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Phytochemical screening and GC-MS analysis of *Skimmia laureola* Decne. leaves from the Kumaon Region of Uttarakhand, India

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Skimmia laureola (DC.) Decne. is one of the most important traditional medicinal plants in the Rutaceae family, found in the Western and Eastern Himalayan regions. Whole plants are well-known for their ethnobotanical and medical uses. Looking at its traditional medicinal uses, the present study aimed to determine the phytochemicals through preliminary phytochemical screening and to profile the bioactive compounds in the different solvent extracts of *S. laureola* using the GC-MS technique. The phytochemical analysis revealed the presence of alkaloids, carbohydrates, flavonoids, coumarins, steroids and terpenoids in all three extracts. The GC-MS analysis identified various phytoconstituents with varying concentrations and molecular masses in each extract. Methanol extract revealed 57 compounds, ethyl acetate extract showed 73 compounds and 53 compounds were obtained from n-hexane extract. The predominant compounds were dictamnine (19.72%), methoxsalen (11.56%) in methanol extract, dictamnine (16.37%), cycloeucalenol acetate (15.27%) in ethyl acetate extract and tetracontane (19.35%), cycloeucalenol acetate (16.16%) in n-hexane extract. Some constituents such as Cycloeucalenol acetate and 13,27-Cycloursan-3-one identified as triterpenoid compounds were observed and had not been previously reported. This suggests the potential of this plant as a cost-effective source of dictamnine and methoxsalen, which are already known for their therapeutic properties. Further detailed investigations are needed to elucidate the therapeutic effects of these phytochemical constituents.

Keywords: Coumarins, Dictamnine, GC-MS, Medicinal plant, Phytochemicals, Therapeutics

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Introduction

Rutaceae is a large family of mainly trees, shrubs, and woody vines with 162 genera and 2085 species found in warm temperate areas and, to some extent, in tropical regions[1]. The family has 29 genera and 114 species in India, most found in the Western and Eastern Himalayas[2]. The family is well-known for its economic value, which includes edible fruits, lumber and essential oils[3]. Members of the Genus *Skimmia* Thunb., are glabrous shrubs with simple, petiolate, alternating, and glandular punctate leaves. Flowers arranged in terminal panicles are polygamous[4]. The genus contains seven to eight species found in the Northwestern Himalayas, East Asia, Japan, and the Phillippines, out of which four species found in India are *S. anquetilia*, *S. laureola*, *S. arborescens* and *S. multinervia*, while only two species *S. laureola* and *S. anquetilia* in Uttarakhand[5,6].

Skimmia laureola (DC.) Decne., locally named 'Nairpati' or 'Kedarpati', is a member of the family Rutaceae. It is an annual, strongly aromatic, perennial evergreen shrub that can grow up to 1.5 m tall[5]. Its range extends from Northern China to the Northern Himalayas, and it is most commonly found in India and Pakistan. In India, the plant occurs throughout the Western Central Himalayas from Jammu and Kashmir to Khasya hills in the East[7]. *S. laureola* grows under shady trees at elevations ranging from 1800 to 3000 m[8]. The leaves are simple and lanceolate and clustered at the ends of the shoots. The flowers are small and appear in dense panicle clusters[9]. All parts of the plant emit a pungent smell when crushed due to the presence of a poisonous compound, skimnianine[10]. The dried leaves are employed as incense in various religious ceremonies[11,12]. The leaves are used as an antitussive and veterinary anthelmintic[6]. The leaves are also useful as insecticides and pesticides. These aromatic leaves treat smallpox, cold, cough, fever, and headache. The roots of this plant are used as medicine for scorpion and snake bites[11]. The smoke produced by burning dry leaves is said to purify the air and can be used to clean the nasal tract[12-14]. Psoriasis and leukoderma are treated using a leaf

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paste mixed with cow's urine[15]. The leaves contain an essential oil that is used to prepare fragrance soap[10,12]. The plant's leaves are used as food-flavoring in the traditional cuisines of some hill tribes[7]. Fruits are used as an abortifacient in India[16]. *S. laureola* is considered an important medicinal and sacred plants.

Alkaloids, coumarins, lupene derivatives and triterpenoids have been reported in the leaves and stembark of the plant[17-20]. Quinoline alkaloids extracted from *S. laureola* exhibited tyrosinase inhibiting, calcium blocking properties and cholinesterase inhibiting activity[21]. Extracts of different plant parts have been screened for their biological activities and exhibited antinociceptive, antipyretic, antifungal, antibacterial and mutagenic activity[22-26]. The silver nanoparticles (AgNPs) were synthesised from aqueous leaf extracts of *S. laureola* and further evaluated for their antioxidant and antibacterial activities[27].

Until now, work has not been done on various crude extracts of *S. laureola* leaves. Hence, this study aims to identify the bioactive compounds present in the various solvent extracts of this medicinal plant originating from the Kumaon region of Uttarakhand, India.

Materials and Methods

Chemicals

The analytical grade chemicals were used in the research study. Methanol, ethyl acetate, and n-hexane were purchased from Merck, Mumbai, India.

Plant sample collection and identification

S. laureola plant material (Fig. 1) was collected in June 2022 from Nainital, Kumaon region of Uttarakhand, India (Altitude-3000 m, Latitude 29.4066°N, and Longitude 79.4395° E). The plants were taxonomically identified by the Botanical Survey of India (BSI), Dehradun. A voucher specimen of *S. laureola* (accession number-1346) was also deposited in the herbarium of BSI.

Fig. 1 — *Skimmia laureola* (DC.) Decne.

Sample extraction

The plant material of *S. laureola* was shade-dried at room temperature. The fully dried leaves were powdered in an electric blender. 50 g of powdered leaves were extracted in 500 mL of solvents (*viz*. methanol, ethyl acetate, and n-hexane) by the soxhlet method. Repetitive extraction was done with the same solvent until a colourless solvent was obtained. The extracts were subsequently evaporated using a rotary evaporator to obtain viscous extract. Extracts were reduced to dryness using a water bath at 60°C for 1 hour. The plant extracts were then stored at 4°C and used for further phytochemical analyses.

