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Isolation of a novel triterpene from the Essential oil of fresh leaves of *Chromolaena odorata* and its *in-vitro* cytotoxic activity against HepG2 cancer cell line

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ABSTRACT

The essential oil from the fresh leaves of *Chromolaena odorata (Asteraceae)* (L.) R.M. King & H. Rob. was obtained by hydro distillation. From the essential oil the compound (I) a triterpene derivative was isolated by column chromatography and it was characterized by IR, ^{1}H-NMR, ^{13}C-NMR and MS spectral data. It was screened for cytotoxicity against HepG2 by MTT assay. It showed significant cytotoxicity with IC_{50} value of 206.7 μg/ml towards HepG2 cancer cell line.

INTRODUCTION

There are approximately 165 species of *Chromolaena* distributed in the tropical and warm temperate regions (Mabberley, 1997). *Chromolaena odorata (Asteraceae)* (L.) R.M. King & H. Rob. (syn.*Eupatorium odoratum* L.), originally ranged from Southern Mexico to Argentina and the Caribbean (Morto, 1987), but has been introduced into the old World tropics where it has become an invasive species (Zachariades *et al*., 2009). The plant has exhibited allelopathic effects and has been reported to cause livestock death (Zachariades *et al*., 2009). Medicinally, the plant decoction is taken as a remedy for coughs and cold or in bath to treat skin diseases (Morto, 1987).

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The plant is used in West African traditional medicine as a wound healing and a local antiseptic agent (Adjanohoun *et al*., 1979; Inya-Agha *et al*., 1987). The chemical composition, insecticidal (Bouda *et al*., 2009), insect repellent (Cui *et al*., 2009), antimicrobial, fungicidal (Inya- Agha *et al*., 1987; Bamba *et al*., 1993) and acaricide activities of *C. odorata* essential oil have been studied by (Iwu *et al*., 1984; Bamba *et al*., 1993; Chowdhury, 2002; Ling *et al*., 200; Inya-Agha *et al*., 1987; Cui *et al*., 2009; Pisutthanan *et al*., 2006; Owolabi *et al*., 2010; Tedonkeng *et al.,* 2004; Cosme Kossouoh *et al*., 2011; Avlessi Felicien *et al*., 2012).

Kurane- type diterpenoids were isolated (Pascal Wafo *et al*., 2011). The present work is to isolate and characterize the compound (I) by IR, ^{1}H-NMR, ^{13}C-NMR and MS spectra. Further, the compound (I) was carried for *in-vitro* cytotoxicity against HepG2, cancer cell lines.

MATERIAL AND METHODS

Plant material

Fresh leaves of *C. odorata* was collected in October, 2009, from Palakkad district, Kerala and the plant species was authenticated in the Department of life science, Karpagam University, Coimbatore-21. Voucher specimen was preserved in the same Department (No. KU11CHE1913).

Isolation of the essential oil

500g of fresh leaves of *C .odorata* was hydro distilled for 4 h in a modified Clevenger-type apparatus to yield 0.2 % of essential oil. The essential oil so obtained was stored in a sealed glass bottle with screw lid cover under refrigeration at 4°C.

Isolation of the compound (I)

In an attempt to isolate components of the essential oil, it was subjected to column chromatography. It was eluted with the solvent petroleum ether followed by a mixture of petroleum ether: ethylacetate. Fractions of 30 ml were collected and monitored by TLC. Fractions 2 to 4 were homogenous by tlc and on concentration after mixing yielded a residue of 80.10 mg.

General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM-400 (400 MHz) instrument; chemical shifts δ in ppm with TMS as internal standard, coupling constants J in Hz. Electrospray Ionization-MS data were recorded on a Bruker Esquire 3000+ Ion-trap mass spectrometer and Electron Impact-MS was performed on a Finnigan MAT-95 mass spectrometer. Perkin –E/model 1650 IR instrument was used to carried the IR spectra.

Compound (I)

IR ν_{max} (KBr) cm^{-1}; 1600, 3400, 1285. ^{1}H-NMR and Spectra ^{13}C-NMR were shown in Table. 1and 2.

