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Active chemical constituents of *Cynanchum viminale* and its cytotoxic effects *via* apoptotic signs on *Allium cepa* root meristematic cells

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Abstract. The present study evaluates the cytotoxic efficacy of methanolic extract of *C. viminale* on *A. cepa* root meristematic cells. DAPI staining was used to study the chromosomal aberrations indued by extract of *C. viminale*. Evans blue staining method was employed to estimate the cell death of root cells of *A. cepa*. The plant extract was found to impart severe cytological damges, specifically chromosomal aberrations at interphase and prophase stage of cell division. Various apoptotic signs such as apoptotic body formation, nuclear budding, micronucleus, nuclear disintegration, nuclear breakage *etc*. were observed in meristematic cells of *A. cepa*. The results suggest the cytotoxic, preferably genotoxic effect of methanolic extract of *C. viminale* as evidenced by various apoptotic symptoms on *A. cepa* root cells.

Keywords. *Cynanchum viminale*, aberrations, *Allium cepa*, cytotoxicity, apoptosis

INTRODUCTION

Plants have long been used for millennia in traditional medicine against various ailments. Instead of a conventional single compound-single target approach, a consortium of bioactive molecules against multiple targets is gaining more attention nowadays. The synergistic action of various phytochemical compounds acts on various target domains, thus increasing therapeutic efficacy and eliminating the side effects (Cilla et al. 2015). The *Sarcostemma* genus (preferably *Sarcostemma acidum*) is considered as Somalata or Somavalli, also known as moon plant. It is a xerophytic, perennial leafless, jointed trailing shrub with green, cylindrical, fleshy glabrous, twining branches having milky white latex, leaves reduced to scales, opposite, flowers white or pale greenish white. The decoction of the plant is useful to gargle for throat and mouth infection, gonorrhoea, muscle pain *etc*. Recent molecular studies resulted in the taxonomic dissolution of *Sarcostemma* into *Cynanchum* (Meve & Liede- Schumann, 2012).

Allium cepa bioassay is an efficient procedure for assessing chromosome damages induced by plant extracts. It is considered as a preliminary cyto-

toxic screening test which shows high sensitivity and good correlation with mammalian test systems. It is also an important tool for environmental monitoring studies, employed to assess the impacts caused by xenobiotics (Leme & Marin-Morales, 2009; Khanna & Sharma, 2013).

The present study is an attempt to evaluate the phytochemical constituents of methanolic extract of *C. viminale* by GC/MS analysis and its cytotoxic screening with special emphasis on apoptotic signs.

MATERIALS AND METHODS

Plant material

Cynanchum viminale (L.) Bassi (1768: 17) subsp. *viminale* was collected from Karnataka, India (Coordinates: 11.8083° N, 76.6927° E). The specimen was authenticated and a voucher specimen (CALI No. 123742) was deposited at the Herbarium of Department of Botany, University of Calicut, Malappuram, Kerala, India.

Plant extract preparation

10 g of the ground plant materials were subjected to sequential extraction in n-hexane to remove non-polar components followed by 100 mL methanol. The extract thus obtained is then completely evaporated to remove the trace amount of methanol so as to avoid toxicity. Stock solution was prepared in water and different concentrations of plant extracts (200, 400, 600, 800 and 1000 µg/mL) were then made from it.

GC/MS analysis

Chemical composition was determined by GC–MS (Shimadzu QP-2010 Plus with Thermal Desorption System TD 20, fitted with a 60 m × 0.25 mm × 0.25 m WCOT column coated with diethylene glycol (AB-Innowax 7031428, Japan). Helium was used as a carrier gas at a flow rate of 1.21 mL/min at a column pressure of 77.6 kPa. Both injector and detector temperatures were maintained at 260 °C. Samples (6 µL) were injected into the column with a split ratio of 10:0. Component separation was achieved following a linear temperature program of 70-260 °C at 3 ◦C/min and then held at 260 °C for 6 min, with a total run time of 44.98 min. The MS parameters used were: electron ionization (EI) voltage 70 eV, peak width 2 s, mass range 40-850 *m/z* and detector voltage 1.5 V. The constituents were identified by comparison of their linear retention indices. The MS fragmentation pattern was checked with those of other compounds of known composition, with pure compounds and by matching the MS fragmentation patterns with National Institute of Standards and Technology (NIST) mass spectra libraries and with those in the literature (Adams, 2001). Finally, their quantification was performed on the basis of their GC peak areas.

Cytotoxic screening on A. cepa

Prior to initiating the test, the outer dry scales of onion bulbs were removed without destroying the root primordia. They were allowed for rooting by placing in distilled water for 1-2 days. Germinated bulbs with healthy roots (1-2 cm) were collected at a period of maximum mitotic activity (between 9 am and 10 am on sunny days) and washed with distilled water. The bases of bulbs were kept in vials containing different concentrations of plant extracts (200, 400, 600, 800 and 1000 µg/mL) in such a way that only roots were suspended in extracts. Positive and negative controls were also kept *viz*., hydrogen peroxide (2%) and distilled water. Root tips were collected from the different vials at 12 h, 24 h, and 48 h intervals. The collected samples were washed in distilled water and immediately fixed in modified Carnoy's fluid for 1 h. Then the root tips were subjected to hydrolysis with 1N HCl for 5-10 min and washed in distilled water followed by incubation in PBS for 15 minutes. Staining was done in DAPI staining solution for 30 minutes in dark condition and washed in PBS by a modified method (Begum & Alam, 2016). Root tips were squashed and mounted in 50% glycerol. Slides were then prepared and the number of damaged cells and total cells were scored in 6 different fields of view using 40X of the fluorescent microscope (Leica DFC 450C, Germany) for cytogenetic effects. Mitotic index (%) and aberration percentage (%) were calculated using the following formulae and values were expressed as mean±SE from at least three independent experiments:

$$\text{Mitotic Index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Aberration percentage (\%)} = \frac{\text{Number of aberrated cells}}{\text{Total number of cells}} \times 100$$

In situ *visualization of cell death*

For the assessment of cell death, control and treated bulbs with intact roots were placed in Evans blue staining solution for 15 min, followed by washing of the roots in running tap water for 30 min (Baker & Mock, 1994). Subsequently, 10 root tips measuring equal length (10 mm) from control and the treated groups were excised and soaked in 3 mL of N, N-dimethylformamide for 1 h at room temperature. The absorbance of Evans blue released was measured spectrophotometrically at 600 nm (Elico SL 218, India).

