Omidiani N *et al.* (2020)
Notulae Scientia Biologicae 12(3):546-555
DOI:10.15835/nsb12310764
Research Article

Anticancer potentials of leaf, stem, and root extracts of *Achyranthes aspera* L.

Nafisehsadat OMIDIANI[1], Kailas D. DATKHILE[2], Rajkumar B. BARMUKH[1]*

[1]*Post Graduate Research Centre, Department of Botany, Modern College of Arts, Science and Commerce, Shivajinagar, Pune-411005, Savitribai Phule Pune University, Pune-411007, Iran; nsomidiani@gmail.com; barmukhbotany@moderncollegepune.edu.in (*corresponding author)*
[2]*Krishna Institute of Medical Sciences University, Department of Molecular Biology and Genetics, Karad, Maharashtra 415539, India; kddatkhile@gmail.com*

Abstract

Achyranthes aspera L. (Amaranthaceae), an herbaceous roadside weed in various parts of India, has several therapeutic uses, including the treatment against cancer. This investigation was undertaken to identify the bioactive compounds conferring cytotoxicity to the extracts from its biomass. The powdered leaf, stem, and root biomass was extracted separately in ethyl acetate, acetone, ethanol, and methanol. Each extract was tested against the HeLa cell line for the cytotoxicity, but the root-acetone extract was the most cytotoxic. This extract revealed eleven bands. The solution obtained from the ninth band (Rf = 0.87±0.06) exhibited more than 90% inhibition of HeLa cells. The LCMS analysis of this solution showed the presence of 37 compounds, out of which few compounds had been reported from different plants to possess cytotoxicity in various systems.

Keywords: *Achyranthes aspera* L.; anticancer activity; cytotoxicity; LC-MS; MTT assay

Introduction

Cancer is one of the most severe global health concerns in developing and developed countries alike. The drugs and therapies used in cancer treatment have several harmful side effects. Therefore, it is essential to search for plant-based natural anticancer agents that are safe and equally efficient. Naturally occurring phytochemicals from different medicinal plants contain a wide range of secondary metabolites with curative properties (Solowey *et al.*, 2014; Greenwell and Rahman, 2015). About 60% of currently used chemotherapeutic drugs are isolated from natural products of more than 3000 plants worldwide (Solowey *et al.*, 2014).

Achyranthes aspera L. (Amaranthaceae) is a herbaceous roadside weed that grows in various parts of India (Singh *et al.*, 2011). Different parts of the plant, as well as the whole plant, have many therapeutic uses. Recent pharmacological investigations have demonstrated antibacterial and anti-inflammatory activity of extracts of *A. aspera* (Valsaraj *et al.*, 1997, Iwalewa *et al.*, 2007), and free radical scavenging and antioxidant properties (Baskar *et al.*, 2012; Alkari and Chaturvedi, 2015).

Various extracts prepared from *A. aspera* have been shown to possess *in vivo* cytotoxic activity (Subbarayan *et al.*, 2012) including killing human colon cancer cells (COLO-205) by triggering the

Received: 22 Jun 2020. Received in revised form: 29 Jul 2020. Accepted: 17 Sep 2020. Published online: 29 Sep 2020.

mitochondrial apoptosis pathway and s phase cell cycle arrest (Arora and Tandon, 2014). However, there is meagre literature on the bioactive compounds conferring cytotoxicity to these extracts. Therefore, the present investigation was undertaken to identify the potential anticancer bioactive molecules from *A. aspera* against HeLa (human cervical carcinoma) cell line.

Materials and Methods

Minimum essential medium (10370-021), fetal bovine serum (2614079), RPMI1640 (11875-085), penicillin-streptomycin (15140-122), were purchased from Thermo Fisher Scientific, DMSO (D2650) from Sigma Aldrich, and MTT (M6494) from Invitrogen. Six well plates (CLS3355), 96 well plates (CLS3599), and 5 mL sterripipettes (CLS4487) were purchased from Corning, India.

Collection of plant material

The healthy plants of *A. aspera* were collected in July and August from the campus of Fergusson College, and the Savitribai Phule Pune University, Pune (accession no. MCASC-BOTLAB-2017-1). The identification of the collected plant was authenticated from the Botanical Survey of India, Western Circle, Pune. The leaves, stem, and roots were separated, washed thoroughly in running tap water, blotted dry, and cut into small pieces. The fresh biomass was layered on sheets of filter paper and dried in the shade at ambient temperature for about one week. The dried biomass was powdered in a mechanical grinder, and the powders were stored at - 20 °C in polythene bags with airtight zip lock.

