Pro-metaphase arrest, polyploidy, micronuclei, and mitotic abnormality inducing agents' isolation from leaf aqueous extract of *Clerodendrum viscosum* Vent.

Sujit Roy, Lalit Mohan Kundu, Gobinda Chandra Roy, Manabendu Barman, Sanjib Ray*

Molecular Biology and Genetics Unit, Department of Zoology, The University of Burdwan, Golapbag, Purba Bardhaman-713104, West Bengal, India.

***Corresponding author.**

E-mail address: sray@zoo.buruniv.ac.in; ray.sanjibray@gmail.com

FAX: 91 0342 2634200 ; Office [0342] 2656566, 2658554, {Ext. - 426}; +919434643512(M)

Abstract:

Clerodendrum viscosum is a traditionally used medicinal plant and the earlier reports indicate its leaf aqueous extract (LAECV) contains metaphase arresting, cell cycle delay, and mitotic abnormality inducing active principles. The present study aimed to isolate pro-metaphase arresting, polyploidy, micronuclei, and mitotic abnormality inducing active principles of LAECV. The LAECV was successively fractionated as petroleum ether (PEF), chloroform (CHF), and ethyl acetate (EAF) fractions. All the extract fractions were tested for *Allium cepa* and *Triticum aestivum* root swelling and root growth inhibition analyses. The petroleum ether fraction was selected for further cytotoxicity analysis on *A. cepa* root tip cells and was processed for detection of the active principles through HPLC, LC-MS, GC-MS, and IR analyses. The comparative seedlings' root growth and swelling patterns indicate the bioactive principles are effectively fractionated in PEF and GC-MS analysis revealed the presence of Clerodin (m/z 434.3), 15-hydroxy-14, 15-dihydroclerodin (m/z 452), 15-methoxy-14, 15-dihydroclerodin (m/z 466), and 14, 15-dihydroclerodin (m/z 436) with a retention time of 14.038, 14.103, 14.480 and 14.655 respectively. Thus the present study explores clerodane diterpenoids of LAECV as pro-metaphase arresting, polyploidy, micronuclei, and mitotic abnormality inducing active principles.

Keywords: Diterpenoids; Mitotic abnormality; Allium cepa; Metaphase arrest.

1. Introduction:

Clerodendrum viscosum (Family: Lamiaceae, common name: bhant, ghentu), a weed of agricultural fields, is widely distributed throughout Asia, Africa, Australia, America, etc. It has gained a reputation in traditional Ayurvedic, Homeopathic, and Unani medicine [1]. The plant's ethanolic extracts have shown to have a bundle of bioactivities including antioxidant, antimicrobial, hepatoprotective, wound healing, and antidiarrheal activities [2]. The whole plant juice is used against worm infection, cough, itching, leprosy, scorpion sting, asthma, bronchitis, fever, etc. [3, 4]. Bark juice is used to relieve indigestion and abdominal pain. The plant is well known for its effectiveness against rheumatism in Unani medicine [5]. It is prescribed to treat post-natal complications, diarrhea, and fresh wounds in the Indian Homeopathic system [6, 7]. The different parts of this plant are used as a remedy for asthma, malaria, cataract, diseases of the skin, blood, and lung by the Indian Tribals of Chotanagpur plateau [4]. Leaf and stem aqueous extract of *C. viscosum* has resulted in significant insecticidal activity against tea pests *Helopeltis theivora* and *Oligonychus coffeae* Nietner when compared to acaricide and *Azadirachta indica* [8]. Chloroform and ethyl acetate extracts of the leaves have shown higher insecticidal activity against *Rhizopertha dominica*, *Sitophiulus oryzae*, and *Tribolium castaneus* than petroleum ether extract [9]. Significant cytotoxic and anthelmintic potential against *Pheretima posthuma* is exhibited by crude methanolic and aqueous extract of its roots [10]. The antihelmintic activity was reported in leaf ethanolic, methanolic, and aqueous extracts against *Pheretima posthuma* [11]. The leaves and roots have great potential against different microbial and fungal strains. Acetone and chloroform extracts of leaves have an inhibitory effect on the growth of *Shigella sp*., *Vibrio cholerae*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, etc. [12] while ethanolic fraction has shown antifungal activity against *Aspergillus niger*, *A. flavus*, and *Candida albicans*[13]. Saponin isolated from petroleum ether extract of leaves is observed to have analgesic activity [14]. In vivo antinociceptive activity is found in methanolic extract comparable to diclofenac sodium drug [15]. Root extract exhibits anti-inflammatory activity against Carrageenan-induced edema in mice [16]. Wound healing activity is exhibited by ethanol and chloroform leaf extracts [17]. Reduction in CCl4-induced hepatotoxicity on rats after treatment with methanolic leaf extract reveals its hepatoprotective activity which is further supported by

biochemical blood parameters [18]. *In vitro* and *in vivo* antioxidant activity is present in leaf ethanolic extract [17]. Its root extract is mixed with mother's milk and given to children for its anthelmintic property while stems are used as an antidote to snake biting [19]. The root extract is found to bind to the acetylcholine receptor leading the inhibition of the snake venom to bind it at the neuromuscular junction and hence exhibit an anti-snake venom property [20]. Cytotoxic lethality is observed in leaf and root methanolic extracts [21, 22]. Leaf methanolic extract exerts a restoring effect on blood glucose levels after streptozotocin treatment [23, 24]. Leaf extract reduces blood glucose level from 130 to 36 mg/dl in two hours and shows a significant acute peripheral analgesic activity at a dose of 500 mg/kg and 200 mg/kg body weight respectively [25, 26]. Allelochemicals from leaf aqueous extract have been found to harm the growth and germination of weeds in agro-ecosystem [27, 28]. A promising positive correlation is established between the plant's parts and their insect repellent and insecticidal activity [29].

Acute toxicity test reveals that these plant parts are safe up to 2000 mg/kg body weight [30]. The crude leaf extracts contain phenolics viz. fumaric acids, acetoside, methyl esters of caffeic acids, terpenoids like clerodin, flavonoids such as apegenin, acacetin, scutellarein, quercitin, cabrubin, hispidulin, steroids such as clerodone, clerodolone, clerodol, clerosterol and some fix oils containing linolenic acid, oleic acids, stearic acid and lingnoceric acid [19].

In our previous study, we have reported that treatment with leaf aqueous extract of *C. viscosum* (LAECV) on root apical meristem cells of wheat and onion gave an increased metaphase frequency along with a reduction in mitotic index, antiproliferative, and apoptosis-inducing effects [31]. The metaphase arrest and cell cycle delay inducing effects were somewhat comparable to Colchicine's actions [32, 33, 34].

Colchicine, an alkaloid isolated from *Colchicum autumnale*, *Gloriosa superba*, and many more medicinally important genera, is used to treat rheumatic complaints nowadays [35]. Like an anti-inflammatory compound, its uses to soothe the pain caused by gout outbreaks by inhibiting neutrophil motility and for the long-term treatment of Behcet's disease and chondritis are also reported [36, 37, 38]. It is also reported to treat the constipation-predominant irritable bowel syndrome in women, severe aphthous stomatitis, and pericarditis [39, 40, 41]. The term 'mitotic or spindle poison' is applied to it due to its potency to destabilize microtubules and consequent

suppression of the cell division by arresting mitosis [42]. This property of colchicine is used extensively in observing the metaphase stages of a cell under microscopes, karyotyping, and inducing the polyploidy in the cytogenetic study and modern agriculture [35]. Colchicine inhibits spindle formation in cells which leads to generating signals delaying the transition from metaphase to anaphase [42]. Later overtime when the concentration of colchicine decreases in the environment, the chromatids separate abnormally and the plant cells become polyploidy [43]. Moreover, polyploid plants have great agricultural importance due to their various advantageous characters such as increased organ sizes, blooming time, drought tolerance, pest resistance, etc. [44]. Colchicine treatment to onion root apical meristem cells, the root growth was inhibited, roots were swelled, and chromosomes were condensed and arranged haphazardly along with an increased frequency in metaphase [34]. In another comparative study, treatment of colchicine and LAECV on *Allium cepa* root tip cells reveals their similar cytogenetic effects concerning an increased frequency in mitotic abnormalities and micronucleus [33]. The metaphase arrest and cell cycle delay inducing effects of LAECV raised the key question about its active principle(s) [34]. Therefore, the present investigation aimed to detect the active molecule(s) responsible for metaphase arrest, cell cycle delay, polyploidy, micronuclei, and mitotic abnormalities induction in apical meristem cells. The leaf aqueous extract of *C. viscosum* was extracted and then successively fractionated with petroleum ether, chloroform, ethyl acetate, and all the extract fractions were tested for *Allium cepa* and *Triticum aestivum* root swelling and onion root growth inhibition analyses. The petroleum ether fraction, PEF, was selected for further cytotoxicity analysis on *A. cepa* root tip cells and was processed for the detection of bioactive principle(s).

2. Materials and methods

2.1.*Chemicals*

Orcein, glacial acetic acid, and methanol were obtained from Merck, Germany. Petroleum ether, Chloroform, and Ethyl acetate were obtained from Thermo Fisher Scientific, The USA. Other analytical grade chemicals were obtained from reputed manufacturers.

2.2.Plant collection and aqueous extraction

After collection of fresh *C. viscosum* leaves from Burdwan University campus, West Bengal, India, it was taxonomically identified by Professor Ambarish Mukherjee, Department of Botany,

The University of Burdwan and a voucher specimen (No. BUTBSR011) is maintained in the Department of Zoology, B.U., for future reference.

Fresh leaves were washed in tap water, dried in shade, ground by Philips Mixer Grinder HL1605, and the obtained powder was stored in a tightly sealed container for further use. 100 g of this pulverized material was extracted in 2.5 L of boiling distilled water for 2-3 h and after that, the extract was filtered with filter paper.

2.3.*Organic solvent fractions preparation*

Leaf aqueous extract of *C. viscosum* (LAECV) was fractioned by petroleum ether with the help of a magnetic stirrer for 10-12 h and the resulting yellow colored petroleum ether (PEF) solution was concentrated by rotary vacuum evaporator and stored in a glass bottle. The remaining aqueous extract was then successively fractioned with chloroform and ethyl acetate in the same way as petroleum ether fraction preparation and the resulting chloroform (CHF) and ethyl acetate (EAF) fractions were also concentrated and stored in glass containers.

