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ANALYSIS OF COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *SKIMMIA LAURIOLA* FROM GARHWAL, HIMALAYA

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ABSTRACT

The chemical composition of the essential oils of *Skimmia lauriola* growing in Northern Garhwal, Himalaya has been studied. The wildly growing plants were collected from Dhanolti region of Tehri Garhwal. Essential oil was extracted by hydrodistillation methods and analyzed by GC/MS. Thirty seven components were identified, which constitutes 86.33% of the total oil. The major constituents are linalool acetate (26.40 %), L-linalool (14.18 %), β-phellendrene (9.03 %), prejeijerene (7.06 %), α-terpineol (6.25 %), geranyl acetate (3.89 %) and myrcene (2.18 %)**.** The essential oil was evaluated for antibacterial activity. The activity was more pronounced against *Pseudomonas aurens* with 8 mm zone of inhibition followed by *Escherichia coli* with 5 mm inhibition while *Bacillus subtilis* and *Staphylococcus aureginosa* were totally unaffected.

Keywords: *Skimmia lauriola*, Hydrodistillation, β-Phellendrene, Linalool acetate, *Staphylococcus aureginosa*

INTRODUCTION

Skimmia lauriola is strongly aromatic perennial shrub distributed throughout temperate Himalaya from Kashmir eastward and also in Khasi, Jantia, Garhwal and Kumaun hills between 1800 to 3000 m altitude. It is a very pretty aromatic gregarious evergreen shrub[1]. Leaves are aromatic, oblong-lanceolate, and acute attenuated at base, entire up to 12.5 cm long, and crowded near the end of the branches, dark green above and yellowish beneath. *Skimmia lauriola* locally known as "Nair" in Garhwal Himalaya; leaves cooked are used as condiment[2]. The dried leaves are used as incense[3]. Strongly aromatic leaves are used in curries or as a flavoring for other food[4].

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The leaves are used in treatment of smallpox[5]. Genus skimmia and skkimia lauriola have been investigated earlier by different workers[6-8] and isolated quinolone alkaloids; reevesianine-A and B, furoquinoline alkaloids; skimmianine, haplopine, evodine, evoxine, furanocoumarin glucosides. *Skimmia lauriola* was not investigated in this region for essential oil composition and hence, it prompted us for detailed phytochemical investigation of the plant.

EXPERIMENTAL

Material and methods

Plant material

The fresh aerial parts of *Skimmia lauriola* were collected from Dhanolti region of Tehri Garhwal in the month of December 2009. A voucher specimen was deposited at Department of Botany, HNB Garhwal University Campus Badshahi thaul, Tehri.

Isolation of the essential oil

The fresh aerial parts (200 g) were dried at 25 °C in the shade and subjected to hydrodistillation, using a Clevenger-type apparatus for 4 h. The oil was dried with anhydrous sodium sulphate, weighed and stored at 4 – 6 °C until use.

Gas chromatography/ mass spectrometry

FID – GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane, 25 m. 0.25 mm i.d., 0.25 μm film thickness); carrier gas, He; split ratio, 1 : 25, and flame ionization detector. Temperature programme: 60 °C (2 min) rising to 240 °C at 4 °C/min; injector temperature, 250 °C, detector temperature, 260 °C. GC–MS was performed using a Hewlett-Packard 6859 with a quadrupole detector, on a HP-5 column (see GC), operating at 70 eV ionization energy, using the same temperature programme and carrier gas as above. Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions according to Van Den Dool method.

Antibacterial activity

The essential oil of *Skimmia lauriola* was tested for antimicrobial activity using agar disc diffusion method on solid media[9,10]. Luria agar was used as basal medium for *Escherchia coli* and *Bacillus subtilis*; and nutrient agar was used as basal medium for *Pseudomonas aeroginosa* and *Staphylococcus aureus*. 5 g of luria broth and 4 g of agar

powder; 3.25 g of nutrient broth and 4 g of agar powder was weighed and 250 mL of water was added, separately. The mixture was heated to dissolve the components. Luria agar and nutrient agar was sterilized in an autoclave[11]. Luria agar and nutrient agar was poured in the sterile petri plates. Mother culture of each organism was set up 24 h before the assays in order to reach stationary phase of growth[12]. The tests were assessed by inoculating petri dishes from the mother cultures, which had been surface spread with 0.1 mL of each bacterium, with the aim of obtaining microorganism concentration of 10^5 colony forming units (CFU/mL)[13]. Sterile dilutions of essential oil were deposited on the sterile Whatmann filter paper No. 1 discs (5 mm disc diameter), which were subsequently placed in inoculated petri plates. Therefore, the petri plates were then incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the diameter of zone of inhibition surrounding bacterial growth[14].