Table.1: ^{13}C-NMR spectral data of the compound isolated from *C. odorata* oil

Carbon	Signal (δ)	Carbon	Signal (δ)	Carbon	Signal (δ)
1	37.17	17	29.11	1'	133.54
2	22.73	18	53.02	2'	32.20
3	41.42	19	78.02	3'	31.64
4	31.99	20	45.3	4'	45.2
5	53.02	21	31.64	5'	51.9
6	20.77	22	39.12	6'	124.87
7	34.73	23	22.69	7'	29.76
8	39.78	24	22.63	8'	11.43
9	47.00	25	22.63	9'	148.91
10	36.15	26	18.78	10'	105.08
11	25.33	27	16.45	11'	133.94
12	124.07	28	26.98	12'	113.99
				13'	32.81
13	135.63	29	14.11		
14	40.79	30	14.11		
15	29.42				
16	34.57				

Table. 2: ^{1}H-NMR spectral data of the compound isolated from *C.odorata* oil.

proton	No of protons	Signal (δ)	Multiplicity	Coupling constant(J in Hz)
6'	1H	4.91	m	
8'	3H	0.87	t	
9'	1H	6.50	t	10.5
10'	2H	5.93,5.75	dd,d	10.5
11'	1H	5.60	t	10.5
12'	2H	5.30,5.23	d,dd	10.5
23,24	6H	0.84-0.83	m	
25	3H	0.85	s	
26	3H	0.88	s	
27	3H	1.19	s	
28	3H	1.19	s	
29,30	6H	0.97-0.95	m	

MTT assay for Cytotoxicity screening

The Hepatocellular carcinoma cells (HepG2), were obtained from National Centre for Cell Science (NCCS), Pune. The HepG2 cells were grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS).

For screening experiment, the cells were seeded into 96-well plates in 100µl of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of essential oil. The essential oil was solubilized in dimethylsulfoxide and diluted in respective medium containing 1% FBS. After 24 h, the medium was replaced with respective medium with 1% FBS containing the oil at various concentration (12.5, 25, 50, 100, 200, 300 µg/ml etc…) and incubated at 37^{0}C, 5% CO_2, 95% air and 100% relative humidity for 48h. Triplicate was maintained and the medium containing without oil served as control. After 48h, 10µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then measured the absorbance at 570 nm using micro plate reader.

Determination of IC_{50}

% cell inhibition was determined using the following formula and graph was plotted between % Cell inhibition and concentration and from this IC_{50} was calculated.

% Cell Inhibition = 100 – Abs (drug)/Abs (control) x100.

RESULTS AND DISCUSSION

The compound I isolated as a colourless gum the *C.odorata* was identified as a derivative of 19-hydroxy amyrin analysed for $C_{43}H_{68}O$ evidenced by the EI-MS by showing a molecular ion peak, $[M]^+$ at 600.3145 and a base peak at m/z 234 indicating of amyrin type of compound. The IR spectra showed strong absorption at 3400 cm^{-1} for hydroxyl group1600 and 1285 cm^{-1} indicating the presence of C=C and another moity C-O in the molecule.

The ^{1}H NMR exhibited nine methyl signals at δ 1.19, 0.97, 0.95, 0.88, 0.85 (2x CH_3), 0.84, 0.83 and a distorted triplet at δ 0.87 which was accounted for the terminal methyl of the long chain hydrocarbon. The signal at δ 5.23 is due to the double bond at C-12, where as the signal at δ 4.51 accounted for H-19. The compound showed molecular ion peak, $[M]^+$ at m/z 600 and a base peak at m/z 234 and ions at m/z 218, 203, 189 arises by the retro-Diels Alder fragmentation (Scheme–1). Based on this and the ^{13}C-NMR spectral value at, δ 78.02 the hydroxyl group was placed at C-19.

This indicated the presence of a 19-hydroxy α/β amyrin type of compound. Apart from this the ^{1}H-NMR exhibited seven signals at δ 6. 50 (1H, t, J = 10.5Hz, H-9'), 5.93 (1H, dd, J = 10.5, Hz, H-10'a), 5.75(1H, d, J = 10.5Hz, H-10'b), 5.60(1H, t, J = 10.5Hz, H-11'), 5.30(1H, d, J = 10.5Hz, H-12'a), 5.23 (1H, dd, J = 10.5Hz, H-12'b), 4.91(1H, m, H-6') suggesting the presence of seven protons. It was complemented by six ^{13}C-NMR signals in between δ 105.95 and 148.91 (Table-1) confirming the presence of three more double bonds in compounds I with two terminal methylene groups.

