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Biologically active Naneoicglycolate of *Aristolochia littoralis* Parodi seed extract with anti-bacterial activity induces cytotoxicity and apoptosis in A431 human skin cancer cell line

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Aristolochia littoralis Parodi is an important traditional medicinal plant known for centuries. In this study, antibacterial and anti-cancerous properties of seeds of this plant were investigated. Experimentally chloroform, ethanol, and aqueous extracts were screened for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The extracts were further analyzed for anticancer activity against human skin carcinoma A431 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide (MTT). The fraction comprising the biomolecule responsible for anticancerous activity was purified by TLC and HPLC. It was subjected to ^{1}H NMR, ^{13}C NMR, and Mass spectrophotometer for structural elucidation. The purified active principle was quantitatively evaluated for inducing apoptosis in A431 skin cancer cell line by flow cytometry analysis. Phytochemical analysis of solvent extracts revealed the presence of alkaloids, steroids, carbohydrates, tannins, and resins. Antibacterial activity was observed in the aqueous and ethanol extracts against the test pathogenic bacteria. Among the solvent extracts, chloroform extract revealed a highly significant anticancer/cytotoxic activity on A431 cell lines. The data revealed that the bioactive principle present in the *A. littoralis* Parodi seed is naneoicglycolate with molecular formula $C_{20}H_{34}O_4$ and was found to be highly cytotoxic at IC_{50} 81.02 µg\mL against A431 cell line. The present study is successful in identifying a novel bioactive principle from an underutilized plant/weed with significant anticancer activity.

Keywords: A431 cell line, Annexin, Apoptosis, *E. coli*, NMR, *Staphylococcus aureus.*

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Introduction

Skin is the first line of defense which is generally damaged through genetic and environmental factors such as heat, radiation, infection, exposure to xenobiotics and other factors out of which most cases of skin cancer are caused by overexposure to ultraviolet rays and other radiations[1-2]. Basal cell carcinoma, squamous cell carcinoma and melanoma are the three types of skin cancers and the first two are referred as non-melanoma cancers. Melanoma of the skin is the 19th most commonly occurring cancer and every year more than one million skin cancers are reported[3]. In general, it is also reported in 2018 that 18.1 million new cancer cases excluding 17 million nonmelanoma skin cancer and 9.6 million cancer deaths excluding the same[4].

Considering the highest mortality and incidence of cancer world over[3,4], there is a continuous search for anticancer molecules from different sources. The data from Food and Drug Administration revealed that 40% of the approved anticancer molecules are of natural origin and out of which, 74% are employed in anticancer therapy. Due to their natural origin, they are considered less toxic to normal cells compared to synthetic drugs. Among the natural sources, plant metabolites in particular such as alkaloids, diterpenes, diterpenoquinone, purine-based compounds, macrocyclic polyethers, lactonic sesquiterpene, peptide, cyclic depsipeptide and proteins are reported to possess anticancer property, and are serving as a promising drug for many cancer treatments[5]. These biomolecules are known to inhibit cancer cells by inhibiting signaling pathways, enzymes, and other cancer cell activating proteins or by activating DNA repair mechanism, inducing antioxidant activity along with stimulating protective enzymes[6]. These biomolecules are considered to be of much importance due to its natural origin and low cost of extraction, which can be further synthesized/ altered

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chemically. Screening of plants for anticancer activity against skin cancer is usually carried out with A431 cell line, which is a model human epidermoid carcinoma cell line widely used in biomedical research with abnormal expression of high levels of the epidermal growth factor receptor (EGFR).

Aristolochia has been considered as an underutilized plant species/ weed but has long been known for their extensive use in traditional medicine in China and African countries[7]. *Aristolochia* sp. is extensively researched and reviewed for its biological activities from 2005 to 2021[8]. *Aristolochia littoralis* Parodi is aggressive woody climber occupying desirable vegetation and its invasion is faster in the natural habitat. Its habitat is reported in Western Ghats, India, which is a due concern for loss of plant diversity. There are several methods developed to weed out this plant in several parts of the world. In the present study, this plant was selected to evaluate its antibacterial and anticancer activity to add medicinal value for this plant which can be exploited for human welfare with due scientific validation.

Materials and Methods

Collection and authentication of plant samples

The seeds of *A. littoralis* plant were collected from the Western Ghats region of Karnataka region. The identified plant was taxonomically authenticated and was submitted to Government Science College, Hassan (Ref. no. 194/2018). The seeds were stored in an airtight container at ambient temperature for further experimental studies.