2.4.*Experimental plants*

Allium cepa and *Triticum aestivum* root apical meristem were used as plant models for determining the root growth retardation and root swelling pattern for selecting the most effective extract fraction and then its cell cycle modulation and metaphase arresting activities were analyzed.

2.5.*Root growth retardation and root swelling effects of the extract fractions of LAECV*

2.5.1. *Culture and treatment of Allium cepa roots*

1% sodium hypochlorite mediated surface-sterilized *A. cepa* bulbs were placed in 6-well plates containing distilled water and kept in the environmental test chamber for germination (25-27 °C, humidity 50%). The 48 h aged similar-sized *A. cepa* roots (2-3 cm root length) were treated with 12.5, 25, 50, 100, and 150 µg/mL concentrations of PEF, CHF, and EAF continuously for 24, 48, and 72 h. The experiments were performed in triplicate. For root swelling pattern analysis, the 24 h aged onion roots were treated with the extract fractions for 4 h and allowed to grow for another 16 h in water and root tip swelling patterns were analyzed.

2.5.2. *Culture and treatment to wheat seedlings for root swelling pattern analysis*

The similar-sized seeds of *T. aestivum* were surface sterilized with 1% sodium hypochlorite solution and five replicas of each with 30 seeds were prepared for each treatment. Seeds were placed on filter paper kept in sterilized Petri dishes (90 mm), were covered then incubated at 25°C in a dark culture room, for germination. For the determination of active fractionation, LAECV was sequentially fractionated into PEF, CHF, and EAF extract fractions. The LAECV (4 mg/mL) and its fractions (4 mg/mL crude equivalent quantity) were treated to 24 h aged wheat roots initially for 4 h and then allowed for another 16 h as recovery and the root swelling patterns were observed.

2.6. Cell cycle delay, metaphase arresting and mitotic abnormality inducing effects *of PEF* in *A. cepa root apical meristem cells*

2.6.1. *Allium cepa root sprouting.*

Allium cepa bulbs with the same size were surface sterilized by 1% sodium hypochlorite and used for root sprouting. Bulbs were placed in 6-well plates containing distilled water and kept in dark at 25-27 °C within an environmental test chamber. The *A. cepa* roots with root length (48h) were used for experimental purposes.

2.6.2. *Treatment and preparation of mitotic phases from root apical meristem cells*

Mitotic abnormalities, micronuclei, and polyploidy frequency were analyzed for the elucidation of PEF induced cytogenotoxic effects on *A. cepa* root-tip cells. The 48 h aged similar-sized *A. cepa* roots (2-3 cm) were treated with 50, 100, 150, and 200 µg/mL of PEF for 2 and 4 h. After 2 and 4 h exposure, 8-10 roots were fixed and processed for squash preparation following the standard procedure [45]. The remaining roots were allowed to grow further for another 16 h in distilled water and subsequently, root tips were fixed. The control group, which had not received any treatment, maintained in distilled water simultaneously with the treatment groups. The treated and untreated root tips were fixed in aceto-methanol (3 parts methanol: 1 part glacial

acetic acid) for 24 h and then hydrolyzed for 10 min in 1 N HCl at 60˚C. Furthermore, roots were stained with 2% aceto-orcein and finally squashed in 45% acetic acid [46, 34]. The well-spread areas of squashed roots were focused under the bright field light microscope for observation and scoring the cellular abnormalities.

2.7.*Preliminary phytochemical detection of organic solvent extract fraction of LAECV*

Preliminary phytochemical detection of the PEF, CHF and EAF for the presence of flavonoids, alkaloids, terpenoids, anthraquinones, tannins, saponins, steroids, phlobatannins, glycosides, and carbohydrate was done by following the standard method of Harbourne 1973, Trease and Evans 1989 and Sofowara 1993 [47, 48, 49].

2.8.*Characterization of PEF by UV-Vis spectrophotometer, FT-IR Spectroscopy, HPLC, LC-MS, and GC-MS*

The PEF was dissolved in petroleum ether and UV-Vis spectrophotometric analysis was done by UV-Vis Spectrophotometer (Shimadzu Corporation). Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out with a 600 series pump and C-18 column (Hitachi). The separation was done in isocratic mode with HPLC-grade acetonitrile and water as a mobile phase at the ratio of 70:30, with a flow rate of 1mL/min. The samples were filtered through a 0.22 μm syringe filter (Himedia) and a 10 μL volume of sample was injected via the injector. The PEF was air-dried in a sterile Petri dish to get a yellowish powdery material which was then subjected to FT-IR spectroscopy by IR Prestige, Shimadzu. The Sample was dissolved in HPLC-graded methanol and filtered using a 0.22 μm syringe filter (Himedia). LC-MS was carried out using a mass spectrometer (AB-Sciex) and GC-MS was performed using a mass spectrophotometer of Agilent Technologies from TCG life science private limited, Kolkata, India.

2.9.*Scoring and statistical analysis*

Allium cepa root growth retardation effect of the different organic solvent fractions of LAECV was performed in triplicate and data analyzed with student t-test. In the case of squash preparation of *A. cepa* root apical meristem cells, at least three randomly coded slides were observed under the light microscope. Calculation of the mitotic index was done by counting the

number of dividing cells per total cells scored for each concentration. Aberrant cell frequencies were calculated by counting the number of abnormal cells scored per slide for each concentration [50]. Different cell phase frequencies, mitotic index, and mitotic abnormalities were analyzed by 2X2 contingency χ2-test.

3. Results

3.1. *Allium cepa root growth retardation effects of extract fractions of LAECV*

Data indicate that PEF, CHF, and EAF induced concentration-dependent *A. cepa* root growth retardation effects. The root growth and growth rate retardation effect was maximum in the case of PEF treated samples. Pooled data indicate that root length retardation for PEF, CHF, and EAF were 68.51±0.56, 60.02±0.94, and 51.20±0.50% respectively at a concentration 150 µg/mL and 72 h treated samples (Table S 1). Similarly, the root growth rate retardation percentages were 91.01±1.51, 89.86±1.60, and 89.85±2.09 % respectively for PEF, CHF, and EAF at a concentration of 150µg/mL for 72 h treatment. IC50 values for root growth rate retardation were 23.68±5.5, 62.78±3.26, and 106.15±4.03 µg/mL respectively for PEF, CHF, and EAF at 48 h of treatment (Figure 1).

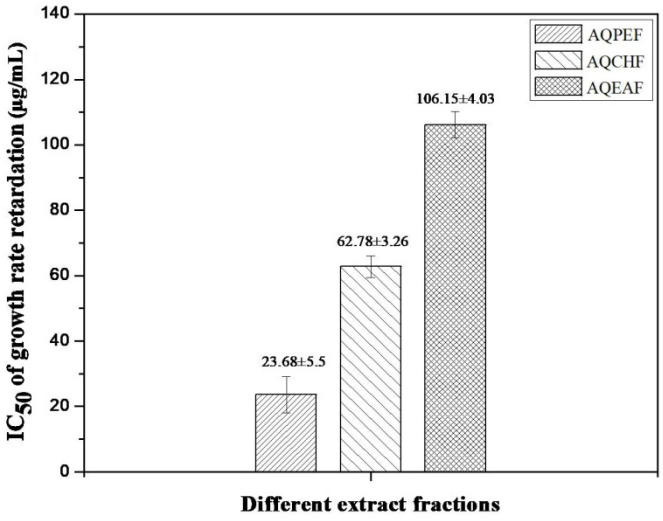


Figure 1: IC50 values (µg/mL) on onion root growth rate retardation of PEF, CHF, and EAF. Data represented as mean ± sem. [a]significant at $p<0.001$ and [b]significant at $p<0.01$, [c]significant at $p<0.05$ as compared to their respective control by t-test with respective df = 1.

Therefore, in the second set of experiments where our previously standardized procedure wheat roots were exposed with LAECV and its extract fractions for 4 h and followed 16 h by a recovery phase, LAECV, PEF, and CHF showed a characteristic swelling pattern. Amongst the extract fractions, the petroleum ether fraction seemed to the most active fraction in terms of root swelling (Figure 2).

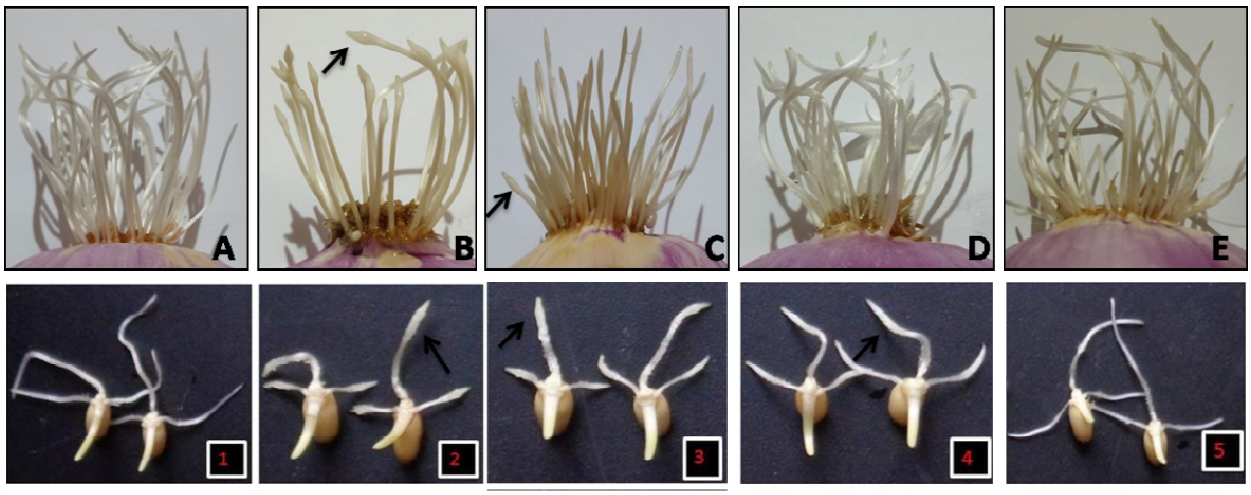


Figure 2: Photograph showing comparative root swelling effect of the extract fractions from LAECV on onion and wheat root tip cells treated for 4 h and followed by16 h water recovery. Onion root tip swelling pattern induced by LAECV (B, 4mg/mL), PEF (C, 100µg/mL), CHF (D, 100µg/mL), EAF (E, 100µg/mL) and control (A); (1) CONTROL, (2) LAECV, (3) PEF, (4) CHF, (5) EAF, treatment respectively at 4 h treatment followed by 16 h recovery in distilled water.

