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Cytotoxic potential and phytochemical profiling of *Andrographis atropurpurea* (Dennst.) Alston an endemic species from Southern Western Ghats

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Abstract
Allium cepa is the most apt test organism for the preliminary cytological studies. In the present study *A. cepa* root tips were treated with different concentration of aqueous plant extract *viz.,* 0.005%, 0.01%, 0.05% and 0.1% at different time intervals (1/2h, 1h, 2h and 3h). Abnormality percentage incremented and mitotic index decremented with increase in the concentration of extract and time interval. Plant extract induced clastogenic, non clastogenic and cytoplasmic abnormalities. Presence of clastogenic abnormalities *viz.* nuclear lesion, nuclear erosion, chromosome stickiness, nuclear extrusion, nuclear disintegration, nuclear fragmentation,nuclear peak and cytoplasmic shrinkage designates the apoptotic potential of the plant extract. While spindle fibre abnormalities observed *viz.,* C-metaphase, polyploidy, vagrants, partial C-metaphase, cytostasis, ball metaphase and stellate metaphase disclosed the C-mitotic efficacy of plant extract. Phytochemical profiling via GC-MS and HR/LC-MS analysis exposed the cytotoxic components of the plant extract.

Keywords: *Andrographis atropurpurea, Andrographis paniculata*, cytotoxicity, C- mitosis, apoptosis, GC-MS, HR/LC-MS

1. Introduction
Cytotoxicity is the process of imparting disparity to the normal functioning of cells. Cytotoxic studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants (McGraw *et al*., 2014) [38]. *Allium cepa* assay is the most felicitous preliminary analysis for the detection of toxicity of the test substance (plant extract). Cytotoxic potential of the plant extract are due to the presence of cytotoxic chemical compounds of the extract. Phytochemicals are plant derived chemicals; also known as natural products, that exhibit many beneficial effects on human health. It has been demonstrated to constitute a key event in antitumor activities via the modulation of apoptotic pathways (Fulda, 2010) [20].
Andrographis atropurpurea (Dennst.) Alston is an endemic representative of Southern Western Ghats. It is named as '*atropurpurea'* due to its dark purple colour in the lower lip of corolla. It is commonly known as 'katu karuvi' and belongs to the family Acanthaceae. Recent studies revealed that *A. atropurpurea* is utilized in lieu of *A. paniculata,* a well known medicinal plant for the preparation of medicines in Ayurvedic system. *Andrographis paniculata* has many reported anticancer (Kumar *et al*., 2004) [30], antitumor (Sheeja & Kuttan, 2007) [54] and cytotoxic potentials (Siripong *et al*., 1992) [56]. Aerial parts of the plant are traditionally utilized for the treatment of cancer (Kritikar & Basu 1975) [29]. Since *A. atropurpurea* is utilized as a substituent of *A. paniculata*, it may also have expected antitumor and cytotoxic potentials. The present study disclosed the cytotoxic potential and phytochemical constituents of *A. atropurpurea*.

2. Materials and methods
2.1 Plant material
The plant material was collected from Vaikom of Kottayam district. The plant material was identified with the avail of type and protologue.

2.2 Cytotoxic assay
Fresh aqueous leaf extract was prepared in distilled water with the help of mortar and pestle. The stock solution was prepared by grinding 1 g of leaf tissue in 100 ml of distilled water. Lower concentrations of extracts 0.005, 0.01, 0.05 and 0.1 were prepared from stock solution utilizing distilled water. Uniform sized healthy bulbs of *Allium cepa* were planted in sterilized soil without manures to obviate cytological aberrations.

Germinated bulbs with healthy roots were washed in distilled water and treated with different concentrations of the extract. Treatment was done during a period of peak mitotic activity (8: 30 am to 10: 30 am). Onion bulbs treated with distilled water and 0.1% H_2O_2, were taken as positive and negative controls. After treatment in various concentrations at different time intervals (1/2, 1, 2 and 24), a few salubrious root tips were excised and washed exhaustively in distilled water. Washed root tips were immediately fixed in Carnoys fluid for 1 hour. After fixing, root tips were hydrolyzed in 1N HCL for 15 minutes at room temperature. Mitotic squash preparations were prepared with improved techniques (Sharma and Sharma, 1980) [53] utilizing 2% acetocarmine. All the slides were examined, tabulated and photomicrographs were taken with Leica ICC 50 digital camera attached to LEICA DM 500 research microscope. Chromosomal aberrations were determined by counting cells with, abnormalities in arbitrarily selected six fields from slides prepared with treated six onion bulbs for each concentration and time duration. Mitotic index and abnormality percentage were calculated using the following formulae:

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

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

Data obtained on mitotic index and chromosomal aberrations were subjected to statistical analysis. Duncan's Multiple Range test and one way ANOVA was performed to determine mean separation and significance of treatments utilizing SPSS version 20, SPSS Inc., Chicago, USA.