Determination of extraction yield (%)

The per cent yield (w/w) of all the extract was calculated as:

$$(\%)\ \text{yield} = \frac{weight\ of\ \ dry\ extract\ after\ solvent\ evaporation\ (g)}{weight\ of\ dried\ plant\ sample} \times 100$$

Preliminary phytochemical screening

Preliminary phytochemical screening was performed on methanol, ethyl acetate, and n-hexane extracts to ascertain the presence of metabolites such as alkaloids, carbohydrates, flavonoids, cardiac steroidal glycosides, coumarin, tannin, proteins and amino acids, steroids and terpenoids. The phytochemical screening was done to determine the presence or absence of the particular phytochemical groups using standard methods[28-30].

Gas Chromatography-Mass Spectroscopy analysis

To determine the various bioactive compounds present in each solvent extract, GC-MS analysis was conducted using Shimadzu, GC-MS-QP2010 Plus system employing the following conditions: The injecting temperature at 260°C, pressure at 81.9 kPa, column flow 1.21 mL/min, total flow 16.3 mL/min, purge flow 3.0 mL/min, linear velocity 40.5 cm/sec, split ratio: 10.0, ion source temperature 220°C, interface temperature 270°C and scan mass range of m/z 40-650. The oven temperature was programmed at an initial temperature of 80.0°C (hold for 2 min) to 280.0°C as a final temperature at an increasing rate of 10°C/min (hold for 20 min).

Identification of compounds

The compounds were identified by comparing their mass spectra with data from NIST 14 (National

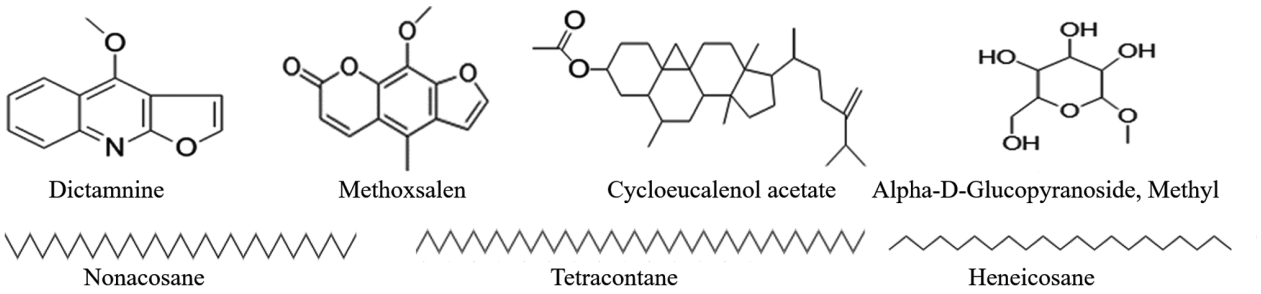

Fig. 2 — Chemical structure of major phytochemical compounds of *Skimmia laureola* identified by GC-MS analysis.

Table 1 — Phytochemical screening of various extracts of *Skimmia laureola* leaves.

Phytoconstituent	Name of the test	Methanol	Ethyl acetate	n-Hexane
Alkaloids	Mayer's test	++	++	+
	Wagner's test	++	++	+
	Dragendorff's test	++	++	+
	Hager's test	++	++	+
Carbohydrates	Benedict's test	++	+	-
	Molish test	++	+	-
	Fehling's test	++	+	-
	Shinoda test	+	+	-
Flavonoids	Zn. hydrochloride test	+	+	-
	Lead acetate test	+	+	-
Proteins and amino acids	Biuret test	-	-	-
	Ninhydrin test	-	-	-
Tannins	$FeCl_3$ test	-	-	-
	Potassium dichromate test	-	-	-
Coumarins	Alcoholic NaOH test	++	++	+
Cardiac steroidal glycosides	Keller–Kiliani test	-	-	-
Steroids and Terpenoids	Salkowski test	++	++	++
	Liebermann-Burchard test	++	++	++

(+) Indicates the presence, (−) indicates the absence, + low, ++ high

Institute of Standards and Technology, US) and WILEY 8 library. The structure of the compounds (Fig. 2) has been drawn using Chem Draw Ultra 8.0 software. A literature survey identified the nature of compounds and their biological activities.

Results and Discussion

Extraction yield

Approximately 50 g of powdered sample yielded a yield of 2.1% of n-hexane extract, 2.9% of methanol extract and a high yield of 4.8% of ethyl acetate extract. Ethyl acetate extract showed the highest extraction yield. This highlights that ethyl acetate is efficient in extracting phytochemicals from *S. laureola* more than the other extraction solvents.

Phytochemical screening

The phytochemical screening showed the presence of alkaloids, coumarins, steroids and terpenoids in all three extracts. Carbohydrates and flavonoids were present in methanol and ethyl acetate extracts but were absent in n-hexane extracts. Cardiac steroidal glycosides, tannin, proteins and amino acids were absent in all three extracts.

The results of Mayer's test revealed that the concentration of alkaloids was high in methanol and ethyl acetate extracts and low in n-hexane extract. Likewise, Wagner's, Dragendorff's, and Hager's tests were high in methanol and ethyl acetate and low in n-hexane extract. The concentration of carbohydrates was high in methanol and low in ethyl acetate extract but absent in n-hexane extract. Coumarins were found at high concentrations in methanol and ethyl acetate extracts and low in n-hexane extract. Flavonoids were found to have a low concentration in methanol and ethyl acetate extracts and were absent in n-hexane extract. Steroids and terpenoids were present in high concentrations in all three extracts. Table 1 shows the results of the phytochemical screening. Previous studies on the phytochemical screening showed similar results for the same

and different solvent extracts for the plant leaf. In the case of different plants, the phytoconstituent extraction efficiency of different solvents varies greatly[31].