3.2.*PEF induced cell cycle delay and mitotic abnormalities in Allium cepa root tip cells*

3.2.1. *Mitotic index and mitotic phase-frequency*

Data indicate that the mitotic index percentage in root apical meristem cells varies with the growing periods and extract concentrations. In the present study, the highest MI (13.25±0.72%) scored at 4 h of PEF (150 µg/mL) treatment and the lowest MI (5.67±0.30%) at 2 h untreated

controls. At 2 h, MI% were gradually increased- 5.67±0.30, 6.63±0.41, 7.58±0.5, 9.76±0.26, and 10.51±0.80% respectively for 0, 50,100, 150, and 200 μg/mL. The MI also increased in 4 h treated samples - 7.09±0.62, 10.3±0.56, 10.3±0.29, 13.25±0.72, and 9.95±0.80% respectively for 0, 50,100, 150, and 200 μg/mL of PEF. In the case of 16 h recovery samples, MI% were 10.06±0.92, 7.00±0.29, 8.45±1.64, 6.51±0.77, and 7.43±0.38% respectively for 0, 50, 100,150 and 200 μg/mL of PEF.

The maximum increase (13.25±0.72) in MI % was scored from the 150 μg/mL concentration of PEF treatment at 4 h. In contrast to these MI% increasing tendencies, a decreased percentage was observed in PEF treatment at 4 h followed by 16 h recovery samples. The lowest MI % was found in the case of 150 μg/mL (6.51±0.77%) treatment followed by 50 μg/mL (7.00±0.29 %)(Table 1).

3.2.2. *Frequency of Prophase*

Data indicates that PEF (50, 100, 150, and 200 μg/mL) treatment for 2 h could not significantly alter the frequency of prophase, though a decreasing tendency was observed. Statistically significant ($p<0.05$, 0.001) reduction in prophase frequency was observed in the case of 4 h of PEF treatment and 4 h treatment followed by 16 h recovery samples. The lowest prophase percentage (8.72±2.61) was observed in 50 μg/mL concentration of PEF treatment for 4 h (Table 1).

3.2.3. *Frequency of Metaphase*

The PEF treatments (50, 100, 150, and 200 μg/mL) induce an increased frequency of metaphase at 2 and 4 h treatments, and 4 h treatment followed by 16 h recovery samples. The highest metaphase cells percentage (81.44±1.61) was scored from 4 h PEF treated (100 μg/mL) samples and that was followed by the concentration of 150 μg/mL (78.48±2.41%). However, in the case of 16 h recovery samples, the metaphase frequency showed a reducing tendency for 4 h treated samples and the metaphase frequencies were scored as 65.69±3.87 % and 52.42±6.02 % respectively for the concentration of PEF 100 and 150 μg/mL(Table 1).

3.2.4. *Frequency of Anaphase*

The percentage of anaphase cells decreased significantly ($p<0.0001$) at 4 h of PEF (50, 100, 150 & 200 µg/mL) treated samples. The lowest anaphase percentage (6.67±1.04%) was observed in 100 µg/mL concentration of PEF at 4 h. The decreased anaphase frequency was also observed in 2 h treated samples, though there was no significant difference as compared to the control group. In the case of 16 h recovery samples, the anaphase percentage reducing tendency was observed for the concentration of 100 (12.23±0.72%) and 150 µg/mL (18.64±2.49%), whereas the remaining concentrations, 50 and 200 µg/mL shown an increased anaphase percentage respectively 24.58±0.94% and 29.68±2.72% (Table 1).

3.2.5. *Frequency of Telophase*

The PEF (50, 100, 150 & 200 µg/mL) treatment showed a decreased tendency of telophase frequency in each of the treatment hours. However, the significant difference ($p<0.0001$) was observed at 4 h treated samples, where the 0, 100, 150, and 200 µg/mL concentrations of PEF induced decreased telophase percentages 12.45±0.51, 1.83±0.52, 3.39±0.76, and 2.26±0.48 % respectively. Except in 150 µg/mL concentration, the reduced anaphase frequency was also observed at 2 h and 4 h followed by 16 h treated samples, though the differences in the frequencies were not significant as compared to control (Table 1).

Table 1: Effects of PEF on the mitotic index and mitotic phase frequency in *A. cepa* root apical meristem cells

H	Conc(µg/mL)	Tc	Tdc	MI%	Pro%	Meta%	Ana%	Telo%
2	0	1959	111	5.67±0.30	21.95±4.90	36.86±2.97	25.87±3.32	15.13±0.70
	50	2181	144	6.63±0.41	16.67±1.27	42.41±1.35	25.64±4.45	15.25±2.48
	100	2445	183	7.58±0.5 [c]	15.93±2.63	56.52±1.96	18.48±3.42	11.09±0.94
	150	2404	234	9.76±0.26 [a]	16.32±1.01	63.67±3.27 [c]	14.95±1.28	5.05±1.76[b]
	200	2389	251	10.51±0.80 [a]	17.74±1.90	53.67±2.05	17.96±2.23	10.63±1.13
4	0	2914	207	7.09±0.62	19.02±0.97	37.72±2.60	30.77±2.07	12.45±0.51
	50	3423	352	10.3±0.56 [a]	8.72±2.61 [b]	66.47±5.70 [a]	14.54±1.98 [a]	10.24±2.27
	100	2162	222	10.3±0.29 [a]	10.04±1.61 [c]	81.44±1.61 [a]	6.67±1.04 [a]	1.83±0.52 [a]
	150	3035	403	13.25±0.72 [a]	9.34±1.11[c]	78.48±2.41 [a]	8.76±2.53 [a]	3.39±0.76 [a]
	200	3525	352	9.95±0.80 [a]	14.33±2.74	76.43±2.61 [a]	8.00±0.27 [a]	2.26±0.48 [a]
4+16	0	2449	241	10.06±0.92	24.63±2.84	38.95±0.78	23.27±0.93	13.11±2.99
	50	3946	276	7.00±0.29 [a]	14.83±0.34 [c]	45.32±0.66	24.58±0.94	15.23±0.43

	100	2130	177	8.45±1.64	12.77±3.18 [c]	65.69±3.87 [b]	12.23±0.72 [c]	9.28±1.71
	150	2068	136	6.51±0.77 [a]	20.08±0.92	52.42±6.02	18.64±2.49	8.82±3.99
	200	2162	161	7.43±0.38 [b]	25.00±2.85	35.78±2.46	29.68±2.72	9.51±2.22

[a]Significant at p<0.0001 and [b] significant at p<0.001, [c] significant at p<0.05 as compared with their respective control by 2x2 Contingency $\chi 2$ -test with respective df = 1. H; Hours, Comp; Compound, Conc; Concentration, Tc; Total no of cells, Tdc; Total no of dividing cells, MI; Mitotic index, Pro; Prophase, Meta; Metaphase, Ana; Anaphase, Telo; Telophase.

3.3.Aberrant cell percentage

A concentration-dependent and statistically significant level (*p*<0.05, 0.001& 0.0001) increased aberrant cells percentage was observed in 100, 150 & 200 µg/mL concentration of PEF treatment at 2 h and 4 h samples. The highest aberrant cells percentage (10.45±0.46%) was scored from 150 µg/mL followed by100 µg/mL (8.75±0.26%) concentration at 4 h treated samples. However, in 16 h recovery samples, the PEF induced aberrant cell frequency was considerably decreased compared to 2 h and 4 h treated samples (Table 2).

3.4.Mitotic abnormalities

The mitotic abnormalities like c-metaphase, vagrant chromosome, laggard chromosome, stickiness, anaphase bridges, micronucleus, and polyploidy were induced by PEF treatment in root tips cells of *A. cepa*.

3.4.1. *C-metaphase frequency*

The study result showed that the C-metaphase was found to be the most frequent type of abnormalities induced by PEF in *A. cepa* root apical meristem cells. The PEF treatment for 2 and 4 h induces a significant (*p*<0.001& 0.0001) increase in the frequency of C-metaphase in *A. cepa* root apical meristem cells. In the case of PEF treatments for both 2 and 4 h, the C-metaphase frequency increased in a dose-dependent manner. The increased percentage of C-metaphase induced by 100, 150 & 200 µg/mL of PEF were scored respectively as 39.2±4.05, 55.62±2.03, and 45.15±3.86% at 2 h and 80.08±1.65, 70.86±2.74, and 68.88±1.67% at 4 h treated samples. In the case of 2 h treatment, 150 µg/mL concentration of PEF showed the highest C-metaphase frequency (55.62%) but in the case of 4 h treatment, 100 µg/mL concentration of PEF induced

the highest (80.08%) C-metaphase. After 16h recovery treatment, a slightly decreased C-metaphase frequency was observed for those respective concentrations (Table 2, Graph 4 & Figure 19, 21, 22).

3.4.2. *Anaphase Bridge*

The PEF treatment induced an increased percentage of anaphase bridges in *A. cepa* root apical meristem cells. In the case of 2 h treated samples, the anaphase bridge percentages 7.0±1.5, 5.62±1.08, 7.24±0.63, and 7.23±0.51% were observed respectively for the concentrations of 50, 100, 150, and 200 µg/mL and these were correspondingly decreased to 5.63±0.41, 3.60±0.47, 5.30±2.20, and 5.50±0.86 % at 4 h and 2.17±0.06, 3.23±1.64, 5.83±1.72 and 7.38±0.71% at 4 h followed by 16 h recovery samples, except for the concentration of 200 µg/mL, which showed the highest (7.38±0.71 %) anaphase bridge percentage (Table 2).

