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Original Article

GC-MS ANALYSIS AND ANTICANCER ACTIVITY OF METHANOL EXTRACT OF LEAVES OF HYPERICUM HOOKERIANUM WIGHT & ARN

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ABSTRACT

Objective: The present investigation was an attempt to study the anticancer activity of the methanol extract of the leaves of H. *hookerianum.* GC-MS analysis revealed that the methanol extract of leaves contained important secondary metabolites viz. oxygenated hydrocarbons and phenolic hydrocarbons. These phytochemicals were presumed responsible for various pharmacological actions like antioxidant, and anticancer activities. Cytotoxic changes observed in MCF 7 cell line by the treatment of methanol extract included cell aggregation, cell rounding and cell death. The overall result indicates that the methanol extract of leaves of *H. hookerianum* showed promising baseline information for its potential use as an anti cancer agent.

Keywords: GC-MS, H. *hookerianum,* Anticancer and MCF7 Cellline.

INTRODUCTION

The plants of the genus Hypericum are widely used in folk medicine [1] and for the treatment of tumors [2]. *H. perforatum* [3], *H. mysorence*, *H. patulum* [4], *H. polyanthemum* [5], *H. drummondii* [6] are reported to possess strong cytotoxic and anticancer properties [7]. Several constituents including hypericin isolated from *H. perforatum* have been studied in detail for their potent anticancer properties [8]. *Hypericum hookerianum* Wight & Arn (Hypericaceae) is a round topped evergreen shrub of Asia with weakly spreading and non-erect branches scarcely distributed in the high altitude forests (2200 m) of Sikkim, Khasi and Jaintia hills of north eastern India, Kodaikonal and Nilgiri hills of southern India. Mostly known for its ornamental value due to its golden yellow flowers, the shoot extracts of this ethno medicinal plant are used by the Toda tribe of the Nilgiri hills as an antimicrobial agent to ward of skin infections and to treat wounds, burns and conditions like anxiety and inflammation (The Wealth of India 1995; Mukherjee and Suresh 2000; Vijayan *et al*., 2004). This shrub grows to a height of 6-8 feet producing attractive flowers and innumerable seeds during the months of July to February. The species occur well exposed in flowing rivers and streams and in river banks of the Shola forests. The leafy shoots may be ocassionally red in color, due to synthesis and accumulation of hypericin, a naphthodianthrone.

MATERIALS AND METHODS

Plant materials

All the leaves of *H.hookerianum* were shade dried at room temperature for 15 days. The dried leaf materials were then powdered separately and stored in the airtight container. Voucher specimen of *H. hookerianum* collected from Kodaikonal hills in the Western Ghats and was identified by Prof .N. Raaman, University of Madras, Tamilnadu, India.

Preparation of crude extract

Powdered leaves (350 g) were soaked in methanol (1 L) for 72 hrs. The supernatant was filtered through whatmann filter paper and the filtrate was concentrated at room temperature. Finally, crude extract was obtained and stored at 4 °C.

Gas chromatography–mass spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Chromatographic conditions were as follows: helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. MS conditions were as follows: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Name, molecular weight and structure of the components of the test materials were ascertained. [9]

Cytotoxicity analysis of methanol extract of *H. hookerianum* on MCF 7 (Human breast cancer) cell line

Chemicals and reagents

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) and Acridine orange and other fine chemicals were obtained from Sigma-Aldrich, USA including media constituents.

Cell culture

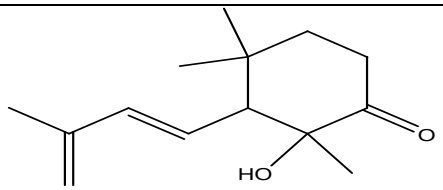
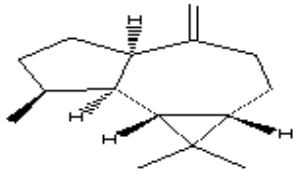
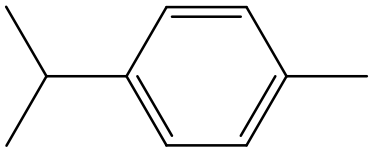
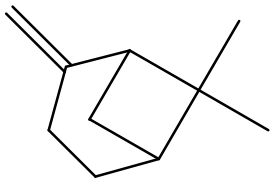
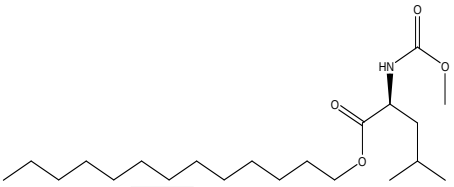
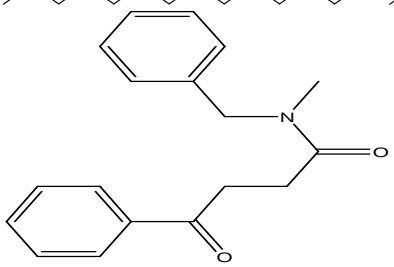
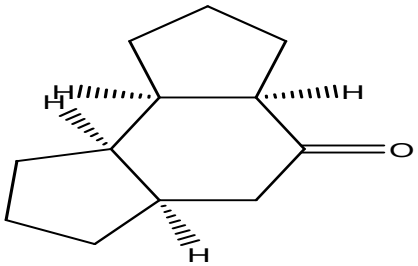
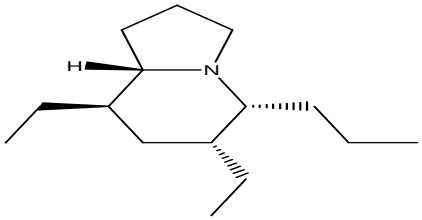
Cell viability was measured with the conventional MTT reduction assay method, as described by Mossman [13] with slight modification. Briefly, MCF 7 cells were seeded at a density of 5×103 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (10-150 µg/mL) of test compound was added and incubated for 48 h. After treatment, cells were incubated with MTT (10µl, 5mg/mL) at 37 ◦C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer

Cell growth inhibition by MTT assay

Cell viability was measured with the conventional MTT reduction assay method, as described Mossman [13] with slight modification. Briefly, MCF 7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (10-150 µg/mL) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10µl, 5mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595nm on a scanning multi-well spectrophotometer.

Cell viability (%) = (Test OD/ Control OD) X 100 **Cytotoxicity (%) = 100 – % of Viability**

Table 1: Active compounds identified from methanol extract of leaves of *Hypericum hookerianum* by GC-MS analysis

S. No	Structures	IUPAC	Molecular formula	Molecular weight
1.		2-Hydroxy-2,4,4-trimethyl-3-(3-methylbuta-1,3-dienyl)cyclohexanone	$C_{14}H_{22}O_2$	222.32
2.		(1aS,4aR,7S,7aS,7bR)-1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene [10]	$C_{15}H_{24}$	204.35
3.		1-Isopropyl-4-methylbenzene [11]	$C_{10}H_{14}$	134.21
4.		6,6-Dimethyl-2-methylenebicyclo[3.1.1]heptane [12]	$C_{10}H_{16}$	136.23
5.		1-Leucine N-methoxycarbonyl tridecylester	$C_{21}H_{41}NO_4$	371.55
6		N-Benzyl-N-methyl-4-oxo-4-phenylbutanamide	$C_{18}H_{19}NO_2$	281.35
7.		cis-anti-cis-Tricyclo[7.3.0.0(2,6)]dodecan-7-one	$C_{12}H_{18}O$	178.27
8.		(5R,6R,8R,8aS)-6,8-Diethyl-5-propyloctahydroindolizine	$C_{15}H_{29}N$	223.4

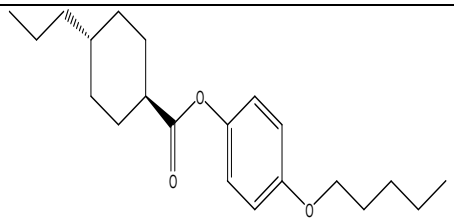
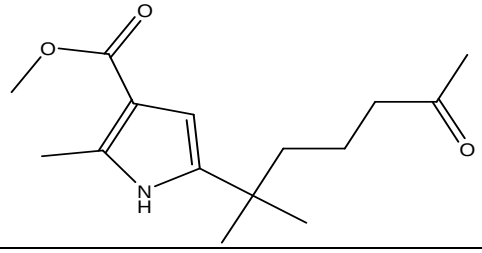
9.		p-Pentyloxyphenyl-trans-4-propylcyclohexanecarboxylate	$C_{21}H_{32}O_3$	332.48
10.		5-(1,1-Dimethyl-5-oxohexyl)-2-methyl-1H-pyrrole-3-carboxylic acid, methyl ester	$C_{15}H_{23}NO_3$	265.35

Table 2: Cytotoxicity of methanol extract of leaves of *Hypericum hookerianum* on MCF7 cell line

S. No.	Concentration, µg/mL	% of viability	% of cytotoxicity
1	0 (control)	100	0
2	10	75.0497	24.95
3	20	68.53877	31.46
4	50	61.72962	38.27
5	100	55.76541	44.23
6	150	41.57	58.43

Table 3: IC_{50} of methanol extract of leaves of *Hypericum hookerianum* on MCF7 cell line