2.3 Phytochemical profiling

2.3.1 Plant extraction

A. atropurpurea leaves were shade dried. The dried sample was powdered and subjected to methanol extraction using soxhlet apparatus. 10 g of powdered plant material was extracted for 6 hours in 100 ml of 100% methanol. The extract so obtained was cooled, filtered and concentrated to dryness in water bath. GC-MS and HR/LC-MS analyses were carried out using this methanol extract.

2.3.2 GC-MS analysis

Volatile phytochemicals were determined by GC-MS (Shimadzu QP – 2010 plus) with Thermal Desorption TD 20, fitted with a 60 m x 0.25 mm x 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 coloumn pressure of 73.2 kPa. Both injector and detector temperature were maintained at 260 ^{0}C. Samples (6µL) were injected into the column with a split ratio of 10:0. Component separation was achieved following a linear temperature program of 70-260 ^{0}C at 3^{0}C/min and then held at 260 ^{0}C for 6 min, with a total run time 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 retention indices. The MS fragmentation pattern was checked with those of other compounds of known composition, with pure compounds by matching the MS fragmentation pattern with National Institute of Standards and Technology (NIST) mass spectra libraries. Finally, their quantification was performed on the basis of their peak areas.

2.3.3 HR/LC-MS analysis

Identification of non-volatile components of *A. atropurpurea* leaf extract was done by HR-LC/MS analysis. Experiments were performed by using an Agilent 1290 Infinity UHPLC with Chipcube, 6550 iFunnel Q-TOFs (palo Alto, CA, USA). The chromatographic separation was achieved on a reverse-phase Agilent Zorbax –C 18 110 A^0 analytical column (250 x 4.6 mm ID., 5µm) operated at 40^{0}C (Thermo electron, Auchtermuchy, UK). Elution was achieved with a gradient mobile phase consisting of methanol (A) and 0.5 µm ammonium formate (B) (9:91) in water (C) at a flow rate of 1ml/min. The gradient system used was as follows: Mobile phase C was held constant throughout the run and a linear gradient of B was maintained for 30 min. Mode of injection was ALS (automatic liquid sampling). Electrospray ionization (ESI) was used as the interface and was operated in positive selected ion monitoring (SIM) mode. The analytes were monitored by tandem-mass spectrometry with positive electrospray ionization. The injection volume was 20µl. The probe temperature was set at 500 ^{0}C and needle voltage was set at 20 V. The cone voltage was set at 50 V for all selected ion monitoring (SIM) scans.

3. Results

3.1 Cytotoxic assay

A. cepa assay disclosed the cytotoxic potential of the plant. Mitotic index (%) decremented and abnormality (%) incremented with the increase in the concentration of extract and time interval (Table 1). Clastogenic, non clastogenic and cytoplasmic abnormalities were observed. Clastogenic abnormalities include nuclear lesion, nuclear budding, nuclear erosion, chromosome stickiness, chromosome bridges, abnormal condensation of chromosomes, nuclear extrusion, nuclear disintegration, chained chromosomes, nuclear fragmentation, pulverised chromatin, giant cells, strap cell formation, chromosome coagulation *etc*. Non clastogenic abnormalities include the spindle aberrations *viz.*, chromosome laggards, stathmo anaphase, macro and micro cell formation, binucleate cell, C-metaphase, stellate arrangement of chromosomes, misorientation of chromosomes, polyploidy, hypoploidy, scattering of chromosomes, ball metaphase, pole to pole arrangement of chromosomes, cytostasis, early movement of chromosomes, vagrant chromosomes, early cell plate formation, tropokinesis, *etc.* Cytoplasmic shrinkage is the only one cytoplasmic abnormality observed.