3.4.3. *Chromosomal stickiness*

Similar to anaphase bridge frequency, an increased frequency of chromosomal stickiness was also observed in the PEF treated onion root tip cells at 2 h and successively decreased at 4 h and 4 h followed by 16 h recovery samples. The PEF treatment to onion root tip cells induced the highest (6.42±0.52) frequency of chromosomal stickiness at 2 h in a concentration of 200 µg/mL. In the case of 4 h treatment and 16 h recovery treatment, sticky chromosomes containing cell percentages reduced for PEF treatment (Table 2, Figures 3 & 4).

3.4.4. *Polar Deviation*

Polar deviation percentage increases in root apical meristem cell treated with PEF, at 2h and but decreases at 4h and 4+16 h treatment. In the case of untreated control root tip cells, the polar deviation was not observed. The percentages of polar deviation were scored as 2.06±0.03, 5.32±1.08, 3.42±1.48, and 3.54±1.24% respectively at 50, 100, 150, and 200 µg/mL concentrations of PEF at 2 h. At 4 h and 4 h followed by 16 h recovery samples, the frequency was consequently decreased as compared to 2 h treated samples (Table 2& Figure 4).

3.4.5. *Vagrant and laggard chromosome*

The increased frequencies of vagrant and laggard chromosomes were not observed for all the treatment concentrations of PEF at 2 h, 4 h, and 4 h treatment followed by 16 h recovery samples. However, an increased percentage of the vagrant chromosomes were observed in the case of 4 h followed by 16 h recovery samples than 2 h and 4 h. The frequency of vagrant chromosome was scored to be 3.69±0.91, 5.16±0.68, and 5.04±0.83 % respectively for the concentration of 100, 150, and 200 μg/mL of PEF in 16 h recovery samples (Table 4). The PEF treatment induced an increased percentage of cells with laggard chromosomes at early hours (2 h) of treatment. Here, 50, 100, 150, and 200 μg/mL concentration of PEF induced respectively 1.03±0.60, 2.29±0.80, 0.40±0.40, and 0.81±0.41 % of laggard chromosome containing cells at 2 h of treatment (Table 2 & Figure 4).

3.4.6. *Micronucleus*

Based on the analysis carried out in root tip cells from *A. cepa*, it was observed that at 16 h water recovery treatment after distinct concentrations of PEF treatment, several cells were found to contain micronuclei. The PEF treatment induced a significant ($p<0.0001$) increase in micronuclei frequency and were scored 4.31±0.33, 5.08±0.13, 5.05±0.22&3.05±0.37% respectively for the concentrations 50, 100, 150, and 200 μg/mL. However, the incidence of micronuclei was not observed in the case of early hours (2 h and 4 h) PEF treated samples (Table 2& Figure 4).

3.4.7. *Polyploidy*

Data indicates that the PEF treatment of onion root tip cells could induce a significantly ($p<0.0001$) increased frequency of polyploidy cells. The polyploidy was induced only in the case of 4 h followed by 16 h water recovery samples. The highest polyploid frequency (20.14±0.68 %) was found in 100 μg/mL followed by 150 μg/mL (18.43±0.21 %) and 200 μg/mL(12.42±0.81 %) concentrations of PEF treatment (Table 2& Figure 4).

Table 2: PEF induced mitotic abnormalities in *A. cepa* root apical meristem cells.

H	Conc(µg/mL)	Percentage of								
2		Ac	Sti	Bri	Pd	C-Meta	Vag	Lag	MN	POL
	0	0.30±0.09	1.78±0.89	1.83±1.83	0.00±0.00	1.66±1.66	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	50	2.58±0.24[a]	6.23±1.24	7.0±1.5	2.06±0.03	22.18±1.52[a]	0.00±0.00	1.03±0.60	0.00±0.00	0.00±0.00
	100	4.59±0.33[a]	5.49±1.36	5.62±1.08	5.32±1.08	39.2±4.05 [a]	2.67±0.35	2.29±0.80	0.00±0.00	0.00±0.00
	150	7.13±0.31[a]	3.84±0.69	7.24±0.63	3.42±1.48	55.62±2.03[a]	2.50±0.63	0.40±0.40	0.00±0.00	0.00±0.00
	200	6.65±0.10[a]	6.42±0.52	7.23±0.51	3.54±1.24	45.15±3.86[a]	0.84±0.42	0.81±0.41	0.00±0.00	0.00±0.00
4	0	0.20±0.05	0.00±0.00	3.09±1.09	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	50	6.28±0.59 [a]	1.76±1.31	5.63±0.41	1.43±0.58	49.38±5.71[a]	2.17±0.84	0.60±0.30	0.00±0.00	0.00±0.00
	100	8.75±0.26 [a]	0.00±0.00	3.60±0.47	0.00±0.00	80.08±1.65[a]	1.35±0.05	0.00±0.00	0.00±0.00	0.00±0.00
	150	10.45±0.46[a]	1.59±0.80	5.30±2.20	0.21±0.21	70.86±2.74[a]	1.16±0.52	0.00±0.00	0.00±0.00	0.00±0.00
	200	7.61±0.76 [a]	1.66±0.76	5.50±0.86	0.35±0.35	68.88±1.67[a]	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4+16	0	0.25±0.03	0.00±0.00	2.49±0.10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	50	1.05±0.9	0.00±0.00	2.17±0.06	0.36±0.36	0.36±0.36 [a]	0.00±0.00	0.00±0.00	4.31±0.33[a]	11.96±0.48[a]
	100	4.37±0.86 [a]	1.16±0.63	3.23±1.64	0.59±0.59	43.05±13.28[a]	3.69±0.91[b]	0.00±0.00	5.08±0.13[a]	20.14±0.68[a]
	150	3.84±0.59 [a]	1.04±1.04	5.83±1.72[c]	0.00±0.00	33.73±1.91 [a]	5.16±0.68[a]	0.00±0.00	5.05±0.22[a]	18.43±0.21[a]
	200	3.13±0.52 [a]	4.33±1.51[b]	7.38±0.71[c]	0.00±0.00	24.81±5.00 [b]	5.04±0.83[b]	0.00±0.00	3.05±0.37[a]	12.42±0.81[a]

[a] significant at $p<0.0001$ and [b] significant at $p<0.001$, [c] significant at $p<0.05$ as compared with their respective control by 2x2 Contingency $\chi 2$ -test with respective df = 1. H; Hours, Comp; Compound, Conc; Concentration, Tc; Total number of cells, TDC; Total dividing cells, Ac; Aberrant cells, Sti; Stickiness, Bri; Bridge, Pd; Polar deviation, C-Meta; Colchicine like Metaphase, Vag; Vagrant chromosome, Lag; Laggard chromosome, MN; Micronucleus, POL; Polyploidy.

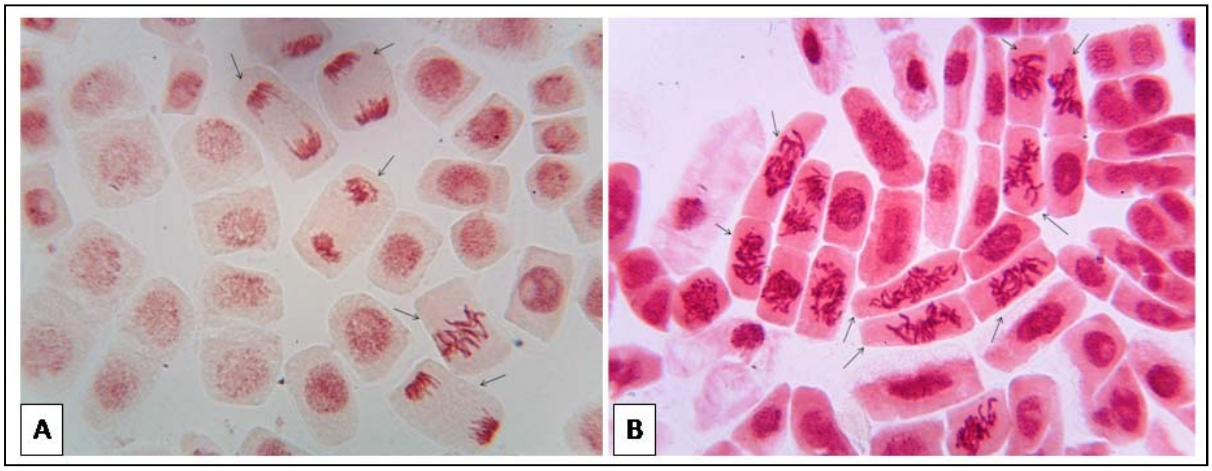


Figure 3: PEF induced increased C-metaphase frequency in root apical cells of *A. cepa* at 4 h continuous treatment (B); A, Untreated.

