CSD-2019 Special Issue

J. Indian Chem. Soc.,
Vol. 97, No. 12c, December 2020, pp. 2874-2883

In vitro assessment of apoptosis induced by phytochemicals from *Ampelocissus latifolia* (Roxb.) Planch.

Anwesa Chaudhuri*[a], Saumabha Chatterjee[b], Ananya Chaudhuri[c] and Sanjib Ray[d]

[a]Department of Zoology, Lady Brabourne College, Suhrawardy Avenue, Beniapukur, Kolkata-700 017, India

[b]Department of Zoology, Durgapur Govt. College, Jawahar Lal Nehru Road, Amarabati Colony, Durgapur-713 214, West Bengal, India

[c]Department of Chemistry, Sidho-Kanho-Birsha University, Purulia-723 104, West Bengal, India

[d]Molecular Biology and Genetics Unit, Department of Zoology, The University of Burdwan, Golapbag, Burdwan-713 104, West Bengal, India

**E-mail:* anwesa.1@rediffmail.com*

Manuscript received online 03 December 2020, accepted 28 December 2020

This study evaluated the apoptotic and cytotoxic activities of aerial parts' hydro-methanolic extract of *Ampelocissus latifolia* (Roxb.) Planch. (AHMEAL) on *in vitro* cultured Dalton's lymphoma (DL) cells. Here, *in vitro* cultured DL cells were exposed to AHMEAL for 24 h. Cell viability assays like trypan blue assay, succinate dehydrogenase activity assay (MTT assay), live/dead assay through fluorescence microscopy, and flow cytometric analysis were done for the study of *in vitro* cytotoxic activity. Results indicated a concentration-dependent decreased survivability, increased percentages of apoptotic cells along with increasing sub-G_1 cell populations in treated DL cells. Steroids, carbohydrates, flavonoids, terpenoids, alkaloids, tannins, glycosides, anthraquinones, and saponins are also present in AHMEAL. Thus, the outcomes of this study reveal that the phytochemicals from AHMEAL hold a significant apoptotic potential and can be targeted for the invention of chemotherapeutic drugs.

Keywords: Apoptosis, fluorescence microscope, hydro-methanol, phytochemicals, sub-G_1.

Introduction

Phytochemicals are of special interest from the time immemorial for their various therapeutic values. They are used globally in cardiac ailments, hypertension, nerve disorders, neurodegenerative diseases, immune-deficiency syndromes etc.[1–3]. Various plants possess antiproliferative, cytotoxic, anticancer, anti-tumor, and antioxidant properties[4–6]. Plant-based health care system relies on the abundance of various phytoconstituents[7,8]. Uvaribonin, camptothecin, chalcone, paclitaxel, vinblastine, 22-epicalamistrin etc. provide an insight into the efficiency of these compounds in having a noteworthy anticancer activity[9,10]. Therefore, the breakthrough and the investigation of these natural products for the management of targeted diseases have become the burning area of interest. Plants may contain phytochemicals, of which some may possess carcinogenic or some have anti-cancer properties[11,12]. So, scientific confirmation and proper exploration of traditional plants is a prerequisite for their therapeutic application. For this, simple bench-top assays as well as *in vitro* bio-assays are used widely to validate their targeted pharmacological activities[13]. The increased interest in apoptosis has cemented the way towards the improvement in cancer research[14]. Apoptosis is a major therapeutic mechanism for different commercially available cytotoxic drugs. Therefore, the screening and scientific exploration of phytochemicals with potential apoptotic activities are of renewed interest for chemotherapeutic purposes[15].

Ampelocissus latifolia (Roxb.) Planch. [Family: Vitaceae] is used to treat gout, dysentery, ulcers, indigestion, tuberculosis etc.[16–18]. *A. latifolia* is a rich source of various phytoconstituents like hexadecanoic acid, tetracosane, heneicosane, β-sitosterol, γ-sitosterol, squalene, uvaribonin,

22-epicalmistrin, chalcone etc.[10,19,20]. This plant possess cytogenotoxic[4,5], allelopathic[21–23], antibacterial[24], anti-inflammatory[25], and antioxidant activities[6]. Here, we did a qualitative analysis of phytochemicals of the hydro-methanolic extract of *A. latifolia* and also evaluated the apoptotic as well as antiproliferative potentials of this extract on Dalton's lymphoma cells.

Materials and methods

Chemicals:

Glacial acetic acid, as well as, methanol was procured from the BDH chemicals Ltd., UK. RPMI-1640 medium, fetal bovine serum (FBS), antibiotic solution, trypan blue, and MTT were purchased from Himedia, India. Ethidium bromide, dimethyl sulfoxide (DMSO), and ferric chloride ($FeCl_3$) were purchased from Sigma, USA. Sodium hydroxide (NaOH) was collected from the MERCK Specialities Pvt. Ltd., Mumbai, India. Acridine orange stain was obtained from the S.D. Fine-Chem. Ltd., India. Other chemicals were purchased from reputed manufacturers.

Collection of plant products:

Fresh plant materials were collected from the Golapbag Campus, The University of Burdwan, during May 2011 and identified by Taxonomist Ambarish Mukherjee of that University. The voucher specimen (Ref. No. BUGBAC012) is kept therein.

Preparation of hydro-methanolic extract:

The collected plant parts were washed, shade-dried, and pulverized. 50 g of the powdered material of *A. latifolia* was extracted with 1 L of solvent (methanol:water, 7:3) in a Soxhlet apparatus for 72 h. Then the solvent was evaporated to obtain a green residue and dissolved in 1% DMSO. Before application, the peak concentration of DMSO was fixed at 0.10%.

