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Research Article

IN-VITRO CYTOTOXIC ACTIVITY OF SKIMMIA ANQUETILIA TAYLOR & AIRY SHAW ESSENTIAL OILS ON VARIOUS HUMAN CANCER CELL LINES

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ABSTRACT

The present study aimed to determine the chemical composition of essential oils extracted from leaf, stem-bark and root-bark of *Skimmia anquetilia* and to evaluate the in-vitro cytotoxic activity. Gas chromatography and mass spectroscopy analysis (GC–MS) identified trans-geraniol (43.5%), linalool (20.73%) and vetiverol (38.59%) as the major constituents of leaf, stem-bark and root-bark essential oils respectively. The tested oil samples exhibited in vitro cytotoxic activity against four different cancer cell lines viz MCF-7 (Breast), HeLa (cervix), PC-3 (Prostate) and Caco-2 (Colon) using sulphorhodamine (SRB) assay. Stem-bark essential oil was most active on all the tested human cancer cell lines with IC50 values ranging from 2.71 to 6.21 µg/ml. Leaf essential oil (IC50¬ 3.01 to 114.50 µg/ml) and root-bark essential oil (IC50 14.88 to 49.04 µg/ml) also showed cytotoxic activity against tested human cancer cell lines. The essential oils from *Skimmia anquetilia* can be a source of anticancerous molecules in near future.

Keywords: Skimmia anquetilia, essential oils composition, anticancer, in-vitro cytotoxicity.

INTRODUCTION

Skimmia anquetilia N.P. Taylor & Airy Shaw (Rutaceae) is an aromatic shrub commonly grows in the Western Himalayas. It is also found growing in forests and shrubberies of Afghanistan to Western Nepal. The plant finds its major use as ethnic medicine for various ailments. The leaf infusion is used for the treatment of a headache, general fever and freshness[1]. The roots are used as an antidote to snake bite and scorpion bite in Indigenous System of Medicine. The dried leaves find their use as insecticide and pesticide agents and for the treatment of cold, fever and headache. The whole plant is used in curing burns and for small pox [2,3]. In Pir-Panjal Range of Himalayas, the plant is used in paralysis, pneumonia, lung cancer and anesthesia by local people. The methanolic extract of leaves showed potential anti-inflammatory activity [3]. Essential oil composition of *Skimmia anquetilia* showed that linalyl acetate, linalool and germacrene-B are major compounds in leaf essential oil, whereas, essential oil of flowers is rich in geijerene and β-phellandrene[4]. Essential oil studies of other *Skimmia* species revealed that linalool and linalyl acetate are the major constituents in leaf essential oil of *S. laureola* and *Skimmia japonica* ssp. *reevesiana*, and β-phellandrane and α-pinene are major compounds in *Skimmia japonica* ssp.*japonica*[5,6].

There are few reports on its essential oil constituents from leaf and flowers, but essential oil from stem-bark and root-bark has

not been studied till date. As per extensive literature survey, there are no reports available documenting *in vitro* cytotoxic activity of the essential oils from leaf, stem-bark and root-bark of *Skimmia anquetilia*. The present investigation thus confers explicit account on the chemical composition of leaf, stem-bark and root-bark essential oil and *in vitro* cytotoxic activity.

MATERIAL AND METHODS

Plant material

The plant material was collected from Lundi-Dohok area of Poonch district from Pir-Panjal Range of the Himalayas at an altitude of 2000-2500 m in the months of June-July, 2014. The plants were identified at CSIR-IIIM, Jammu and the voucher specimen was submitted to JanakiAmmal Herbarium of IIIM (RRL), Jammu bearing voucher specimen number 22230 for future reference.

Extraction of the essential oil

The collected plant material was properly cleaned, and the leaves, stem-bark and root-bark were separately air dried. From each part, viz leaf, stem-bark and root-bark, 200 g material was subjected to hydro-distillation using Clevenger-type apparatus. The essential oils obtained were dried over anhydrous sodium sulphate and after filtration stored at 4°C until tested and further analysed.

GC–MS analysis conditions of the essential oil

GC-MS analyses of the essential oils were performed on Varian with MS detector, using CP-Sil-8 capillary column (30 m x 0.25 mm x 0.25u) with a temperature program from 50°Cto 280°C @1.5°C/min, and finally held at 280°C. Helium gas was the carrier (flow rate 1 ml/min). The MS were recorded under EI conditions with a split ratio 1:150 column oven temperature 50°C for 5 minutes. Sample components were identified by comparing their mass spectra with those in the NIST Library and by comparison with literature data and GC retention indices.