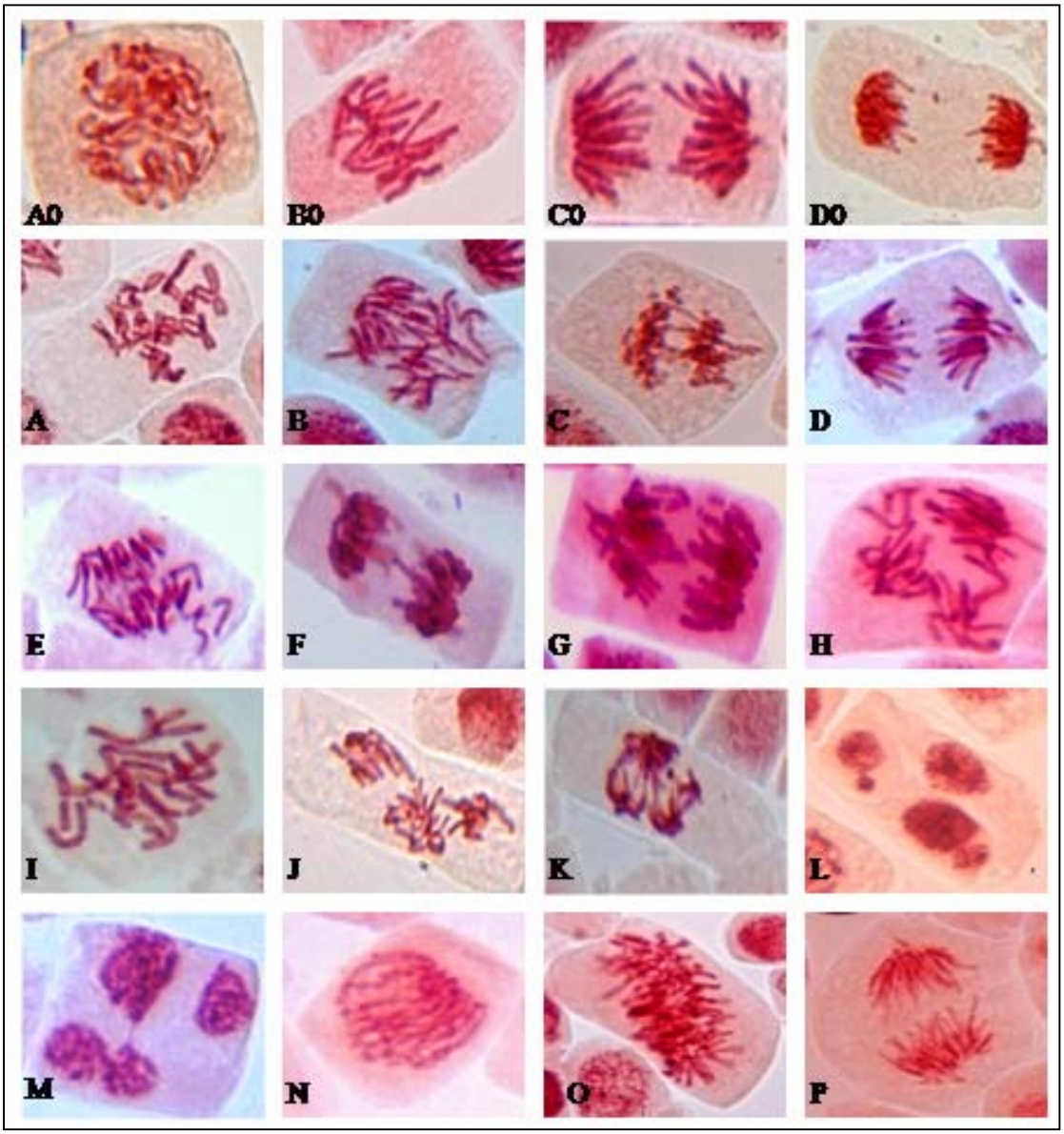


Figure 4: PEF induced polyploidy, micronuclei, and mitotic abnormalities in *A. cepa* root apical meristem cells. Untreated (A0-D0): A0; Prophase, B0; Metaphase, C0; Anaphase, and D0; Telophase of *A. cepa* root apical meristem cells. A; C-metaphase, B; Anaphase bridge, C; Decondensed anaphase bridge, D; Polar deviation, E; Vagrant chromosome, F; Sticky with anaphase bridge, G; Chromatid Break, H; Disrupted anaphase, I; Disrupted metaphase, J; Multipolar anaphase, K; Decondensed multipolar anaphase, L; Micronucleus with multiple nuclei, M; Multi nuclei with decondensed anaphase bridge, N; Polyploid prophase, O; Polyploid metaphase, P; Polyploid anaphase.

3.5.*Phytochemical analysis*

Preliminary phytochemical analysis indicates the presence of terpenoids in all fractions. PEF and CHF contain a minute amount of saponins and carbohydrates are present only in CHF. Alkaloids, flavonoids, steroids, tannins, glycosides are absent in all organic solvent fraction (Table 3).

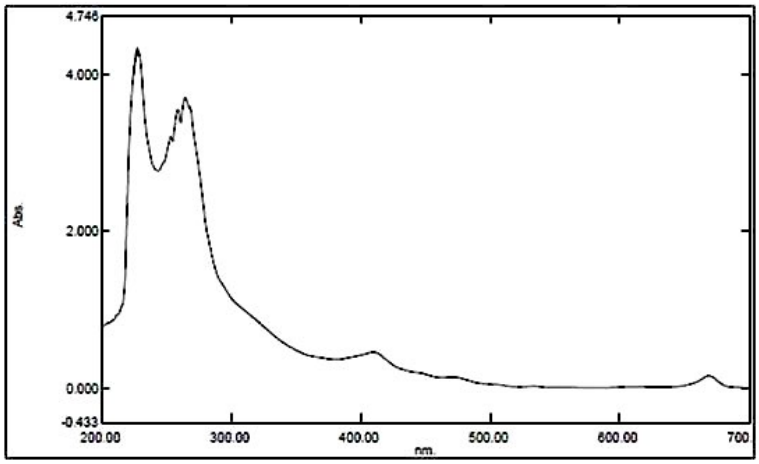
Table 3: Showing the phytochemical constituent of PEF, CHF, and EAF.

Phytochemicals tests Performed		PEF	CHF	EAF
Alkaloids	Mayer's test	-	-	-
	Wagner's test	-	-	-
Flavonoids (Alkaline reagent test)		-	-	-
Anthraquinones (Borntrager's test)		-	-	-
Terpenoids (Kantamreddi et. al. 2010)		+++	++	+
Steroids (Kantamreddi et. al. 2010)		-	-	-
Tannins	$FeCl_3$ test	-	-	-
	Alkaline reagent test	-	-	-
	Phlobatannins (HCl test)	-	-	-
	Saponins (Froth test)	+	+	-
Glycosides (Alkaline reagent test)		-	-	-
Carbohydrates (Fehling's test)		-	+	-

3.6. Chemical characterization

3.6.1. *UV-Vis spectrum* analysis

Data obtained from UV-Vis spectrophotometer (Shimadzu, UV 2600) shows that PEF has some UV absorbance with the maxima at 227nm. It also shows a significant UV absorbance at 258 and 264nm (Figure 5).



No.	P/V	Wavelength	Abs.
1	P	669.00	0.149
2	P	610.50	0.015
3	P	561.00	0.003
4	P	533.00	0.020
5	P	471.50	0.132
6	P	409.50	0.454
7	P	264.00	3.702
8	P	258.50	3.555
9	P	227.00	4.314
10	V	629.50	0.006
11	V	580.00	-0.002
12	V	548.00	0.001
13	V	523.00	0.010
14	V	463.00	0.126
15	V	381.00	0.360
16	V	261.00	3.389
17	V	243.00	2.773

Figure 5: Showing the UV spectrum and the relative absorbance of PEF.

3.6.2. FT-IR Spectroscopy analysis

Fourier Transform Infrared Spectrophotometer (FT-IR) is the most authentic tool for the characterization of functional groups. Here, the FT-IR spectrum of PEF shows that it contains the major peak at 2924.09, 1732.08, 1616.35, 1454.33, 1365.60, 1247.94, 1085.92, 1008.77, 800.46 cm− 1 which implies the presence of alkane group (C-H), aldehyde group (C=O), conjugated alkane (C=C), methyl group (C-H), alcohol group (O-H), alkyl aryl ether group (C-O), aliphatic ether / primary alcohol group (C-O), alkene group (C-C), alkene group (C-C) respectively (Figure 6).

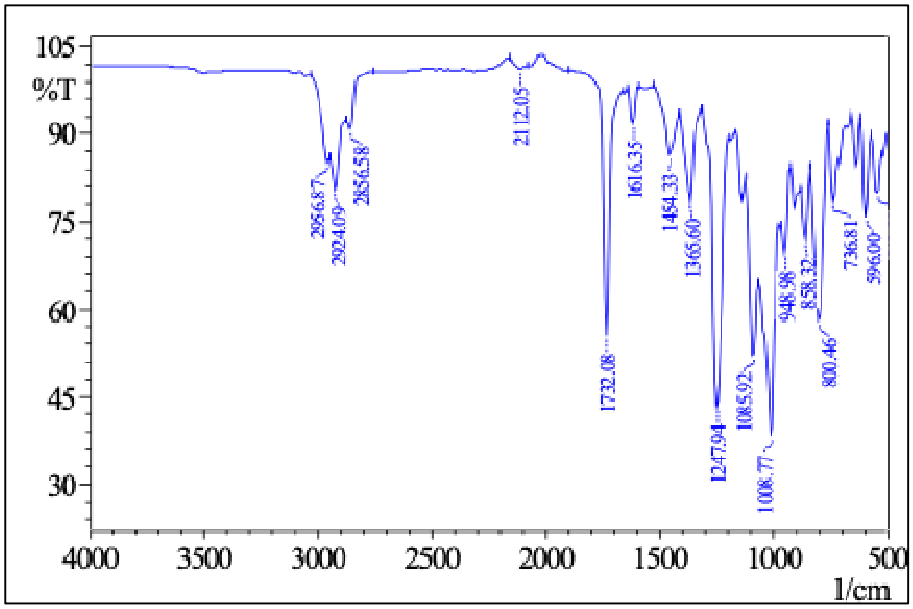


Figure 6: Showing the FT-IR spectrum of PEF.

3.6.3. HPLCanalysis

High performance liquid chromatographic separation of PEF, using the C-18 column (Hitachi) and acetonitrile-water with a ratio of 70:30 as the mobile system at a flow rate of 1 mL/min, shows six main peaks with the retention time of 3.950, 4.637, 5.803, 7.440, 9.507 and 9.907 (Figure 7).