In vitro culture of Dalton's lymphoma (DL) cells:

DL cells were obtained from the North-Eastern Hill University, Shillong, India and maintained *in vivo* in Swiss albino mice (20–25 g)[26]. After 7–8 days, the fluid was drawn and approximately 2×10^6 cells were set for culture in the medium (RPMI-1640 medium, penicillin-streptomycin antibiotic solution and 10% heat-inactivated FBS). Each cell suspension was treated for 24 h (AHMEAL, 0.50–4 mg/mL) in a sterile humified CO_2 incubator (37°C). Culture with 0.10% DMSO was used as the control. After 24 h, trypan blue and MTT assays, fluorescence microscopic analysis of apoptosis, and flow cytometric analysis were done with the cultured cells. Experiments were done following the Rules of the Animal Ethical Committee.

Trypan blue assay:

Trypan blue is chemically known as 3,3′-[(3,3′-dimethyl(1,1′-biphenyl)-4,4′-diyl)bis(azo)]bis(5 amino-4-hydroxy-2,7-naphthalene di sulfonic acid) tetra sodium salt. This dye exclusively labels the dead cells as live cells possess an undamaged cell membrane and exclude it[27]. Here, the cell suspension (50 μL) was mixed with 50 μL of 0.40% trypan blue solution, and the numbers of cells were counted using a hemocytometer within 3–5 min to prevent dye uptake by the live cells. Total cells counted were 200–250. Cytotoxicity was calculated by dividing the number of dead cells with total number of cells and expressed in terms of percent values.

Succinate dehydrogenase activity assay (MTT assay):

The extract-induced cytotoxicity was studied using MTT assay[28]. Here, the metabolic function of the cells is considered as a viability indicator. In this assay, the reduction of yellow-colored water-soluble compound MTT {3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide} to purple colored insoluble formazan crystal {(*E*,*Z*)-5-(4,5-dimethylthiazole-2-yl)-1,3-diphenylformazan} is catalyzed by mitochondrial succinate dehydrogenase (Fig. 1). Hence, this assay works according to the cellular respiration and assesses the viability of a cell. Here, the cell suspensions were allowed to react with 100 μL MTT reagent (5 mg/mL) for 4 h and incubated at 37°C. After that, the culture medium was removed, the crystals were allowed to dissolve in 2 mL raw DMSO (solubilizing agent), incubated for 30 min at 37°C and the absorbance value was noted at 570 nm. OD values of the AHMEAL-treated cells were compared to the controls and the results are represented as cell survivability percentages.

Live/dead assay using a fluorescence microscope (FM):

A live/dead assay is an informative and useful tool for detecting cell viability. In principle, after combined staining with ethidium bromide (EB)-acridine orange (AO), cells are

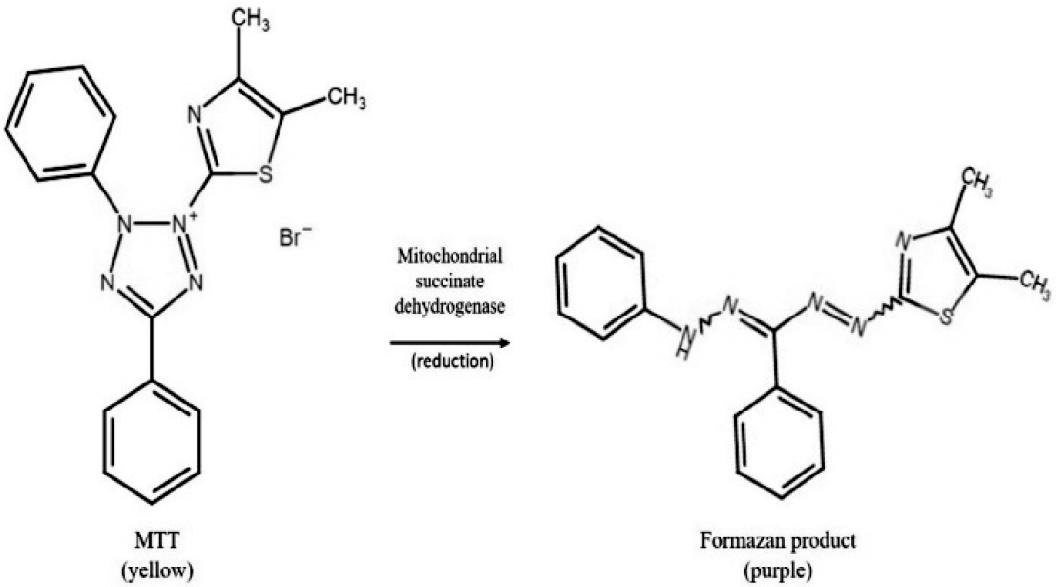

Fig. 1. The chemical reaction in MTT assay.

differentiated following their characteristic color pattern. Live cells (normal intact nuclei) fluoresce green, early apoptotic cells (moderate nuclear condensation) fluoresce greenish to yellowish and late apoptotic cells (nuclear fragmentation) fluoresce dark red. Necrotic cells with round nuclei fluoresce red. Here, the cultured cells, after washing in 1X PBS were stained with 1:1, v/v of ethidium bromide (EB)-acridine orange (AO) and examined under the fluorescence microscope following the procedure as described earlier[4,29] with little modifications. For each concentration, 50 microscopic fields were scored and the observations were repeated thrice in different non-overlapping fields.

Flow cytometric study using fluorescence-activated cell sorter (FACS):

Flow cytometric analysis of cell cycle kinetics of the harvested cells was done based on the procedures described by Kang and Alvarado[30]. Briefly, after rinsing the cells (about 1×10^6 cells) in PBS (1X), they were fixed in 70% alcohol. Then, these cells were again suspended in PBS, reacted with 1% Triton X-100, 1 μg/mL RNase A, and 1 mg/mL propidium iodide for 20 min and analyzed using FACS. The results were plotted in a graph, where the X-axis denotes fluorescence intensity (red fluorescence; λ_{em}: 585; nm FL-2) and the Y-axis denotes cell counts.

