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Research Article

GC-MS Analysis of Phytocomponents in the Various Extracts of *Shorea robusta* Gaertn F.

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ABSTRACT

The current investigation was carried out to determine the possible phytocomponents present in the different extracts of *Shorea robusta* using gas chromatography-mass spectrometry (GC-MS). The dried powdered resin of *Shorea robusta* was extracted exhaustively by Soxhlet apparatus with different solvents such as methanol, ethanol and chloroform. The prepared extracts were analyzed by GC-MS to identify and characterize the phytocomponents present in the crude extracts. Qualitative determination of different phytocomponents from crude extracts of *Shorea robusta* using GC-MS revealed different types of high and low molecular weight phytoconstituents with varying quantities present in each of the extracts. The GC-MS analysis provided a variety of peaks determining the presence of different compounds in various extracts of *Shorea robusta* namely Caryophyllene (1.50%), Caryophylline oxide (6.65%), Ledene oxide (11.17%), Calarene epoxide (5.15%), Alloaromadendrene oxide-(1) (8.72%), Beta-amyrin (7.99%), Alpha-amyrin (1.40%), Cycloisolongifolene (2.54%), Isolongifolene (4.73%), Silane (2.64%). The three extracts possess major phytoconstituents that were identified and characterized spectroscopically. The abundance of phytoconstituents was found to decrease in the order: methanol extract>ethanol extract>chloroform extract.

Keywords: GC-MS analysis, *Shorea robusta*, Phytocomponents, Caryophyllene.

INTRODUCTION

Medicinal plants are an expensive gift from nature to human. The approval of traditional medicine as an alternative form of health care and the development of microbial resistance to the existing antibiotics has lead researchers to scrutinize the antimicrobial compounds from nature. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate[1]. Medicinal plants are at great interest to the researcher in the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds[2]. The chosen medicinal plant namely *Shorea robusta* resin belongs to the Dipterocarpaceae family. *Shorea robusra* Gaertn.f. is widely distributed in India, Nepal and Bhutan. In India, the species are distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, eastern districts of Madhya Pradesh extending further to the eastern ghats of Andhra Pradesh. Different parts of the plant are traditionally used for the treatment of diverse purposes. The leaves are used to treat wounds, ulcers, itching, leprosy, gonorrhea, cough, earache and headache[3]. The oleoresin exuded from the cut bark has astringent and detergent properties. The bark is also used to treat diarrhea, dysentery, wounds, ulcers and itching[4]. In Unani system of medicine, the resin is used for treating menorrhagia[5], enlargement of spleen[6] and for relieving eye irritations[7]. In Ayurveda the leaves are used as anthelmintic and alexiteric. *Shorea robusta* leaf extract has been found to possess significant anti-inflammatory activity[8]. A combination of cow ghee, flax seed oil, *Phyllanthus emblica* fruits, *S. robuta* resin and Yashada bhasma has been demonstrated to possess wound healing activity[9]. With this background the present study was aimed to identify the phytoconstituents present in different extracts of *Shorea robusta* using GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

Pure *Shorea robusta* resin was purchased from local market of Delhi, India. *Shorea robusta* resin was identified by Department of Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), Delhi, India (Voucher specimen number: NISCAIR/RHMD/Consult/2016/2954/147, Dated 06/01/2016). The powdered material was stored in air tight polythene bags until use.

Preparation of extracts

The above powdered material was successively extracted with methanol, ethanol and chloroform in Soxhlet

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Table 1: Phytoconstituents of Methanol extract from *Shorea robusta* resin.