Table 1: Mitotic index and abnormality percentage of treated *A. cepa* root tips

Concentration of the extract	Duration	Total number of cells ± SE	Mitotic index % ± SE	Abnormality %± SE
0.005	½	132 ± 5.16^{a}	67.36 ± 0.89^{c}	41.55 ± 0.68^{a}
	1	142 ± 3.75^{a,b}	65.56 ± 0.59^{c}	43.75 ± 0.61^{b}
	2	150 ± 5.10^{b}	63.63 ± 0.59^{b}	45.30 ± 0.42^{b}
	3	154 ± 2.76^{b}	61.73 ± 0.37^{a}	48.13 ± 0.57^{c}
0.01	½	140 ± 4.21^{a}	62.13 ± 0.73^{c}	44.21 ± 0.5^{a}
	1	150 ± 3.63^{a}	61.81 ± 0.3^{c}	44.63 ± 0.18^{a}
	2	162 ± 3.04^{b}	59.61 ± 0.24^{b}	46.26 ± 0.18^{b}
	3	167 ± 2.56^{b}	57.93 ± 0.25^{a}	49.03 ± 0.14^{c}
0.05	½	161 ± 1.78^{a}	54.61 ± 0.29^{c}	52.90 ± 0.23^{a}
	1	161 ± 1.73^{a}	53.48 ± 0.26^{b}	55.71 ± 0.85^{b}
	2	156 ± 2.64^{a}	51.11 ± 0.36^{a}	57.41 ± 0.4^{c}
	3	161 ± 2.53^{a}	50.85 ± 0.15^{a}	59.96 ± 0.36^{d}
0.1	½	159 ± 3.00^{b}	47.08 ± 0.54^{d}	62.58 ± 0.27^{a}
	1	138 ± 2.33^{a}	45.38 ± 0.35^{c}	63.31 ± 0.31^{a}
	2	138 ± 2.42^{a}	43.81 ± 0.29^{b}	64.80 ± 0.48^{b}
	3	155 ± 1.62^{b}	42.45 ± 0.34^{a}	66.63 ± 0.28^{c}
C−ve	½	146 ± 5.55^{a}	76.81 ± 1.26^{a}	1.14 ± 0.24^{a}
	1	152 ± 6.13^{a}	78.76 ± 1.29^{a}	1.55 ± 0.44^{a}
	2	149 ± 7.40^{a}	76.6 ± 1.26^{a}	2.05 ± 0.43^{a}
	3	145 ± 7.23^{a}	77.03 ± 0.7^{a}	1.59 ± 0.4^{a}
C+ve	½	127 ± 4.90^{a}	36.31 ± 1.01^{c}	53.80 ± 1.45^{a}
	1	142 ± 6.90^{a,b}	34.95 ± 1.36^{c}	57.38 ± 0.93^{b}
	2	154 ± 4.33^{b}	30.55 ± 0.41^{b}	60.13 ± 0.53^{c}
	3	147 ± 4.17^{b}	26.76 ± 1.06^{a}	65.06 ± 0.18^{d}

C^{+ve} –positive control (0.01% H_2O_2), C^{-ve} – Negative control, SE – Standard error, Means within a column followed by the same letters are not significantly different at $P < 0.05$ as determined by Duncan's multiple range tests.