Preparation of seed sample for crude extraction

Exactly 25 g of dried seeds was pulverised and subjected to maceration (cold extraction by ethanol, chloroform, and distilled water) and hot extraction by soxhlet apparatus[9]. Fine powdered seeds (100 mg) were infused in 3 mL of solvents (HPLC grade, Merck) with increasing order of polarity chloroform, methanol, ethanol and water respectively for four days at ambient temperature. The extracts obtained from the infusion were filtered using Whatman no. 1 filter paper and was concentrated by a rotary vacuum evaporator. The vacuum dried extracts were preserved in dark until further use.

Phytochemical analysis

Preliminary phytochemical analysis for alkaloid, steroid, carbohydrate, tannin, flavonoids, saponins, terpenoid, phenols, carboxylic acid, amino acids, resin, and quinone was done for the obtained extracts[10].

Analysis of the extracts using TLC and HPLC

A 100 mg of the chloroform extract was used for Liquid-Liquid (L-L) partition to obtain chloroform, methanol and hexane fractions using separating funnel. TLC plates were prepared using silica gel 0.25 mm with fluorescent indicator F_{254}. Sample containing 10 mg/mL of L-L fractions were prepared with respective solvents, 2.5 µL of samples were spotted on TLC plate and allowed to dry. Samples were eluted using chloroform:methanol (9.5:0.5) mobile phase. Plates were removed after an optimal development time and dried. The spots/zones were detected using UV chamber at 366, 254 nm and visible light. Rf value was calculated using the standard formula. The HPLC analysis was carried out in Shimadzhu LC-Prominence 20 AT using C18 column 250 mm x 4.6 mm, 5µ particle. Test samples (10 mg/mL) were prepared from stock respectively in HPLC grade solvents i.e., methanol, chloroform, and water. Mobile phase was prepared using a mixture of HPLC grade methanol (50%) and milli-Q water (50%). Flow rate of the sample was maintained at 1 mL/min and injection volume was 10 mL.

Antibacterial activity assay

Antibacterial assay of the obtained extracts of *A. littorolis* seeds was performed *in vitro* by agar well diffusion method on Mueller Hinton Agar (MHA) plates. *E. coli* and *S. aureus* (characterized laboratory native isolates of clinical origin) at 0.5 McFarland standard turbidity were lawn cultured onto a sterilized media. Six of 5 mm wells were bored using sterile cork borer on the inoculated media. Different volume of all the extracts at 50, 40, 30, 20, 10, and 5 µL were pipetted to separate wells created in the inoculated media, air dried and were incubated at 37°C for 24 h. After incubation, the zone of inhibition if any was measured in millimeter and ampicillin (10 µg) was maintained as a positive control.

Cell line and *In vitro* cytotoxicity assay

A431 cell line[11] was procured from ATCC, stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 5% CO_2 at 37^{o}C until confluent. The cell was dissociated with cell dissociating solution [0.2% trypsin, 0.02% EDTA,

0.05% glucose in Phosphate Buffer Saline (PBS)]. The viability of the cells was determined and centrifuged. Further, 50,000 cells/well were seeded in a 96 well plate and incubated at 37°C, for 24 h in 5% CO_2 incubator.

For the *in vitro* cytotoxicity, the monolayer cell culture of A431 cell line was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/mL using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100 µL of the diluted cell suspension (50,000 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was aspirated and the monolayer was washed once with medium. To this, 100 µL of different concentrations of test drugs were added in microtiter plates. The plates were then incubated at 37°C for 24 h in 5% CO_2 incubator. After incubation, the test solutions in the wells were discarded and 100 µL of MTT (5 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µL of Dimethyl sulfoxide (DMSO) was added and the plates were gently shaken to solubilize the formazan formed. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values was generated from the dose-response curves for each cell line.

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{Optical density of the sample}}{\text{Optical density of the control}}\right) \text{X } 100$$

Statistically, IC_{50} values for cytotoxicity tests performed were derived from nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, SanDiego, CA, USA).

Characterization of the active principle

The isolated compound was subjected to flash EA1112 series CHN thermo Finnigan analysis to obtain elemental analysis. ^{13}C, ^{1}H Nuclear Magnetic Resonance (NMR)[12] and LC-MS analysis was carried out using Shimadzu make spectrophotometer.