Chemical constituents present in the extracts

The analysis of GC-MS results led to the identification of different types of phytoconstituents from all the extracts, and the results are shown in Figs. 3-5 The names of various compounds with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (peak area %) are presented in Tables 2-4. In total, 203 compounds were identified from various extracts of *S. laureola*. The highest number of compounds were detected in the ethyl acetate extract (73 compounds), followed by the methanol extract (57 compounds) and the hexane (53 compounds) extract of *S. laureola.*

In the methanolic extract, 57 compounds were identified. The main compounds based on their relative contents were dictamnine (19.72%, RT-16.118 min), followed by methoxsalen (11.56%, RT-17.103 min), cycloeucalenol acetate (7.49%, RT-31.383 min), alpha-D-glucopyranoside, methyl (6.74%, RT-13.386 min), bergapten (4.8%, RT-17.334 min), 13,27-cycloursan-3-one (3.14%, RT-33.124 min) and pabulenol (2.00%, RT-21.404 min). At the same time, the rest had a peak area composition of less than 2%. Most compounds extracted with methanol were furanocoumarins (Fig. 3 and Table 2).

The 73 constituents were identified in the ethyl acetate extract and the major constituents were dictamnine (16.37%, RT-16.140 min), cycloeucalenol acetate (15.27%, RT-31.497 min), methoxsalen (8.46%, RT-17.104 min), nonacosane (6.75%, RT-24.051 min), 13,27-cycloursan-3-one (4.79%, RT-33.201 min), bergapten (4.46%, RT-17.334 min), tetratetracontane (4.08%, RT-26.578 min), tetradecanoic acid (2.47%, RT-13.173), heneicosane (2.44%, RT-22.250 min), phytol (2.38%, RT-17.437 min), (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl dodecanoate (2.18%, RT-28.200 min), cyclopropa[5,6]-33-norgorgostan-3-ol,3',6-dihydro-,

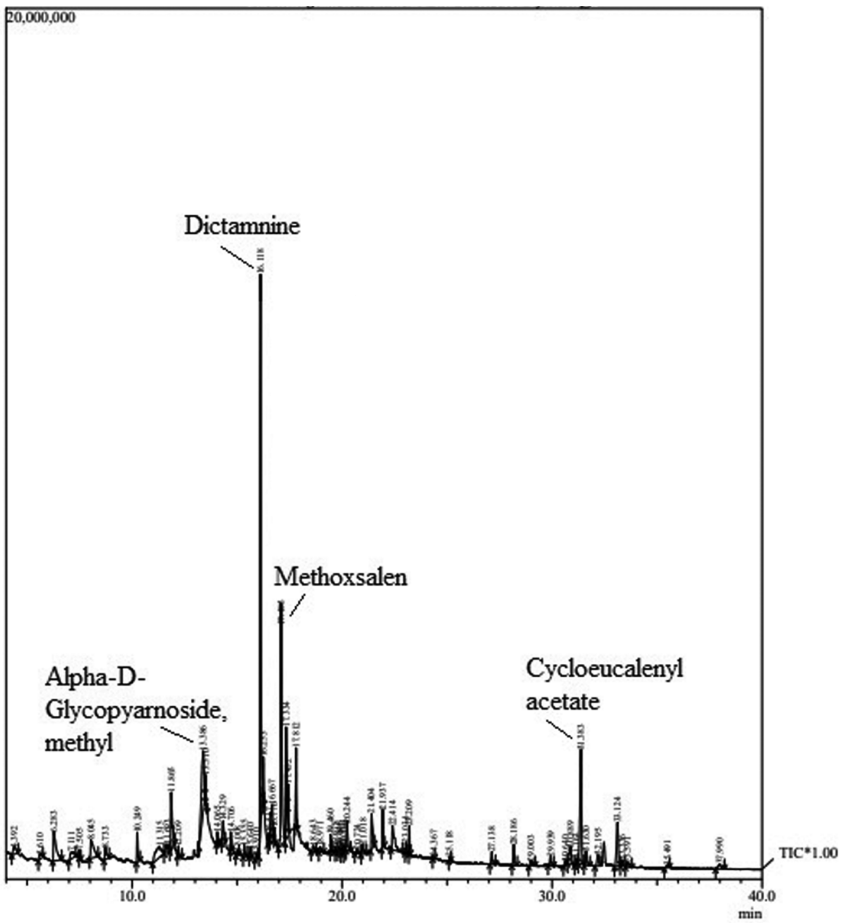

Fig. 3 — GC-MS Chromatogram of methanol extract of *Skimmia laureola*.

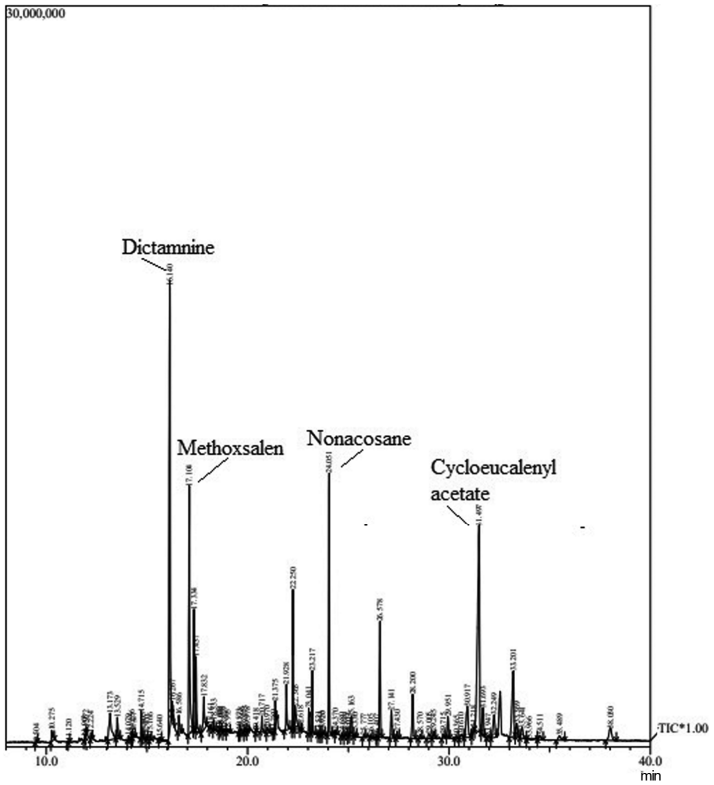

Fig. 4 — GC-MS Chromatogram of ethyl acetate extract of *Skimmia laureola*.