RESULTS

GC/MS analysis

The volatile composition of methanolic extract of *C. viminale* was determined by GC/MS. A total of 26 compounds were detected in the methanolic extracts of *C. viminale* by GC/MS. These compounds belonged to various classes *viz*., terpenoids, aldehydes, fatty acids, phenolics, fatty acid esters *etc*. The compounds identified in the methanolic extract of *C. viminale* by GC-MS analysis are enlisted in Table 1 and gas chromatogram is given as Fig. 1. The major compounds detected were carvone (31.57%), hexadecanoic acid (29.56%) and 9-cis-octadecenoic acid (10.57%). Terpenes were the predominant class of compounds present in the extract; also aldehydes and alcohols in significant quantities. 2-hexyl-2-decenal, pentadecanal, and myristaldehyde were the aldehydes present in the extract. Coniferyl alcohol, 2,4,4-trimethyl-2-penten-1-ol, (E)-2-nonenol and 1-heptanol were the alcohols present in the extract. Nonanoic acid methyl ester, heptadecanoic acid methyl ester, isopropyl pentadecanoate and methyl docosanoate were the fatty acid esters present in the extract. Phenolic compounds like p-vinylguaiacol, 3-tert-butyl-4-methoxyphenol and allylsyringol were detected in negligible amounts. An alkaloid, 6-bromo-5-methoxy-Nb methoxycarbonyltryptamine was also detected in the analysis. The extract contained fatty acids such as myristic acid,

Table 1. Chemical composition of *C. viminale* as analysed by GC/MS.

Sl No.	RT	Compounds	Class	Content (%)
1	6.58	Limonene	Terpene	8.06
2	**9.68**	**Carvone**	**Terpene**	**31.57**
3	10.98	p-vinylguaiacol	Phenol	0.37
4	14.25	3-tert-butyl-4-methoxyphenol	Phenol	0.43
5	15.85	Allylsyringol	Phenol	0.96
6	16.33	Coniferyl alcohol	Alcohol	5.34
7	16.35	2-nitropropane	Alkane	0.12
8	17.24	Myristaldehyde	Aldehyde	1.64
9	17.69	Nitrous acid, butyl ester	Carboxylic acid ester	0.13
10	18.12	Nonanoic acid, methyl ester	Fatty acid ester	0.47
11	18.45	Myristic acid	Fatty acid	0.56
12	**18.55**	**Hexadecanoic acid**	**Fatty acid**	**29.56**
13	18.82	2-methyl 1-butanol nitrite	Organic compound	0.1
14	18.91	Acetic acid, methyl ester	Carboxylic acid ester	0.11
15	18.95	6-bromo-5-methoxy-Nb methoxycarbonyltryptamine	Alkaloid	0.81
16	19.97	Phytol	Diterpene alcohol	0.95
17	**20.28**	**9-cis-octadecenoic acid**	**Fatty acid**	**10.57**
18	22.33	Pentadecanal	Aldehyde	0.37
19	22.63	Isopropyl pentadecanoate	Fatty acid ester	0.9
20	24.11	2,4,4-trimethyl-2-penten-1-ol	Alcohol	0.33
21	24.23	Methyl docosanoate	Fatty acid ester	0.12
22	24.27	(E)-2-nonenol	Alcohol	0.25
23	24.58	4-methyl pentanoic acid	Carboxylic acid	0.66
24	36.20	1,5-diazabicyclo[5.4.0]undec-5-ene	Amide	0.73
25	37.33	2-hexyl-2-decenal	Aldehyde	0.34
26	39.38	1-heptanol	Alcohol	4.55