Phytochemical screening:

A qualitative study of phytochemicals **(**flavonoids, anthraquinones, phlobatannins, tannins, carbohydrates, glycosides, steroids, terpenoids, saponins, alkaloids etc.) present in AHMEAL was done following the standard protocols[31–34], with little modifications[35].

Statistical analysis:

Data were assessed in three sets and the results are shown as Mean±SEM. Student's t-test and 2×2 contingency χ^2-test were done for the comparison of the statistical differences between the control and the treated groups. The level of statistical significance was considered at $p < 0.05$–$p < 0.001$. IC_{50} value calculations were done using probit analysis.

Results and discussion

Trypan blue assay:

To reveal the potential apoptotic activity, we did *in vitro* screening of AHMEAL by measuring its cytotoxicity on the DL cell line. Amongst the various cytological assays, a trypan blue assay was done, which discriminates the dead and live cells by differential color pattern. Here, data indicated distinct cytotoxicity in a concentration-wise manner towards DL

cells as shown by the live cells percentages to be decreased and the dead cells percentages to be increased with the IC_{50} value of 1.82±0.10 mg/mL (Table 1).

Table 1. Trypan blue assay showing the effect of AHMEAL on the viability of DL cells

Treatment	Conc. (mg/mL)	Live cells (%) (Mean±SEM)	Dead cells (%) (Mean±SEM)	Dead cells (%) % Increase
Control	00	74.64±1.11	25.36±1.11	0
AHMEAL	0.5	64.61±1.75	35.39±1.75[a]	39.55
	2	48.07±3.77[a]	51.93±3.77[a]	104.77
	4	22.27±2.89[a]	77.73±2.89[a]	206.51

[a]Significant at $p < 0.001$ as compared to the control by 2×2 contingency χ^2-test (d.f. = 1).

Previous studies explore that the crude extracts of different plants of currently available anticancer drugs exhibits a significant growth inhibitory property on cancer cells[36,37]. The crude ethanolic extract of *Catharanthus roseus* was reported possessing antitumor activities in Ehrlich ascites carcinoma (EAC) cells and tumor-bearing mice[37]. The crude extracts of *Artocarpus camansi, Premna odorata*, *Gliricidia sepium* and their hexane and ethyl acetate fractions also possess cytotoxicity against some human cancer cell lines[38]. Dichloromethane fraction of methanolic extract of *Artocarpus* sp. was promoted apoptosis in hepatocellular carcinoma cells[39].

Succinate dehydrogenase activity assay (MTT assay):

MTT test is the colorimetric measurement based assay, familiar with the assessment of cytotoxicity and cellular proliferation. AHMEAL-induced cytotoxicity was examined by the MTT assay which is an effective way to detect extract-induced cytotoxicity. Here, the treatment with AHMEAL significantly ($p < 0.05$–$p < 0.001$) inhibited the propagation of DL cells by the concentration-wise mode which was reflected in OD values and cell viability (Table 2). The IC_{50} value was estimated to be 0.99±0.29 mg/mL. There is a previous study that reports the *Solanum pseudocapsicum*-induced antitumor activity on the DL cells[40]. It was also reported previously that *Ephemerantha* sp. extract caused reduced viability of HCT-116 and HT-29 cells in a concentration-dependent manner as measured by this assay[7].

Table 2. MTT assay showing the effect of AHMEAL on DL cell viability

Treatment	Conc. (mg/mL)	OD at 570 nm	Cell survivability (%) (Mean±SEM)	Cell death (%) (Mean±SEM)
Control	00	1.57±0.08	100±0	00
AHMEAL	0.5	0.86±0.19	54.56±7.29[a]	45.44±7.29[a]
	2	0.62±0.08[c]	39.60±3.32[a]	60.40±3.32[a]
	4	0.56±0.04[b]	35.42±1.90[a]	64.58±1.90[a]

[a]Significant at $p < 0.001$, [b]at $p < 0.01$, [c]at $p < 0.05$ by Student's t-test. Conc.: Concentration.

Cellular morphology analysis by fluorescence microscope:

Fluorescence microscopic analysis is used for determining the apoptotic activity[41]. During apoptosis DNA is cleaved into smaller oligonucleosomal fragments, causing the formation of chromosomal condensation, nuclear fragmentation, cell shrinkage, membrane blebbing, and the apoptotic bodies[42]. It is a significant and greatly enviable feature of the anticancer drugs[43]. We have used, EB-AO combined staining to recognize AHMEAL-induced apoptotic activity, where live cells showed green fluorescence with undamaged nuclei and treated cells showed typical patterns of apoptotic morphologies (Fig. 2).

Data revealed that in the treated group of cells the apoptotic cells (%) increased significantly in a concentration-reliant manner, with a concomitant decrease in the live cells (Table 3). Compared to the apoptotic cells, the proportions of necrotic cells were very low. Thus the outcomes of our present study suggest the apoptotic activity of AHMEAL on DL cells.