Extraction of phytochemicals

The phytochemicals from the powdered leaf (L), stem (S), and root (R) biomass were extracted in a Soxhlet extractor separately in ethyl acetate (EA), acetone (Ac), ethanol (Et), and methanol (Me). Twenty-gram biomass was wrapped in Whatman No. 1 filter paper and extracted for five h in 180 mL solvent. The extract was suction filtered through a Whatman No. 1 filter paper disc placed in the Buchner funnel. The clear extract was evaporated to dryness in a 250 mL beaker placed in a hot water bath. The mass of dried residue was measured on an electronic weighing balance (CAS-250, Contech Instruments Ltd., India). The residue was suspended in 5.0 mL of solvent for eight h, after which it was centrifuged at 5000 × g. The clear supernatant was collected in glass vials and stored at - 20 °C until further use. Thus, four types of leaf extract L-EA, L-Ac, L-Et, L-Me; four types of stem extracts S-EA, S-Ac, S-Et, and S-Me; and four types of root extracts R-EA, R-Ac, R-Et, and R-Me were prepared. Each extract was diluted with the solvent used for extraction to yield a working solution with concertation of 500 micrograms per milliliter (µg/mL).

Cell lines and cell culture

In vitro cytotoxic effects of all twelve extracts made from *A. aspera* leaves, stem and roots were studied on human cervical adenocarcinoma (HeLa) cell line. The HeLa cell line was procured from National Centre for Cell Sciences (NCCS) Pune, India. The cell lines were maintained in T-25 flasks containing MEM essential media along with 10% heat-inactivated fetal bovine serum (FBS) and Penicillin-Streptomycin at 100 U/mL and 100 µg/mL, respectively. Cells were maintained under an atmosphere of 5% CO_2 and 95% humidity at 37 °C.

In vitro cytotoxicity assay

In vitro cytotoxicity of each extract on HeLa cells was determined by using MTT assay. The cells were maintained in MEM medium supplemented with 10% FBS, 100 U/mL Penicillin, and 100 µg/mL Streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Ten thousand cells in the respective medium were seeded in a 96 well plate and incubated at 37 °C in the atmosphere of 5% CO_2. After 24 h incubation, the

confluent cells were exposed to the respective treatment of each of the 12 extracts at the concentrations of 0, 25, 50, 100, 200, and 400 µg/mL in culture medium without FBS and incubated for further 48 h at 37 °C and 5% CO_2 atmosphere. After 24 and 48 h of treatments, cells were washed with sterile phosphate-buffered saline, and the cell proliferation was determined using MTT dye. Ten µl MTT (5 mg/mL) was added to each well, and the plates were further incubated at 37 °C in a 5% CO_2 atmosphere. After four h incubation, the MTT solution was carefully removed, and 200 µL of DMSO was added to each well. The absorbance of purple color developed was measured at 560 nm using a UV-Vis spectrophotometer (Model 1800, Shimadzu, Japan). The results were expressed as a percentage of cell inhibition as compared to the control.

The extract showing the highest cytotoxicity was subjected to TLC separation, and the solution made from each eluted TLC band was checked for cytotoxicity at the concentrations of 0, 2.5, 5, 10, 25, 50, and 100 µL/mL against the HeLa cells as described earlier.

Cell morphology

Cancer cells ($1x10^6$ cells/well) were seeded in a six-well culture plate and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. Later, the cells were treated with 0, 25, 50, 100, 200, and 400 µg/mL concentrations of acetone root extract of *A. aspera* in respective culture medium without FBS and further incubated for 48 h. The morphology of the cells was then examined under an inverted phase-contrast microscope (Primovert, Carl Zeiss).

Separation of the extract by using Thin Layer Chromatography (TLC)

The extract was subjected to TLC for the separation of phytochemicals. The pre-coated silica plates (TLC Silica Gel 60 $F_{254,}$ Merk, India) were used for the separation. Analytical separation of extract containing 25 µg dissolved residue was carried out in various solvent systems differing in polarity. A preparative TLC plate of 200 mm × 200 mm was used to separate 350 µg dissolved residue in the best solvent system (20% acetone in ethyl acetate) identified from the separation on analytical plates. Two such plates were simultaneously developed in one chromatography chamber. At the end of separation, the solvent front was marked, and the Rf value of each visible band was calculated.