Quantitative apoptosis analysis

A431 skin cancer cell line were maintained in tissue culture petri dishes as monolayer in DMEM medium supplemented with 10% heat inactivated foetal bovine serum (FBS). The cultured cells seeded with 1 X 10^6 A431 cells were passaged twice each week and the medium was replaced every alternate days. The cultures were maintained at 37°C in 5% CO_2 humidified chamber. Cell viability was determined, where 70–85% confluent cultures were utilized for the experiment.

Acridine orange (AO) and propidium iodide (PI) double staining were performed. The day before induction of apoptosis, 1 X 10^6 cells of A431 cell line per well was seeded for a 6-well plate using DMEM cell culture medium. After 18 h of incubation, floating (dead) cells are removed from the wells by pipetting and are replaced with new culture medium to the original volume. The treated cells were induced for apoptosis with 80 and 160 µg/mL of sample and incubated for 24 h. The incubated cells detached from the dish were centrifuged and the supernatant was discarded. These cells were washed twice with cold PBS and then resuspended in 1 mL of 1X binding buffer at a concentration of ~1 x 10^6 cells/mL. A 500 µL of cell suspension was aliquoted and 10 µL of PI and 5 µL annexin V was added. The cell suspension was incubated for 15 min at room temperature in the dark. Post incubation, the cells were analyzed by flow cytometer (BD FACS Caliburr, 488 nm, Cell quest PRO software) as early as possible/within 1 h of treatment.

Results

Phytochemical constituents

Among the seed extracts obtained from the hot and cold extraction process, highest yield was obtained for chloroform extract (Table 1). Phytochemical analysis of *A. littorolis seed* extracts of ethanol, chloroform and aqueous showed the presence of assorted phytochemical constituents. The alkaloids, steroid, tannin and resin were present in aqueous ethanol and chloroform extracts. Carbohydrate was determined in chloroform, whereas terpenoid was concentrated in ethanol extracts of the samples analyzed. Flavonoids, saponins, coumarins, phenols, carboxylic acid, amino acids and quinone was found be absent in the tested solvent extracts (Table 2).

TLC and HPLC analysis for the crude extracts

Soxhlet based solvent extracts of the *A. littoralis* seed were subjected to both normal TLC and HPLC systems. The TLC Plates were examined under light at 254, 366, and 620 nm for the visualization of

Table 1 — Liquid –Liquid extraction (L-L extraction) of *A. littoralis* seed and yield of solvent fractions of sample

Sample	Sample taken for L-L extraction (mg)	Solubility solvent	Sample fractions	Yield (mg)
Hot extract	100	Methanol	L-L Methanol	21.6
			L-L Chloroform	55.4
			L-L Hexane	0.0
Aqueous extract	100	Distilled water	L-L Methanol	9.7
			L-L Water	25.4
			L-L Hexane	0.0
Chloroform extract	100	Chloroform	L-L Chloroform	70.1
			L-L Methanol	25.6
			L-L Hexane	0.0

Table 2 — Phytochemical constituents analyzed in *A. littoralis* seed extracts

Phytochemical constituents	Extracts		
	Aqueous	Ethanol	Chloroform
Alkaloid	+	+	+
Steroid	+	+	+
Carbohydrate	-	-	+
Tannin	+	+	+
Flavonoids	-	-	-
Saponins	-	-	-
Terpenoid	-	+	-
Coumarins	-	-	-
Phenols	-	-	-
Carboxylic acid	-	-	-
Amino acids	-	-	-
Resin	+	+	+
Quinone	-	-	-

+: Positive, -: Negative

coloured spots (Fig. 1a-c). The bands separated with their corresponding retention factors had significant diversity of compounds separated representing secondary metabolites. The chloroform extract of the chloroform fraction had an excellent separation for the group of bands. TLC analysis of the L-L fractions of the chloroform extract revealed the presence of two bands at Rf 1.00 with blue colour and 0.33 with light colour under UV light (Fig. 1b). HPLC analysis of chloroform fraction of chloroform extract revealed the presence peak at retention time 10.24 and with highest purity ranging from 87-91% (Fig. 1d). This band was purified and subjected for spectral analysis.