Flow cytometric study using fluorescence-activated cell sorter (FACS):

Flow cytometric study reveals the percentages of cells at different phases of the cell cycle. Cytotoxic agents cause cellular damages and thus, the normal cell cycle kinetics is altered. Here, flow cytometric study showed that there is a concentration-wise elevation in the sub-G_1 cell population after AHMEAL (0.5, 2, and 4 mg/mL) treatment (Fig. 3), keeping in line with the results of the fluorescence microscopic analysis showing a concentration-wise increase in cytotoxi-

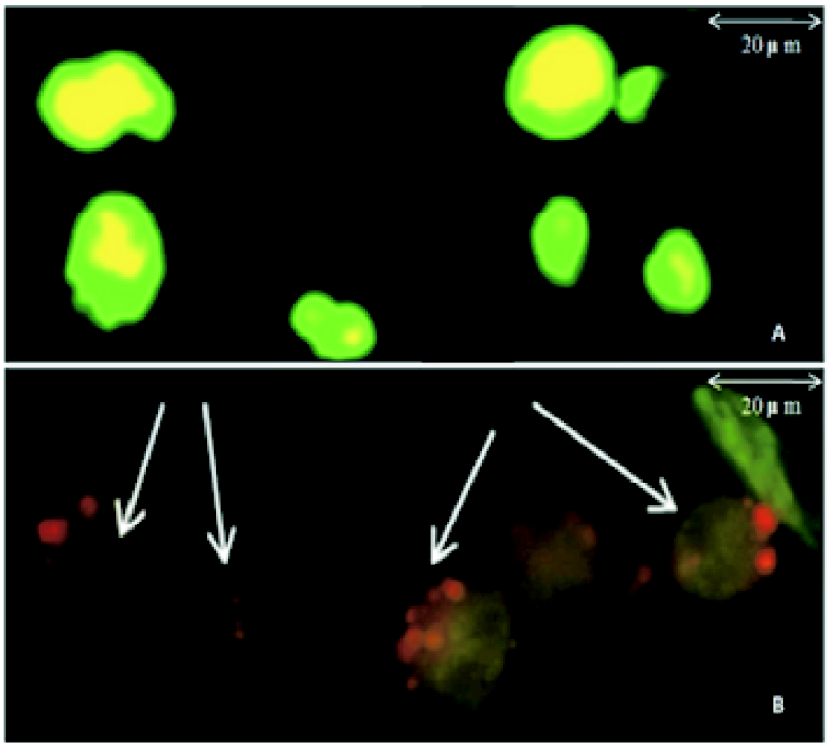

Fig. 2. Photomicrographs showing the morphologies of normal cells (A) vs apoptotic cells (B) under the fluorescence microscope. Photomicrographs (200X) are magnified (12x) using the Microsoft Power Point. The cells showing apoptotic bodies and membrane blebbing are indicated by white arrows.

city in terms of apoptotic activity (Table 3; Fig. 2). Several anticancer medicines block cell cycle progression and cause an increase in the sub-G_1 cell population. Crude methanolic extract of four plants (*Uvaria longipes*, *Artabotrys burmanicus*, *Marsypopetalum modestum*, and *Dasymaschalon* sp.) from the family Annonaceae was found to arrest cell cycle at sub-G_1 phase causing induction of apoptosis[13,44]. Moscatilin, extracted from *Dendrobium* sp. also caused cell cycle arrest at sub-G_1 phase in the HCT-116 cells[7].

Phytochemical analysis

Screening and phytochemical characterization of the commonly used medicinal plants having chemo-preventive properties and the scientific validation of their therapeutic perspectives lead to the detection of new chemicals with increased efficiency against cancer. Due to the cost-effectiveness, as well as a low toxicity, some plants have gained a renewed attraction in cancer therapeutics[45]. Phytochemicals like steroids, terpenoids, glycosides, flavonoids, alkaloids,

Table 3. Analysis of the fluorescence microscopic observations showing the influence of AHMEAL on DL cells

Conc.	TC	NVC		AC		NC	
(mg/mL)		TC	Mean±SEM	TC	Mean±SEM	TC	Mean±SEM
00	6164	5495	89.94±1.19	669	10.86±0.01	0	0
0.5	3003	2350	78.33±1.02[a]	652	21.73±0.08[a]	1	0.03±0.02
2	2406	1130	47.30±2.40[a]	1271	52.83±0.08[a]	5	0.21±0.04[b]
4	2326	796	34.15±1.08[a]	1509	64.88±0.07[a]	21	0.88±0.29[a]

Conc.: Concentration; TC: total cells; NVC: normal viable cells; AC: apoptotic cells; NC: necrotic cells. [a]Significant at $p < 0.001$, [b]at $p < 0.01$ in comparison with the control by 2×2 contingency χ^2-test (d.f. = 1).