Elution of separated bands

The TLC plate was air-dried, and each band was scraped off from the plate by using a scalpel, and the powder of two identical bands from two plates was pooled and suspended in 500 µL of acetone for eight h. The suspension was then centrifuged to remove the silica powder, and the clear supernatant was stored in glass vials. The solution thus prepared from each band was subjected to cytotoxicity testing on a v/v basis (2.5 to 100 µL/mL) against the HeLa cell line. The solution showing the highest percent inhibition was subjected to LC-MS analysis.

LC-MS analysis

LC-MS profiling of TLC band that showed the highest cytotoxic activity was performed on Agilent G6540B Quadrupole Time of Flight (QTOF) binary LC system equipped with Dual Agilent Jet Stream Electrospray Ionization (AJS ESI) and separated on Agilent Zorbax column (50 × 2.1 mm, 1.8 µm) using aqueous 0.1% formic acid (Solvent A) and acetonitrile (Solvent B). A stepwise increasing polarity gradient of solvent A and solvent B was applied at the flow rate of 0.3 mL/min for 30 minutes. The capillary voltage, cone voltage, and fragmentor voltage were 3.5 kV, 45 V, and 150 V, respectively. The gas temperature was set to 325 °C. Electroscopy mass spectra data were recorded in a positive ionization mode. The data was acquired at a scan rate of 2 spectra/sec in the mass range of 60-1700 m/z and analysed with Mass Hunter Qualitative Software and METLIN database. The class of the putatively identified compounds was deduced from the websites of the metabolomic workbench (www.metabolomicsworkbench.org) and the Lipidomics Gateway (www.lipidmaps.org).

Statistical analysis

All experiments were performed in three replicates, and the values were expressed as mean ± SD or mean ± standard error (SE) of means.

Results

Isolation and characterization of phytoconstituents from different parts of A. aspera

The result of the yield of extractable constituents is presented in Table 1. The percentage yield of extractable constituents was different in different solvents as well as source biomass. The stem biomass gave higher yield in all the solvents used except ethyl acetate, which gave the highest yield from leaf biomass.

Table 1. The percentage yield of extractable constituents from the biomass of *A. aspera*

Solvent	Leaf biomass (L)	Stem biomass (S)	Root biomass (R)
Ethyl acetate (EA)	1.47±0.17	1.13±0.13	0.64±0.06
Acetone (Ac)	0.91±0.08	3.07±0.42	2.80±0.44
Methanol (Me)	3.72±0.58	5.51±0.74	3.09±0.49
Ethanol (Et)	1.63±0.18	4.65±0.61	3.10±0.48

Values represent mean ± SD of 3 experiments

In vitro cytotoxicity of A. aspera extracts

To analyse the *in vitro* cytotoxic effects of ethyl acetate, acetone, ethanol and methanol extracts of root, stem, and leaf biomass of *A. aspera*, cell viability, and cell proliferation was determined by using MTT assay. The cytotoxic activity of these extracts was tested *in vitro* at the concentrations between 25 - 400 µg/mL against the cervical adenocarcinoma cell line (HeLa) exposed to the extracts for 24 h and 48 h. It was observed that the acetone and ethanol extracts of root biomass exhibited profound cytotoxic activities in a dose-dependent manner. In contrast, all other extracts of leaf and stem biomass showed comparatively lower cell growth inhibition even at higher concentrations.

At 400 µg/mL concentration, L-EA, S-EA, and R-EA extracts showed comparatively less inhibition of cell growth (Figure 1A). At the same concentration, L-Ac, S-Ac, and R-Ac extracts showed cell growth inhibition by (69.88 ± 1.11), (79.78± 1.11), and (91.15 ± 2.84) percent respectively after 24 h exposure (Figure 1B). The ethanol extracts of leaf and stem also showed relatively less inhibition of cell growth (Figure 1C). The L-Me, S-Me, and R-Me extracts showed (79.23 ± 0.56), (70.27 ± 0.27), and (73.72 ± 4.38) percent growth inhibition, respectively after 24 h exposure to these extracts (Figure 1D).