When the signals of the ^{13}C-NMR spectra in the region δ 11.43 and 53.02 is compared with some spectra of known compounds in the literature suggested the presence of a pentacyclic triterpenoid. Some of the signals closely match with the signals of amyrin. Based on the above data the structure of the compound is shown in Fig.1.

We employed MTT assay, a simple and reliable technique, which measures cell viability for screening the anticancer activity and the results are shown in table 3.

Table. 3: *In vitro* cytotoxicity activity of the compound (I) from *C. odorata* against HepG2 cancer cell line.

Name of the cell lines	IC_{50} µg/ml
HepG2	206.7

The viability of cancer cells after incubation with different concentrations of compound (I) are depicted in Fig.2 (HepG2 cancer cell line). The incubation with different concentration of compound (I) affected the viability of hepatocellular carcinoma cells (HepG2). The compound (I) showed cytotoxic effect on the HepG2 cancer cell line in dose dependant pattern and the IC_{50} value was determined as 206.7µg/ml. With HepG2 below 206 µg/ml concentration no activity was observed but at 206 µg/ml there is a sharp increase in the inhibition and (90%) was observed. This is the first report of its kind to test the compound (I) from the essential oil of *C.odorata* for anticancer activity. Previous report showed that the Togos essential oil exhibited moderate cytotoxicity activity against human cell line HaCaT with an IC_{50} value 700 $\mu L.mL^{-1}$(Kofi koba *et al.*, 2009). We have reported moderate cytotoxicity activity of the essential oil against HeLa, NIH 3T3 and Hep-2 cell lines and its IC_{50} value 60.3, 67.5, 72.0 µg/ml (Velliangiri Prabhu *et al.*, 2011).

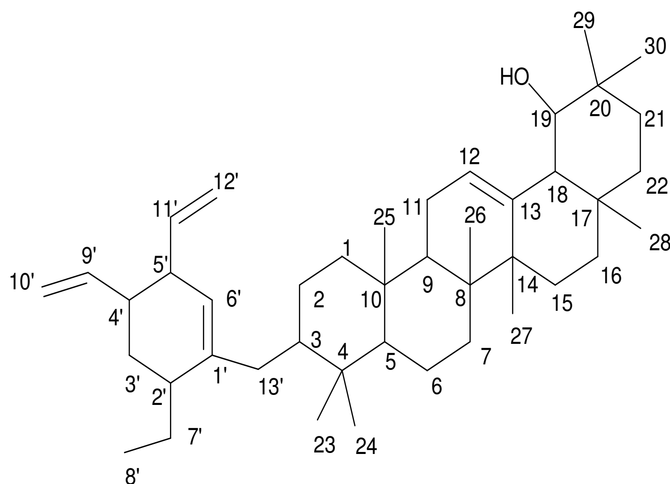


Fig .1: The structure of compound (I).