Antibacterial activity for the extracts

The solvent extracts of *A. littoralis* seeds were further analyzed for antibacterial activity against *S. aureus* and *E. coli*. The results showed the presence of antibacterial activity for the fraction ethanol and water extracts in comparison to standard antibiotic ampicillin (50 mg). The growth inhibition zone was measured which ranged from 10 to 20 mm for test bacteria that were sensitive to the fraction (Table 3).

Cytotoxicity assay

To measure the anticancer activity of *A. littoralis* seed extracts, A431 cell line was treated with increasing concentrations (10 to 320 µg/mL) of different solvent extracts for 24 h. The standard MTT assay results are described in Fig. 2. Among the different extracts tested, chloroform extract recorded a highly significant anticancer activity compared to control along with aqueous extract followed by ethanol extract. An increase in activity was observed with increase in concentration of extract from 10 to 320 µg/mL (Fig. 2a). Maximum activity was observed at the highest concentration tested (320 µg/mL). For crude aqueous, chloroform, ethanol extracts obtained through soaking and hot extraction had respective IC_{50} value of 239.4, 238, 105.5, and 118.9 µg/mL (Fig. 2b).

Structural elucidation of the isolated active compound

Structure of the isolated bioactive compound based on the data is as shown in (Fig. 3) with molecular formula $C_{20}H_{34}0_4$, the isolated bioactive compound is nomenclatured as Naneoicglycolate (Fig. 3a). The ^{13}C and ^{1}H NMR revealed the following assignments for the predicted compound ^{1}H NMR ($CdCl_3$); δ 5.29 (m, 4H), 4.31 (t, 4H), 2.34 (t, 4H), 2.31 (d, 4H), 2.02 (d, 4H), 1.62 (m, 4H), 1.3 (m, 4H), 0.9 (t, 6H), ^{13}C NMR ($CdCl_3$); δ 173.29, 130.0, 129.68, 129.7, 62.1, 68.90, 34.0, 29.6, 24.6, 22.6, 14.09. The CHN analysis showed N; 6.45%, C; 82.14% and Hydrogen 11.32%, (Fig. 3b) whereas predicted elemental analysis was C; 70.97, H; 10.12, O; 18.91. The positive mode ESI-MS showed a pseudo molecular in