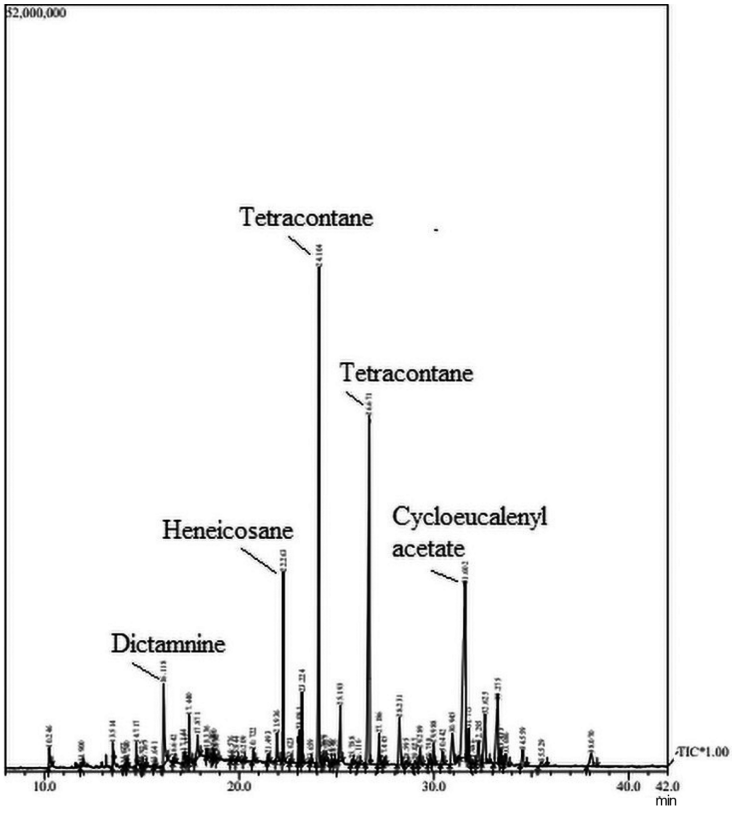

Fig. 5 — GC-MS Chromatogram of n-hexane extract of *Skimmia laureola*.

Table 2 — Phytochemical compounds identified in the methanolic extract of *Skimmia laureola* leaves by GC-MS analysis

S. no.	Retention time (min)	Peak area %	Compound name	MF	MW
1	4.392	0.83	Isosorbide Dinitrate	$C_6H_8N_2O_8$	236
2	5.610	0.49	3-Acetoxydodecane	$C_{14}H_{28}O_2$	228
3	6.283	3.18	1,5-Anhydro-6-Deoxyhexo-2,3-Diulose	$C_6H_8O_4$	144
4	7.111	0.94	L-Glyceraldehyde	$C_3H_6O_3$	90
5	7.530	0.18	1,5-Dimethyl-1-Vinyl-4-Hexenyl 2-aminobenzoate	$C_{17}H_{23}NO_2$	273
6	8.045	2.40	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134
7	8.733	0.88	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150
8	10.249	1.15	Doconexent	$C_{22}H_{32}O_2$	328
9	11.315	2.66	Sucrose	$C_{12}H_{22}O_{11}$	342
10	11.693	0.74	Unidentified	-	-
11	11.865	3.39	3',5'-Dimethoxyacetophenone	$C_{10}H_{12}O_3$	180
12	12.209	0.48	Megastigma-3,7(Z),9-Triene	$C_{13}H_{20}$	176
13	13.386	6.74	Alpha-D-Glucopyranoside, Methyl	$C_7H_{14}O_6$	194
14	13.510	0.72	3,7-Dimethyl-2,6-Octadienyl Hexofuranoside	$C_{16}H_{28}O_6$	316
15	14.065	0.29	Tetradecanoic acid (Myristic Acid)	$C_{14}H_{28}O_2$	228
16	14.329	1.32	4-Butoxy-N-[2-(2-Thienyl) Ethyl] Benzamide	$C_{17}H_{21}NO_2S$	303
17	14.706	0.34	Undec-10-ynoic acid, 3-methylbut-2-en-1-yl ester	$C_{16}H_{26}O_2$	250
18	15.008	0.17	(Z)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene) propanal	$C_{15}H_{22}O$	218
19	15.355	0.48	Alpha-Copaen-11-ol	$C_{15}H_{24}O$	220
20	15.640	0.21	Eicosanoic Acid, Methyl Ester	$C_{21}H_{42}O_2$	326
21	15.910	0.19	9-Hexadecenoic Acid	$C_{16}H_{30}O_2$	254
22	16.118	19.72	Dictamnine	$C_{12}H_9NO_2$	199
23	16.253	1.35	Cyclopenta[c]Pyran-7-Carboxaldehyde, 4-[(Acetyloxy)Methyl]	$C_{12}H_{10}O_4$	218
24	16.517	0.24	2H-1,2,3,4-Tetrazole, 5-(3-Ethoxy-4-Methoxyphenyl)-2-Methyl	$C_{14}H_{14}N_4O_2$	234
25	16.667	2.07	Scopoletin	$C_{10}H_8O_4$	192
26	16.776	0.22	9-Octadecenoic Acid (Z)-	$C_{18}H_{34}O_2$	282
27	17.103	11.56	Methoxsalen	$C_{12}H_8O_4$	216
28	17.334	4.08	Bergapten	$C_{12}H_8O_4$	216
29	17.432	0.56	Phytol	$C_{20}H_{40}$	296
30	17.812	2.61	cis, cis, cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234
31	18.643	0.42	Suberosin	$C_{15}H_{16}O_3$	244
32	18.971	0.36	5,8-Dimethoxypsoralen	$C_{13}H_{10}O_5$	246
33	19.460	0.40	5-Ethyl-5-phenyl-4-iminobarbituric acid	$C_{12}H_{13}N_3O_2$	231
34	19.846	0.18	7H-Furo[3,2-G][1] Benzopyran-7-One, 4-[(3-Methyl-2-Butenyl) Oxy]	$C_{16}H_{14}O_4$	270
35	19.982	0.14	Skimmianine	$C_{14}H_{13}NO_4$	259
36	20.103	0.23	2H-1-Benzopyran-2-One, 7-Methoxy-6-(3-Methyl-2-Oxobutyl)	$C_{15}H_{16}O_4$	260
37	20.244	1.55	(.+/-.)-Platydesmine	$C_{15}H_{17}NO_3$	259
38	20.724	0.32	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376
39	21.018	0.72	4-(3-Methyl-2-oxobutoxy)-7H-furo[3,2-g][1] benzopyran-7-one	$C_{16}H_{14}O_5$	286
40	21.404	2.00	Pabulenol	$C_{16}H_{14}O_5$	286
41	21.937	1.21	Auraptene	$C_{19}H_{22}O_3$	298
42	22.414	2.35	4-(2,3-Dihydroxy-3-methyl butoxy)furo(3,2-g)chromen-7-one	$C_{16}H_{16}O_6$	304
43	23.209	0.64	Squalene	$C_{30}H_{50}$	410
44	24.367	0.09	Ethanol, 2-(3,3-dimethylcyclohexylidene)-, (Z)-	$C_{10}H_{18}O$	154
45	27.138	0.62	(+)-Alpha-Tocopherol	$C_{29}H_{50}O_2$	430
46	28.186	1.06	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl dodecanoate	$C_{27}H_{48}O_2$	404
47	29.003	0.45	3,11-Dihydroxypregnan-20-one	$C_{21}H_{34}O_3$	334