Chemicals used

The chemicals utilized in this study include RPMI-1640, minimum essential medium, fetal calf serum, trypsin, trypan blue, ethanol, penicillin, streptomycin, gentamycin, dimethyl sulfoxide (DMSO), sulforhodamine B (SRB), 5-fluorouracil (5-FU; Sigma Chemical Co., St Louis, MO, USA), phosphate buffer saline (Merck, Darmstadt, Germany); trichloroacetic acid (TCA), distilled water, sodium hydroxide, Tris-EDTA buffer, Tris buffer (Hi-Media, Mumbai, India), acetic acid, sodium bicarbonate, hydrochloric acid (Rankem, New Delhi, India), isopropanol (Sisco, Mumbai, India), and Tris-acetate-EDTA buffer. All other chemicals used in this study were purchased locally and were of analytical grade.

Cell lines, growth medium and treatment conditions

The human cancer cell lines viz MCF-7 (breast), HeLa (cervix), Caco-2 (colon), and PC-3 (Prostate) were procured from European Collection of cell culture (ECACC), UK. Cells were grown in RPMI-1640 medium & Minimum Essential Medium (MEM) supplemented with 10 % fetal calf serum (FCS) and 1 % penicillin. Penicillin was dissolved in phosphate buffer saline (PBS) and sterilized by filtering through a 0.2µm filter in laminar air flow hood. Cells were cultured in a CO_2 incubator (New Brunswick, Galaxy 170R, Eppendrof) with an internal atmosphere of 95 % air and 5 % CO_2 gas and the cell lines were maintained at 37 °C. The media was stored at low temperature (2-8 °C). The medium for cryopreservation contained 20 % FCS and 10 % dimethylsulphoxide (DMSO).

Cell cytotoxicity assay

The *in vitro* cytotoxicity was determined by a semi-automated assay using SRB. Cytotoxicity assay of essential oils based on SRB was performed on MCF-7, HeLa, PC-3 and Caco-2 cell lines. Adriamycin and 5-Flurouracil were used as positive controls (1µg/ml each). Optimum cell density was seeded and exposed to 100µl of each concentration (3.72, 6.25, 12.5, 25, 50 and 100 µg/ml) of leaf, stem-bark and root-bark essential oils in the culture medium. The cells were incubated with different concentrations of samples for 48 hr and then fixed in ice-cold trichloro-acetic-acid (TCA) for 1 h at 4 °C. The plates were washed five times with distilled water and allowed to air dry. Then 0.4 % SRB solution was added to each well of the air dried 96-well plates and kept for staining at room temperature for 30 min. Then plates were quickly washed with 1 % v/v acetic acid to remove unbound SRB dye. The bound SRB dye was solubilized by adding 10 mM buffered tris base (pH 10.5) to each 96 well plate on a shaker platform. The absorbance was recorded at 540 nm. Dissolve essential oil samples in sterile water to give a concentration of 1 mg/ml. Mix 40.5 ml dibasic sodium phosphate Na_2HPO_4 200 mM and 9.5 ml monobasic sodium phosphate NaH_2PO_4 200 mM to achieve sodium phosphate buffer, pH 7.4. Cofactor A solution: dissolve magnesium chloride, $NADP^+$ and glucose-6-

phosphate (all from Sigma–Aldrich, UK) in the sodium phosphate buffer solution to give concentrations of 50, and 10 mM, respectively.

RESULTS AND DISCUSSION

Essential oil analysis

The oil yield from leaf, stem-bark and root-bark was 0.75%, 0.15% and 0.05% respectively on dry weight basis. GC–MS analysis of the three oil samples obtained from leaf, stem-bark and root-bark showed variation in chemical constituents. Trans-geraniol (43.5%) was the major compound followed by linalool (20.73%), β-Fenchol (9.5%), geranyl acetate (5.30%) and pregeijerene (5.11%) in the essential oil of leaf. In the stem-bark, linalool (21.67%) was the major constituent of the oil followed by vetiverol (17.81%), pregeijerene (10.51%) and neryl acetate (6.77%). In root, vetiverol (38.59%) was the major constituent of the oil followed by linalool (18.29%), pregeijerene (10.91%), β-fenchol (9.51%) and geranyl acetate (5.30%) (Table 1). The components were classified as monoterpenes and sesquiterpenes— in leaf oil (90.58 and 7.51%), in stem-bark oil (48.07 and 49.5%) and root-bark oil (54.49 and 36.02%) respectively.

