.* were observed in both extracts at higher concentrations in higher frequency. Aneugenic aberrations like micronuclei, chromosome bridges and loops were also common in hexane extract in longer exposure (24h). It has been noticed that hexane extract induced an alteration in the shape and size of the cells in 1 h as well as in 24 h exposure. Cells with abnormal mono nucleus whose nuclear volume was twice (or more) larger than a normal cell were scored as giant cells [18]. Induction of giant cells has clinical significance during *in vitro* studies in cancer biology [19]. In hexane extract, nuclear extrusion was observed in higher test concentration. The phenomenon of material extruded from the nuclei observed in interphase cells had been described earlier [20]. This kind of nuclear dissolution described in the present study can probably present a correlation with cell death and constitute an important effect in the studied system.

The nature and occurrence of aberrations indicated that the crude extract contains certain components which were S phase independent since all the phases of mitosis is affected by the treatment [21]. Chromosomal aberrations and chromatid aberrations were noticed in both extracts. Single and multiple lesions were the most frequent aberrations in prophase and telophase cells. Lesions are the result of inhibition in S phase of the cell cycle where duplication of DNA strands happens. Formation of prophase lesions are common in irradiated cells, but the site of lesions or its repair mechanisms are not yet explored in detail. Earlier studies revealed a similar situation where UV irradiated lesions were found to be repairable in the cell [22]. MN was frequently observed in higher concentrations of both extracts which clearly indicate the presence of compounds that can silence cell cycle checkpoint genes [23]. BN cells, especially non-synchronized BN cells were typical of hexane extract except in low concentration. BN stem from dicentric chromosomes, which may occur due to the misrepair of DNA breaks or telomere end fusions, or as a result of the defective separation of sister chromatids at anaphase due to decatenation failures of DNA. Stickiness was frequently observed in metaphase chromosomes as well as anaphase chromosomes. Hexane higher concentrations showed intense stickiness involving the entire genome causing the clumping of the chromosome into a single mass making it difficult or impossible for normal disjunction. Chromosome stickiness is a clear evidence of geno toxicity, usually of an irreversible type leading to cell death [24]. Non-clastogenic aberrations like multipolar orientation and migration of chromosomes in metaphase and anaphases indicated the presence of compounds that can affect the spindle formation or/and assembly.

Polyploidy and aneuploidy were common incidences in all concentrations, but the frequency was high in higher concentrations of both extracts. The frequency of polyploidy was reduced after allowing the roots to recover, which indicates that the process of polyploidy is a reversible one [25]. Physiological chromosome aberrations like C-metaphase, vagrant chromosomes, laggards in metaphase and anaphases were also noticed. C-metaphase was very frequently seen even in lower concentrations of plant extracts. Metaphase, anaphase, and telophase bridges were more frequently observed in hexane extract. Bridges are the result of the translocation of the dicentric chromosomes causing unequal exchange and structural chromosome mutation. The results showed that higher concentrations were able to inhibit cell division significantly. Nuclear buds were prominent in the higher concentration of hexane extract in interphase as well as telophase

nuclei. Single and multiple buds were visible in highest concentrations which indicate the loss of amplified DNA, DNA repair complexes and/or excess chromosome accumulation from aneuploid cells. Recently nuclear buds also are gaining much attention along with MN and BN and regarded as biomarkers of genotoxic events [26].

The breakage–fusion–bridge (BFB) cycle model, which was originally proposed by B. McClintock, explains the amplification of genes and formation of anaphase nucleoplasmic bridges which cause the formation of BN, nuclear buds and MN as a consequence of the cycle [27]. According to this theory, nucleoplasmic bridges between BN usually break unevenly resulting in the unequal distribution of genetic material in daughter cells-one receiving extra copies of genes while the other loses genes. There is a chance that broken chromosomes which lack the telomerase can fuse with their replicas after DNA synthesis resulting in further amplification of genes adjacent to the break or fusion point. These amplified genes can form aberrant looped structures forming minute chromosomes or nuclear buds which can be eliminated by budding. Sometimes the interlinking DNA strand between the nucleus and buds breaks before being excluded from the cell and the bud becomes MN [28, 29]. BFB cycle model has been used to explain the generation of a variety of gene amplifications or genomic instabilities related to cancer. In the present study, highest test concentration (300 ppm) of hexane extract induced BN, MN and nuclear buds in root tip cells of *A. cepa* confirming the genomic instability caused by the crude extract at high concentration.

The incidence of structural, as well as numerical aberrations, affirmed the potential genotoxicity of plant extract. Both solvent extracts exhibited a significant genotoxic potential in *in vitro* as well as *in vivo* studies in the present investigation. Primary phyto-chemical analysis by spectroscopic method revealed the presence of bioactive compounds in the crude extract. The UV-Vis spectrum of extracts in different solvent extracts were recorded (Data is not shown). Methanol extract in chloroform (1:5) showed sharp absorption peaks in 205.7 (1.1 Abs), 248.8 (2.35 Abs), 285.2 (2.2 Abs). Hexane extract showed prominent absorption peaks in methanol (1:10) and chloroform (1:10) in 282 (1.7 Abs), 280.4 (1.05 Abs), 413.4 (1.2 Abs), 416.2 (0.8 Abs). The UV-Vis spectrum analysis points to the presence of bioactive compounds. The results are supported by the FT-IR spectroscopic analysis of the extracts. FT-IR studies enable the identification of the chemical constituents and elucidation of the structures of compounds. In the present study, FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids and aromatics in both extracts. UV-Vis and FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [30].

## CONCLUSION

The present study reveals the cytotoxicity of *O. thymiflorus* methanol as well as hexane extract in cell lines and *Allium cepa*. It is the first time report on the biochemical constitution of a bioactive crude extract of the plant by spectroscopic analysis. The phytochemicals present in the crude extract, from the spectroscopic analysis, showed the presence of a spectrum of bioactive compounds which have established therapeutic effects. The plant is used in folk medicines and Ayurvedic preparations. The result of the investigation points to the necessity of further studies in an animal system and isolation and characterization of the active compounds in the extract. The effectiveness of the crude extract over isolated compounds should also be analyzed since most of the biological activities in plant extract are due to a synergistic effect of compounds present in crude extracts. Further studies in the cytotoxic/geno protective effect of the plant *in vivo* animal model are in progress.

## CONFLICT OF INTERESTS

Declared none

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