S.No.	Peak	R.Time	Area%	Name of the Compound	Molecular Formula	Molecular weight
1	3	6.732	0.03	Nitro-L-arginine	$C_6H_{13}N_5O_4$	219.20
2	5	8.268	0.06	Hexanoic acid	$C_6H_{12}O_2$	116.16
3	14	13.921	1.50	Caryophyllene	$C_{15}H_{24}$	204.36
4	18	16.097	6.65	Caryophyllene oxide	$C_{15}H_{24}O$	220.3505
5	19	16.422	11.17	Ledene oxide-(II)	$C_{15}H_{24}O$	220.3505
6	21	18.082	5.15	Calarene epoxide	$C_{15}H_{24}O$	220.3505
7	22	18.542	8.72	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220.3505
8	23	19.259	13.68	Gamma-Gurjunenepoxide-(2)	$C_{15}H_{24}O$	220.356
9	25	20.807	2.19	Spiro[2.5]octane	C_8H_{14}	110.2
10	26	21.244	1.63	Isocaryophillene	$C_{15}H_{24}$	204.35106
11	28	22.579	0.62	Anthracene	$C_{14}H_{10}$	178.2292
12	29	23.207	1.48	Culmorin	$C_{15}H_{26}O_2$	238.36574
13	30	25.607	0.49	Butanoic acid	$C_4H_8O_2$	88.11
14	35	29.028	0.15	Corticosterone	$C_{21}H_{30}O_4$	346.47
15	37	29.678	0.16	2-ethylacridine	$C_{15}H_{13}N$	207.2704
16	43	32.998	0.39	Ursa-9(11), 12-dien-3-one	$C_{30}H_{46}O$	422.68564
17	46	34.535	0.48	Coumarin	$C_9H_6O_2$	146.1427
18	48	35.611	7.99	Beta-amyrin	$C_{30}H_{50}O$	426.729
19	50	36.565	1.40	Alpha-amyrin	$C_{30}H_{50}O$	426.729
20	57	38.942	2.81	Taraxasterol	$C_{30}H_{50}O$	426.7174
21	65	42.049	1.64	Aminoglutethimide	$C_{13}H_{16}N_2O_2$	232.283
22	67	42.946	1.77	Neoisolongifolene, 8-bromo-	$C_{15}H_{23}Br$	283.253
23	68	44.056	2.13	4-azapyrine	$C_{15}H_9N$	203.244
24	69	44.370	0.53	Cycloisolongifolene	$C_{15}H_{24}$	204.357
25	74	47.280	0.25	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.4618

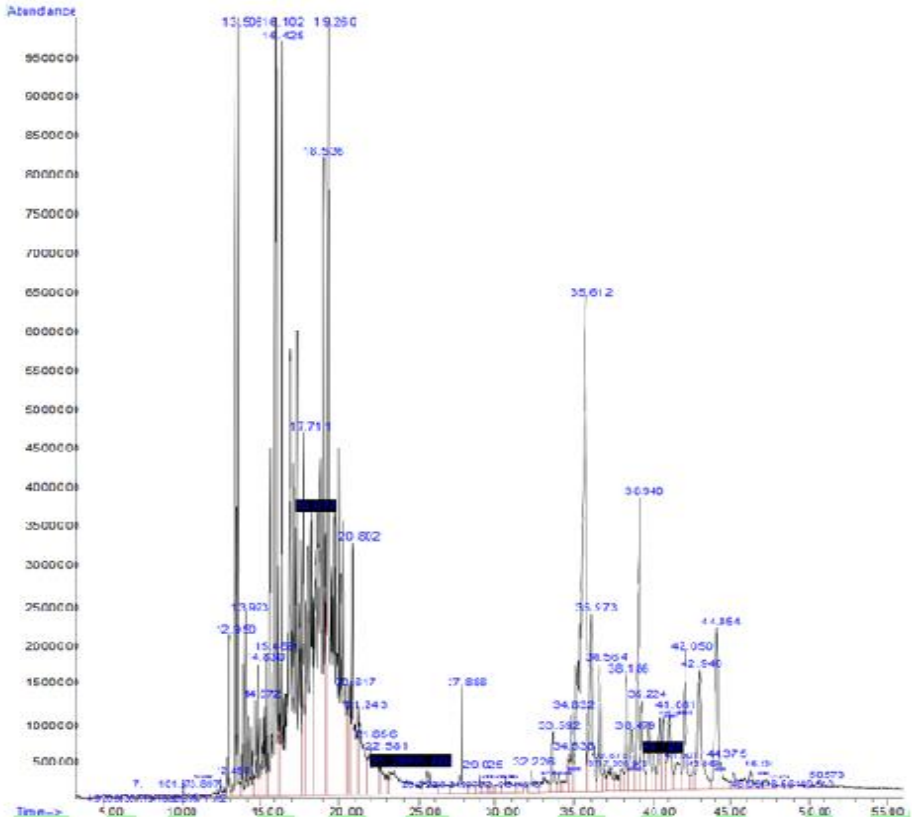

Figure 1: GC-MS chromatogram of methanol extract of *Shorea robusta*.

apparatus for 48 hrs. The extracts were concentrated by evaporation and subjected to freeze drying till dry powder was obtained. The final residue thus obtained was then subjected to GC-MS analysis[10, 11].