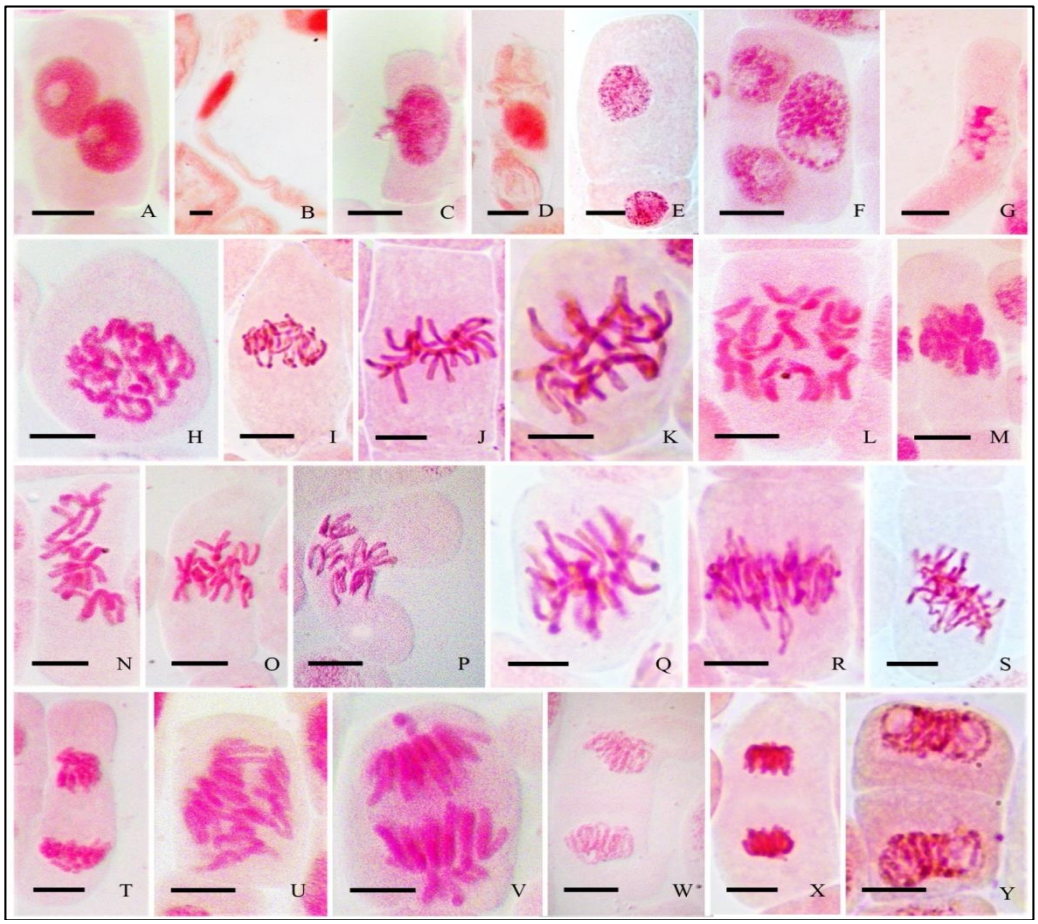

Fig 1: Major cellular aberrations observed in *Allium cepa* root tip meristem after treatment with *A. atropurpurea* extract. A-E: Interphase; A Binucleate cell showing single and double nuclear lesions, B Strap cell showing shrinkage, C Nuclear lesion and extrusion, D Ghost cell formation, E Micro and macro cell formation, F-G: Prophase; F Triple nucleate cell showing lesion and erosion, G Ghost cell formation showing disintegration of chromatin, H-T: Metaphase; H Ball metaphase, I Chained early metaphase, J Partial C- metaphase, K C- metaphase, L Cytostasis at sticky C-metaphase, M Coagulated metaphase, N Pole to pole metaphase, O Scattered metaphase, P Pulverized chromosome at C-metaphase in a hypoploid cell, Q Sticky stellate metaphase, R Pulverized chromosome in hyperploid cell, S-V: Anaphase; S Diagonal stathmo anaphase, T Non synchornized movement, U Diagonal anaphase showing multiple bridges, V Diagonal anaphase showing vagrants, W-X Telophase; W Pulverized chromosome, X Coagulated chromosome at telophase, Y Lesion at late cytokinesis.

3.2 Phytochemical profiling

3.2.1 GC-MS analysis

GC-MS analysis exposed 26 volatile components in the plant extract. These compounds belong to various classes' *viz.*, terpenoids, aldehydes, fatty acids, phenolics, hydrocarbons and esters. The prevailing compounds detected were cis-vaccenic acid (49.47%), hexanoic acid (9.19%), methyl oleate (4.83%), methyl linoleate (3.2%), phytol (3.11%) and octadecanoic acid (2.52%). Fatty acids (60.18%) were the predominant class of compounds present in the extract and esters were the second major class of compounds (Table 3).

Table 2: Volatile chemical composition of *Andrographis atropurpurea* as analyzed by GC-MS

Peak	R. Time	Compound	Class	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1	7.797	2-Ethyl-1-hexanol	Alcohol	$C_8H_{18}O$	130.231	1.24
2	16.586	2,4-DI-Tert-butylphenol	Phenol	$C_{14}H_{22}O$	206.329	0.41
3	17.937	Methyl salicylate	Ester	$C_8H_8O_3$	152.149	0.53
4	19.480	Phenol,-(1,1-dimethylpropyl)	Phenol	$C_{11}H_{16}O$	164.248	0.86
5	19.987	7-Hexadecenal, (Z)	Aldehyde	$C_{16}H_{30}O$	238.415	0.80
6	21.201	Neophytadiene	Terpene	$C_{20}H_{38}$	278.5	2.41
7	21.759	Phytol	Terpene	$C_{20}H_{40}O$	296.539	3.11
8	22.201	1,E-11,Z-13-Octadecatriene	Hydrocarbon	$C_{18}H_{32}$	248.454	0.42
9	22.326	Methyl palmitate	Ester	$C_{17}H_{34}O_2$	270.457	1.32
10	22.838	Hexanoic acid	Fatty acid	$C_6H_{12}O_2$	116.16	9.19
11	24.363	Methyl linoleate	Ester	$C_{19}H_{34}O_2$	294.479	3.2
12	24.441	Methyl oleate	Ester	$C_{19}H_{36}O_2$	296.495	4.83
13	24.738	Methyl stearate	Ester	$C_{18}H_{36}O_2$	284.484	0.68
14	24.996	cis-Vaccenic acid	Fatty acid	$C_{18}H_{34}O_2$	282.468	49.47
15	25.211	Octadecanoic acid	Fatty acid	$C_{18}H_{36}O_2$	284.484	2.52
16	26.121	6-Dodecyloxathiane 2,2-dioxide	Hydrocarbon	$C_{16}H_{32}O_3S$	304.489	0.27
17	26.622	Glycidyl palmitate	Ester	$C_{19}H_{36}O_3$	312.494	0.74
18	27.989	2,4-Bis(1-phenylethyl)phenol	Phenol	$C_{22}H_{22}O$	302.417	1.58
19	28.806	Glycidyl oleate	Ester	$C_{21}H_{38}O_3$	338.532	3.36
20	32.542	Glyceryl 2-linoleate	Ester	$C_{21}H_{38}O_4$	354.531	1.33
21	32.629	Glyceryl monooleate	Ester	$C_{21}H_{40}O_4$	356.547	1.13
22	35.754	2,4,6-Tris(1-phenylethyl)phenol	Phenol	$C_{23}H_{32}O$	324.508	0.48
23	36.788	beta-Sitosterol acetate	Ester	$C_{31}H_{52}O_2$	456.755	3.66
24	39.470	gamma-Sitosterol	Ester	$C_{29}H_{50}O$	414.718	3.88
25	42.126	Isoaromadendrene epoxide	Hydrocarbon	$C_{15}H_{24}O$	220.356	1.35
26	45.524	Z,E-2,13-Octadecadien-1-ol	Alcohol	$C_{18}H_{34}O$	266.4620	1.23