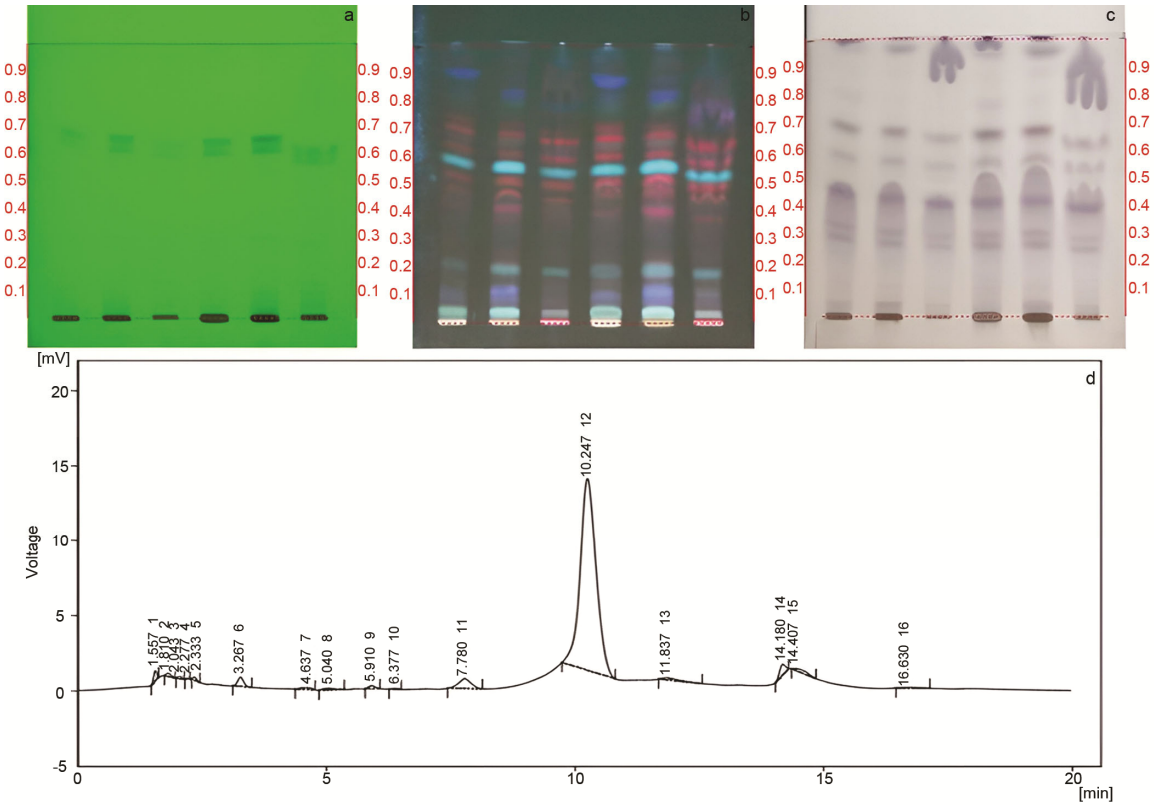


Fig. 1 — Chromatographic profiling for *A. littoralis* seed extracts in different organic solvents. Solvent System Toluene: Ethyl acetate (7.0: 1.0). a) TLC at 254 nm; b) TLC at 366 nm and, c) TLC at 620 nm. Track 1- Hot extraction ethanol - 4µL, Track 2 - Cold extraction ethanol – 4 µL, Track 3 - Cold extraction chloroform – 4 µL, Track 4 - Hot extraction ethanol – 8 µL, Track 5 - Cold extraction ethanol – 8 µL, Track 6- Cold extraction chloroform – 8 µL; d) HPLC chromatograph for the chloroform fraction of chloroform extract obtained at highest purity at retention time 10.24.

peak at m/z 377 for [M+K]$^+$ ion suggesting molecular formulae of C_{20} H_{34} O_4 and a molecular weight of 338, m/z:338.25 (100%), 339.25 (22.2%), 340.25 (3.1%).

Apoptosis assay

The apoptotic effect of the isolated active principle naneoicglycolate was explored by double staining the cells with Annexin-V/PI dye and evaluation by flow cytometry (Fig. 4). The cytotoxicity induction was observed that occurred through the mechanisms associated with apoptosis in a concentration-dependent manner. The cell cycle distribution along with their representative histograms of A431 cell are presented in Fig. 4 (Panel A: Untreated, B: Treated with 80 µg/mL of naneoicglycolate; C: Treated with 160 µg/mL of naneoicglycolate). A reduction in the number of proliferating cells at S-phase for all the cells tested with naneoicglycolate revealed highly significant anticancer activity when compared to control. Maximum activity was observed at 320 µg/mL concentration with IC_{50} 81.02 µg/mL (Table 4). An increase in activity was observed with increase in concentration of the compound from 10 to 320 µg/mL. The 80,160 µg/mL treatment of sample has induced early and late apoptosis in A431 with 2.18, 0.33 and 7.42, 16.83% apoptotic cells, respectively (Fig. 4; Panel D). The sample treated with 80 and 160 µg/mL has induced cell death in A431 with 26.83 and 40.56% necrotic cells when compared to control cells with 5.08% (Table 5).

Discussion

Aristolochia is a member of the family Aristolochiaceae and consists of more than 500 species[13]. *A. elegans* Mast is a synonym of *A. littoralis*[14] and it is commonly called as Indian birthwort. It is a perennial climbing vine with large pipe-shaped resembling Dutchman's pipe. It is widely distributed all over world and reported as threat to biodiversity in Australia, Tahiti Island, it is declared as class 3 weed meaning its sale and supply is prohibited and removal may be required if it is present in environmentally significant areas in Queensland and recently Verma *et al.*[15], reported the invasion of