RESULTS AND DISCUSSION

The hydrodistillaton of the dried flowering aerial parts of *Skimmia lauriola* gave light yellowish oil with yield of 1.2 % (v/w). Thirty seven components were identified in the oil, representing 86.33 % of the total composition. The major constituents were linalool acetate (26.40 %), L-linalool (14.18 %), β-phellendrene (9.03 %), prejeijerene (7.06 %), α-terpineol (6.25 %), geranyl acetate (3.89 %) and myrcene (2.18 %) **(**Table 1). The presence of oil was found more or less similar with other in respect of the presence of linalyl acetate, β-phellendrene, prejeijerene, as major compounds[15-17] but different in respect of citronellol, linalool, geraniol for their absence and presence of nerol not report earlier. These variations in the chemical composition may be due to chemical races, altitudinal variation and differences in maturity.

Table 1: The chemical composition of the essential oil from *Skimmia lauriola*

No.	Components	Retention time (sec.)	KI	Area percentage
1	2, 3-Butenediol	2.57	769	0.10
2	Trans-2-Hexanal	3.99	854	0.05
3	2,3-Hexenol	4.01	857	0.07
4	n-Hexanol	4.45	867	0.05

Cont...

No.	Components	Retention Time (sec.)	KI	Area percentage
5	α-Pinene	6.60	939	1.34
6	Sabinene	8.29	976	0.74
7	Myrcene	9.20	991	2.18
8	α-Phellendrene	9.65	1005	0.38
9	α-Terpinene	10.22	1010	0.04
10	p-Cymene	10.62	1026	0.11
11	β-Phellendrene	10.78	1031	9.03
12	t-β-Ocimene	11.42	1040	1.39
13	Benzyl acetaldehyde	11.57	1043	0.07
14	Trans-β-Ocimene	11.88	1050	1.52
15	γ-Terpinolene	12.25	1062	0.05
16	Cis Linalool oxide	12.93	1074	0.08
17	Terpinolene	13.63	1088	0.43
18	L-linalool	14.47	1098	14.18
19	Geijerene	15.90	1144	1.53
20	4-Terlineol	17.86	1177	0.17
21	α-Terpineol	18.59	1189	6.25
22	Nerol	20.42	1228	1.30
23	Linalyl acetate	21.84	1257	26.40
24	Preijeijerene	22.84	1288	7.06
25	Methyl geranate	24.75	1323	0.06
26	Neryl acetate	26.55	1365	1.83

Cont...

No.	Components	Retention Time (sec.)	KI	Area percentage
27	Geranyl acetate	27.39	1383	3.89
28	Dimethyl anthranilate	28.11	1410	0.09
29	β-caryophyllene	28.48	1418	0.08
30	Alloaromadendrene	31.12	1461	0.42
31	Germacrene –D	31.66	1480	0.51
32	Elemol	33.86	1549	0.40
33	Nerolidol	34.55	1564	0.45
34	Eremophyllene	38.50	1655	2.48
35	Z, E-Fernesol	40.37	1697	0.25
36	Z, E-Fernesyle acetate	44.54	1818	1.26
37	Phytol	53.07	1949	0.09

The method described earlier was studied for its antibacterial activity for bacterial strains viz. *Bacillus subtilis*. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerugenosa.* The oil was found to be active against three bacterial strains i.e. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, in most sensitive with 8 mm zone of inhibition against E. coli while it is least sensitive with *Staphylococcus aureus*, 4 mm of inhibition (Table 2).

Table 2: Antibacterial activity of essential oil composition of *Skimmia lauriola* DC

Bacterial strains	Group concentration (mL)		Zone of inhibition
Bacillus subtilis	(Gram +)	0.1	-ve
Staphylococcus aureus	(Gram +)	0.1	4 mm
Escherichia coli	(Gram -)	0.1	8 mm
Pseudomonas aeruginosa	(Gram -)	0.1	6 mm

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