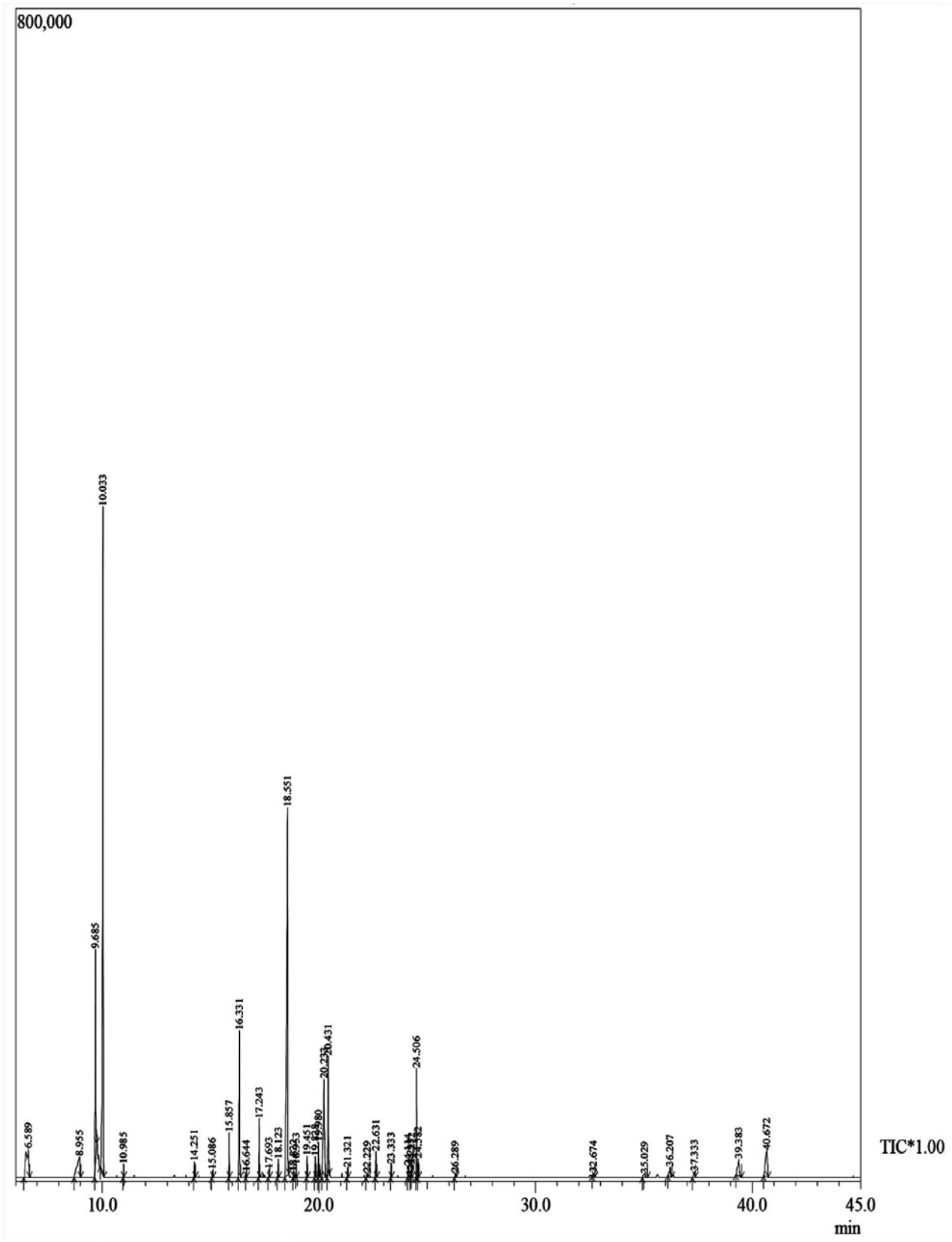

Fig. 1. GC chromatogram of methanolic extract of *C. viminale* [Total ion current (TIC) chromatogram].

9-cis-octadecenoic acid, tridecanoic acid and hexadecanoic acid. Among these, hexadecanoic acid was the predominant one. Negligible quantity of carboxylic acid esters *viz*., nitrous acid butyl ester and acetic acid methyl ester were also present in the extract. Other compounds belonging to amides, alkanes, alkenes, alkynes and heterocyclic organic compounds *etc*. were also detected in trace quantity.

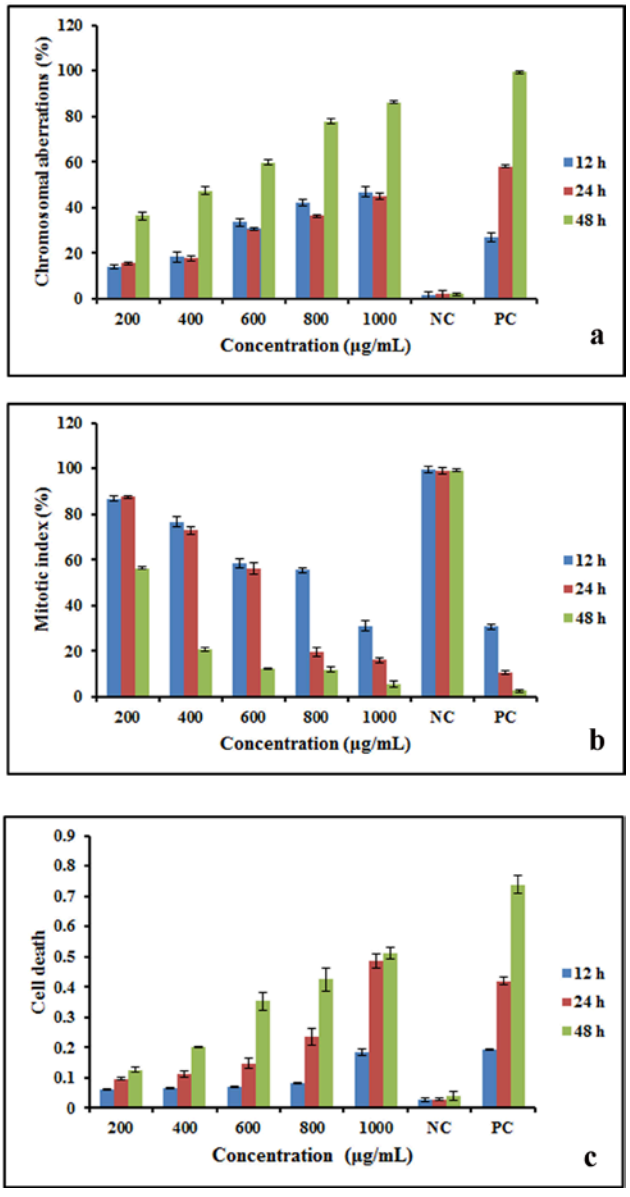

Fig. 2. a: Determination of chromosomal aberrations on *A. cepa* by *C. viminale;* b: Mitotic index; c: Spectrophotometric determination of cell death by Evans blue staining.

Cytotoxic evaluation on A. cepa *root meristem*

Cytotoxic potential of *C. viminale* in terms of mitotic index and chromosomal aberrations were tested on *A. cepa* root tips. The concentrations of methanolic plant extracts of 200, 400, 600, 800 and 1000 µg/mL as well as incubation period of 12 h, 24 h, and 48 h were taken as the experimental conditions. Time-and dose-dependent increase in chromosome aberrations were observed in *A. cepa* as visualized by DAPI staining.