Table 2: Phytoconstituents of Ethanol extract from *Shorea robusta* resin.

S.No.	Peak	R.Time	Area%	Name of the Compound	Molecular Formula	Molecular Weight
1	5	13.955	1.49	Caryophyllene	$C_{15}H_{24}$	204.36
2	9	16.153	5.23	(-)-Spathulenol	$C_{15}H_{24}O$	220.35046
3	10	16.467	2.54	Cycloisolongifolene	$C_{15}H_{24}$	204.357
4	11	17.005	4.73	Isolongifolene	$C_{15}H_{24}$	204.35106
5	12	17.185	1.57	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220.3505
6	14	17.768	2.32	(-)-Neoclovene-(I), dihydro-	$C_{15}H_{26}$	206.373
7	15	18.306	6.21	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220.35046
8	19	20.874	3.24	Longifolenaldehyde	$C_{15}H_{24}O$	220.356
9	20	21.334	3.81	Spiro[2.5]octane	C_8H_{14}	110.2
10	21	22.938	0.76	Epiglobulol	$C_{15}H_{26}O$	222.37
11	24	27.727	0.32	Beta –Humulene	$C_{15}H_{24}$	204.36
12	26	28.512	0.28	Beta -Guaiene	$C_{15}H_{24}$	204.36
13	30	32.449	0.17	Lanosterol	$C_{30}H_{50}O$	426.71
14	31	33.032	0.46	Ursa-9(11),12-dien-3-one	$C_{30}H_{46}O$	422.68564
15	32	33.615	0.44	Ursa-9(11),12-dien-3-ol	$C_{30}H_{48}O$	424.713
16	35	35.791	6.56	Beta -amyrin	$C_{30}H_{50}O$	426.729
17	37	36.722	1.43	Alpha –amyrin	$C_{30}H_{50}O$	426.729
18	40	38.314	0.51	Humulane-1, 6-dien-3-ol	$C_{15}H_{26}O$	222.36634
19	42	39.133	1.54	Taraxasterol	$C_{30}H_{50}O$	426.7174
20	43	39.391	0.39	Fluoranthene	$C_{16}H_{10}$	202.26
21	49	41.712	0.24	Lupeol	$C_{30}H_{50}O$	426.73
22	50	42.251	0.79	Glucosamine per-TMS	$C_{24}H_{61}NO_5Si_6$	612.264
23	52	44.303	1.65	9-anthracenecarbonitrile	$C_{15}H_9N$	203.244