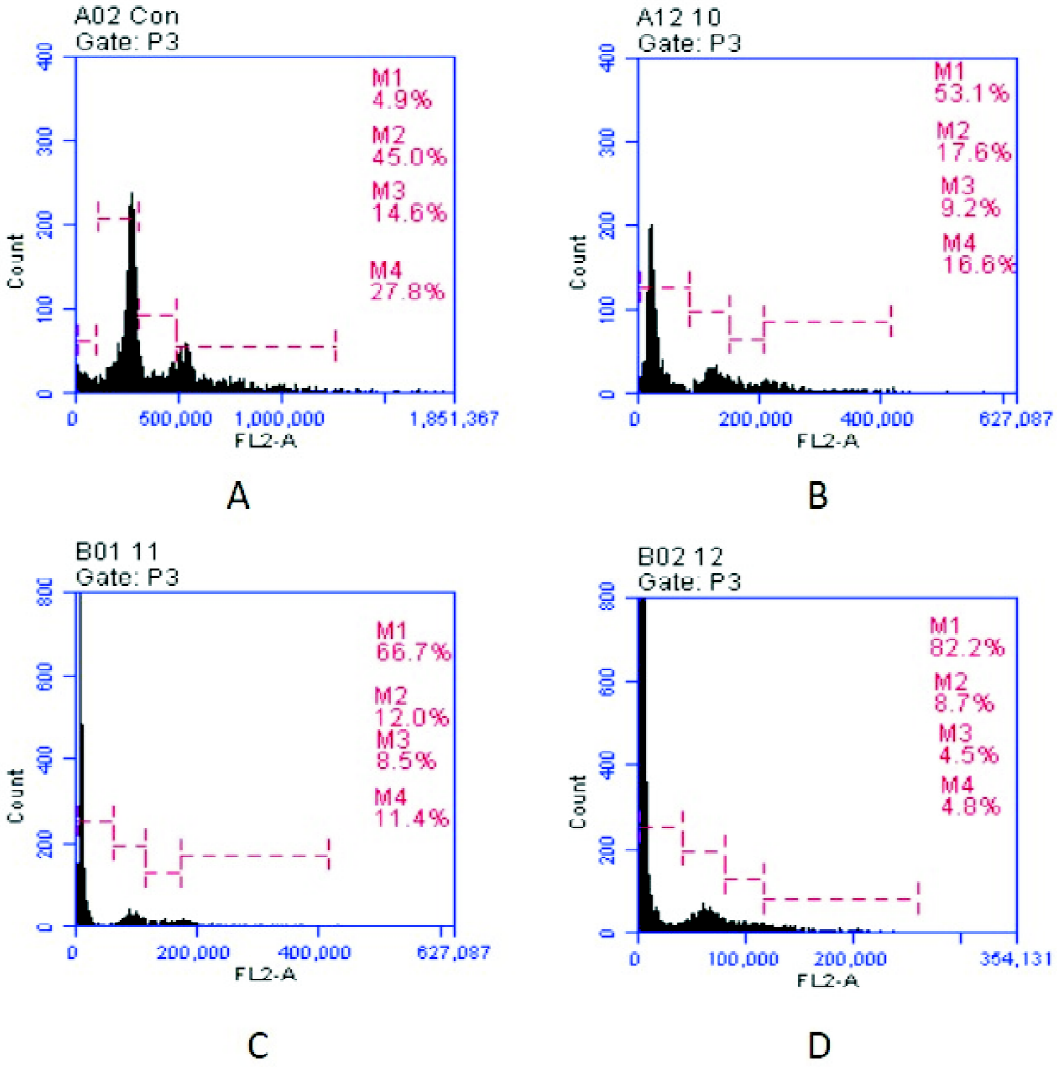

Fig. 3. Flow cytometric study of DL cells treated with AHMEAL (A, B, C, and D for 00, 0.5, 2, and 4 mg/mL respectively) **(**M1, M2, M3, and M4 represents sub-G_1, G_0/G_1, S, and G_2/M phase cell populations respectively).

saponins etc. are known since time immemorial for their immunomodulatory activity especially as anticancer and anti-inflammatory agents[8,12,45,46]. The plant *Ampelocissus latifolia* has been used since ancient times in traditional medication. In the present study, detailed qualitative analysis of AHMEAL had shown the presence of different phytochemicals (Table 4) which possess multiple health benefits including the treatment of cancer. Saponins possess targeted apoptotic activity specifically in cancer cells[46–48]. Various steroidal saponins isolated from *Paris polyphylla* rhizome showed remarkable cytotoxicity and apoptosis induction in T739 inbred mice and LA795 lung adenocarcinoma[49]. Anticancer alkaloids vinblastine and taxol obtained from the plant *Catharanthus roseus* and *Taxus brevifolia* respectively introduced a new era in cancer therapeutics[50,51]. Alkaloids like matrine, piperine, tetrandrine, berberine etc. were also found to be effective as antineoplastic agents in different cancer cell lines[52]. Plant phenolics and flavonoids are also renowned for their apoptosis-inducing and anticancer activities. Various scientific studies reveal the anticancer and apoptosis-inducing activities of quercetin in head, neck, stomach, breast, and ovarian cancer cell lines[53–55]. Catechins from green tea were

Table 4. Phytochemicals present in AHMEAL

Phytochemicals	Tests performed	AHMEAL	Ref.
Steroids	H_2SO_4 and glacial acetic acid reagent	+	34, 35
Carbohydrates	Benedict's test	+	32, 35
	Fehling's reagent	+	
Flavonoids	Shinoda's test	+	31
	Alkaline solution test	+	
Terpenoids	H_2SO_4 and glacial acetic acid reagent	+	34, 35
Alkaloids	Mayer's reagent	+	33
	Wagner's reagent	+	
Tannins	$FeCl_3$ reagent	+	31, 35
	Alkaline reagent	+	
Glycosides	Aqueous NaOH solution	+	32, 35
	Fehling's reagent	+	
Anthraquinones	Borntrager's reagent	+	32
Saponins	$NaHCO_3$ Froth test	+	33
Phlobatannins	HCl test	–	32, 35

"+" and "–" symbols denote the presence and absence of corresponding phytochemicals respectively.

also reported to have antitumor and apoptotic activities[56,57]. Theaflavins and epigallocatechin gallate were also reported to possess matrix metalloproteinase inhibitory action which is considered to be one of the key roles in the blockage of metastatic events[57]. Cerberin, a cardiac glycoside, isolated from *Cerbera odollam,* was also reported to exert anticancer activity via growth inhibition and apoptosis induction through the inhibition of PI3K/AKT/mTOR signal transduction pathways in human cancer cell lines[58]. Terpenoids-rich leaf ethanolic extract of the edible fruit *Annona cherimola* was found to exhibit the proapoptotic and antiproliferative effects on the Acute Myeloid Leukemia cell line[59]. Moreover, the *Aloe arborescens* extract was proved to be fruitful in conventional cancer therapy[60–62]. Aloe-emodin, an anthraquinone, present in this plant showed a noteworthy antiproliferative activity against various human cancers and also on mice models[63,64]. Another anthraquinone, aloin, was also found to induce apoptosis of A549 cells[65]. It also induced bone metabolism, enhanced alkaline phosphatase activity and the enhancement of osteogenic differentiation of bone marrow-derived mesenchymal stem cells[66]. Thus, the various research findings suggest that different groups of phytochemicals hold promising roles as anti-carcinogenic agents with potential therapeutic values.