Effect on mitotic index

Reduction in mitotic index is an important factor concerning the cytotoxicity of plant extracts on *A. cepa*. At 12 h period of incubation, the percentage of dividing cells was 86.66 ± 1.07 in the 200 µg/mL concentration of *C. viminale* (Fig. 2b). On increasing concentration, mitotic index is found to be gradually declined with respect to concentration and exposure time. At the highest concentration, 1000 µg/mL of *C. viminale,* mitotic index was observed as 31.11 ± 2.24%. In *C. viminale* extract treatment, mitotic index was found to be even lower than the positive control. The decrease in mitotic index was positively correlated with an increasing concentration of plant extracts. In addition to concentration, the time period is an important factor in genotoxicity and reduction of mitotic index. At the final time period of 48 h, mitotic index (5.47 ± 0.62%) was declined to much lower percentage in all concentrations tested than other two time periods considered. In the case of positive control, mitotic index was found to sharply decreased to 2.55 ± 0.56% at 48 h where was in negative control group, no reduction in mitotic index was observed. The progressive reduction in the number of dividing cells at increasing concentrations of plant extracts suggests that the plant extract has a mitodepressive effect on the cell division of *A. cepa*.

Effect on chromosomal aberrations

Chromosomal aberration percentage is also an endpoint parameter considered for cytotoxicity assays. Time-and dose-dependent increase in chromosome aberrations was observed in *A. cepa* exposed to plant extracts (Fig. 2a). At the lowest concentration 200 µg/mL, chromosome aberrations were 13.98 ± 1.74%. As observed in the case of mitotic index of *A. cepa* root cells treated with plant extract, dose- and time-dependent variation of chromosome aberrations were also observed. During 12 h treatment period and at the high-

est concentration of methanolic extracts of *C. viminale,* chromosome aberrations observed were 46.88 ± 0.68%. Hydrogen peroxide was used as the positive control, which induced serious clastogenic aberrations in *A. cepa* root cells in the form of prominent nuclear lesions. However, the plant extracts at 600, 800 and 1000 µg/mL concentrations induced more aberrations than the positive control. In the case of chromosomal aberrations, it was increased up to 86.24 ± 0.68 % for *C. viminale* at 1000 µg/mL concentration for 48 h.

Wide spectra of chromosomal aberrations were induced by the plant extract, more specifically numerous apoptotic symptoms were found to be prominent. The major chromosomal aberrations observed in the study was lesions, nuclear budding, nuclear peak, nuclear extrusion, nuclear fragmentation, bridged binucleate cell, giant cells, nuclear disintegration, nuclear erosion, hyperchromasia, fragmentation, cytoplasmic vacuolation *etc.* (Fig. 3-4). Nuclear buds were observed as frequent chromosomal aberration observed in higher concentration of plant extract and its various stages of development were also observed (Fig. 5). It is noteworthy to observe the apoptotic symptoms such as apoptotic bodies, nuclear disintegration, micronucleus *etc.* in *A. cepa* cells treated with different concentrations of *C. viminale* plant extract. Most of the damages were multiple aberrations such as bridged binucleate cell, giant cell with cytoplasmic shrinkage, shrunken and twisted cell with nuclear diminution, double budding and lesion, chromosome fragmentation in the hypoploid cell *etc.* which indicated the acute cytotoxic potential of the species of *Cynanchum*. These results suggested the significant cytotoxic potential or more specifically, genotoxic potential of methanolic extracts of *C. viminale* on *A. cepa* meristematic cells mediated by apoptotic signs.

In-situ *visualization of cell death*

Visualization of cell death of *A. cepa* root cells was performed by Evans blue staining and their corresponding estimation of cell death was carried out spectrophotometrically at 12, 24 and 48 h of treatment periods. N, N-dimethylformamide was the solvent used to release Evans blue from root cells and the solvent containing Evans blue was then quantified by noting their absorbance. Spectrophotometric determination of cell death suggested that severe cytotoxicity was observed in the higher concentration of plant extracts at 48 h of the incubation period.

At 12 h of incubation of *A. cepa* root cells with methanolic extracts of *C. viminale*, absorbance was found to be gradually increasing with respect to the concentration. Furthermore, cell death was highest in positive control and minimum for negative control. Dose and time served as an important factor concerning the cell death of *A. cepa* by methanolic extracts of *C. viminale*. Dosage and exposure time was found to be directly proportional to cell death. Incubation of *A. cepa* root cells with methanolic extracts of *C. viminale* for 24 h resulted in the cell death of maximum absorbance 0.48 ± 0.02 (Fig. 2c). Finally, incubation of *A. cepa* with methanolic extracts of *C. viminale* for 48 h caused a profound cell death. The absorbance read was 0.51 ± 0.01, which corresponds to the cell death. Negative control exerted negligible cell death and positive control treated *A. cepa* showed extremely severe cell death in terms of 0.73 ± 0.03 absorbance.