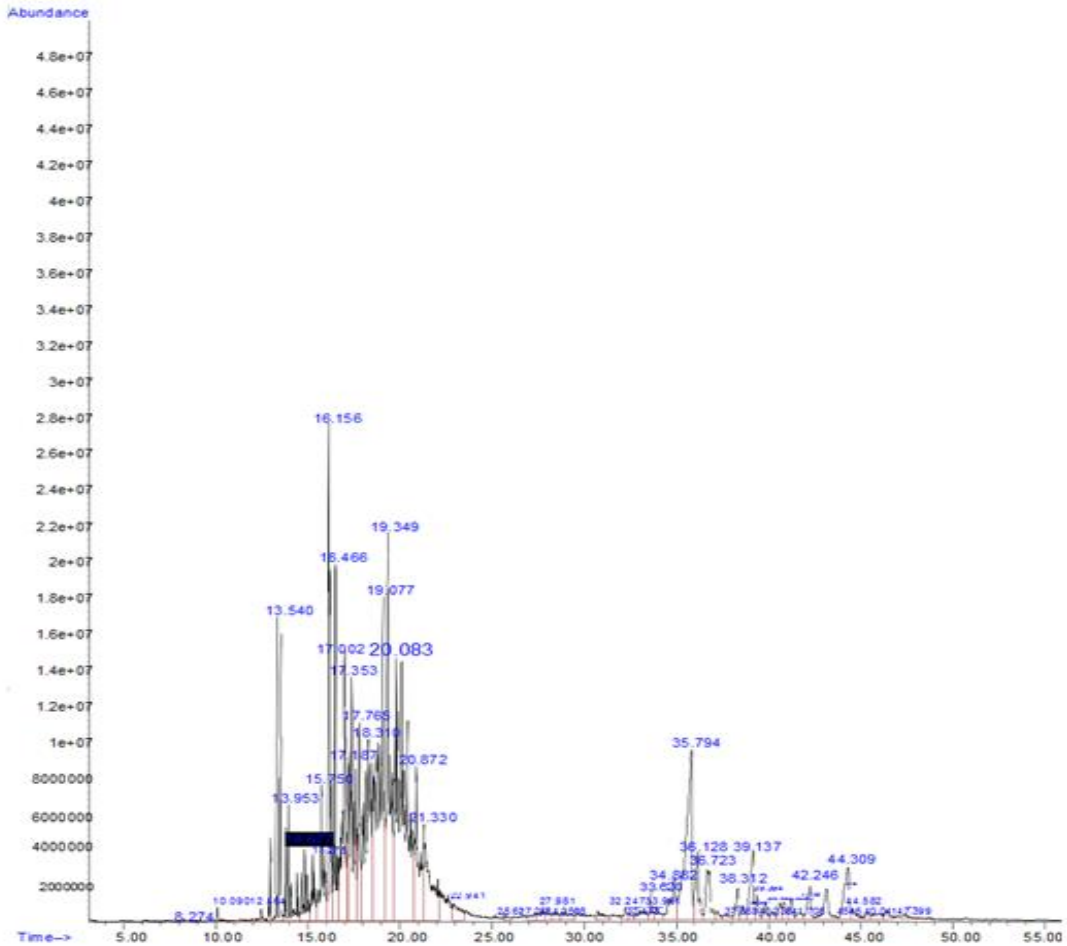

Figure 2: GC-MS chromatogram of ethanol extract of *Shorea robusta.*

Table 3: Phytoconstituents of Chloroform extract from *Shorea robusta* resin.

S.No.	peak	R.Time	Area%	Name of the Compound	Molecular Formula	Molecular Weight
1	10	19.226	0.49	Cytisine	$C_{11}H_{14}N_2O$	190.24
2	12	20.033	0.55	2,3-dimethylamphetamine	$C_{11}H_{17}N$	163.264
3	19	27.323	3.79	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	310.687
4	25	32.673	2.68	Silane	H_4Si	32.12
5	29	38.752	6.37	Cyclotrisiloxane hexamethyl-	$C_6H_{18}O_3Si_3$	222.462
6	36	43.787	3.60	Anthracene	$C_{14}H_{10}$	178.2292