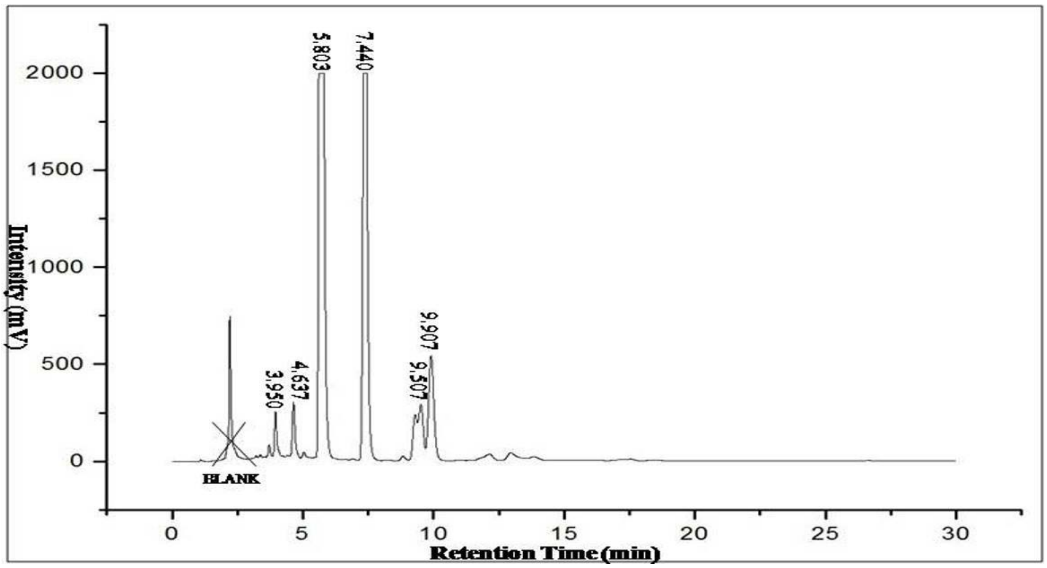


Figure 7: Showing the HPLC chromatogram of PEF.

3.6.4. **LC-MS analysis**

The liquid chromatogram of PEF shows that it contains two major peaks with a retention time of 1.66 and 1.74 min. The mass spectrum of the compound with a retention time of 1.66 min shows molecular ion peak [M+Na] at m/z 460 and the compound with a retention time of 1.74 shows molecular ion peak [M+Na] at m/z 458. Molecular weight obtained from the mass spectrum reveals that PEF contains Dihydroclerodin [M+Na, m/z 460] and Clerodin [M+Na, m/z 458] respectively (Figure 8).

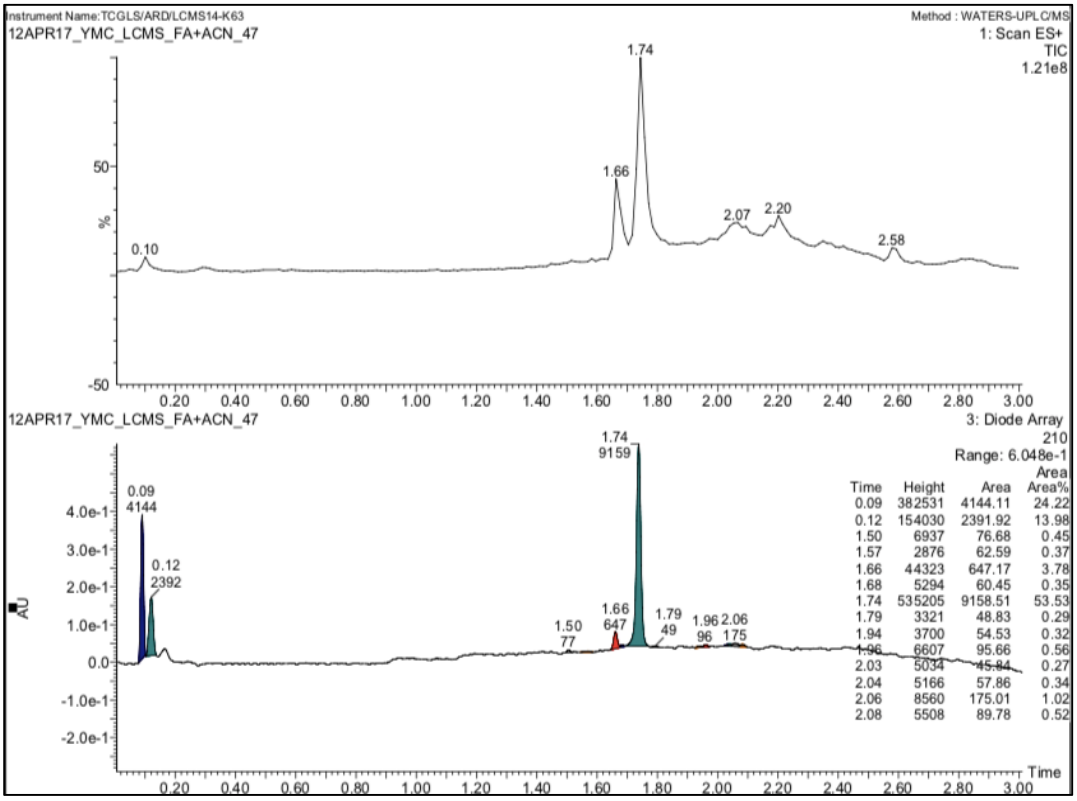


Figure 8: Showing the liquid chromatogram of PEF.

3.6.5. GC-MS analysis

Gas chromatogram of PEF indicates the presence of four (4) compounds with the retention time of 14.038, 14.103, 14.480, and 14.655 respectively. Analysis of the major peak obtained in gas chromatogram with the retention time of 14.038, recorded to have the molecular weight of **m/z 434.3** is similar to Clerodin (Mass fragments of clerodin - m/z(55, 69, 81, 95, 111,133, 145, 159, 173, 187, 204, 221, 233, 247, 264, 286, 301, 319, 321, 331, 349, 361, 374, 391, 405, 417 and **434**). Rest of the peaks with the retention time of 14.103, 14.480 and 14.655 has clear coordination with 15-hydroxy-14, 15-dihydroclerodin (Mass fragments m/z (55, 81, 111, 133, 175, 204, 229, 264, 286, 314, 349, 374, 406, 434 and **452**), 15-methoxy-14, 15-dihydroclerodin (Mass fragments m/z (55, 81, 111, 147, 175, 204, 234, 264, 286, 314, 349, 391, 431, and **466**), 14, 15-dihydroclerodin (Mass fragments m/z (436)69, 91, 113, 133, 173, 204, 233, 263, 288, 307, 331, 351, 376, 393, 417, and **436)**.

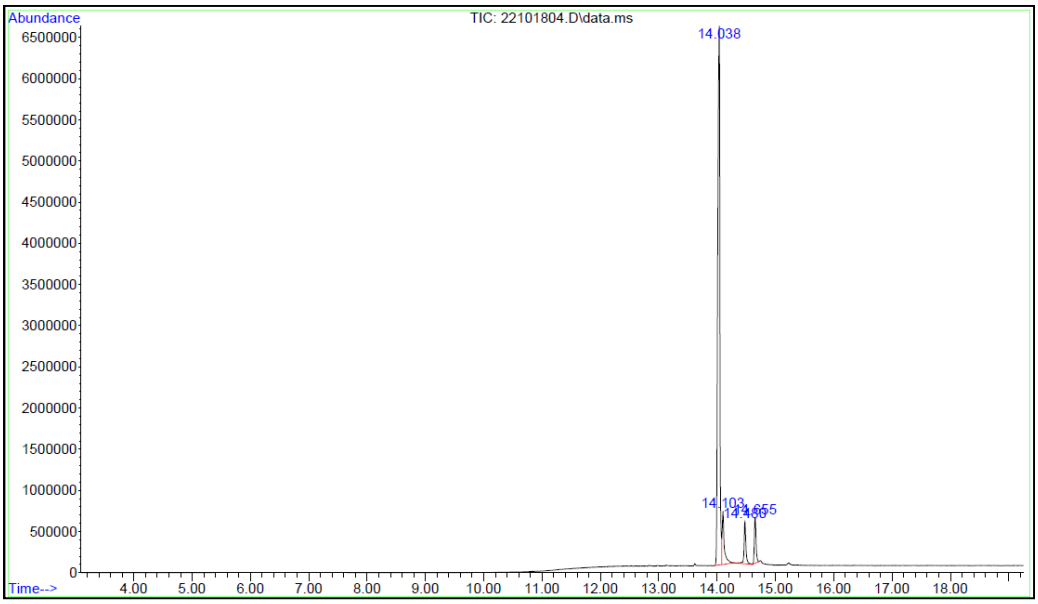


Figure 9: Gas chromatogram of PEF.

4. Discussion:

The different extracts of *Clerodendrum viscosum* have anti-septic, anti-helminthic, anti-inflammatory, anti-leprosy, anti-pyretic, anti-tumor, anti-bacterial, antiproliferative, metaphase arresting, apoptosis-inducing, antifeedant activities, etc. [51, 52, 53, 17, 54, 31, 34, 33]. This study is in continuous with the previous investigation that indicated antiproliferative, metaphase arresting, mitotic abnormality, and micronuclei inducing potentials of leaf aqueous extract of *C. viscosum* (LAECV) [34, 33]. The focus of the present investigation was to identify the active principle(s) responsible for metaphase arrest and mitotic abnormalities in *A. cepa* root apical meristem cells.