DISCUSSION

Methanol has a higher dielectric constant than ethanol; which enables to extract more polar compounds in comparison with ethanol. As a safety concern, methanol content is completely removed by evaporating the extract and thus the further studies were carried out with various concentration of extracts prepared in water. The GC/MS analysis revealed the presence of 26 constituents in the methanolic extract of *C. viminale* (Table 1). The peak with a maximum area of intensity of 31.57% corresponds to carvone followed by hexadecanoic acid (29.56%) and 9-cis-octadecenoic acid (10.57%). Carvone is a monoterpene found as an important constituent of essential oil of spearmint, clove, syzygium *etc.* (Kokkini et al. 1995; Chaieb et al. 2007) and found to have insecticidal and genotoxic activity. Apart from these, limonene is another monoterpene that occupied 8.06% of the total area. The cytotoxic activity of limonene was evaluated in amelanotic melanoma C32, renal cell adenocarcinoma ACHN, hormone-dependent prostate carcinoma LNCaP, and MCF-7 breast cancer cell lines by the sulfo rhodamine B assay (Loizzo et al. 2007). p-vinyl guaiacol is a phenolic component present in 0.37% peak area. Also, the compound is a major constituent of almost all essential oils from plants (*Bituminaria*, *Ferula*, *Torreya etc.*) as reported before. 3-tert-butyl-4-methoxyphenol is a phenolic constituent and a potential antioxidant compound which was recognized from the essential oil of *Dictamnus dasycarpus* which showed significant antimicrobial activity and cytotoxicity towards ACHN, MCF-7, ZR-75-30, MDA-MB-435S, Hep-G2 and Bel-7402 cell lines (Lei et al. 2008).

Coniferyl alcohol is present as 5.34% of the peak area of the total area of intensity. It is synthesized *via*

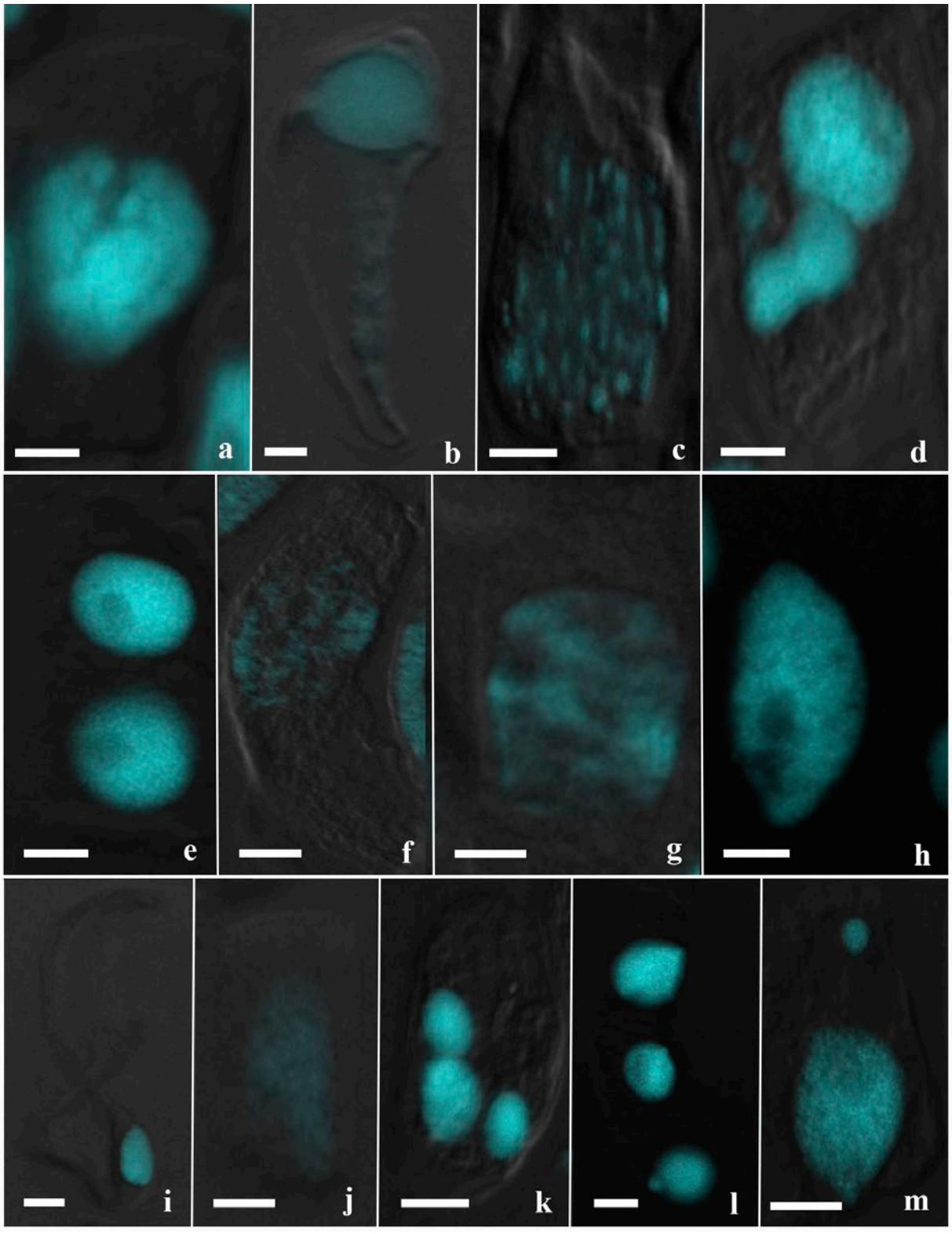

Fig. 3. Chromosomal aberrations induced by extract of *C. viminale* on *A. cepa*. a: apoptotic breakage of nucleus at interphase; b: cytoplasmic vacuolation; c: apoptotic fragmentation of nucleus; d: binucleate cell showing micronuclei; e: binucleate cell with lesions; f: nuclear disintegration; g: apoptotic nuclear disintegration; h: nuclear peak; i: shrunken and twisted cell with nuclear diminution; j: nuclear disintegration; k: trinucleate cell; l: trinucleate cell showing different stages of nuclear budding; m: nuclear budding and micronucleus; Bar: 10 μm.