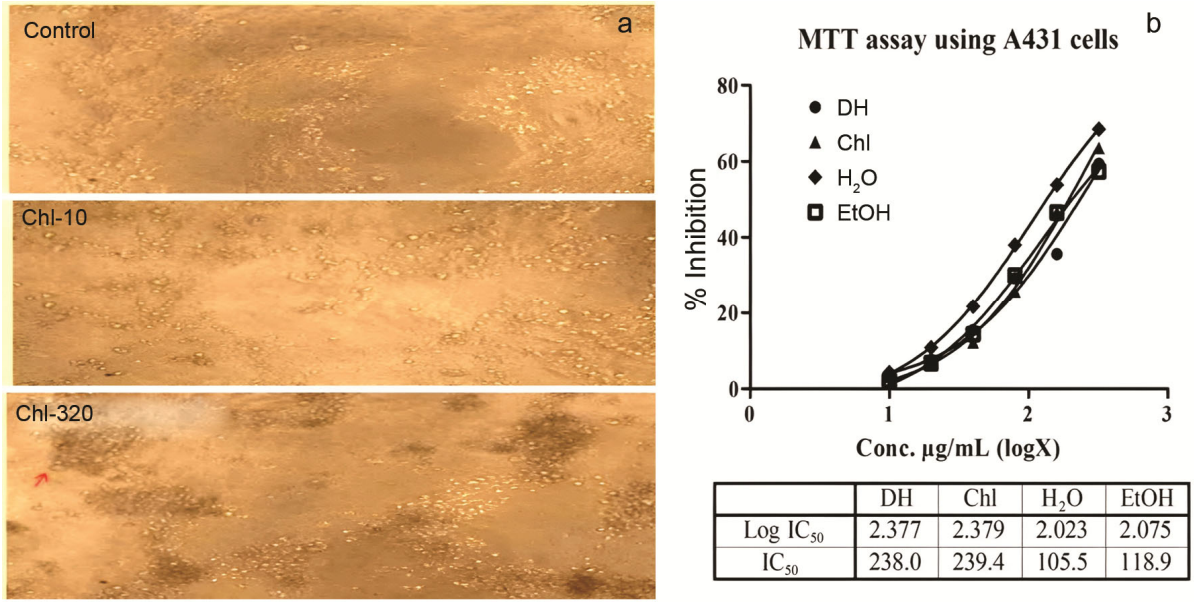
Table 3 — Antibacterial activity of crude extracts

Extract	Concentration (µL)	Zone of inhibition measured in mm	
		S. aureus	*E. coli*
Hot extract	50	10±0.22	R
	40	10±0.11	R
	30	12±0.32	R
	20	14±0.16	R
	10	14±0.25	8±0.11
	05	16±0.11	8±0.11
Chloroform extract	50	R	R
	40	R	R
	30	R	R
	20	R	R
	10	R	R
	05	R	R
Ethanol extract	50	20±0.21	R
	40	10±0.33	R
	30	10±0.11	8±0.12
	20	10±0.15	R
	10	16±0.15	R
	05	R	R
Aqueous extract	50	R	8±
	40	R	8±
	30	R	10±0.21
	20	R	8±0.11
	10	R	8±0.11
	05	R	8±0.11

R- Resistant; mm- millimeter; values are mean of triplicates ± SE

this plant to Kailash sacred landscape under Pithoragarh district of Uttarakhand and cautioned the threat to flowering plants due to its invasive nature. Even in other parts of the world it is considered as a weed and there are several eradication strategies developed to weed it out. On the other hand, few species of *Aristolochia* are widely exploited as traditional and local medicine in many regions of the world[16]. Antimycobacterial activity of fargesin and cubebin isolated from hexane extract of rhizome of this plant was reported by Jimenez-Arellanes *et al.*[17], hexane extract and another compound eupomatenoid-1 were also reported to be inhibitory to *E. histolytica* and *G. lamblia* and other two compounds mentioned earlier was found moderately active. Methanol extracts of aerial organs and roots of micro propogated *A. elegans* were reported to possess antitoxin activity against scorpion poisoning[18].

Considering limitations of the anticancer drugs available in the market such as gastrointestinal, cardiovascular, hemato, pulmonary and nephrotoxicity, along with nonspecificity, toxicity, limited bioavailability and restriction in metastasis search for alternative drugs from natural origin is in progress[19,20]. In particular plants and its metabolites which are used in medicinal practice since ancient time is considered to be better alternative. There are promising reports on anticancer activity of different



	DH	Chl	H_2O	EtOH
Log IC_{50}	2.377	2.379	2.023	2.075
IC_{50}	238.0	239.4	105.5	118.9

Fig. 2 — Cell cytotoxicity determination through MTT dye reduction assay. a) Microscopic visualization of morphological changes of A431 cells (100 X magnifications) treated with chloroform extracts of *A. littoralis* seed at 10 µg/mL and 320 µg/mL concentration. Arrow indicative of cell blebbing; b) Graphical representation of % inhibition of the A431 cells with IC_{50} values for the solvent extracts.

