Research Article

Isolation, Characterization and Cytotoxic activity of Diterpenoid and Flavonoids of *Aerva lanata*

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ABSTRACT

Phytochemical investigation of methanol extract of seeds of the *Aerva lanata* led to the isolation of a new diterpenoid and three known compounds. Their structures were established on the basis of NMR and mass spectroscopic analysis. In addition, all the isolates were tested for their cytotoxicity against leukemia K562, lung cancer (H522), breast cancer (MCF-7/ADR) and prostate cancer (DU145) cancerous cell lines. The new Compound 1 has shown good cytotoxic activity.

Keywords: *Aerva lanata,* phytochemical investigation, diterpenoid, methanolic extract.

INTRODUCTION

Aerva lanata belongs to the family, Amaranthaceae and is extensively used as a condiment in local medicines and as a flavor. Plants belonging to the genus *Aerva* were used traditionally in India and the Republic of China for the treatment of cold, in reducing swellings and circulatory-system invigoration.[1] Essential oils, extracts and their constituents of plants are greatly valued in ayurveda[2] and have been also reported to exhibit a wide range of biological activities of therapeutic importance that include antiseptic activity,[3] antimicrobial, antitumor, antiulcer,[4] certain heart problems, central nervous system disorders, gastrointestinal disorders, cough and bronchitis anti HIV, and anticancer.[5-18] The common name of *A. lanata* is galangal and is mostly used in India, Thailand and China. It is also known to possess other medicinal properties such as anti-diarrhea and anti-carminative along with use in curing stomachaches. The rhizomes of *lanata* showed antimicrobial properties towards bacteria, parasites, yeast and fungi.

The compounds that were previously isolated from *Aerva* include flavonoids, phenyl propanoids, oxygenated sesquiterpenoids and diterpenoids. Almost all the parts of the plants belonging to the genus *Aerva* have some medicinal use. It is widely cultivated in India and South East Asian countries. The seeds, for example, are known to possess stomachic properties, especially in Japan and China. The extracts, constituents and essential oils of the plants are known to possess valuable Ayurvedic and biological activities that include antitumor, antiulcer, anthelmintic activity, diabetic, antimicrobial, antiseptic, osteroarthritis, gastrointestinal disorders, cough, CNS disorders and bronchitis. With many plants still remaining unexplored, there is the need to continue research in this area. Hence, we focused our research on *Aerva* since it is known to possess several medical uses. In this manuscript we described the phytochemical investigation and Cytotoxic activity of methanol extract and isolated compounds. The phytochemical analysis resulted in the isolation of one new labdane type diterpene along with three known compounds. Here in, we report the isolation and anticancer activity of the constituents from the seeds of *Aerva lanata* it was found that the methanol extract of seeds of the *Aerva lanata* showed the cytotoxic activity[19] against against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cancerous cell lines.

MATERIALS AND METHODS

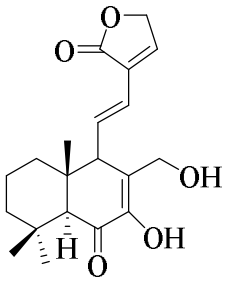
Plant material

The seeds of *Aerva lanata* were collected from the forest of area of Rourkela, Odisha, during the first week of January, 2014, was identified by Dr. Abhijith Chauhan, Taxonomist, Dept. of Taxonomy, BITU, Rourkela, Odisha. Collected seeds, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

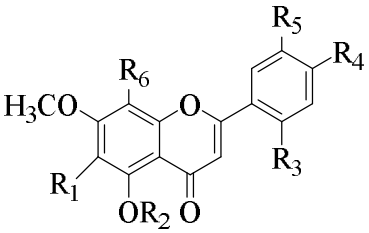
Isolation method

The dried seeds (500 g) were ground and extracted three times with methanol. The combined extracts were concentrated under vacuum. The portion of active methanol extract (10 g) was subjected to column chromatography (silica gel, 60–120 mesh) using step gradient of chloroform/methanol to yield six major fractions (F1-F4). Fraction F1 was subjected to repeated silica gel (100–200 mesh) column chromatography (CC) by eluting with MeOH/$CHCl_3$ (3:97) to yield compound 4 (3.01). Fraction F2 was subjected to silica gel column chromatography eluting with MeOH/$CHCl_3$ (7:93) to get compound 6 (1.22 g). A portion of fraction F3 was subjected to silica gel column chromatography with MeOH/$CHCl_3$ (11:89) to yield 0.070 g of compound 1, with MeOH/$CHCl_3$ (13:87) to yield 0.98 g of compound 3. Similarly, Fraction F4 was subjected to repeated column chromatography eluting with MeOH/$CHCl_3$ (15:85) to yield 0.85 g of compound 2.

Compound 1 is new compound and compounds 2-4 are known Flavonoids. These structures were confirmed by NMR and mass spectroscopic studies.