With a more prolonged exposure for 48 h, all the extracts showed varying degrees of increase in the cytotoxicity (Figures 1E to 1H). At the highest concentration of 400 µg/mL, the minimum increment of 4.65% was observed from R-Ac extract (Figure 1F) whereas a maximum of 68.66% increment was shown by L-Et extract (Figure 1G).

Thus, the R-Ac extract of *A. aspera* exhibited the maximum cytotoxic activity against HeLa cells after 24 h exposure. Morphological features of treated cells observed under an inverted phase-contrast microscope revealed distinct cellular morphological alterations such as loss of membrane integrity. Such cells, therefore, appeared shrunken and had cytoplasmic condensation indicating unhealthy nature. On the contrary, the untreated control cells appeared normal. These results indicate that the cells treated with higher concentrations of R-Ac extract had died, whereas untreated cells remained alive. The R-Ac extract subjected to TLC separation showed 11 distinct bands. The Rf value and the cytotoxicity potential of the solutions constituted from each band are presented in Table 2 and Figures 2A and 2B.

Solutions prepared from all eleven bands separated on a TLC plate showed cytotoxicity against HeLa cells. The cell growth inhibition ranged from about 29% to 86% after 24 h exposure to the solutions. There was not much difference between cell toxicity observed after 24 h and 48 h treatments. The maximum difference

(~14-15%) was observed in the cells treated with the solutions prepared from the 1st and the 11th bands. In contrast, a minimum of 0.9% difference was observed from the solution of the second band. The 9th band solution showed the maximum cytotoxicity against HeLa cells. The acetone solution prepared from the 9th band when subjected to LC-MS separation revealed 37 putatively identified compounds with match scores higher than 90 (Table 3).

LC-MS analysis of the phytochemicals from the solution prepared from the 9th band on the TLC plate thus reveals its heterogeneous nature. It contained 32% lipids, 11% organic acids, 7.9% benzenoids, 2% sterols, and 2.6% each of terpenes, quinones, and alkaloids.

Table 2. Rf value and cytotoxicity of compounds eluted from TLC bands of R-Ac extract (100 µL/mL) of *A. aspera*

Band No.	Rf	Percentage cell growth inhibition of HeLa cells after 24 h	Percentage cell growth inhibition of HeLa cells after 48 h
1	0.07±0.01	70.46± 1.13	84.53 ± 1.57
2	0.10±0.03	46.77 ± 1.14	47.67 ± 1.01
3	0.13±0.04	43.27 ± 1.26	44.67 ± 1.27
4	0.17±0.05	78.10 ± 0.22	84.29 ± 1.23
5	0.31±0.08	34.51 ± 1.18	37.92 ± 0.73
6	0.42±0.12	40.90 ± 0.99	46.69 ± 1.51
7	0.52±0.15	28.95 ± 0.29	31.26 ± 2.32
8	0.75±0.17	55.87 ± 3.01	58.95 ± 0.52
9	0.87±0.06	86.41 ± 1.96	94.30 ± 0.62
10	0.91±0.09	31.77 ± 3.05	36.30 ± 4.60
11	0.94±0.07	72.92 ± 1.04	87.37 ± 1.18

Values represent Mean ± SEM of three experiments

Table 3. Compounds detected in the acetone solution of 9th TLC band of R-Ac extract of *A. aspera*

Sr. No.	Name	Class	Formula	RT	m/z	Mass	Score
1.	Nε,nε,nε-trimethyllysine	Organic acid	$C_9H_{21}N_2O_2$	7.95	171.15	189.16	93.5
2.	Naproxen	Benzenoid	$C_{14}H_{14}O_3$	8.84	213.09	230.09	97.4
3.	Butorphanol	Benzenoid	$C_{12}H_{29}NO_2$	9.31	202.22	219.22	93.33
4.	2-benzoyl-5-methoxybenzoquinone	Benzenoid	$C_{14}H_{10}O_4$	10.02	225.05	242.06	97.06
5.	C16 sphinganine	Sphingolipid	$C_{16}H_{35}NO_2$	10.65	256.26	273.27	94.44
6.	10-keto tridecanoic acid	Fatty acyl	$C_{13}H_{24}O_3$	10.9	228.20	228.17	92.71
7.	P-Hydroxymexiletine	-	$C_{11}H_{17}NO_2$	10.98	178.12	195.13	93.38
8.	Cuscohygrine	Alkaloid	$C_{13}H_{24}N_2O$	11.3	225.20	224.19	94.76
9.	2E,4Z,6Z,8Z-Decatetraenedioic acid	Fatty acid	$C_{10}H_{10}O_4$	11.38	177.05	194.06	96.01
10.	Rhapontin	Aromatic polyketide	$C_{21}H_{24}O_9$	11.39	403.14	420.14	93.74
11.	Eupatorin	Flavonoid	$C_{18}H_{16}O_7$	12.4	327.09	344.09	96.36