(*contd.*)

Table 2 — Phytochemical compounds identified in the methanolic extract of *Skimmia laureola* leaves by GC-MS analysis (*contd.*)

S. no.	Retention time (min)	Peak area %	Compound name	MF	MW
48	30.640	0.26	Alpha.-Levantenolide	$C_{20}H_{30}O_3$	318
49	30.889	1.44	3-(1-[2-(1,2-Dimethylpropyl)cyclopropyl]ethyl)-3a,5b-dimethylhexadecahydro-1H-cyclopenta[a]cyclopropa[k]phenanthren-8-ol	$C_{30}H_{50}O$	426
50	31.383	7.49	Cycloeucalenol acetate	$C_{32}H_{52}O_2$	468
51	32.195	0.94	3,7,11,15-Tetramethyl-2,6,10,14-Hexadecatetraenyl acetate	$C_{22}H_{36}O_2$	332
52	33.124	3.14	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	424
53	33.336	0.40	9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E)	$C_{30}H_{50}O_2$	442
54	33.591	0.33	3.alpha.,7.beta.-dihydroxy-5.beta.,6.beta.-epoxycholestane	$C_{21}H_{46}O_3$	418
55	35.491	0.20	Betulin	$C_{30}H_{50}O_2$	442
56	37.990	0.54	Phytol, acetate	$C_{22}H_{42}O_2$	338

MF: Molecular formula, MW: Molecular weight

Table 3 — Phytochemical compounds identified in the ethyl acetate extract of *Skimmia laureola* leaves by GC-MS analysis

S. no.	Retention time (min)	Peak area%	Compound name	MF	MW
1	9.504	0.04	Trans-7-Pentadecene	$C_{15}H_{30}$	210
2	10.275	0.58	Unidentified	-	-
3	11.120	0.03	Phenol, 3,5-Bis(1,1-Dimethylethyl)-	$C_{14}H_{22}O$	206
4	11.903	0.08	Arachidonoyl Ethanolamide	$C_{22}H_{37}NO_2$	347
5	11.972	0.06	2,2,4-Trimethyl-1,3-Pentanediol Diisobutyrate	$C_{16}H_{30}O_4$	286
6	12.224	0.25	1-(1-Adamantyl)-1-Phenylethanol	$C_{18}H_{24}O$	256
7	13.173	2.47	Tetradecanoic Acid	$C_{14}H_{28}O_2$	228
8	13.529	0.88	Farnesol	$C_{15}H_{26}O$	222
9	14.256	0.14	10-Heneicosene (c,t)	$C_{21}H_{42}$	294
10	14.327	0.16	Beta-D-Glucopyranose, 1,6-Anhydro-	$C_6H_{10}O_5$	162
11	14.715	0.59	6-Octen-1-ol, 3,7-Dimethyl-, Acetate	$C_{12}H_{22}O_2$	198
12	14.805	0.06	2-Pentadecanone, 6,10,14-Trimethyl-	$C_{18}H_{36}O$	268
13	14.970	0.08	Neophytadiene	$C_{20}H_{38}$	278
14	15.166	0.13	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$	296
15	15.640	0.06	Eicosanoic Acid, Methyl Ester	$C_{21}H_{42}O_2$	326
16	16.140	16.37	Dictamnine	$C_{12}H_9NO_2$	199
17	16.267	0.53	Cyclopenta[c]pyran-7-carboxaldehyde,4-[(acetyloxy)meth	$C_{12}H_{10}O_4$	218
18	16.586	0.72	4,7-Di(methyloxy)coumarin	$C_{11}H_{10}O_4$	206
19	17.104	8.46	Methoxsalen	$C_{12}H_8O_4$	216
20	17.334	4.46	Bergapten	$C_{12}H_8O_4$	216
21	17.437	2.38	Phytol	$C_{20}H_{40}O$	296
22	17.832	1.65	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234
23	18.600	0.04	Farnesol	$C_{15}H_{26}O$	222
24	18.654	0.09	2H-1-Benzopyran-2-one, 7-methoxy-6-(3-methyl-2-butenyl	$C_{15}H_{16}O_3$	244
25	18.829	0.11	1-(1-P-Tolylcyclohexyl) Piperidine	$C_{18}H_{27}N$	257
26	18.995	0.13	5,8-Dimethoxypsoralen	$C_{13}H_{10}O_5$	246
27	19.573	0.05	4,8,12,16-Tetramethylheptadecan-4-Olide	$C_{21}H_{40}O_2$	324
28	19.668	0.19	2H-1-Benzopyran-2-One, 7-Methoxy-6-(3-Methyl-2-Oxobutyl)	$C_{15}H_{16}O_4$	260
29	19.875	0.22	1-Dodecanethiol	$C_{12}H_{26}S$	202
30	19.978	0.13	Skimmianin	$C_{14}H_{13}NO_4$	259
31	20.418	0.03	1-Dimethyl (Butyl)Silyloxypentane	$C_{11}H_{26}OSi$	202
32	20.717	0.54	Carbonic Acid, Pentadecyl Prop-1-en-2-yl ester	$C_{19}H_{36}O_3$	312