Different species classified under the genus *Ampelocissus* have shown various medicinal properties like antibacterial, antiviral, antiprotozoal, antifungal, anti-inflammatory activities[10,67]. Phytochemical analysis of the leaf ethanolic extract of *A. araneosa* showed the abundance of steroids, terpenoids, flavonoids, alkaloids, glycosides, tannins, terpenoids[68]. Methanolic extract of this plant was found to exhibit antipyretic function on rabbit[69]. *A. araneosa* possess 2*R*-acetoxymethyl-1,3,3-trimethyl-4*t*-(3-methyl-2-buten-1-yl)-1*t*-cyclohexanol; 2,4,4-trimethyl-3-hydroxymethyl-5*A*-(3-methyl-but-2-enyl)-cyclohexene; 2-methyl-3-(3-methylbut-2-en-1-yl)-2-(4-methylpent-3-en-1-yl)oxetane in *A. araneosa*[68]. Earlier studies have reported different biological activities like antibacterial[70], antioxidant[70], anti-inflammatory[71], anticancer[72] attributed to these phytoconstituents. Phytoconstituents like chalcone, uvaribonin, and 22-epicalamistrin present in Phillippine *Ampelocissus* sp. held a significant role in cancer cell growth inhibition[10]. *Ampelocissus tomentosa* exhibited broad-spectrum antimicrobial activity along with antiviral, antifungal, antileishmanial, and antimalarial activities[67,73]. In the present study, the phytochemical-rich crude AHMEAL showed significant apoptotic and antiproliferative activity on DL cells. Thus, in view of the observed results and the previous study reports, this plant can be recognized as a probable resource

of anticancer substances. However, further research on this plant species is needed for the separation and structural elucidation of the principal phytoconstituents along with their pharmacological assessment.

Conclusions

Our present research signifies that the hydro-methanolic extract of *A. latifolia,* a plentiful resource for different phytochemicals, significantly repressed the proliferation of DL cells. The apoptotic and cell growth repression effect was in accordance with decreased cell survivability, increased cytotoxicity, and arrest of cell cycle, with an amplified population of sub G_1 cells. The extract-induced growth inhibitory effect was too associated with an increased proportion of apoptotic cells as shown by fluorescence microscopic analysis. These results depict that the phytochemicals impart a significant biological efficiency to *A. latifolia*, which may be a rich source of chemopreventive principles. Thus, our study initiates an opportunity for the detailed exploration and isolation of chemotherapeutic agents from AHMEAL.

Acknowledgements

Authors acknowledge the State-Funded Fellowship [FC(Sc.)/RS/SF/ZOO./2011-12/96(3) dated 30.01.2012], infrastructural support of the Department of Zoology, The University of Burdwan, and Lady Brabourne College, Calcutta University. The authors also acknowledge Professor A. Bhattacharyya, Calcutta University for extending the flow cytometric facility.