Table 3: Percentage compositions of various classes of compounds detected in the methanolic extract of *Andrographis atropurpurea* revealed through GC-MS analysis

Sl. No.	Class of compounds	Peak area %
1	Alcohols	2.47
2	Aldehydes	0.80
3	Esters	22.17
4	Fatty acids	60.18
5	Phenols	3.33
6	Terpenoids	3.11
7	Hydrocarbons	4.06
8	Steroids	3.88
Total		100

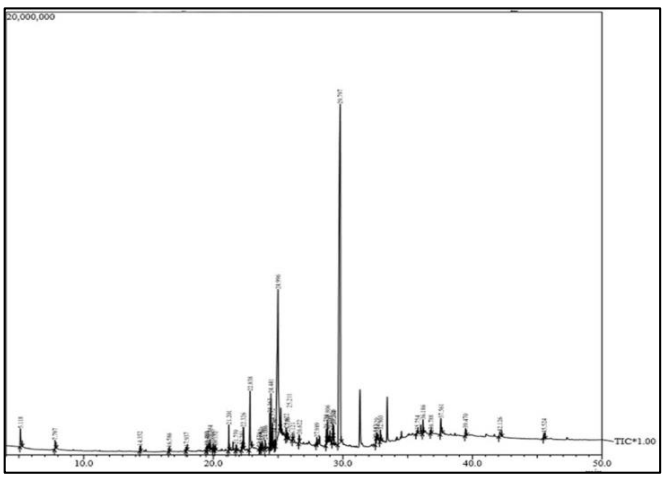

Fig 2: Gas chromatogram of methanolic extract of *Andrographis atropurpurea* (DEnnst.) Alston.

3.2.2 HR/LC-MS

HR/LC-MS analysis disclosed 22 non volatile compounds present in the plant extract. The compounds belong to various classes like peptides, terpenoids, amides, alkaloids, esters, flavonoids and alcohols.

Table 4: Non-volatile chemical constituents of *Andrographis atropurpurea* as analyzed by HR-LC/MS

SI No.	RT(min)	Compounds	Class	Mass	Molecular formula
1	5.072	Lys leu Glu	Peptide	388.2373	$C_{17}H_{32}N_4O_6$
2	5.481	Quassin	Terpene	388.18	$C_{22}H_{28}O_6$
3	5.887	Lys Thr Thr	Peptide	348.197	$C_{14}H_{28}N_4O_6$
4	6.094	Neoquassin	Terpene	390.2043	$C_{22}H_{30}O_6$
5	6.095	His Asn His	Peptide	406.178	$C_{16}H_{22}N_8O_5$
6	6.096	Gibberellin A_{12}	Terpene	332.2007	$C_{19}H_{24}O_2$
7	6.097	Geranyl cinnamate	Ester	284.1797	$C_{19}H_{24}O_2$
8	6.394	Ile Arg Cys	Peptide	390.2052	$C_{15}H_{30}N_6O_4S$
9	6.708	Sericetin	Flavonoid	404.1634	$C_{25}H_{24}O_5$
10	6.746	His His Lys	Peptide	420.2144	$C_{18}H_{28}N_8O_4$
11	6.902	Panaxydol	Alcohol	260.1828	$C_{17}H_{24}O_2$
12	6.921	Asn Gln Asn	Peptide	374.1535	$C_{13}H_{22}N_6O_7$
13	6.947	Arg Asp Asp	Peptide	404.1642	$C_{14}H_{24}N_6O_8$
14	7.097	Farnesylthioacetic acid	Terpene	396.1802	$C_{17}H_{28}O_2S$
15	7.189	Ginkgolide C	Terpene	440.1379	$C_{20}H_{24}O_{11}$
16	7.534	Gln Asn Gln	Peptide	388.1693	$C_{14}H_{24}N_6O_7$
17	7.686	Eupatorin	Flavonoid	344.0933	$C_{18}H_{16}O_7$
18	8.625	Arg Met Val	Peptide	404.2218	$C_{16}H_{32}N_6O_4S$
19	16.655	Arg Met His	Peptide	442.2163	$C_{17}H_{30}N_8O_4S$
20	17.808	Steramide	Amide	283.2908	$C_{18}H_{37}NO$
21	18.3	Dihydrogambogic acid	Terpene	630.3126	$C_{38}H_{46}O_8$
22	26.625	Phenylethylamine	Alkaloid	121.0907	$C_8H_{11}N$