(*contd.*)

Table 3 — Phytochemical compounds identified in the ethyl acetate extract of *Skimmia laureola* leaves by GC-MS analysis (*contd.*)

S. no.	Retention time (min)	Peak area%	Compound name	MF	MW
33	21.280	0.04	1-Octanol, 2,2-Dimethyl-	$C_{10}H_{22}O$	158
34	21.375	0.87	Pabulenol	$C_{16}H_{14}O_5$	286
35	21.928	1.47	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy]-	$C_{19}H_{22}O_3$	298
36	22.250	2.44	Heneicosane	$C_{21}H_{24}$	296
37	22.365	1.52	4-(2,3-Dihydroxy-3-methylbutoxy) furo(3,2-g) chromen-7-one	$C_{16}H_{16}O_6$	304
38	22.618	0.05	Palmitic acid, neryl ester	$C_{26}H_{48}O_2$	392
39	23.041	0.94	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate	$C_{31}H_{56}O_2$	460
40	23.217	1.38	Squalene	$C_{30}H_{50}$	410
41	23.521	0.07	8-Hexadecene, 8,9-diheptyl-	$C_{30}H_{60}$	420
42	23.653	0.04	2-Methyltetracosane	$C_{25}H_{52}$	352
43	23.720	0.12	8-Hexadecene, 8,9-diheptyl-	$C_{30}H_{60}$	420
44	24.051	6.75	Nonacosane	$C_{29}H_{60}$	408
45	24.370	0.16	Geranyl linolenate	$C_{28}H_{46}O_2$	414
46	24.680	0.07	Eicos-9-ene-1,20-diacetate	$C_{24}H_{44}O_4$	396
47	24.849	0.07	Heptadecane, 3-methyl-	$C_{18}H_{38}$	254
48	25.013	0.02	Bicyclo [3.3.0] oct-2-en-7-one, 6-methyl-	C_9H_{12}	136
49	25.330	0.09	Tricosyl acetate	$C_{25}H_{50}O_2$	382
50	25.777	0.10	i-Propyl 5,9,19-octacosatrienoate	$C_{31}H_{56}O_2$	460
51	26.105	0.08	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate	$C_{31}H_{56}O_2$	460
52	26.407	0.03	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,	$C_{15}H_{22}O$	218
53	26.578	4.08	Tetratetracontane	$C_{44}H_{90}$	618
54	27.141	1.16	(+)-.Alpha.-tocopherol	$C_{29}H_{50}O_2$	430
55	27.430	0.28	Phytol, acetate	$C_{22}H_{42}O_2$	338
56	28.200	2.18	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl dodecanoate	$C_{27}H_{48}O_2$	404
57	28.570	0.22	(22e)-Ergosta-4,7,22-Trien-3-One	$C_{28}H_{42}O$	394
58	29.005	0.37	3-Hydroxypregn-5-En-20-One	$C_{21}H_{32}O_2$	316
59	29.245	0.36	Hexacosanal	$C_{26}H_{52}O$	380
60	30.365	0.09	2-methyloctacosane	$C_{29}H_{60}$	408
61	30.610	0.30	Lanosterol	$C_{30}H_{50}O$	426
62	30.917	2.44	Cyclopropa[5,6]-33-norgorgostan-3-ol, 3',6-dihydro-, (3.beta.,5.beta.,6.alpha.,22.xi.,23.xi.)	$C_{30}H_{50}O$	426
63	31.218	0.28	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	424
64	31.497	15.27	Cycloeucalenol acetate	$C_{32}H_{52}O_2$	468
65	31.947	0.14	Cycloprop [7,8] ergost-22-en-3-one, 3',7-dihydro-, (5.alpha., 7.beta.,8.alpha.,22E)	$C_{29}H_{46}O$	410
66	33.201	4.79	13,27-Cycloursan-3-one	$C_{30}H_{48}O$	424
67	33.369	0.81	1-(1,5-Dimethylhexyl)-8-Methoxy-10a,12a-Dimethyl-5-Methylenehexadecahydrocyclobuta[M]Cyclo Penta[A]Phenanthren-6(4H)-One	$C_{31}H_{50}O_2$	454
68	33.641	0.81	Unidentified	-	-
69	33.966	0.17	2,3,3-Trimethyl-2-(3-Methyl-Buta-1,3-Dienyl)-6-Methylene-Cyclohexanone	$C_{15}H_{22}O$	218
70	34.511	0.28	Hexacosanal	$C_{26}H_{52}O$	380
71	35.489	0.46	Cycloartanyl acetate	$C_{32}H_{54}O_2$	470
72	38.030	1.31	Phytyl decanoate	$C_{30}H_{58}O_2$	450

MF: Molecular formula, MW: Molecular weight

Table 4 — Phytochemical compounds identified in the n-hexane extract of *Skimmia laureola* leaves by GC-MS spectral analysis