Compound 1



2: $R_1 = R_2 = R_3 = R_4 = R_5 = H, R_6 = OCH_3$

3: $R_1 = R_2 = H, R_3 = OCH_3, R_4 = R_5 = H, R_6 = OCH_3$

4: $R_1 = R_2 = R_4 = H, R_3 = R_5 = R_6 = OCH_3$

Fig. 1: Compounds isolated from the seeds of *Aerva lanata*

Compound **1** was isolated as yellow oil. The ESI-MS of compound **1** revealed a molecular ion peak corresponding to $(M+H)^+$ at m/z 361.18 indicating the molecular formula $C_{20}H_{24}O_6$. The ^1H NMR spectrum of compound **1** showed all the features of labdane diterpene. The IR spectrum displayed absorption bands at 3318 cm^{-1} (OH), 1648 cm^{-1} (α, β-unsaturated carbonyl) and 1756 cm^{-1} (α, β-unsaturated γ-lactone). The ^1H NMR spectrum displayed three quaternary methyl signals each integrating for three protons as singlets at δ 0.99, 1.14 and 1.21. It has displayed a singlet at δ 2.21 for one proton (H-5) indicating the presence of one methane adjacent to the carbonyl (C-6) carbon atom. A sharp singlet integrated for 1H at δ 5.94 is due to methine proton (H-14) in the lactone ring. The presence of one trans double bond at δ 6.08 (1H, dd, *J* = 15.8 Hz, 9.8 Hz) and δ 6.36 (1H, d, *J* = 15.8 Hz) was suggested by the ^1H NMR, and NOESY spectrum. And comparison of NMR data with that of yunnacoronarin D8 indicates the presence of trans double bond at C-11/C-12 position. Another sharp singlet integrated for two protons at δ 4.92 (2H, s) is assigned to CH_2 group in the lactone ring. Furthermore, the ^1H NMR spectrum also revealing that the trans double bond (C-11/C-12) is conjugated with lactone ring. The ^{13}C NMR spectrum of compound **1** showed the presence of 20 carbon atoms. The DEPT experiment indicated the presence of three methyls, four CH_2, five CH groups, and eight quaternary Carbons. The ^{13}C NMR spectrum of compound 1 also showed all the features of labdane diterpene. The ^{13}C NMR spectrum indicated the presence of α, β -unsaturated ketone (δ 200.74), tetra substituted olefin (δ 158.92 and 150.24) and three methyl signals (δ 32.82, 21.25 and 15.59). Further, it also displayed signal at δ 173.46 is due to C=O of lactone ring, δ 136.85, 129.94 are corresponding to disubstituted trans olefin, and δ 68.92 is assignable to methylene carbon in the lactone ring. A complete assignment of protons and carbons was assisted by HMBC, COSY and HSQC experiments. The HMBC correlations also suggested that α, β -unsaturated γ-lactone ring (δ 136.82, 121.43, 68.92 and 173.46) is attached to the decalone nucleus through the trans double bond (δ 136.85, 129.94). In addition, the relative configuration of 1 was proposed on biogenetic basis and by inspection of NOESY spectrum, which showed the correlation between the

following proton pairs (19-H_3 and 20-H_3; 5-H and 18-H_3). Based on these data, compound **1** was identified a new labdane diterpene.

In vitro cytotoxicity evaluation

All the isolates obtained in this investigation of *Aerva lanata* were tested for their in vitro cytotoxicity against different cancerous cell lines, against against lung cancer (H522), leukemia K562, breast cancer (MCF-7/ADR) and prostate cancer (DU145) cancerous cell lines using the MTT assay according to the method of Mosmann *et al* . Cisplatin was considered as the positive control. IC_{50} values were determined with each cell line after four individual observations (Table 1). The small structural differences of labdane diterpenes influenced the Cytotoxic activity. As evident from cytotoxic activity results compound 1 exhibited significant activity on DU145, and MCF-7 cell lines and moderate activity on H522 and k562 cell lines. The Cytotoxic activity of compound 1 on the cell lines followed the following order: DU145 > MCF-7 > K562 > H522. Compound 2 also shown moderate activity on H522, MCF-7 and significant activity on K562 cell lines. Remaining Flavonoids **3** and **4** are inactive with tested cell lines. Hence, the new compound 1 was found to possess significant cytotoxic activity among all the isolates. The cytotoxic experiments were repeated for four times, and the IC_{50} values were expressed as mean ± standard error.

Table 1: Cytotoxicity effects of methanol extract and isolated compounds (1-4) of *Aerva lanata* against cancer cell lines

	Cell lines (IC_{50} µM)[a]			
Compound	**H522**	**K562**	**MCF-7/ADR**	**DU145**
1	17.85±3.50	16.15±3.35	13.82±2.56	8.17±1.15
2	27.56±2.14[b]	59.55±2.95	18.30±2.75	10.56±2.75
3	49.85±2.45	41.98±2.85	30.65±3.65	27.50±2.89
4	34.35±1.45	33.98±2.12	NA	NT
Methanol extract	20.15±3.30	15.90±3.55	23.85±5.45	10.96±2.85
cisplatin[c]	4.74±0.50	3.76±0.85	9.55±1.25	5.54±1.35

[a] Concentration of compound required to inhibit cell growth by 50% as determined by MTT assay;
[b]data are expressed as mean±standard deviation;
[c]Cisplatin was used as positive control; NA- not active; NT- not tested;

CONCLUSION

In summary, *Aerva lanata*, of the Amaranthacea family has been used as a traditional medicine for the treatment of cancer. The compounds responsible for this activity have yet to be determined. These results encourage us to continue our future research of this series by synthesizing additional labdane-type diterpenoid derivatives with the aim of obtaining cytotoxic compounds that are more potent and selective toward cancer cells.

Spectral data of new compound (1)

Yellow oil, ^{1}H NMR ($CDCl_3$): δ 0.99, 1.14, 1.21 (3H each, all s, CH_3), 1.28 (2H, m), 1.49 (2H, m), 1.61 (2H, m), 2.21 (1H, s), 4.92 (1H, s), 5.94 (1H, s), 6.08 (1H, d, J = 15.6 Hz), 6.36 (1H, dd, J = 9.8,15.6 Hz), 6.48 (1H, s, H-14). ^{13}C NMR ($CDCl_3$): δ 15.99, 17.88, 21.25, 32.71, 32.82, 39.58, 42.97, 42.98, 64.39, 68.92, 72.36, 121.43, 129.94, 136.82, 136.85, 150.24, 158.92, 173.96, 194.48, 200.74. EIMS: m/z 331 (M+H)+,EIMS m/z 361.18.

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