12.	Cetylpyridinium	-	$C_{21}H_{38}N$	12.75	304.30	304.30	97.08
13.	(Z)-N-(2-hydroxyethyl) icos-11-enamide	Fatty acyl	$C_{22}H_{43}NO_2$	12.98	354.34	353.33	93.98
14.	Byssochlamic acid	Maleic anhydride	$C_{18}H_{20}O_6$	13.23	337.10	332.12	91.62
15.	Argphegln	Organic acid	$C_{20}H_{31}N_7O_5$	13.25	432.24	449.24	93.93
16.	N-(2-hydroxyethyl) icosanamide	Fatty acyl	$C_{22}H_{45}NO_2$	13.75	356.35	355.35	98.51
17.	6b,11b,16a,17a,21-Pentahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide	Terpene	$C_{24}H_{32}O_7$	13.87	415.21	432.22	98.85
18.	Cetrimonium	Quaternary ammonium cation	$C_{19}H_{42}N$	14.07	284.33	284.33	92.66
19.	Leupeptin	Organic acid	$C_{20}H_{38}N_6O_4$	14.82	409.29	426.29	94.7
20.	Glucosylsphingosine	Sphingolipid	$C_{24}H_{47}NO_7$	14.87	444.33	461.33	94.12
21.	3,7-epoxycaryophyllan-6-one	-	$C_{15}H_{24}O_2$	14.98	237.19	236.18	94.15
22.	Lactone of PGF-MUM	Quinone	$C_{16}H_{24}O_5$	15.15	279.16	296.16	97.38
23.	C8-dihydroceramide	Sphingolipid	$C_{26}H_{53}NO_3$	15.67	428.41	427.40	98.26
24.	10-deoxymethymycin	Polyketide	$C_{25}H_{43}NO_6$	16.06	476.30	453.31	97.38
25.	Phytosphingosine	Sphingolipid	$C_{18}H_{39}NO_3$	16.89	300.29	317.29	99.5
26.	26,27-diethyl-1alpha,25-dihydroxy-20,21-didehydro-23-oxavitamin	-	$C_{30}H_{48}O_4$	17.08	455.35	472.35	92.71
27.	3-Deoxy-3-azido-25-hydroxyvitamin D3	Secosteroid	$C_{27}H_{44}N_3O$	17.08	409.35	426.35	92.78
28.	Asp Lys Trp	Organic acid	$C_{21}H_{29}N_5O_6$	17.15	470.20	447.21	96.58
29.	1-hexadecanoyl-sn-glycerol	Glycerolipid	$C_{19}H_{38}O_4$	18	353.27	330.28	95.36
30.	1,2-ditetradecanoyl-sn-glycero-3-phospho-(1'-sn-glycerol)	phospholipid	$C_{34}H_{68}O_{10}P$	18.59	690.44	667.45	94.3
31.	N-(2-hydroxyethyl) oleamide	Fatty acid	$C_{20}H_{39}NO_2$	18.77	308.29	325.30	93.67
32.	1-octadecanoyl-rac-glycerol	Monoradylglycerol	$C_{21}H_{42}O_4$	19.76	381.30	358.31	98.91
33.	Docosanedioic acid	Fatty acid	$C_{22}H_{42}O_4$	21.01	371.32	370.31	96.99
34.	N-Stearoyl-D-sphingosine	Sphingolipid	$C_{36}H_{71}NO_3$	21.04	566.55	565.54	96.1

35.	3-Hydroxylidocaine glucuronide	Steroid	$C_{20}H_{30}N_2O_8$	21.68	431.18	426.20	91.01
36.	Hexacosanedioic acid	Fatty acid	$C_{26}H_{50}O_4$	23.65	427.38	426.37	98.4
37.	N-(3-oxo-octanoyl)-homoserine lactone	Fatty acyl	$C_{12}H_{19}NO_4$	28.74	224.13	241.13	95.6