The LAECV was successively fractionated with petroleum ether (PEF), chloroform (CHF), and ethyl acetate (EAF). The root growth retardation analysis in *A. cepa* indicates that petroleum ether fraction (IC50 = 23.68 ± 5.5 µg/mL) has better root growth retardation/antiproliferative activity than chloroform (IC50 = 62.78 ± 3.26 µg/mL) and ethyl acetate fraction (IC50 = 106.15 ± 4.03 µg/mL). Many authors suggest that antiproliferative and cytotoxic effects of plant extracts or chemical substances can be evaluated using *A. cepa* root tip cells and it is very commonly used in the assessment and monitoring of environmental toxicants [55, 50, 56, 57, 58]. The percentage of root growth retardation depends on the antiproliferative potentials of the treated substances. The PEF treatment for 4 h +16 h recovery shows a dose-dependent increase in *A. cepa* root apical meristem swelling. The PEF induced root swelling both in *A. cepa* root and *T. aestivum* seedlings, indicating metaphase arresting bioactive principles that were extracted mainly in petroleum ether fraction of LAECV. It can be assumed that the generation of polyploidy is related to the root apical meristem swelling in PEF treatment because of the cellular dimension increases in polyploid cells (Figure 2,3, and 4). The presence of terpenoids in LAECV and its successive fractions indicate that terpenoids may be responsible for the root growth retardation effect [31]. Our previous investigation indicated that LAECV has colchicines-like metaphase arrest and mitotic abnormalities inducing potentials [31, 33] and this study indicates that the successive petroleum ether fraction (PEF) of LAECV contains the active principles responsible for metaphase arrest and mitotic abnormalities. Earlier we observed similar root swelling patterns in seedlings after colchicine and LAECV exposure [34]. Further investigation by squash preparation of *A. cepa* root apical meristem cells revealed that PEF

induced increased metaphase frequencies in a dose-dependent manner at both 2 h and 4 h treated samples. In the present investigation, the metaphase arresting and mitotic abnormality inducing effects indicate that PEF has definite metaphase arresting activity at 2 h, 4 h, and 4+16 h treated *A. cepa* root tip cells. The PEF treatment for 2 h and 4 h in *A. cepa* root tip cells results in decreased frequencies of prophase, anaphase, and telophase in a dose-dependent manner. Subsequently, the frequencies of prophase, anaphase, and telophase showed an increasing pattern in 4+16 h recovery treated root tip cells as compared to 2 h and 4 h treated cells. The PEF treatment for 2 h and 4 h increases by the MI%, whereas MI% decreased in 4 h treatment followed by 16 h recovery root tip cells. The probable reason for mitotic index elevation in 2 h and 4 h treated cells may be due to an increase in metaphase frequencies [59]. Similarly, reduction in metaphase frequencies and cytotoxicity exerted by the PEF may lead to mitotic index depression in 4 h treatment followed by 16 h recovery *A. cepa* root tip cells.

Cytogenotoxic potentials of various chemical substances can be deciphered by studying the mitotic abnormalities [60]. Analysis of mitotic abnormalities induced by PEF shows that it has aberrant cells producing capabilities in 2 h and 4 h treated *A. cepa* root apical meristem cells. In the case of 4 h treatment followed by 16 h recovery treatment, the aberrant cell frequencies reduced in comparison to 2 h and 4 h treated cells but PEF still induces more aberrant cells than untreated ones. Reports of Kundu and Ray (2016) revealed that LAECV has colchicine like mitotic abnormalities (sticky chromosome, c-metaphase, anaphase bridge, vagrant chromosome, micronucleus) inducing capabilities in 4 h and 4 h treatment followed by 16 h recovery *A. cepa* root tip cells [33]. Similarly, the PEF has produced similar types of mitotic abnormalities in all the treated hours. Colchicine is a well-known spindle poison that destabilizes the microtubule network in cells and is responsible for microtubule destabilization and can induce c-metaphase, anaphase-bridge, polar deviation, vagrant chromosome, laggard chromosome, and sticky chromosome, etc. [42, 37, 36, 55]. An investigation by Mercykutly and Stephen 1980 states that decondensation of DNA, nucleoprotein disaggregation, and removal of protein coat from DNA may result in a sticky chromosome [61]. The basis for the onset of the sticky chromosome may be due to sub chromatid association among chromosomes or dissolution of nucleoprotein or decondensation of DNA [62, 63, 64, 65]. Results from the present investigation indicate that PEF has induced increased frequencies of sticky chromosomes at 2 h treated root tip cells but it was reduced at 4 h and 4 h treatment +16 h recovery. Formation of anaphase-bridge, a major mitotic

abnormality, may occur due to fission and fusion of the chromatids and chromosomes. The PEF induced Anaphase Bridge at 2 h, 4 h, and 4+16 h treated *A. cepa* root tip cells. PEF induces multipolar anaphase in condensed and decondensed form and such multipolar anaphases might be formed from the chromosomal bridge and sticky chromosome. Formation of sticky chromosomes and Anaphase Bridges may results in cell death, as they exert an irreversible toxic outcome to the cells. Anaphase Bridge can be seen in squash preparation and chromatid or chromosome fusion and fission may be the driving force for the formation of Anaphase Bridge. The polar deviation is another type of mitotic abnormality, that was evident by many scientists and Ray et al. 2016, state that LAECV and Colchicine have a similar effect on the induction of polar deviation in *A. cepa* root apical meristem cells [33]. The present investigation shows that PEF could induce polar deviation at 2 h and 4 h treatments, whereas, the frequency of polar deviation reduced for all compounds at 4+16 h treatment. The polar deviation becomes evident in microtubule destabilizing drugs i.e. Colchicine. Thus, the preliminary mode of action of PEF is almost certainly as reminiscent of Colchicine. Further investigation shows that the PEF induces c-metaphase at 2 h, and 4 h treatment and 4 h treatment + 16 h recovery in *A. cepa* root tip cells. Formation of c-metaphase or Colchicine-like metaphase is directly correlated with the microtubule disruption within the cell and result from the present investigation indicates that phytochemicals present in PEF may have Colchicine-like microtubule destabilizing activity [66, 67, 68]. These data also correlate with the occurrence of c-metaphase in LAECV and Colchicine treated *A. cepa* root tip cells [33]. Formation of the c-metaphases may result from the spindle poisoning effect of microtubule destabilizing drugs or chemicals (66, 67). Mitotic abnormalities like vagrant chromosomes and laggard chromosomes can also form due to spindle poisoning. Results from the present investigation show that PEF induces both types of abnormalities at 2 h and 4 h treatment in *A. cepa* root tip cells. Frequencies of vagrant and laggard chromosomes decreased at 4 h PEF treatment followed by 16 h recovery in *A. cepa* root tip cells. Squash preparation also reveals that PEF induces micronuclei and polyploid cells at 4+16h treatment. Reconstruction of c-metaphases, vagrant chromosomes, and chromatid breaks result in the formation of micronuclei. Correlation between the formation of c-metaphase, vagrant chromosome, and polyploidy was evident by many investigators in *A. cepa* root tip cells [69, 55]. Disruption of the mitotic spindle also inhibits cytokinesis and such inhibition of cytokinesis and

reconstruction nuclei leads to the formation of polyploid cells [69, 55, 70, 71, 72]. Based on this correlative evidence, we can postulate that PEF has a similar effect on *A. cepa* root tip cells.

The HPLC detection of PEF depicted six peaks with two large peaks at the retention time of 5.803 and 7.440 min. The LC-MS analysis revealed that PEF contains two major compounds a the retention time of 1.66 and 1.74 min and the mass spectrum analysis confirms the presence of Dihydroclerodin (M+Na, m/z 460, Rt-1.66 min) and Clerodin (M+Na, m/z 458, Rt-1.74 min) [73]. Further investigation by GC-MS analysis revealed the presence of clerodane diterpenoids like Clerodin (m/z 434.3), 15-hydroxy-14, 15-dihydroclerodin (m/z 452), 15-methoxy-14, 15-dihydroclerodin (m/z 466), and 14, 15-dihydroclerodin (m/z 436) with the retention time of 14.038, 14.103, 14.480 and 14.655 respectively. Investigation of PEF by UV-Vis spectrophotometer and FT-IR shows that petroleum ether fraction has absorption maxima at 227 nm and contains alkane group (C-H), aldehyde group (C=O), conjugated alkane (C=C), methyl group (C-H), alcohol group (O-H), alkyl aryl ether group (C-O), aliphatic ether / primary alcohol group (C-O), alkene group (C-C) which are in agreement with the obtained data of LC-MS and GC-MS. A previous investigation by various authors also stated that aerial parts of the *C. viscosum* contain Clerodin and many other Clerodane diterpenoids. Terpenoids, the largest class of natural products, contain approximately 25,000 chemical structures and are well known for their use in the fragrance and flavor industries, and also in the pharmaceutical and chemical industries [74]. Terpenoids are divided into several subclasses like monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids, and tetraterpenoids based on their structures. The large group of clerodane diterpenes occurs naturally as secondary metabolites in several hundreds of plant species from various families and in organisms from other taxonomic groups, such as fungi, bacteria, and marine sponges. In recent years, there is increasing attention on clerodane diterpenoids because of their noteworthy biological activities, particularly as insect antifeedant against economically important insect phytophagous pests. The various genera of the Lamiaceae family have been identified as rich sources of clerodane diterpenoids antifeedants. The presence of metaphase arresting, mitotic abnormality, and polyploidy inducing capabilities of both LAECV and PEF and also the presence of clerodane diterpenoids in PEF indicate that clerodane diterpenoids are may be responsible for the above stated biological effects. Thus the present study evidenced clerodane diterpenoids of LAECV may have cell cycle delay, pro-metaphase arresting, and mitotic abnormality inducing potentials.

5. Conclusions

The present study was conducted to identify the chemical compositions of LAECV that are responsible for cell cycle delay, pro-metaphase arrest, and mitotic abnormality induction in *Allium cepa* root apical meristem cells and the findings indicate that PEF is the main bioactive fraction containing clerodane diterpenoids like Clerodin (m/z 434.3), 15-hydroxy-14, 15-dihydroclerodin (m/z 452), 15-methoxy-14, 15-dihydroclerodin (m/z 466), and 14, 15-dihydroclerodin (m/z 436). Further detailed investigation is required for purification of the individual clerodane diterpenes of *Clerodendrum viscosum* and to test their relative pro-metaphase arrest and mitotic abnormality inducing potentials as well as a comparative analysis with the colchicines actions.

Disclosure statement

No conflict of interest was declared.

Acknowledgements

The authors acknowledge Prof. A. Mukherjee for plant species authentication and the financial support of UGC-SRF (FC(Sc)/RS/UGC/ZOO/2018-19/129, w.e.f. 07.04.2018, dated: 04.02.2019), and the DST-PURSE, DST-FIST, and UGC-DRS-sponsored facilities in the Department of Zoology.

References

[1] S. Nandi, L. MawkhliengLyndem, Natural Product Research, 30 (2015) 497-506.

[2] D. Bhattacharjee, A. Das, S.K. Das, G.S. Chakraborthy, Journal of advanced pharmaceutical and Healthcare Research, 1 (2011) 82-5.

[3] S. Bhattacharya, C. Vanaushadhi, Forth ed., *Ananda*, Kolkata, 1981.

[4] K.R. Kirtikar, B.D. Basu, Indian Medicinal Plants, 2nd ed., Dehradun, 1991.