References

1. B. Baharvand-Ahmadi, M. Bahmani, P. Tajeddini, M. Rafieian-Kopaei and N. Naghdi, *J. Renal Inj. Prev.*, 2016, **5(3)**, 123.
2. P. Srivastava and R. S. Yadav, *Adv. Neurobiol.*, 2016, **12**, 107.
3. C. T. Ford, S. Richardson, F. McArdle, S. B. Lotito, A. Crozier, A. McArdle and M. J. Jackson, *Br. J. Nutr.*, 2016, **115(10)**, 1699.
4. A. Chaudhuri and S. Ray, *Int. J. Pharm. Bio. Sci*., 2014, **5(4)**, 225.
5. A. Chaudhuri and S. Ray, *Int. J. Pharm. Bio. Sci*., 2015, **6(2)**, 99.
6. A. Chaudhuri and S. Ray, *J. King Saud Univ. Sci.*, 2020, **32(1)**, 732.
7. T. H. Chen, S. L. Pan, J. H. Guh, C. H. Liao, D. Y. Huang, C. C. Chen and C. M. Teng, *Clin. Cancer Res*., 2008, **14(13)**, 4250. doi: 10.1158/1078-0432.CCR-07-4578.
8. D. K. Suh, E. J. Lee, H. C. Kim and J. H. Kim, *Arch. Pharm. Res*., 2010, **33(5)**, 781. doi: 10.1007/s12272-010-0519-4.
9. G. M. Cragg, P. G. Grothaus and D. J. Newman, *Chem. Rev*., 2009, **109(7)**, 3012.
10. G. R. Pettit, V. J. Mukku, G. Craqq, D. L. Herald, J. C. Knight and C. L. Herald, *J. Nat. Prod.*, 2008, **71(1)**, 130.
11. Y. Wang, J. Wang, H. Wang and W. Ye, *Chem. Biol. Drug Des*., 2016, **88(4)**, 556.
12. G. D. C. Orfali, A. C. Duarte, V. Bonadio, N. P. Martinez, M. E. M. B. Araujo, F. B. M. Priviero, P. O. Carvalho and D. G. Priolli, *World J. Clin. Oncol.*, 2016, **7(2)**, 189. https://dx.doi.org/10.5306/wjco.v7.i2.189.
13. K. Nouri and R. Yazdanparast, *Daru J. Pharmaceut. Sci*., 2011, **19(2)**, 145.
14. C. M. Shachaf and D. W. Felsher, *Cancer Res.*, 2005, **65(11)**, 4471.
15. S. Safe and R. Kasiappan, *Phytother. Res.*, 2016, **30(11)**, 1723.
16. K. J. Patil and S. V. Patil, *Asian J. Pharm. Life Sci*., 2012, **2(2)**, 144.
17. A. B. Prusti and K. K. Behera, *Ethnobot. Leaflets*, 2007, **11(1)**, 122.
18. S. Swarnkar and S. S. Katewa, *Ethnobot. Leaflets*, 2008, **12(1)**, 647.
19. S. Mitra, D. Banerjee and M. Mandal, *J. Economy Environ. Soc*., 2017, **1(2)**, 49.
20. K. B. Theng and A. N. Korpenwar*, Int. J. Pharm. Sci. Res.*, 2015, **6(9)**, 3936.
21. A. Chaudhuri, L. M. Kundu, S. Dutta, S. Chatterjee, S. Goswami, G. C. Roy and S. Ray, *Asian J. Plant Sci. Res*., 2015, **5(3)**, 11.
22. A. Chaudhuri and S. Ray, *Euro. J. Exp. Bio.*, 2015, **5(8)**, 1.
23. A. Chaudhuri and S. Ray, *IOSR J. Agric. Vet. Sci*., 2016, **9(7)**, 90.
24. P. A. Pednekar and B. Raman, *Asian J. Pharm. Clin. Res*., 2013, **6(1)**, 157.
25. C. T. Tamilarashi, U. Subasini, S. Kavimani and B. Jaykar, 2000, *Anc. Sci. Life*, **20(1-2)**, 14.
26. S. Chakrabarti, A. Chakrabarti and A. K. Pal, *Cancer Genet. Cytogenet.*, 1984, **11(4)**, 417.
27. K. Grankvist, A. Lernmark and I. B. Taljedal, *Biochem. J.*, 1977, **162(1)**, 19.
28. T. Mosmann, *J. Immunol. Methods*, 1983, **65(1-2)**, 55.
29. J. Anter, Z. Fernandez-Bedmar, M. Villatoro-Pulido, S. Demyda-Peyras, M. Moreno-Millan, A. Alonso-Moranga, A. Munoz-Serrano and M. D. Luque de Castro, *Mutat. Res.*, 2011, **723(2)**, 165. doi: 10.1016/j.mrgentox.2011.05.005.
30. H. Kang and A. S. Alvarado, *Dev. Dyn*., 2009, **238(5)**, 1111. doi: 10.1002/dvdy.21928.
31. G. E. Trease and W. C. Evans, "Pharmacognosy (Brailliar

Tiridel can", 13th ed., Cassell and Collier Macmillian Publishers Ltd., England, 1989.