S. No.	sample	IC_{50}, µg/mL
1	Methanol extract	113.05
2	Cyclophospamide	90.00

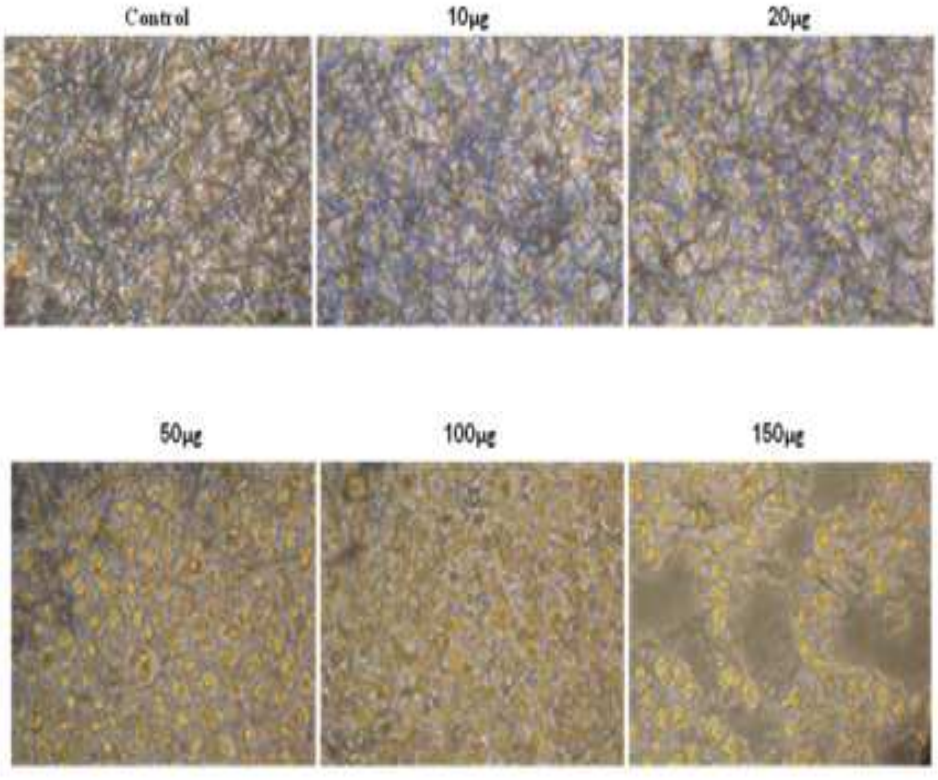


Fig. 2: Morphological representation of Cytotoxicity of methanol extract of leaves of *Hypericum hookerianum* on MCF7 cell line

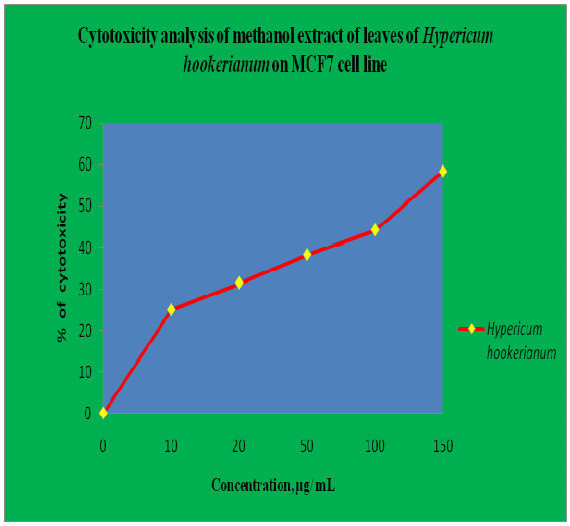


Fig. 1: Graphical representation of Cytotoxicity of methanol extract of leaves of *Hypericum hookerianum* on MCF7 cell line

RESULTS

The active compounds present in methanol extract of leaves of *H. hookerianum* were confirmed by GC-MS chromatogram, compared with NIST library and presented in Table 1. Experiment on the cytotoxicity of methanol extract of the leaves on human breast adenocarcinoma MCF-7 cells showed increasing cytotoxicity with increasing concentrations of extract and the viable cells detected with MTT assay. The results depicted in Figure 1, summarize the cytotoxic effects of the extract on MCF-7 breast cancer cell lines. The concentration dependent cytotoxic effect on this cell line was also vouched for by the data presented in Table 2. The IC50 of methanol extract of leaves of *H. hookerianum* on cytotoxic activity of breast adenocarcinoma (MCF-7) cell line was 113.05 µg/mL concentration. The Morphological representation of cytotoxicity of methanol extract of leaves of H. hookerianum on MCF7 cell line was given in Figure 2.

.DISCUSSION

GC-MS analysis revealed that the methanol extract of leaves of *H. hookerianum* contains important secondary metabolites. The GC-MS data of methanol extract showed that it is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These phytochemicals are responsible for various pharmacological actions like antioxidant, and anticancer activities. Cytotoxic changes observed in MCF 7 cell line by the treatment of methanol extract of leaves of *H. hookerianum* showed cell aggregation, cell rounding and cell death. The overall result indicates that the methanol extract of leaves of *H. hookerianum* showed promising baseline information for the potential uses of as an anti cancer agent.

CONCLUSION

This study is only a preliminary study of the occurrence of certain properties of *H. hookerianum* leaves extract; an in depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above. The phenolic compounds identified by GCMS attribute to the antioxidant property.The results of cytotoxicity of methanol extract of leaves of *H hookerianum* showed significant cytotoxicity against MCF 7 cell line. Thus it shows apoptotic potential, this programmed cell death phenomenon may play a vital role in cancer control. Thus further invivo studies may substantiate this claim.

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