[5] V.K. Singh, Z.A. Ali, M.K. Siddiqui, International Journal of Pharmacognosy, 35 (1997) 194-206.

[6] F. Hamilton. The flora homoeopathica, New Delhi: B Jain (Original Publication, 1852), 1997.

[7] K.M. Nadkarni, A.K. Nadkarni, Indian MateriaMedica, Bombay, 2002.

[8] S. Roy, A. Mukhopadhyay, G. Gurusubramanian, Journal of Pest Science, 83 (2010) 371-377.

[9] M.Z. Haque, M.A. Rouf, M.A. Jalil, M.B. Islam, M.R. Islam, Bangladesh J SciInd Res, 45 (2010) 381-386.

[10] M. Rahman, European Journal of Medicinal Plants, 3 (2013) 127-134.

[11] M.S. Islam, M.M.R. Moghal, S.K. Ahamed, J. Ahmed, M.A. Islam, Int Res J Pharm, 4 (2013) 99-102.

[12] R. Lobo, K.S. Chandrshakar, B. Jaykumar, B. Mamatha, Der Pharmacia Lettre, 2 (2010) 257-260.

[13] A.J. Modi, S.S. Khadabadi, I.A. Farooqui, D.S. Ghorpade, Der Pharmacia Lettre, 2 (2010) 102-105.

[14] D.K. Pal, S. Sannigrahi, U.K. Mazumder, Indian J ExpBiol, 47 (2009) 743–747.

[15] M.M. Rahman, N.N. Rumzhum, K.-E.K. Zinna, Stamford Journal of Pharmaceutical Sciences, 4 (1970) 74-78.

[16] N. Khatry, J. Kundu, S.C. Bachar, M.N. Uddin, J.K. Kundu, Dhaka University Journal of Pharmaceutical Sciences, 5 (1970) 63-66.

[17] K. Gouthamchandra, R. Mahmood, H. Manjunatha, Environmental Toxicology and Pharmacology, 30 (2010) 11-18.

[18] S. Sannigrahi, U.K. Mazumder, D.K. Pal, S.L. Mishra, Pharmacognosy Magazine, 5 (2009) 394-399.

[19] S. Singhmura, Journal of Pharmaceutical & Scientific Innovation, 5 (2016) 80-84.

[20] R. Lobo, I.S.R. Punitha, K. Rajendran, A. Shirwaikar, J Nat Prod, 129 (2006) 153-156.

[21] A.K. Azad, W.S. Wan -Azizi, T.M.F. Syafiq, S. Mahmood, H.A. Almoustafa, Z.K. Labu, Aust J Basic &ApplSci, 7 (2013) 641-647.

[22] M.S. Islam, M.M.R. Moghal, S.K. Ahamed, J. Ahmed, M.A, Islam, Int Res J Pharm, 4 (2013) 99-102.

[23] K. Arvind, R. Pradeep, R. Deepa, V. Mohan, Indian J Med Res, 116 (2002) 163-176.

[24] S. Das, S. Bhattacharya, A. Prasanna, R.B. Suresh Kumar, G. Pramanik, P.K. Haldar, Diabetes Ther, 2 (2011) 92-100.

[25] M.S. Hossain, J. Islam, R. Sarkar, S.M.M. Hossen, Int J Pharmacol, 1 (2014) 449-53.

[26] C. Shekar, Journal of Drug Delivery and Therapeutics, 2 (2012).

[27] O.I. Devi, B.K. Dutta, P. Choudhury, Journal of Applied and Natural Science, 5 (2013) 37-40.

[28] J.R. Qasem, C.L. Foy, Journal of Crop Production, 4 (2001) 43-119.

[29] T. Muh, A. Waliullah, A.M. Yeasmin, I.M. Wahedul, H. Parvez, Academic Journal of Entomology, 7 (2014) 63-69.

[30] R. Gupta, H.K. Singh, Asian Pacific Journal of Tropical Disease, 2 (2012) S465-S470.

[31] S. Ray, L.M. Kundu, S. Goswami, C.S. Chakrabarti, International Journal of Pharmaceutical Research and Development, 4 (2012) 332-345.

[32] R.G. Chandra, R.A.Y. Sanjib, International Journal of Pharma and Bio Sciences, 8 (2017).

[33] L.M. Kundu, S. Ray, Caryologia, 70 (2016) 7-14.

[34] S. Ray, L.M. Kundu, S. Goswami, G.C. Roy, S. Chatterjee, S. Dutta, A. Chaudhuri, C.S. Chakrabarti, Cell Prolif, 46 (2013) 109-117.

[35] R. Ade, M.K. Rai, Bioscience, 2 (2010) 90-96.

[36] I. van Echteld, M.D. Wechalekar, N. Schlesinger, R. Buchbinder, D. Aletaha, Cochrane Database of Systematic Reviews, (2014).

[37] G. Cocco, D.C.C. Chu, S. Pandolfi, European Journal of Internal Medicine, 21 (2010) 503-508.

[38] X. Puéchal, B. Terrier, L. Mouthon, N. Costedoat-Chalumeau, L. Guillevin, C. Le Jeunne, Joint Bone Spine, 81 (2014) 118-124.

[39] S. Porter, C. Scully, Clinical Evidence, 13 (2005) 1687-1694.

[40] S. Alabed, J.B. Cabello, G.J. Irving, M. Qintar, A. Burls, Cochrane Database of Systematic Reviews, (2014).

[41] G.N. Verne, R.H. Davis, M.E. Robinson, J.M. Gordon, E.Y. Eaker, C.A. Sninksy, The American Journal of Gastroenterology, 98 (2003) 1112-1116.

[42] E.D. Salmon, M. McKeel, T. Hays, J Cell Biol, 99 (1984) 1066-1075.

[43] A.D. Caperta, M. Delgado, F. Ressurreição, A. Meister, R.N. Jones, W. Viegas, A. Houben, Protoplasma, 227 (2006) 147-153.

[44] A. Manzoor, T. Ahmad, M.A. Bashir, I.A. Hafiz, C. Silvestri, Plants (Basel), 8 (2019) 194.

[45] A. Chaudhuri, S. Ray, Int J Pharma Bio Sci, 6 (2015) 99-108.

[46] A.K. Sharma, A. Sharma, Hardwood Academic Publishers, Netherlands, 1999.

[47] J.B. Harborne, Phytochemical Methods, Springer Netherlands, 1973, pp. 1-32.

[48] G.E. Trease, W.C. Evans, BrailliarTiridel can, Macmillian Publishers, 13 (1989).

[49] A. Sofowara, Spectrum Books Ltd., Ibadan, Nigeria, 1993, pp. 289.

[50] A.A. Bakare, A.A. Mosuro, O. Osibanjo. J EnvironBiol, 21 (2000) 263–271.

[51] M. Yusuf, J.U. Chowdhury, M.A. Wahab, J.A. Begum, Bangladesh Center for Scientific and Industrial Research, (1994) 192 .

[52] P.K. Warrier, V.P.K. Nambbiar, R.C. Kutty, Arya Vaidyasala Publication, 1996. pp. 160.

[53] L. Jirovetz, G. Buchbauer, C. Puschmann, M.P. Shafi, A. Saidutty. HerbaPolonica Journal, 45 (1999) 87-93.

[54] K.N.A. Aley, S.M. Mathews, P.N. Leena,Int J Pharma Bio Sci, 2 (2011) 182-187.

[55] A. Levan, Hereditas, 24 (2010) 471-486.

[56] A.A. Bakare, A.O. Adeyemi, A. Adeyemi, O.A. Alabi, O. Osibanjo, Caryologia, 65 (2012) 94-100.

[57] A. Bakare, O. Alabi, A. Gbadebo, O. Ogunsuyi, C. Alimba, Challenges, 4 (2013) 169-187.

[58] V.D.-S. Frescura, H.D. Laughinghouse, S.B. Tedesco, Caryologia, 65 (2012) 27-33.

[59] D. Davidson, R.D. MacLeod, M. O'Riordan, Nature, 212 (1966) 1541-1542.

[60] R. Caritá, M.A. Marin-Morales, Chemosphere, 72 (2008) 722-725.

[61] V.C. Mercykutty, J. Stephen, CYTOLOGIA, 45 (1980) 769-777.

[62] I. Klasterska, A.T. Natarjan, C. Ramel, Hereditas, 83 (1976) 153-162.

[63] A. Badr, A.G. Ibrahim, CYTOLOGIA, 52 (1987) 293-302.

[64] J.H. Ford, A.T. Correll, Genome, 35 (1992) 702-705.

[65] H. Babich, M.A. Segall, K.D. Fox, The American Biology Teacher, 59 (1997) 580-583.

[66] G. FiskesjÖ, Hereditas, 102 (2008) 99-112.

[67] E. Bonciu, P. Firbas, C.S. Fontanetti, J. Wusheng, M.C. Karaismailoğlu, D. Liu, F. Menicucci, D.S. Pesnya, A. Popescu, A.V. Romanovsky, S. Schiff, J. Ślusarczyk, C.P. de Souza, A. Srivastava, A. Sutan, A. Papini, Caryologia, 71 (2018) 191-209.

[68] S.A. Shahin, K.H. El-Amoodi, Mutation Research/Genetic Toxicology, 261 (1991) 169-176.

[69] M.S.S. Carvalho, L.F. Andrade-Vieira, F.E.d. Santos, F.F. Correa, M. das Graças Cardoso, L.R. Vilela, ScientiaHorticulturae, 245 (2019) 90-98.

[70] M. Fenech, J.W. Crott, Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 504 (2002) 131-136.

[71] L.K.S. Chauhan, V. Sundararaman, CYTOLOGIA, 55 (1990) 91-98.

[72] L.K.S. Chauhan, T.S.S. Dikshith, V. Sundararaman, Mutation Research/Genetic Toxicology, 171 (1986) 25-30.

[73] G. Abbaszadeh, C. Srivastava, S. Walia, National Academy Science Letters, 35 (2012) 457-464.

[74] J. Gershenzon, N. Dudareva, Nature Chemical Biology, 3 (2007) 408-414.