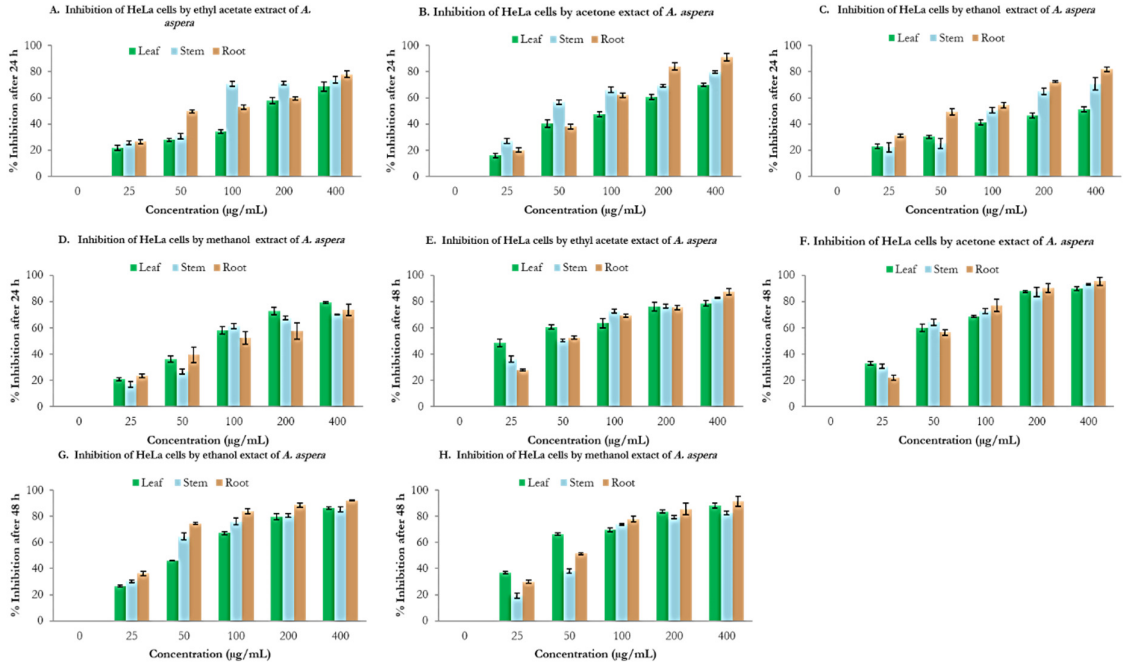

Figure 1. Inhibition of proliferation in HeLa cells exposed for 24 h (A-D) and 48 h (E-H) to extracts prepared from *A. aspera*

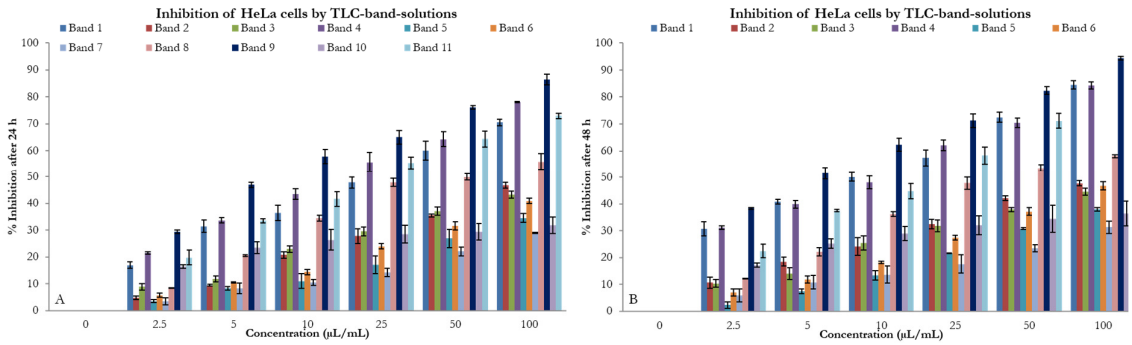

Figure 2. Inhibition of proliferation in HeLa cells exposed to solutions prepared from TLC separated bands of R-Ac extract of *A. aspera* (A) Inhibition of HeLa cells by TLC-band-solutions - 24 h and (B) Inhibition of HeLa cells by TLC-band-solutions - 48 h