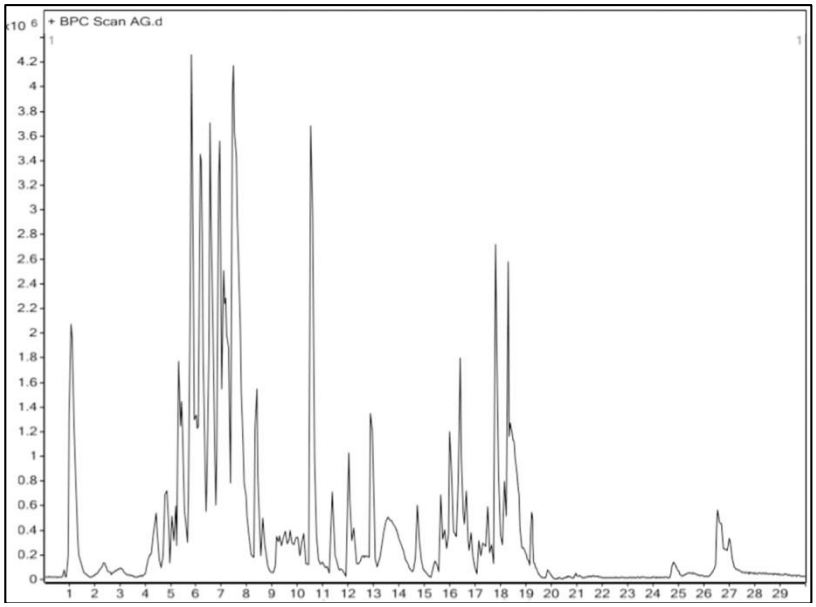

Fig 3: Liquid chromatogram of *Andrographis atropurpurea* (Dennst.) Alston

4. Discussion

4.1 Cytotoxic assay

Decrement of mitotic index with the increment of concentration of extract and time interval is due to the presence of mitodepressive component of extract. A clastogen is a mutagenic agent introducing destruction or breakage to chromosomes or chromatin, here the plant extract act as clastogen that cause cytotoxicity. Aberrations affecting the nucleus are known as clastogenic abnormality. Among the observed clastogenic abnormalities *viz.*, nuclear lesion, nuclear erosion, chromosome stickiness, chromosome fragments, abnormal condensation of chromosomes, nuclear extrusion, nuclear disintegration, nuclear fragmentation, nuclear peak, pulverised chromatin, chromosome coagulation, and the cytoplasmic abnormality designates the apoptotic potential of the plant extract (Prajitha & Thoppil, 2017) [46]. Studies by Pasqualini *et al*. (2003) [44] revealed that nuclear lesions are associated with programmed cell death in plants. Nuclear fragmentation is found to be an identification mark of programmed cell death (Gavrieli *et al*., 1992) [22].

Non clastogenic abnormalities are spindle fiber aberrations that arise due to defect in spindle fiber assembly. Spindle fiber breakage, non-synchronous assembly of microtubules, tubular protein degradation, inhibition of spindle assembly, tilt in spindle apparatus *etc* are the reasons of non clastogenic abnormalities. Spindle fiber abnormalities *viz.*, C-metaphase, polyploidy, vagrants, partial C-metaphase, cytostasis, ball metaphase and stellate metaphase, were found to be similar to that of colchicine which induced anomalies in cells (C-mitotic aberrations). Arresting of cell cycle is considered as