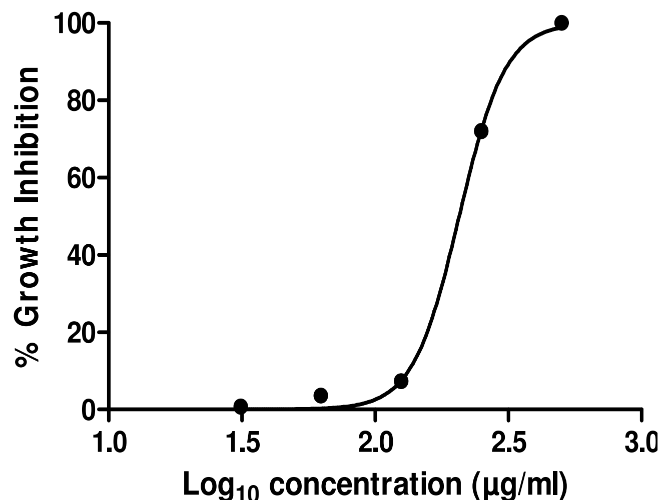
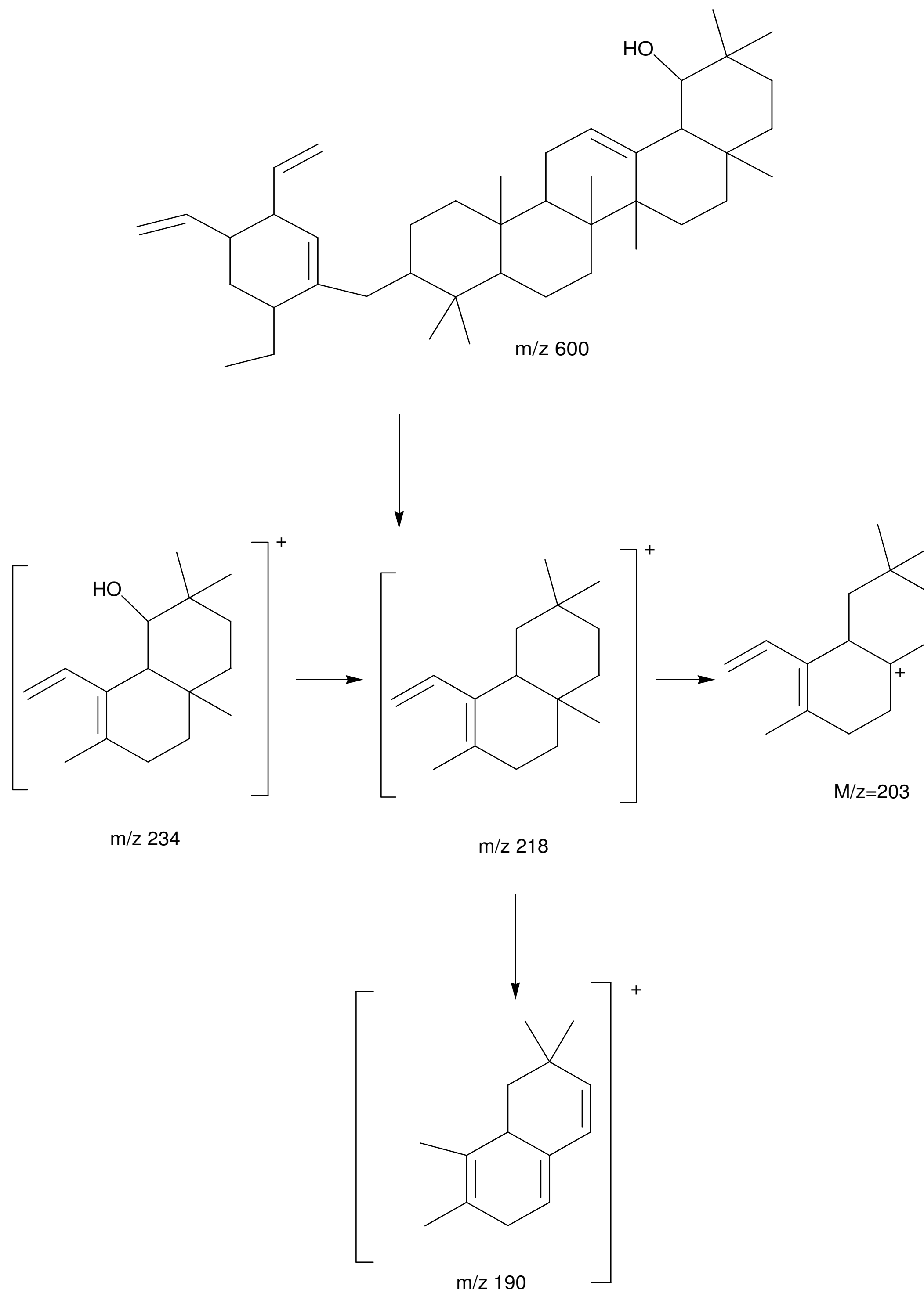


Fig .2: The compound (I) from *C. oodrata* against HepG2 cancer cell line



Scheme . 1: Retro –Diels Alder fragmentation.

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