32. A. Sofowara, "Medicinal plants and Traditional medicine in Africa", Spectrum Books Ltd., Ibadan, Nigeria, 1993, pp. 289.
33. J. B. Harborne, "Phytochemical methods", Chapman and Hall Ltd., London, 1973, 49.
34. V. S. S. N. Kantamreddi, Y. N. Lakshmi and V. V. V. S. Kasapu, *Int. J. Pharm. Bio Sci*., 2010, **1(4)**, 351.
35. S. Ray, S. Chatterjee and C. S. Chakrabarti, *IOSR J. Pharm*., 2013, **3(2)**, 1.
36. V. R. M. Lombardi, I. Carrera and R. Cacabelos, *Evid. Based Compl. Alt. Med*., 2017, **2675631**, 1. https://doi.org/10.1155/2017/2675631.
37. S. R. Ruskin and S. R. Aruna, *Int. Res. J. Pharm. App. Sci*., 2014, **4(6)**, 1.
38. O. A. G. Tantengco and S. D. Jacinto, *Asian Pac. J. Trop. Biomed*., 2015, **5(12)**, 1037.
39. C. W. Tzeng, W. S. Tzeng, L. T. Lin, C. W. Lee, M. H. Yen, F. L. Yen and C. C. Lin, *Am. J. Chin. Med*., 2015, **43(3)**, 559. https://doi.org/10.1142/S0192415X15500354.
40. P. Vijayan, S. V. Kumar, S. A. Dhanaraj, S. Badami and B. Suresh, *Pharm. Biol*., 2002, **40(6)**, 456.
41. S. K. Saha, S. Sikdar, A. Mukherjee, K. Bhadra, N. Boujedaini and A. R. Khuda-Bukhsh, *Environ. Toxicol. Pharmacol*., 2013, **36(1)**, 202. doi: 10.1016/j.etap.2013.03.
42. S. R. Kabir, M. M. Rahman, R. Amin, M. R. Karim, Z. H. Mahmud and M. T. Hossain, *Tumor Biol*., 2016, **37(6)**, 8437. doi: 10.1007/s13277-015-4735-x.
43. B. Sun, S. Geng, X. Huang, J. Zhu, S. Liu, Y. Zhang, J. Ye, Y. Li and J. Wang, *Cancer Lett*., 2011, **301(1)**, 95. doi:10.1016/j.canlet.2010.10.010.
44. K. Pumiputavon, T. Chaowasku, C. Saenjum, M. Osathanunkul, B. Wungsintaweekul, K. Chawansuntati, J. Wipasa and P. Iithanatudom, *BMC Complement. Altern. Med*., 2017, 17, **294**, 1. https://doi.org/10.1186/s12906-017-1811-3.
45. B. R. Cassileth and C. C. Chapman, *Cancer*, 1996, **77(6)**, 1026.
46. R. S. MacDonald, J. Y. Guo, J. Copeland, J. D. Browning, D. Sleper, G. E. Rottinghaus and M. A. Berhow, *J. Nutr*., 2005, **135(5)**, 1239. https://doi.org/10.1093/jn/135.5.1239.
47. S. Man, W. Gao, Y. Zhang, L. Huang and C. Liu, *Fitoterapia*, 2010, **81(7)**, 703. doi: 10.1016/j.fitote.2010.06.004.
48. M. Thakur, M. F. Melzig, H. Fuchs and A. Weng, *Botanics Targets Therapy*, 2011, **1**, 19.
49. L. L. Yan, Y. J. Zhang, W. Y. Gao, S. L. Man and Y. Wang, *Exp. Oncol*., 2009, **31(1)**, 27.
50. F. Guthlein, A. M. Burger, M. Brandl, H. H. Fiebig, R. Schubert, C. Unger and U. Massing, *Anti-Cancer Drugs*, 2002, **13(8)**, 797. doi: 10.1097/00001813 200209000-00003.
51. H. J. Long, *Mayo Clin. Proc*., 1994, **69(4)**, 341. doi: 10.1016/s0025-6196(12)62219-8.
52. J. J. Lu, J. L. Bao, X. P. Chen, M. Huang and Y. T. Wang, *Evid. Based Complement. Alternat. Med*., 2012, **485042**, 1. https://doi.org/10.1155/2012/485042.
53. H. Sharma, S. Sen and N. Singh, *Cancer Biol. Ther*., 2005, **4(9)**, 949.
54. J. H. Yang, T. C. Hsia, H. M. Kuo, P. D. L. Chao, C. C. Chou, Y. H. Wei and J. G. Chung, *Drug Metab. Dispos*., 2006, **34(2)**, 296. doi: 10.1124/dmd.105.005280.
55. M. Hashemzaei, A. D. Far, A. Yari, R. E. Heravi, K. Tabrizian, S. M. Taghdisi, S. E. Sadegh, K. Tsarouhas, D. Kouretas, G. Tzanakakis, D. Nikitovic, N. Y. Anisimov, D. A. Spandidos, A. M. Tsatsakis and R. Rezaee, *Onc. Reports*, 2017, **38(2)**, 819.
56. G. Liang, A. Tang, X. Lin, L. Li, S. Zhang, Z. Huang, H. Tang and Q. Q. Li, *Int. J. Oncol*., 2010, **37(1)**, 111. doi: 10.3892/ijo-00000659.
57. M. Isemura, K. Saeki, T. Kimura, S. Hayakawa, T. Minami and M. Sazuka, *Biofactors*, 2000, **13(1-4)**, 81. doi: 10.1002/biof.5520130114.
58. M. S. Hossan, Z. Y. Chan, H. M. Collins, F. N. Shipton, M. S. Butler, M. Rahmatullah, J. B. Lee, P. Gershkovich, L. Kagan, T. J. Khoo, C. Wiart and T. D. Bradshaw, *Cancer Lett*., 2019, **453**, 57.
59. C. Ammoury, M. Younes, M. E. Khoury, M. H. Hodroj, T. Haykal, P. Nasr, M. Sily, R. I. Taleb, R. Sarkis, R. Khalife and S. Rizk, *BMC Complem. Altern. Med*., 2019, **19**, 365. doi: 10.1186/s12906-019-2768-1.
60. P. Lissoni, F. Rovelli, F. Brivio, R. Zago, M. Colciago, G. Messina, A. Mora and G. Porro, *In Vivo*, 2009, **23**, 171.
61. F. Baronchelli, E. Marin and M. Grison, *Molecules*, 2019, **24(11)**, 2128.
62. N. Ozenver, M. Saeed, L. O. Demirezer and T. Efferth, *Oncotarget*, 2018, **9(25)**, 17770.
63. H. D. Lin, K. T. Li, Q. Q. Duan, Q. Chen, S. Tian, E. S. M. Chu and D. Q. Bai, *Oncol. Lett*., 2017, **13(5)**, 3431.
64. K. Liu, C. Park, S. Li, K. W. Lee, H. Liu, L. He, N. K. Soung, J. S. Ahn, A. M. Bode, Z. Dong, B. Y. Kim and Z. Dong, *Carcinogenesis*, 2012, **33(7)**, 1406.
65. L. Wan, L. Zhang, K. Fan and J. Wang, *Mol. Med. Rep*., 2017, **16(5)**, 5759.
66. P. Li, J. Kong, Z. Chen, S. Huang, G. Lv, B. Wei, J. Wei, K. Jing, J. Quan and J. Chu, *J. Nat. Med*., 2019, **73(1)**, 104.
67. B. Joshi, S. K. Panda, R. S. Jouneghani, M. Liu, N. Parajuli, P. Leyssen, J. Neyts and W. Luyten, *Evid. Based Complementary Altern. Med*., 2020, **1043471**, 1. https://doi.org/10.1155/2020/1043471.
68. V. Sellamuthu and S. Angappan, *Int. J. Res. Pharm. Sci*., 2017, **8(4)**, 549.

69. B. Uma Maheswari and R. S. Meerabai, *Int. J. Curr. Res. Biosci. Plant Biol*., 2015, **2(10)**, 120.
70. S. Sen, N. C. Talukdar and M. A. Khan, *Curr. Sci*., 2015, **108(1)**, 63.
71. Anupama, G. Madhumitha and K. S. Rajesh, *Biomed. Res. Int*., 2014, **512369**, 1. https://doi.org/10.1155/2014/512369.
72. S. J. Naine, C. S. Devi, V. Mohanasrinivasan, C. G. P. Doss and D. T. Kumar, *Appl. Microbiol. Biotechnol*., 2016, **100(6)**, 2869.
73. B. Joshi, S. Hendrickx, L. Magar, N. Parajuli, P. Dorny and L. Maes, *J. Intercult. Ethnopharmacol*., 2016, **5(4)**, 383.