cytostasis, is a part of C- mitotic effect in cell (Neelamkavil and Thoppil, 2013) [40]. Cytostasis is the mechanism of stopping the cell division without killing the cell (Rixe & Fojo, 2007) [47]. Mitodepressive effect seems to be responsible for the cytostatic activity (Ahumada *et al*. 1995) [1]. Partial C-metaphase is considered as the fore step of C-metaphase were a few tubulin polymerization is present. Ball metaphase is a form of C-mitosis with characteristically clumped chromosomes. It is followed by either a complete degeneration of the cell or a state similar to interphase (Barber & Callan, 1943) [6]. Colchicine induced more than 50% of ball metaphase in cell suspension cultures of *Nicotiana plumbaginifolia* (Verhoeven *et al*., 1990) [63]. Lower dose of colchicine induced scattering of chromosomes, while higher dose induced ball metaphase in the Ehrlich ascites tumor cells of mice (Love, 1964) [33]. In the present study, the formation of ball metaphase may be due to the localized activity of spindle apparatus at the center so that the chromosomes were arranged in such a way that their centromeres remain at the equator and arms radiating in different directions and orienting in the form of a ball. Stellate metaphase is formed by decreasing the size of spindle in a fully formed metaphase and drawing the proximal end of the chromosomes towards a common center. Simultaneously the distal end of the chromosomes moves off the equatorial plane so that they finally spread outward in all directions to form star shaped metaphase. Cell division is arrested after the accumulation of chromosomes in star shape at metaphase (Gaulder & Carlson, 1951) [21]. Colchicine was found to induce star metaphase in the testis cells of the grasshopper, *Spathosternum prasniferum* (Manna & Parida, 1965) [37] and also in Newt embryonic cells (Barber & Callen, 1942) [6].

Vagrants are a form of C-mitotic effect (Neelamkavil and Thoppil, 2013) [40]. Vagrants arise by the movement of chromosome or chromosomes ahead from its chromosomal groups towards poles (Khanna & Sharma, 2013) [27]. Vagrants of chromosomes led to the formation of micronuclei (El-Ghamery, 2003) [17], which is a form C-mitotic effect in cells (Majone, 1990) [34]. Polyploidy is the presence of more than two complete sets of chromosomes per cell (Sattler *et al*., 2016) [52]. Caperta *et al*. (2006) [8] observed that tubulin polymerization occurs at C-metaphase cells after treatment with cochicine in *Secale cereale* cells thereby inducing polyploids. Thus the present study revealed the C-mitotic efficacy and apoptotic potential of the plant extract.

4.2 Phytochemical profiling

Phytochemical screening is conducted to expose the chemical constituents responsible for the cytotoxic potential of plant extract.

4.2.1 GC-MS analysis

In the present analysis out of 22 volatile constituents detected, 8 compounds *viz.,* cis vaccenic acid, methyl linoleate, methyl oleate, methyl stearate, methyl palmitate, γ-sitosterol, and phytol have reported antitumor potentials. Field *et al*. (2009) [19] reported the antitumor activity and apoptotic potential of cis-vaccenic acid. Tundis *et al*. (2009) [61] revealed the cytotoxic potential of methyl stearate. Numata *et al*. (1994) [42] isolated four free fatty acids palmitic acid, stearic acid, oleic acid and linoleic acid from seeds of *Coix lachryma-jobi* and reported their antitumor activity in mice. Nishikawa *et al*. (1976) [41] reported the antitumor activity of unsaturated fatty acids and their ester derivatives against Ehrlich ascites carcinoma. Zhu *et al*. (1989) [66] disclosed antitumor activity of methyl esters of oleic acid and linoleic acid on transplanted tumors in mice. γ-Sitosterol is a naturally occurring plant steroid, which is the major compound in the genus Lagerstroemia (Sirikhansaeng *et al*., 2017) [55]. Sundarraj *et al*. (2012) [57] reported that γ-sitosterol isolated from *Acasia nilotica* L. induces cell cycle arrest and apoptosis through c-myc suppression in MCF-7 and A549 cells. It is also an opportune drug for diabetes (Balamurugan *et al*., 2011; 2012) [4, 5]. Gupta *et al*. (2011) [24] reported the antidibetic and antioxidant potential of γ-sitosterol in streptozotocin induced experimental hyperglycemia. Phytol is the terpenoid detected in the present study. It is an acyclic diterpenoid alcohol and a constituent of chlorophyll (Costa *et al*., 2012; Lee *et al*., 2016) [13, 31]. Santos *et al*. (2013) [50] reported the antinociceptive and antioxidant activities of phytol on *in vivo* and *in vitro* models. Phytol has reported antiradical, cytotoxic potential and antimicrobial activities (Pejin *et al*., 2014) [45]. Varsha *et al*. (2015) [62] disclosed antioxidant, antitumor and antifungal potential of 2,4-DI-Tert-butylphenol isolated from *Lactococcus* sp. Choi *et al*., 2013 [11] reported the antioxidant potential of 2,4-DI-Tert-butylphenol isolated from sweet potato. Other bioactive compounds detected were methyl salicylate and neophytadiene. Methyl salicylate is the methyl ester of salicylic acid, an important plant hormone required for the establishment of systemic acquired resistance (Ryals *et al*., 1994) [49]. It has reported antipyretic properties (Higashi *et al*., 2010) [26]. Neophytadiene is an unsaturated hydrocarbon; it is the prevailing phytochemical of tobacco (Rowland, 1957). Palic *et al*. (2012) [48, 43] reported the antimicrobial activity of neophytadiene. It is also reported in *Andrographis paniculata* (Aneesh *et al*., 2018; Thangavel *et al*., 2015) [3, 58].