Discussion

In the present investigation, HeLa cells treated with various extracts for 24 h and 48 h suffered dose-dependent cytotoxicity (25-400 µg/mL) coupled with distinct morphological damage including cellular and

nuclear shrinkage. It was observed that the cancer cells died due to reduced cell proliferation when exposed to extracts prepared from biomass of *A. aspera,* and the extract of roots in acetone had the maximum cytotoxicity against HeLa cancer cell line. Therefore, it can be concluded that at least a few of the 37 compounds identified by the LC-MS analysis of the root-acetone extract possess anticancer property. For example, the polyphenols from *A. aspera* (100 mg/kg, p.o.) were shown to possess synergistic anti-cancerous and cytokine based immunomodulatory role in mice suffering from urethane-induced lung cancer (Narayan and Kumar, 2014). The LC-MS has detected eupatorin ($C_{18}H_{16}O_7$, RT=12.4), a flavonoid polyphenol in the 9th TLC band of R-Ac extract. It has shown an anti-proliferative activity against several cancer cell lines including human uterus carcinoma (HeLa), human gastric adenocarcinoma (MK-1), and murine melanoma (B16F10) (Nagao *et al.*, 2002); liver murine colon carcinoma (26-L5) (Tezuka *et al.*, 2000), and human breast cancer cell line (MDA-MB-468) (Androutsopoulos *et al.*, 2008).

The antitumor effect of ethanolic and aqueous root extracts of *A. aspera* on the growth of colon cancer COLO-205 cells has been reported earlier (Arora and Tandon, 2014). Methanolic extract of *A. aspera* leaves contains anti-proliferative compounds with specific activity against pancreatic cancer (Subbarayan *et al.*, 2010). The methanolic extract of *A. aspera* has shown cytotoxic effects against VERO (monkey healthy kidney epithelial) cells, AGS (human stomach cancer cells), MCF-7 (human breast cancer cell line), A549 (human lung cancer cells), and COLO 320 DM (human colon cancer cell line) with IC_{50} values (µg/mL) of 682±51, 224±17, 315±24, 285±21, and 321±24 after 24 h exposure, and 412±31, 196±14, 286±21, 206±15, 294±22 µg/mL after 48 h exposure, respectively (Baskar *et al.*, 2012). In the present investigation, the 400 µg/mL leaf-methanol extract of *A. aspera* has shown 79.23% and 88.16% inhibition of HeLa cells after 24 h and 48 h exposure, respectively.

Conclusions

The present investigation supports the claims of anticancer properties attributed to *Achyranthes aspera* L. The LC-MS analysis of the acetone extract of its roots has revealed the presence of a few anticancer compounds reported in the literature. The global increase in the number of cancer patients, limitations of conventional therapies, the toxicity of drugs used in cancer treatment, and expensive and time consuming traditional anticancer drug discovery approaches have compelled the researchers to look for inexpensive and efficient alternatives in the kingdom of plants. Medicinal plants like *A. aspera* are natural reservoirs of bioactive phytochemicals that are effective against a wide range of diseases, including cancer. Moreover, they can be incorporated as complementary treatments alongside conventional drugs. Their use in managing or restraining the cancerous growth can be a better alternative to the conventional allopathic line of treatment on various cancers. Several plant-derived biomolecules like alkaloids, flavonoids, terpenes, and other secondary metabolites, have been authorized and subscribed as anticancer medicines. The notable examples that have been through clinical trials are taxol from *Taxus brevifolia* Nutt., vinca alkaloids from *Catharanthus roseus* G. Don., curcumin from *Curcuma longa* L., podophyllotoxin from *Podophyllum emodi* Wall., and camptothecin-derived topotecan from *Camptotheca acuminata* Decne. Their use as eco-friendly, biocompatible, and cost-effective alternative in the treatment of various types of cancers is gaining momentum worldwide. There is a genuine need for many such efficient anticancer drugs with little or no side effects. At the same time, it is necessary to evaluate such anticancer biomolecules for their effectiveness, possible applications, and toxicological studies under *in vitro* conditions and in the clinical trials as well.