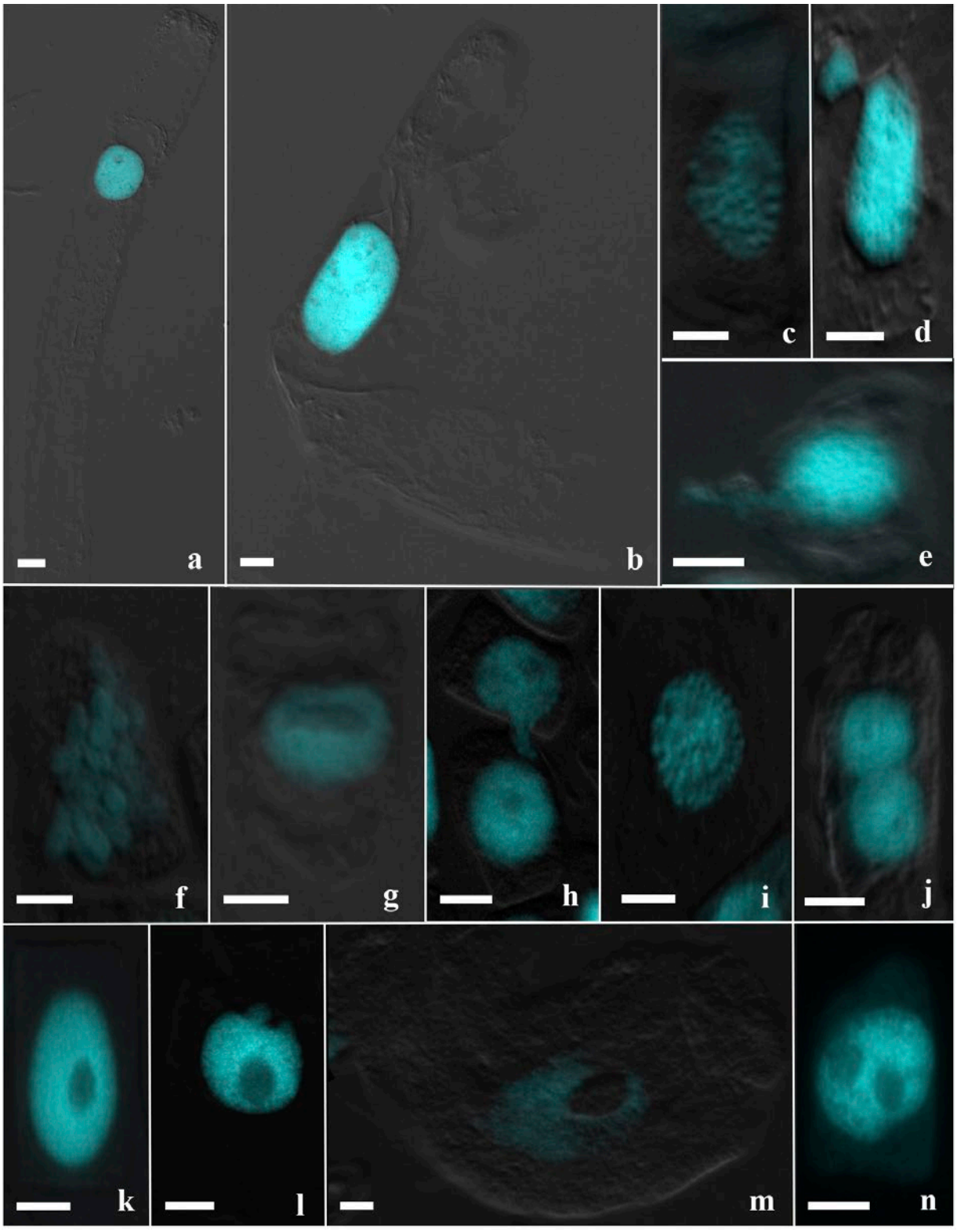

Fig. 4. Chromosomal aberrations induced by extract of *C. viminale* on *A. cepa*. a: giant cell; b: giant cell with cytoplasmic shrinkage; c: formation of apoptotic bodies in the nucleus; d: bridged binucleate cell; e: nuclear extrusion; f: apoptotic body formation; g: nuclear and cytoplasmic lesions; h: cytomictic transfer of nuclear material; i: nuclear disintegration; j: binucleate cell; k: nuclear lesion; l: double budding and lesion; m: giant cell showing nuclear disintegration and lesion; n: double nuclear lesions; Bar: 10 μm.