4.2.2 HR/LC-MS analysis

Non volatile phytochemicals detected by HR/LC-MS *viz*., ginkgolides, dihydrogambogic acid, eupatorin, panaxydol and biopeptides have marked antitumor and cytotoxic potentials. Ginkgolides are terpenoid lactones, the bitter principles of *Ginkgo biloba* (Sasaki *et al*., 2003). Chao *et al*. (2004) [51, 10] reported the cytotoxic and apoptotic potential of ginkgolide in human carcinoma cells. Chan, (2005) [9] disclosed that ginkgolide treatment on mouse blastocysts induces apoptosis, decreases cell numbers, retards early post implantation, blastocyst drtrtevelopment, and increases early-stage blastocyst death. Dihydrogambogic acid is a derivative of gambogic acid, a xanthanoid. Gambogic acid and dihydrogambogic acid are inhibitors of ubiquitin proteasome system in cells and chymotrypsin activity. The inhibitory effects of gambogic acid and dihydrogambogic acid on proteasome function corresponded with apoptosis induction and cell death (Felth *et al*., 2013) [18]. Dihydrogambogic acid silences $CCND_1$ as well as $CCND_2$ and induce cell- cycle arrest and cytotoxicity in myeloma cells (Tiedemann *et al*., 2008). Zhang *et al*. (2004) and Cai *et al*. (2002) [60] reported anticancerous and apoptotic potential of dihydrogambogic acid.

Eupatorin is a naturally occurring flavanone. It is one of the constituent of the medicinal plant *Orthosiphon stamineus*, which has reported antiproliferative potential (Doleckova *et al*., 2012) [16]. It induces cell cycle arrest at G2/M phase and apoptosis in HeLa cells (Lee *et al*., 2016) [31]. Androutsopoulos *et al*. (2008) [2] disclosed the antiproliferative and cytostatic potential of eupatorin on breast cancer cell lines. Panaxydol is the fatty alcohol reported in the present analysis. Hai *et al*. (2007) [25] reported the apoptotic potential of panaxydol. Moon *et al*. (2000) [39] reported that

panaxydol isolated from *Panax ginseng* induces G1 cell cycle arrest. Panaxydol was shown to induce apoptosis through an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), activation of JNK and p38 MAPK, and generation of reactive oxygen species (ROS) initially by NADPH oxidase and then by mitochondria (Kim *et al*., 2011). Panaxydol markedly inhibited proliferation of HL60 cells in a time- and dose-dependent manner via an apoptotic pathway (Yan *et al*., 2011). Guo *et al*. (2009) [64, 23] disclosed that panaxydol inhibits the cell proliferation and induces differentiation of human hepatocarcinoma cell line HepG2.

Biopeptides are the most fascinating bioactive compounds reported in the present study. They are smaller peptides derived from proteins via hydrolysis. Recently researchers have focused their attention on the mechanism of action and potential role of biopeptides in the prevention and treatment of cancer, cardiovascular and infective diseases (Cicero *et al*., 2017) [12]. Peptides can directly target cancer cells without affecting normal cells (targeted therapy), which is evolving as an alternate strategy to conventional chemotherapy. Peptides can be utilized directly as a cytotoxic agent through various mechanisms or can act as a carrier of cytotoxic agents and radioisotopes by specifically targeting cancer cells (Thundimadathil, 2012) [59]. They have reported antidiabetic, antioxidant and antinflammatory properties (Leo *et al*., 2016) [32]. Lunasin characterized by Arg-Gly-Asp sequence, promotes apoptosis in human colon cancer cells by mitochondrial pathway activation and induction of nuclear clustering expression (Dia & de Mejia, 2010) [14]. Food protein-derived bioactive peptides have been shown to exhibit anti-inflammatory activity by inhibiting or reducing the expression of these inflammatory biomarkers and/or by modulating the activity of these transcription factors (Majumder *et al*., 2016) [35]. Some bioactive peptides in cereal foods exert an antihypertensive effect by inhibiting Angiotensin-I Converting Enzyme (Malaguti *et al*., 2014) [36].

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