Authors' Contributions

Conceptualization: RBB; Methodology, data curation and analysis: NO, KDD & RBB; Supervision: KDD & RBB; Writing- Original draft: NO, KDD and RBB; Writing- Review and editing: RBB

All authors read and approved the final manuscript.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors are grateful to Dr. Neeta M. Patil, Head, Department of Botany, and Dr. Rajendra S. Zunjarrao, Principal, Modern College of Arts, Science and Commerce, Shivajinagar, Pune-5, for their continuous support, motivation, and for providing the necessary infrastructure to conduct the experiments.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Alkari S, Chaturvedi A (2015). *Achyranthes aspera* Linn: A reservoir of antioxidants. International Journal of Pharma and Bio Sciences 6(2):55-66. *https://ijpbs.net/abstract.php?article=NDA4MQ==*

Androutsopoulos V, Arroo RRJ, Hall JF, Surichan S, Potter GA (2008). Anti-proliferative and cytostatic effects of the natural product eupatorin on MDA-MB-468 human breast cancer cells due to CYP1-mediated metabolism. Breast Cancer Research 10(3):R39. *https://doi.org/10.1186/bcr2090*

Arora S, Tandon S (2014). *Achyranthes aspera* root extracts induce human colon cancer cell (COLO-205) death by triggering the mitochondrial apoptosis pathway and S phase cell cycle arrest. The Scientific World Journal 2014:129697. *https://doi.org/10.1155/2014/129697*

Baskar AA, Al. Numair KS, Alsaif MA, Ignacimuthu S (2012). *In vitro* antioxidant and anti-proliferative potential of medicinal plants used in traditional Indian medicine to treat cancer. Redox Report 17(4):145-156. *https://doi.org/10.1179/1351000212Y.0000000017*

Greenwell M, Rahman PKS (2015). Medicinal plants: Their use in anticancer treatment. International Journal of Pharmaceutical Science and Research 6(10):4103-4112. *https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12*

Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology 6(25):2868-2885. *https://doi.org/10.5897/AJB2007.000-2457*

Nagao T, Abe F, Kinjo J, Okabe H (2002). Antiproliferative constituents in plants 10. Flavones from the leaves of *Lantana montevidensis* Briq. and consideration of structure-activity relationship. Biological and Pharmaceutical Bulletin 25(7):875-879. *https://doi.org/10.1248/bpb.25.875*

Narayan C, Kumar A (2014). Antineoplastic and immunomodulatory effect of polyphenolic components of *Achyranthes aspera* (PCA) extract on urethane induced lung cancer *in vivo*. Molecular Biology Reports 41:179-191. *https://doi.org/10.1007/s11033-013-2850-6*

Singh BR, Yadav S, Singh S, Brind L (2011). Pharmacognostic investigations of *Achyranthes aspera* Linn. International Journal of Ayurvedic Medicine 2(4):145-152. *https://www.ijam.co.in/index.php/ijam/article/view/76*

Solowey E, Lichtenstein M, Sallon S, Paavilainen H, Solowey E, Lorberboum-Galski H (2014). Evaluating medicinal plants for anticancer activity. The Scientific World Journal 2014:721402. *https://doi.org/10.1155/2014/721402*

Subbarayan PR, Sarkar M, Impellizzeri S, Raymo F, Lokeshwar BL, Kumar P, ... Ardalan B (2010). Anti-proliferative and anti-cancer properties of *Achyranthes aspera*: Specific inhibitory activity against pancreatic cancer cells. Journal of Ethnopharmacology 131(1):78-82. *https://doi.org/10.1016/j.jep.2010.06.002*

Subbarayan PR, Sarkar M, Nagaraja RS, Philip S, Kumar P, Altman N, ... Lokeshwar BL (2012). *Achyranthes aspera* (Apamarg) leaf extract inhibits human pancreatic tumor growth in athymic mice by apoptosis. Journal of Ethnopharmacology 142(2):523-530. *https://doi.org/10.1016/j.jep.2012.05.030*

Tezuka Y, Stampoulis P, Banskota AH, Awale S, Tran KQ, Saiki I, Kadota S (2000). Constituents of the Vietnamese medicinal plant *Orthosiphon stamineus*. Chemical and Pharmaceutical Bulletin 48(11):1711-1719. *https://doi.org/10.1248/cpb.48.1711*

Valsaraj R, Pushpangadan P, Smitt UW, Adsersen A, Nyman U (1997). Antimicrobial screening of selected medicinal plants from India. Journal of Ethnopharmacology 58(2):75-83. *https://doi.org/10.1016/s0378-8741(97)00085-8*