Our report revealed that the concentration of chemical constituents in leaf, stem-bark and root-bark is highly variable. Trans-geraniol, the major compound in leaf was very low in stem-bark (2.70%) and root-bark (2.88%). Similarly, vetiverol (38.59%) is highest in root-bark oil that represented only 2.4% in leaf oil. However, linalool was almost equally distributed in all plant parts. Linalool (21.67%) was the major constituent of stem-bark essential oil, and it was also present in adequate amount in root-bark (18.29%) and leaf (20.73%) oil samples. In previous reports, linalyl acetate/pregeijerenechemotype and germacrene-B rich chemotypes of *S.anquetilia* were reported from different locations[7]. It is also demonstrated that essential oil constituents differ in flowers and leaf essential oils[4].

Table 1: Chemical composition of essential oils obtained from leaf, stem-bark and root-bark of *Skimmia anquetilia*

Compounds	RI*	Essential oils		
		Leaf	Stem	Root
β-pinene	978	0.78	2.57	1
Limonene	1023	1.94	0.71	0.48
β-phellandrene	1025	1.50	2.69	4.52
Cis-ocimene	1040	5.00	2.06	1.14
Terpinolene	1082	2.00	0.61	0.37
Linalool	1086	20.73	21.67	18.29
Pregeijerene	1169	5.11	10.51	10.91
β-fenchol	1200	2.21	1.04	9.51
cis-geraniol	1235	7.60	0.71	0.67
Vetiverol	1240	2.40	17.81	38.59
Trans-geraniol	1284	43.5	2.70	2.88
Terpinyl acetate	1335	1.05	1.31	1.75
Neryl acetate	1342	2.48	6.77	2.16
Geranyl acetate	1362	1.64	t	5.30
trans-2,4-Decadienol	1424	1.89	3.73	0.59
2,3-dichlorobi phenyl	1430	t	1.72	0.47
Dipropyl phthalate	1514	t	1.0	0.32
Thunbergene	1529	t	1.0	0.22
Oil yield (w/w)(% dry weight)		0.75	0.15	0.05
Monoterpenes (%)		90.58	54.49	48.47
Sesquiterpenes (%)		7.52	36.02	49.91

*Retention index (Kovalts) relative to n alkanes (C9–C26).

3.2. Cytotoxic activity

In vitro cytotoxic activity of essential oils based on SRB assay was performed on MCF-7, HeLa, PC-3 and Caco-2 cell lines. Adriyamycin and 5-Flurouracil (5-FU) showed 83% inhibition against MCF-7 and 5-FU showed 58% inhibition against (PC-3) and 75% against (Caco-2) at (1µg/ml) for 48h.

The cytotoxicity of essential oils on human cancer cell lines is shown graphically as percentage survival at various concentrations with different IC_{50} values. Stem essential oil was most active on all the tested human cancer cell lines viz. MCF-7, HeLa, PC-3 and Caco-2 cell lines with IC_{50}as 2.71, 3.60, 3.24, 6.21 µg/ml respectively. Leaf essential oil exerted high activity against MCF-7 cell lines with IC_{50} value as 3.01 µg/ml as compared to HeLa, PC-3 and Caco-2 cell lines with IC_{50} as 22.72, 84.71 and 114.50 µg/ml respectively. Root essential oil exhibited comparatively higher cytotoxic activity on Caco-2 and PC-3 cell lines with IC_{50} of 14.88 and 21.43µg/ml respectively than on the MCF-7 and PC-3 with IC_{50} value of 42.6 and 49.04 µg/ml respectively (Fig.1).