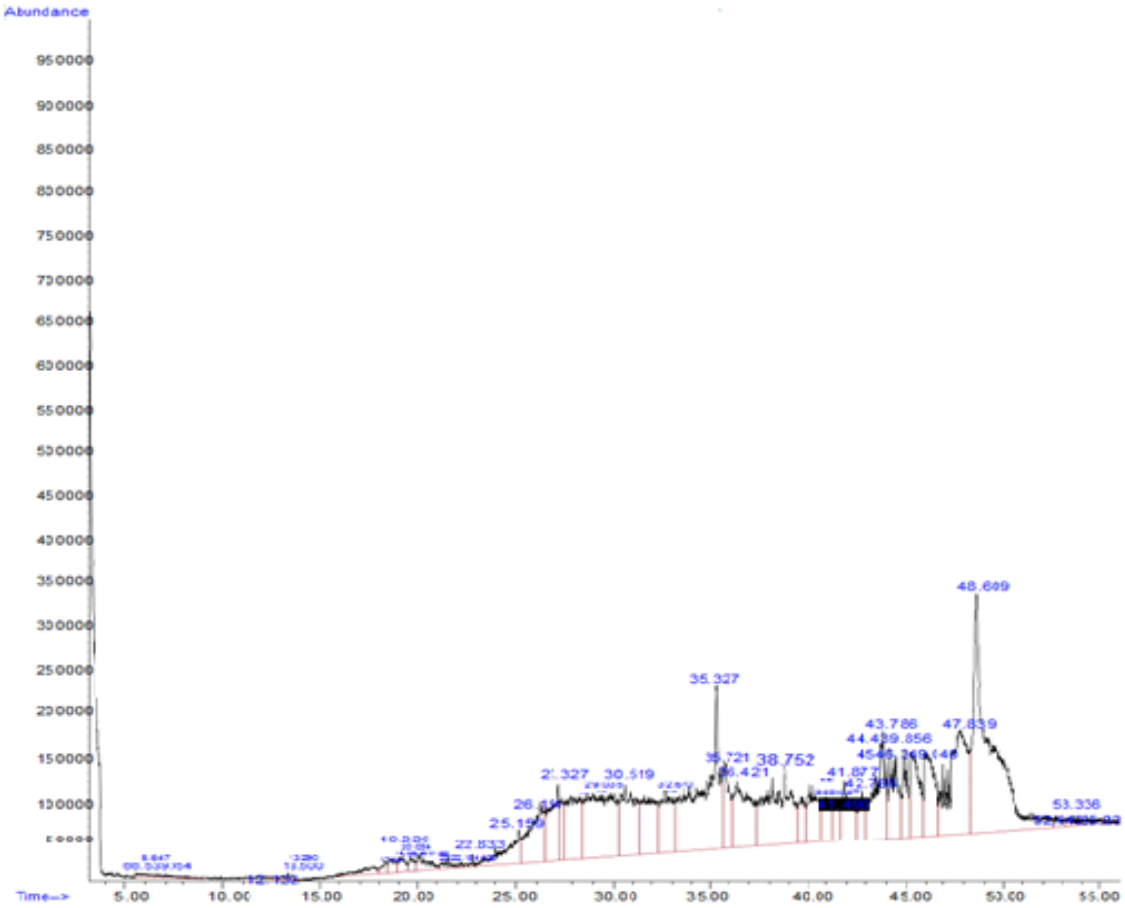

Figure 3: GC-MS chromatogram of chloroform extract of *Shorea robusta*..

The GC-MS analysis

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30 m × 0.25 mm with a film thickness of 0.25 mm composed of 95% dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5 ml/min. Sample injection volume of 1µl was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then increased to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification[12].

Identification of components

The spectra of the components were compared with the database of spectra of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The biological activities listed are based on Dr. Duke's phytochemical and ethno botanical databases by Dr. Jim Duke of the Agriculture Research service/USDA.

RESULTS

Table 4: Biological activity of phytocomponents identified in the methanol, ethanol, and chloroform extracts of *Shorea robutsa*.

S.No.	Compound	Biological activity	Reference
1	Caryophyllene	Anti-inflammatory, antinociceptive, neuroprotective, antioxidant	13
2	Calarene epoxide	Antioxidant, anti-inflammatory, anti-cancer	14
3	Lupeol	Antiprotozoal, antimicrobial, anti-inflammatory, chemopreventive	15
4	Coumarin	Treatment of asthma and lymphedema	16
5	Beta-amyrin, alpha amyrin	Antioxidant, antimalarial, antiulcer	17
6	(-)-Spathulenol	Antibacterial, immunosuppressive	18
7	Beta humulene	Anti-inflammatory, inhibition of tumor necrosis factor-α (TNF-α) & interleukin-1β (IL 1β)	19
8	Alloaromadendrene oxide-(1)	Antimicrobial, anti-inflammatory	20
9	Ursa-9(11),12-dien-3-one	Anti-inflammatory, anti-oxidant	21

The phytocomponents present in methanol, ethanol and chloroform extracts obtained from *Shorea robusta* resin are shown in Tables 1-3. The name, retention time, molecular formula, molecular weight and area percent are also presented in Tables 1-3. The biological activities of these phytocomponents are listed in Table 4. The major compounds present in methanolic extract were caryophyllene (1.50 %), caryophyllene oxide (6.65 %), calarene epoxide (5.15 %), ledene oxide-(I) (11.17 %), gamma-gurjunepoxide-(2) (13.68 %), alloaromadendrene oxide-(1) (8.72 %). The ethanol crude extract contained (-) –Spathulonol (5.23 %), Isolongifolene (4.73 %), Isoaromadendrene epoxide (6.21 %), Longifolenaldehyde (3.24 %). The chloroform crude extract has Cytisine (0.49 %), Tetrasiloxanedecamethyl (3.79 %) and Anthracene (3.60 %). The GC chromatograms of the three extracts are presented in Figures 1-3.

DISCUSSION

In the present study, the GC-MS analysis of different extracts of *Shorea robusta* showed the presence of various compounds. In terms of percentage amounts triterpenoids, sesqueterpenes, and flavonoids were predominant. These three major compounds have been shown to possess antioxidant[22], wound healing, antimicrobial and anti-inflammatory activity[23]. Anti-inflammatory and antioxidant activity are exhibited by caryophyllene and calerene epoxide, while isocaryophillene and alloaromadendrene oxide-(1) have proven wound healing and antimicrobial activity[24,25].

We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. The methanol solvent recovered higher extractable compounds and contained the highest triterpenoid, phenolic and flavonoid content. This type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

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