S. no.	Retention time (min)	Peak area%	Compound name	MF	MW
1	10.246	0.81	Doconexent	$C_{22}H_{32}O_2$	328
2	11.900	0.19	DodecanoicAcid	$C_{12}H_{24}O_2$	200
3	13.514	0.63	Farnesol	$C_{15}H_{26}O$	222
4	14.077	0.10	TetradecanoicAcid	$C_{14}H_{28}O_2$	228
5	14.240	0.08	7-Hydroxyfarnesen	$C_{15}H_{24}O$	220
6	14.717	0.49	6-Octen-1-ol,3,7-dimethyl-, acetate	$C_{12}H_{22}O_2$	198
7	15.165	0.11	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$	296
8	15.641	0.06	HexadecanoicAcid,MethylEster	$C_{17}H_{34}O_2$	270
9	16.118	4.11	Dictamnine	$C_{12}H_9NO_2$	199
10	16.642	0.29	4,7-Di(methyloxy)coumarin	$C_{11}H_{10}O_4$	206
11	17.144	0.48	Methoxsalen	$C_{12}H_8O_4$	216
12	17.327	0.10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292
13	17.440	1.15	Phytol	$C_{20}H_{40}O$	296
14	17.871	1.22	cis,cis,cis-7,10,13-Hexadecatrienal	$C_{16}H_{26}O$	234
15	18.610	0.04	4,8,12-Tetradecatrienal,5,9,13-trimethyl	$C_{17}H_{28}O$	248
16	18.690	0.11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330
17	18.830	0.09	1-(1-Phenylcyclohexyl)-4-Methylpiperidine	$C_{18}H_{27}N$	257
18	19.576	0.06	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324
19	19.844	0.12	Imperatorin	$C_{16}H_{14}O_4$	270
20	20.722	0.34	Octacosane	$C_{28}H_{58}$	394
21	21.936	1.05	2H-1-Benzopyran-2-one, 7-[(3,7-dimethyl-2,6-octadienyl) oxy]-, (E)	$C_{19}H_{22}O_3$	298
22	22.263	3.59	Heneicosane	$C_{21}H_{44}$	296
23	22.623	0.06	Octadecanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	$C_{28}H_{52}O_2$	420
24	23.081	1.18	2-methyloctacosane	$C_{29}H_{60}$	408
25	23.224	1.42	Squalene	$C_{30}H_{50}$	410
26	23.659	0.04	2-Methyltetracosane	$C_{25}H_{52}$	350
27	24.104	17.46	Tetracontane	$C_{40}H_{82}$	562
28	24.299	0.12	3,7,11,15-Tetramethyl-2,6,10,14-hexadecatetraenyl acetate	$C_{22}H_{36}O_2$	332
29	24.385	0.15	Geranyl linolenate	$C_{28}H_{46}O_2$	414
30	24.690	0.06	Eicos-9-ene-1,20-diacetate	$C_{24}H_{44}O_4$	396
31	24.856	0.07	3-Methylheptadecane	$C_{18}H_{38}$	254
32	25.193	2.38	Tetratetracontane	$C_{44}H_{90}$	618
33	25.788	0.19	Hexacosanal	$C_{26}H_{52}O$	380
34	26.116	0.07	Farnesyl palmitate	$C_{31}H_{56}O_2$	460
35	26.671	19.35	Tetracontane	$C_{40}H_{82}$	562
36	27.186	1.18	(+)-Alpha-tocopherol	$C_{29}H_{50}O_2$	430
37	27.445	0.25	Phytol,acetate	$C_{22}H_{42}O_2$	338
38	28.231	2.66	Farnesyl palmitate	$C_{31}H_{56}O_2$	460
39	28.595	0.24	Unidentified	-	-
40	29.027	0.31	6-Nitrocholest-5-en-3-yl acetate	$C_{29}H_{47}NO_4$	473
41	29.289	0.84	Hexacosanal	$C_{26}H_{52}O$	380
42	29.758	0.14	(2E,6E)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yldodecanoate	$C_{27}H_{48}O_2$	404
43	31.602	16.16	Cycloeucalenol acetate	$C_{32}H_{52}O_2$	468
44	31.775	1.43	Cycloeucalenol	$C_{32}H_{50}O$	426
45	31.986	0.13	Lanosterol	$C_{30}H_{50}O$	426

(*contd.*)

Table 4 — Phytochemical compounds identified in the n-hexane extract of *Skimmia laureola* leaves by GC-MS spectral analysis (*contd.*)

S. no.	Retention time (min)	Peak area%	Compound name	MF	MW
46	32.625	3.30	Farnesyl Acetate1	$C_{17}H_{28}O_2$	264
47	33.275	4.97	13,27-Cycloursan-3-One	$C_{30}H_{48}O$	424
48	33.433	0.68	1-(1,5-Dimethylhexyl)-8-Methoxy-10a,12a-dimethyl-methylenehexadecahydrocyclobuta[m]cyclo penta[a]phenanthren-6(4H)-one	$C_{31}H_{50}O_2$	454
49	33.686	0.84	Unidentified	-	-
50	34.559	1.18	Octadecanal	$C_{18}H_{36}O$	268
51	35.529	0.47	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	$C_{32}H_{54}O_2$	470
52	38.070	1.33	Phytyl Decanoate	$C_{30}H_{58}O_2$	450

MF: Molecular formula, MW: Molecular weight

Table 5 — Nature and biological activity of major phytochemical compounds from GC-MS analysis of *Skimmia laureola*

S. No.	Compound name	Nature of compound	Biological activity
1	Dictamnine	Furoquinoline alkaloid	Antifungal, antibacterial, vascular-relaxing, antiplatelet aggregation antihypertension[33]
2	Methoxsalen	Furanocoumarin	Treatment of Psoriasis, eczema, cutaneous (skin) manifestations of T-cell lymphoma and idiopathic vitiligo (Leucoderma)[34]
3	Bergapten	Furocoumarin	Anti-inflammatory, antimicrobial, antifungal, antiviral, anticancer, anti-vitiligo and anti-osteoporosis[35]
4	Scopoletin	Coumarin	Antioxidant, antidiabetic antiinflammation, antihepatotoxicity, antitubercular, antimigratory, antihypertensive, antiproliferative, antibacterial, antithyroid, antifungal, neurological, antidopaminergic, antiadrenergic and Antihyperuricemic[36]
5	Pabulenol	Furocoumarin	Antiproliferative and cytotoxic[37]
6	Auraptene	Coumarin	Antibacterial, antioxidant and melanogenesis inhibitory[38]
7	Squalene	Triterpene	Antioxidant, antitumor against skin carcinogens, skin hydrartion, antibacterial[39]
8	Alpha-Tocopherol	Vitamin E	Antiageing, antidiabetic, antioxidant anti-inflammatory, analgesic, antitumor, antidermatitic, antileukemic, anticancer, antibronchitic, antistroke, hypocholesterolemic, vasodilator, antispasmodic, anticoronary, anticoronary, antiulcerogenic, hepatoprotective[40]
9	13,27-Cycloursan-3-One	Triterpene	Antioxidant, antibacterial[41]
10	Tetracontane	Alkane	Analgesic[42]
11	Doconexent	Omega 3 Fatty acid	Antiinflammatory, anemiahematotoxic, hyperthermic, hypercholesterolemic, antineoplastic, pulmonary oedema[43]
13	Phytol	Diterpene alcohol	Antifungal, anti-inflammatory, anticancer, anti-diuretic, anti-diabetic and immunostimulatory[44]
14	Heneicosane	Alkane	Antimicrobial[42]
15	Nonacosane	Alkane	Antibacterial, antioxidant[45]

(3.beta.,5.beta.,6.alpha.,22.xi.,23.xi.) (2.44%, RT-30.917 min). The remaining compounds present in this extract have a peak with a composition of less than 2%. Most compounds extracted with ethyl acetate were furoquinoline alkaloids and cycloartane-type triterpenes (Fig. 4 and Table 3).