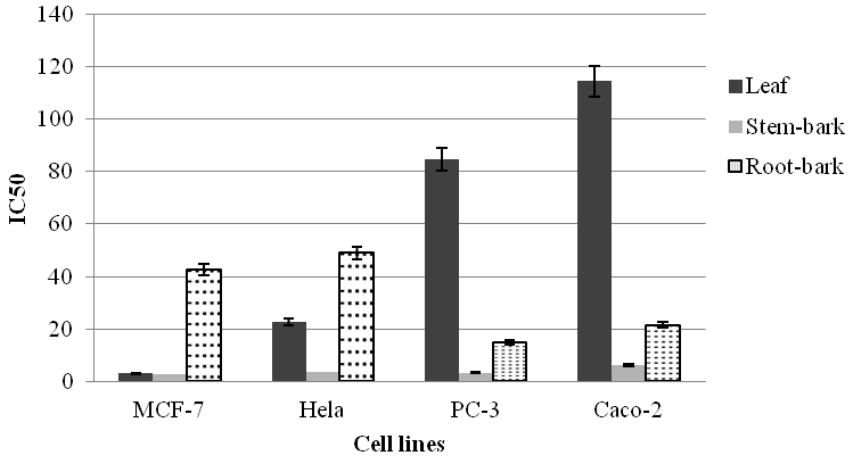

Fig.1

Fig. 1: IC_{50}Valued of essential oils

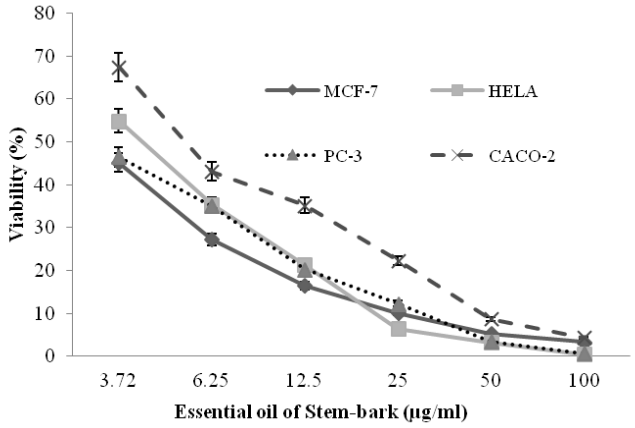

Fig.2

Fig. 2: Cytotoxicity of stem-bark essential oils

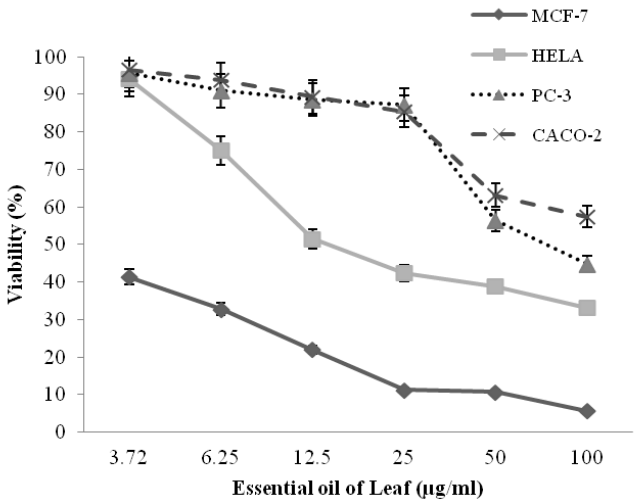

Fig.3

Fig. 3: Cytotoxicity of leaf essential oils

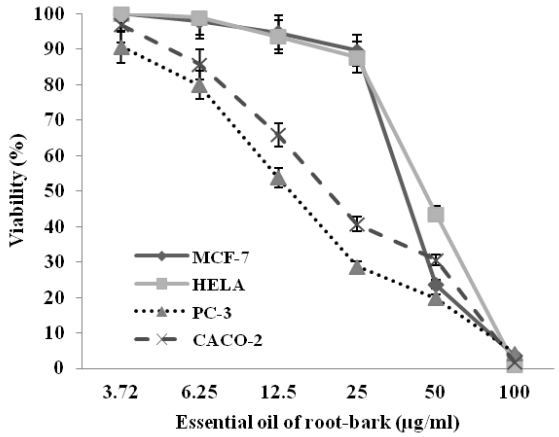

Fig.4

Fig. 4: Cytotoxicity of root-bark essential oils

Stem oil exhibited the most potent biological activity on all the tested human cancer cell lines (Fig. 2). Whereas, cytotoxic activity of leaf oil showed high activity against MCF-7 and HeLa cell lines (Fig. 3), and root showed considerable cytotoxic activity against PC-3 and Caco-2 human cancer cell lines (Fig. 4). These activities can be attributed to the different composition of essential oils of leaf, stem-bark and root-bark with regard to the predominant compounds. Our results are of importance since data on cytotoxic activities of essential oils of *S.anquetilia* are not available till date. The findings above showed that stem-bark oil of *S.anquetilia* is a better cytotoxic agent. Our results also demonstrated that stem-bark and leaf essential oils were potent cytotoxic on MCF-7 cell line. Generally, the major constituents are found to reflect quite well biological features of the essential oils[8]. However, it is possible that the activity of the main components is modulated by other minor molecules[9].

CONCLUSION

Among all the three oils analysed, stem-bark oil showed potent cytotoxic activity upon all the four human cancer cell lines. The essential oils from *Skimmia anquetilia* can be a source of anticancer molecules in near future.

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