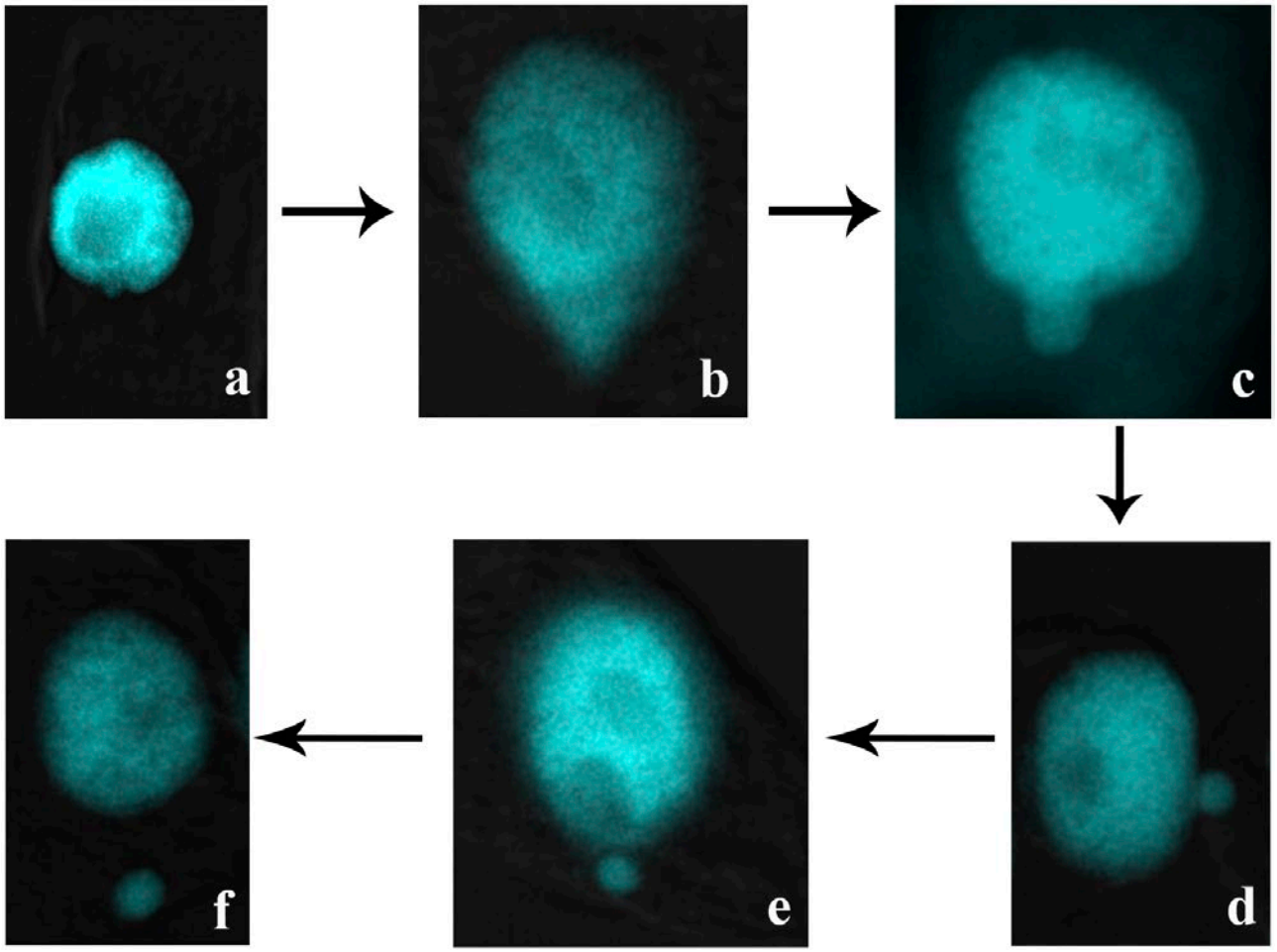

Fig. 5. Various stages of nuclear budding induced by extract of *C. viminale*. a: initiation of bud; b: growth of bud; c: protruding as fully formed bud; d: bud with a basal notch; e: detachment of bud from the nucleus; f: micronucleus.

the phenylpropanoid biochemical pathway and it is an intermediate in the biosynthesis of eugenol, stilbenoids, and coumarin (Kadir et al. 2015). Myristic acid is another fatty acid component present in 0.56% in the whole content of plant extract. Earlier the antioxidant and larvicidal activity against malaria and filariasis vectors were studied using the bioactive fraction of myristic acid from *Ammannia baccifera* aerial extract (Suman et al. 2013). Phytol is another compound detected (0.95%) and it is a diterpene alcohol found ubiquitously in many plant species. It was well documented that phytol is having cancer preventive properties irrespective of their concentration in the plant (Hema et al. 2011).

In this study, *C. viminale* contained a bioactive fatty acid hexadecanoic acid which was found to be predominant (29.56%). Hexadecanoic acid, a palmitic acid type compound was detected in extracts of various plants and have shown to possess hemolytic, antioxidant, anticancer, nematicide, 5-alpha reductase inhibition properties *etc*. (Jananie et al. 2011; Kalaivani et al. 2012). A systematic study with respect to the fatty acid composition of *Sarcostemma viminale* has been carried out earlier, where hexadecanoic acid and octadecanoic acid were the major components (Girme et al. 2014). 9-cis-octadecenoic acid, another fatty acid present as 10.57% of the total content of volatile compounds present in *C. viminale*. The derivatives or their esters have the potential to act against cancer or prevent cancer at the very initial stage itself (Farina & Chodahry, 2011).

The present study evaluates cytotoxic efficacy of *C. viminale* mediated by acute apoptotic symptoms in *A. cepa* root cells. Several researchers had demonstrated the efficacy of *A. cepa* bioassay for validating the cytotoxic potential of plants. The present observations showed a sharp decline in the mitotic index of *A. cepa* root cells as a result of treatment with different concentrations of the

plant extract, which is a clear indication of the mitotic depressive effect of the crude plant extracts. The positive control used for the study was H_2O_2, a serious clastogen which directly interacts with genetic material and result in prominent nuclear lesions in *A. cepa* which suggest that it interfere with cell cycle mechanism at the initial stage itself; so cells couldn't be passed onto the next stages of cell cycle.

The aberrations induced by plant extract had the potential to affect all phases of cell cycle. Henceforth, these results suggest that the tested concentrations of *C. viminale* extract is inhibitory, turbagenic and mitodepressive on cell division of *A. cepa*, which is in agreement with Akintonwa et al. (2009). The genotoxic effect of *C. viminale* was evidenced by a remarkable lowering of mitotic division in vegetative cells of *A. cepa*. In the experiments, mitotic activity showed a tendency to decrease to 5.47 ± 0.62% respectively for *C. viminale* at the highest concentration (1000 µg/mL) of plant extract at 48 h treatment. This reduction in the mitotic activity could be attributed to inhibition of DNA synthesis or blockage of the cell cycle in G2 phase, thus preventing the cells from entering into mitosis (Sudhakar et al. 2001).