The GC-MS analysis of n-hexane extract identified 53 chemical constituents (Fig. 5 and Table 4). The major compounds that were found in n-hexane extract are tetracontane (19.35%, RT-26.671 min), tetracontane (17.46%, RT-24.104 min), cycloeucalenol acetate (16.16%, RT-31.602), 13, 27-cycloursan-3-One (4.97%, RT-33.275 min), dictamnine (4.11%, RT-16.118 min), heneicosane (3.59%, RT-22.263 min), farnesyl palmitate (2.66%, RT-28.231min), tetratetracontane (2.38%, RT-25.193 min), and cycloeucalenol acetate (2.30%, RT-30.945 min). Many other compounds were also identified in the extracts comparatively in fewer amounts, as shown in Table 4.

Some of the identified compounds have been reported to possess various biological activities, as summarised in Table 5. The phytochemical analysis by GC-MS revealed the presence of useful chemical constituents *viz*. furoquinoline alkaloid (19.86%), furanocoumarins (18.18%), coumarin (3.79%),

triterpenoids (4.31%), cycloartane-type triterpenes (8.38%), fatty acid (1.19%), diterpene (0.80%) as the predominant compounds in methanolic extract. In contrast, furoquinoline alkaloid (16.50%), furanocoumarin (4.59%), furocoumarin (9.33%), coumarin (1%), hydrocarbon (14.85), diterpene (2.90%), sesquiterpene (1.34%), triterpenoid (6.75%), cycloartane-type triterpenes (17.29%), fatty acids (3.21%) and fatty aldehyde (2.29%) were the major constituents in ethyl acetate extract and furoquinoline alkaloid (4.11%), furanocoumarin (0.6%), coumarin (1.34%), cycloartane-type triterpenes (21.28%), triterpenoid (7.36%), diterpene (1.72%), fatty acid (1.1%), fatty acid, methyl ester (2.05%), fatty aldehyde (3.5%), sesquiterpene (3.93%) and hydrocarbon (44.16%) were the major constituents in hexane extract.

A total of 60 compounds were isolated from *S. laureola* based on the previous study[11]. The compounds isolated from the roots are coumarins[5], and the aerial part contains quinoline alkaloids, coumarins, lupene derivatives, steroids and triterpenoids[17-20]. Previous phytochemical reports indicate that the main constituents in *S. laureola* included dictamnine, skimmianine, bergapten and scopoletin, which are also identified in this present report[11,16]. The common compounds found in other *Skimmia* species are dictamnine, skimmianine, auraptene and 6,7-dimethoxycoumarin[11,16].

GC-MS analysis of the methanol, ethyl acetate and n-hexane extracts revealed the presence of various bioactive compounds. The common constituent found in all three extracts is dictamnine, which is present in methanol (19.72%), ethyl acetate (16.37%) and n-hexane extracts (4.11%), but in different quantities. Additionally, methoxsalen (11.56% in methanol,8.46% in ethyl acetate and 0.48% in n-hexane), phytol (0.56% in methanol, 2.38% in ethylacetate and 1.15% in n-hexane), phytol, acetate (0.54% in methanol, 0.48% in ethyl acetate and 0.41% in n-hexane), 13,27-cycloursan-3-one (3.47% in methanol, 5.07% in ethyl acetate and 4.97% in n-hexane), cycloeucalenol acetate (8.47% in methanol, 16.83% in ethyl acetate and 18.46% in n-hexane), squalene (0.64% in methanol, 1.38% in ethyl acetate and 1.42% in n-hexane), alpha-tocopherol (0.62% in methanol, 1.16% ethyl acetate and 1.18% in n-hexane extract), tetradecanoic acid (0.29% in methanol, 2.63% in ethyl acetate and 0.10% in n-hexane) are present in different solvent extracts. Some compounds that were not present in the methanol extract but were present in the other two solvents *i.e.* ethyl acetate and n-hexane are phytyl decanoate, lanosterol, farnesyl palmitate, aurapten, tetratetracontane, hexacosanal, 2-methyltetracosane, cis, cis, cis-7,10,13-hexadecatrienal, heneicosane and 2-methyloctacosane whereas pabulenol, skimmianine, suberosin, bergapten, (2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl dodecanoate, methoxsalen, and baldrinal are compounds that were absent in n-hexane but common in methanol and ethyl acetate solvents.

Dictamnine in methanol extract, dictamnine in ethylacetate extract and tetracontane in hexane extract, with their respective areas of 19.72, 16.37, and 19.35%, were major constituents. Based on studies, some of the chemical constituents revealed by GC-MS are biologically active compounds. They have been shown to possess pharmacological properties, which may be linked to the healing potential of the plant[32].

Conclusion

The phytochemical study of *S. laureola* leaf extract was studied for the first time using a polarity-based extraction in three different solvents. This is the first available information about the phytoconstituents of *S. laureola* from India. Considering the above data, methanol and ethyl acetate are better solvents than hexane for extracting the active ingredients of this plant. GC-MS spectral analysis of the various solvent extracts revealed that *S. laureola* plants contain a high concentration of phytoconstituents such as furoquinoline alkaloid, furanocoumarins, cycloartane-type triterpenes and hydrocarbons. Various phytochemical components have justified the use of the whole plant for medicine. Therefore, further studies on the bioactivity, toxicity profile, isolation and identification of individual constituents are needed. Further, its pharmacological properties should also be investigated at the molecular level using various biotechnological techniques in the future.

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Conflict of interest

The authors declare no conflict of interest.

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