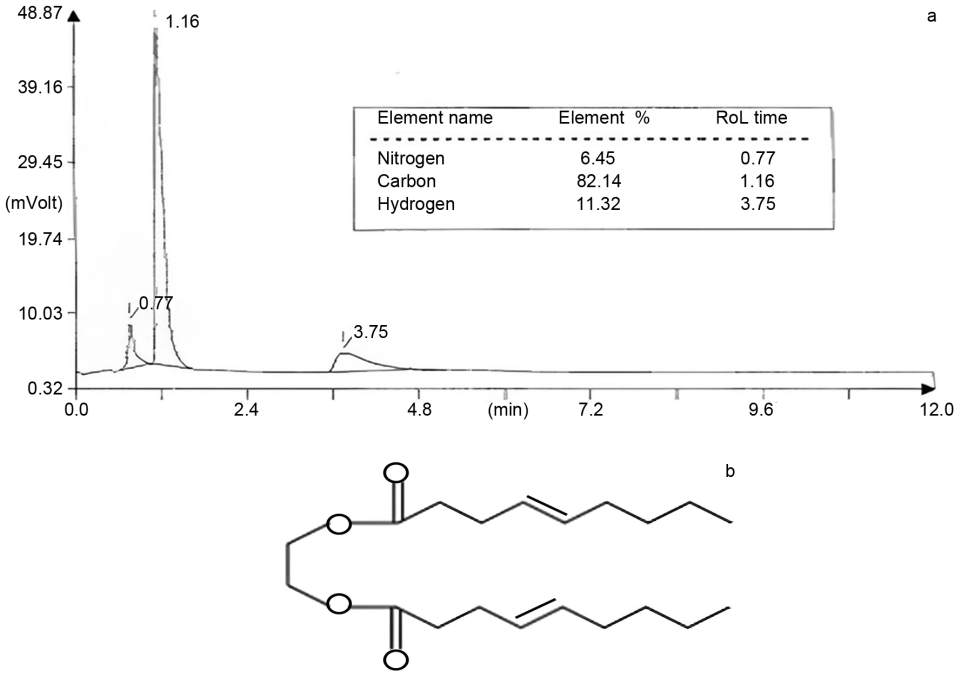


Fig. 3 — Spectroscopy analysis for *A. littoralis* active principle, a) LC-MS profile chromatography for the eluted purified compound; b) Naneoicglycolate molecular structure.