Many serious chromosomal aberrations were observed as a result of treatment with various concentrations of plant extract. Of these, 90% of the damages were contributed to the genotoxic aberrations. Treatment of *A. cepa* with *C. viminale* extract resulted in various apoptotic symptoms like nuclear buds, micronuclei, nuclear fragmentation, nuclear blebbing *etc.* Most of the damages were nucleotoxic, whereas other aberrations were caused by the disturbance on the formation of spindle fibers during cell division. Nuclear buds are one of the prominent aberrations observed in the bioassay experiment. Four models have been proposed for the generation of nuclear buds. Nuclear buds are formed in the S-phase, representing the expulsion of excess genetic material derived from the polyploidization process, which may subsequently lead to micronucleation (Fernandes et al. 2007; Lindberg et al. 2007)micronucleus-like bodies attached to the nucleus by a thin nucleoplasmic connection, have been proposed to be generated similarly to micronuclei during nuclear division or in S-phase as a stage in the extrusion of extra DNA, possibly giving rise to micronuclei. To better understand these phenomena, we have characterized the contents of 894 nuclear buds and 1392 micronuclei in normal and folate-deprived 9-day cultures of human lymphocytes using fluorescence in situ hybridization with pancentromeric and pantelomeric DNA probes. Such information has not earlier been available for human primary cells. Surprisingly, there appears to be no previous data on the occurrence of telomeres in micronuclei (or buds, whose chromatin replication has failed. Nuclear bud formation from broken anaphase bridges (Gisselsson and Pettersson 2000) would appear to be an clear explanation, assuming that the typically stalked structure of a bud results from the collapse of the bridge when it is resolved.

The mechanisms responsible for micronucleus have not been yet fully understood. It may have originated during anaphase from lagging acentric chromosomes or chromatid fragments caused by misrepair of DNA breaks or unrepaired DNA breaks (Fenech et al. 2011; Bonciu et al. 2018). Nuclear blebs were also observed in *A. cepa* cells, consisting of nuclear material, with bud-shaped excrescences on the main nucleus, protruded from the nucleus, but without an obvious constriction or bridge between the protruding nuclear material and nucleus (Wang et al. 2014). Nuclear lesions and erosions are a type of nuclear disintegration, observed frequently in *A. cepa* cells as a result of treatment with *C. viminale* extract. These may suggest the direct action of phytochemical components on DNA synthesis and it is a cytological evidence for the inhibitory action on DNA biosynthesis and nuclear poisoning (Saghirzadeh et al. 2008; Ngozi, 2011). Nuclear erosion, which may result from the disintegration of chromatid proteins, represents irreversible toxicity (Karaismailoglu et al. 2013). Nuclear extrusion or sometimes nucleolar extrusion was another type of clastogenic event frequently observed in *A. cepa* cells. It is known that the nuclear pore complex (NPC) was the most important channel for nuclear material transport. The phenomenon that the nucleolar material was extruded from the nucleus into the cytoplasm could be explained by the fact that the proteins were affected after plant extract treatment, causing the NPC to lose selectivity (Qin et al. 2010). The fragmentation of nuclei may indicate cell death process and this may ultimately result in aneuploidy and then to cell death. This pattern of nuclear degeneration of nucleus were also observed in programmed cell death in the nucellus of *Tillandsia* presenting various signals of degeneration like deformed shape, chromatin condensation, plasmalemma detachment etc. (Brighigna et al. 2006). Binucleate and trinucleate cells were the frequent aberrations observed in the study, due to the inhibition of cytokinesis in any of the control points of the cell cycle (Özkara et al. 2015). Moreover, shrunken root cells, nuclear blebs, marked nuclear chromatin condensation, fragmentation *etc.* clearly indicate the possibilities to tend towards apoptosis. These clastogenic, as well as apoptotic signs of aberrations, provide a clue that the plant *C. viminale* can be effectively utilized for anticancer studies.

In addition, it is interesting to highlight the high frequency of multiple chromosomal aberrations [bridged

binucleate cell, giant cell with cytoplasmic shrinkage, chromosome fragmentation in a hypoploid cell, giant cell showing nuclear disintegration and lesion, double nuclear lesions *etc.*] in cells of *A. cepa* rather than single aberration by treatment with *C. viminale* extracts. The above results point to the phytochemicals present in the extracts which might have disrupted the cell cycle mechanism since various cytotoxic compounds such as carvone, limonene *etc.* were detected in GC/MS analysis. They might have possibly interfered with the normal cell cycle process and led to cell death. The present results thus support the notion that the cytotoxic effect of plant extracts is due to the synergistic action of a broad array of phytochemicals, the total activity of which may result in health benefits. Moreover, multiple aberrations of chromosomes might have attributed by the multiple compound-multiple target mechanism of interaction between phytochemical constituents of *C. viminale* and *A. cepa* cells.

Cytotoxic efficacy of *C. viminale* was then confirmed by estimating the cell death of *A. cepa* root cells. Evans blue staining method works on the basis of its penetration to non-viable cells (Panda et al. 2011). Evans blue staining of treated and control roots of *A. cepa* points is considered as an indirect evidence of cell death by visualising the intensity of Evans blue taken up by roots, suggesting the loss of viability of cells. The intensity of dye absorbed by root cells was directly proportional to the cell death; this could be seen within few minutes after the treatment, in corroboration with the result reported earlier (Achary et al. 2008). Cell death can be positively correlated with an increase in the concentration of plant extract and increase in duration of treatment.

CONCLUSION

The cytotoxic effects were found to increase proportionately with the concentration of plant extract. The chromosomal aberrations observed in this study are evidently caused by the chemical constituents in the extract since no aberration was observed in the negative control. The above obtained cytotoxic results may account for the severe cell death and this observation provides a plausible basis for its further use in anti-proliferative studies on *in vitro* cancer cell lines. However, the mechanism of action remains to be investigated in plant test system and further studies are necessary to clarify the fact.

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