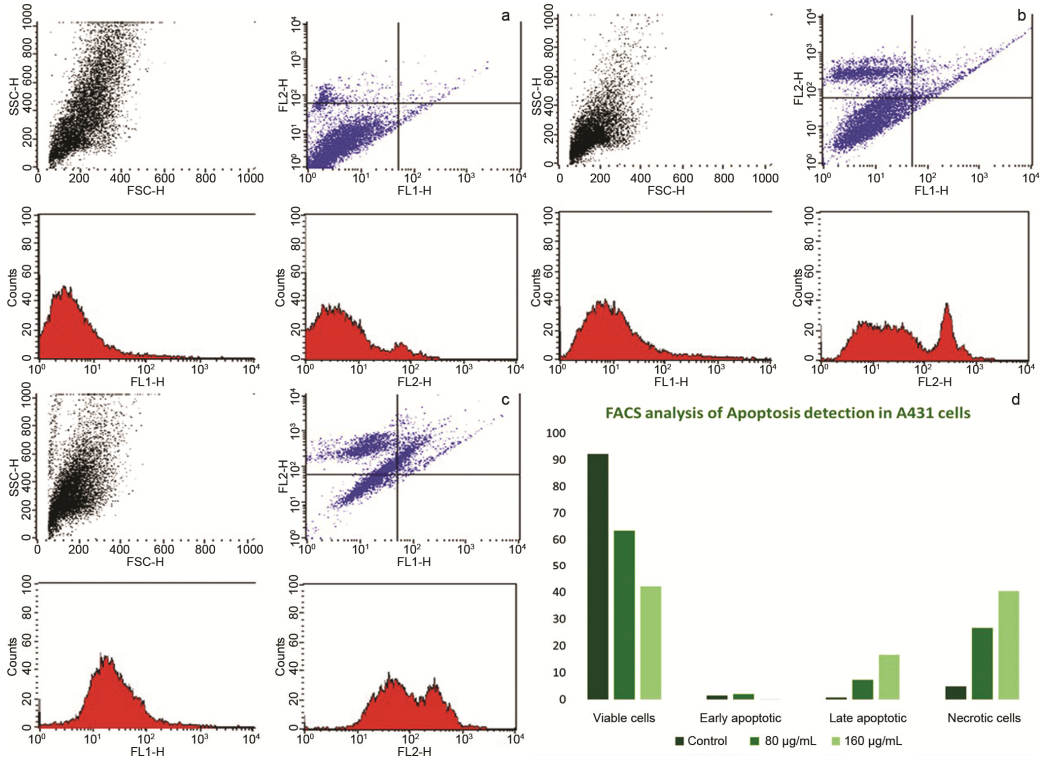


Fig. 4 — Apoptotic cell death of A431 cells measured using the Annexin V−FITC and propidium iodide staining assay represented through scattered dot and histogram plots. a) Untreated cells; b) Cells exposed to 80 µg/mL of Naneoicglycolate; c) Cells exposed to 160 µg/mL of Naneoicglycolate. Values are reported as mean±SD (n = 3); d) Graph representing effects of Naneoicglycolate in A431 cell cycle progression through Fluorescence activates cell sorting (FACS based flow cytometry).

Table 4 — Anticancer activity of different concentrations of Naneoicglycolate

Compound name	Concentration (µg/mL)	OD at 590 nm	% Inhibition	IC_{50} (µg/mL)
Control	0	0.7413	0.00	
Naneoicglycolate	10	0.6830	7.87	81.02
	20	0.6026	18.71	
	40	0.5174	30.21	
	80	0.3994	46.12	
	160	0.2723	63.27	
	320	0.1755	76.32	

Table 5 — Flow cytometry analysis of Apoptosis detection of A431 cells

Cell line	Sample (µg/mL)	Viable cells	Early apoptotic	Late apoptotic	Necrotic cells
A431	Control	92.39	1.67	0.86	5.08
	80	63.58	2.18	7.42	26.83
	160	42.28	0.33	16.83	40.56

constituents on A431 cell line such as the Growth Inhibitory (GI_{50} value) of the acetone extract of *Cissus quadrangularis* L. was reported to be 8 mg/mL,[21] callus of *Chonemorpha fragrans* (Moon) Alston with topo as well as DNA polymerase inhibitory activity[22], grape seed extract with IC_{50} value 480 µg/mL[23], curcumin/neem loaded polycaprolactone nanofibres[24], caffeic acid n-butyl ester with a half-maximal inhibitory concentration of 20 µM and it was reported to have cell cycle arrest and apoptosis of the cell line[25].

Isolating and characterizing the biomolecule from a weed plant becomes necessary to identify the bioactive component of the solvent extract for its potential use in therapy. HPLC and ^{13}C and ^{1}H NMR analysis of the chloroform fraction of the *A. littoralis* seed extract revealed the presence of a compound namely naneoicglycolate. The compound having methyl, long chain methylene and carbonyl group is the first reported molecule for having anti-microbial and anti-cancer activity determines its pharmacological importance in skin cancer therapy. Further studies on its effect on L6 cell lines revealed that the molecule is safe for normal cells and its anticancer activity analyzed through flow cytometry revealed cell death due to apoptosis.

In the present study, anticancer efficacy of isolated bioactive principle naneoicglycolate on A431 cell line was determined and evaluated by flow cytometry. It was found that the tested active compound reduced cell viability in a concentration dependent manner with IC_{50} as 81.02 µg/mL. At this concentration, the treated cells were vacuolated with extensive blebbing and reduction in normal cell morphology, indicative of autophagic cell death pathway. A similar effect was observed in other extracts with lower or equivalent efficiency on A431 cells (water and ethanol extract). The obtained anti-cancer efficacy was further confirmed by MTT assay and flow cytometry. Henceforth, bioactive effects of active compounds in the *Aristolochia* sp. plant extracts depend on the extraction procedure and the characteristics of the solvent used. Therefore, the study on *A. littoralis* may pave a way for excavation of new molecules as targeted therapy for melanogenesis and apoptosis of skin carcinoma.

Conclusion

To the best of the authors' knowledge, the present study is the first to highlight the anticancer activity of *A. littoralis* seeds. The chloroform extracts exhibited highest anticancer capacities against the A431 skin cancer cell line in comparison to ethanol and aqueous extracts. The study was successful in isolating and characterizing an anticancer active principle naneoicglycolate from the weed *Aristolochia littoralis* on A431 skin cancer cell line.

Conflict of interest

The authors have no conflict of interest.

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