

GC-MS Analysis of Phytochemicals in the Methanolic Extract of *Shorea Robusta*

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Abstract: Plants have been an important source of medicine with qualities for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the whole plant methanolic extract of *Shorea robusta* by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like 9,12-Octadecadienoic acid (Z,Z)-, Tetra decanoic acid myristic acid, Nonanedioic acid, dibutyl ester, Azelaic acid, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Heptadecanoic acid, Phytol isomer, Oleic acid 9-octadecenoic acid (Z), Octadecanoic acid stearic acid, Cis-11,14-Eicosadienoic acid, methyl ester and Hexadecanoic acid in the methanolic extract of *Shorea robusta*. These findings support the traditional use of *Shorea robusta* in various disorders.

Keyword: Gas chromatography and Mass spectroscopy, *Shorea robusta*, Phytochemistry

1. Introduction

Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines^[1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations^[2].

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function^[3].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates)^[4]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as

research uncovers more of their remarkable benefits^[5]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals^[6].

The chosen medicinal plant namely as *Shorea robusta* leaves L belongs to the Dipterocarpaceae family. *Shorea robusta* Gaertn.f. (Dipterocarpaceae) is widely distributed in India, Nepal and Bhutan. In India, the species is 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^[7]. The literature survey revealed that no biological activity and phytochemical works has been done so far with the oleoresin of this plant. The biological activity was screened against the micro organisms causing skin allergies, diarrhea and dysentery. A recent study with methanol extract of mature leaves reported anti-inflammatory and antinociceptive activity^[8-13]. The aim of this study is to determine the organic compounds present in the *Shorea robusta* extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. Material and Methods

2.1 Plant Materials

The fully mature *Shorea robusta* leaves were collected in April 2013 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr.S.John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu, India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

2.2 Preparation of Extracts

The collected *Shorea robusta* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

2.3 GC –MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydioxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200°C, then 5°C/min to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0

3. Results and Discussion

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions^[14].

3.1 Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. The biological activities listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

3.2 GC-MS Analysis

Thirty compounds were identified in *Shorea robusta* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were 9,12-Octadecadienoic acid (Z,Z)-, Tetra decanoic acid \$\$ myristic acid, Nonanedioic acid , dibutyl ester \$\$ Azelaic acid, 1-(+)-Ascorbic acid 2,6-dihexadecanoate, Heptadecanoic acid, Phytol isomer, Oleic acid \$\$ 9-octadecenoic acid (Z), Octadecanoic acid \$\$ stearic acid, Cis-11,14-Eicosadienoic acid, methyl ester and Hexadecanoic acid.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

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Table 1: Shows the components identified in methanolic extract of *Shorea robusta* (GC MS study)

Peak	R.TIME	Area%	Name of the Compound	Molecular Formula	Molecular Weight
1	6.984	0.71	1-Dimethyl(octyl)silyloxypropane	C ₁₃ H ₃ OOS	230
2	6.984	4.04	1,2,3-PROPANETRIO	C ₃ H ₈ O ₃	92
3	16.358	0.62	Silane, dimethyldi(but-2-enyloxy)-	C ₆ H ₁₆ O ₂ Si	148
4	16.819	0.41	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200
5	17.535	0.16	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222
6	18.965	0.24	2-Propenal, 3-(2,4,5,6,7,7A-Hexahydr	C ₃ H ₆ O ₃	90
7	19.650	1.07	Tetradecanoic acid \$\$ Myristic acid	C ₂₄ H ₄₈ O ₂	368
8	20.620	0.95	2,6,10-Trimethyl,14-ethylene-14-PE	C ₂₀ H ₃₈	278
9	20.722	0.28	2-Pentadecanon, 6,10,14-Trimethyl	C ₁₈ H ₃₆ O	268
10	20.910	0.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol -	C ₂₀ H ₄₀ O	296
11	21.135	0.42	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296
12	21.848	1.17	Oleic Acid \$\$ 9-Octadecenoic acid (Z)	C ₁₈ H ₃₄ O ₂	282
13	21.892	0.76	Nonanedioic acid, dibutyl ester	C ₁₇ H ₃₂ O ₄	300
14	22.032	18.38	1-(+)-Ascorbic acid 2,6-dihexadecanoate	C ₃₈ H ₆₈ O ₈	562
15	23.095	1.11	Heptadecanoic acid \$\$ Potassium	C ₁₇ H ₃₄ O ₂	270

16	23.724	1.80	Phytol isomer	C ₂₀ H ₄₀ O	296
17	23.808	0.22	Octadecanoic acid, methyl ester	C ₁₉ H ₃₈ O ₂	298
18	24.00	25.84	Oleic Acid	C ₁₈ H ₃₄ O ₂	282
19	24.242	25.86	Octadecanoic acid \$\$ Stearic acid	C ₁₈ H ₃₄ O ₂	282
20	24.542	3.06	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280
21	25.047	0.56	9,12-Octadecadienoic acid	C ₂₁ H ₄₂ O ₄	358
22	25.556	0.77	Nonadecanoic ACID	C ₁₉ H ₃₆ O ₂	296
23	26.125	0.46	Hexadecanoic acid, 2-hydroxy-1,3	C ₃₇ H ₇₄ NO ₈ P	691
24	26.817	0.80	cis-13-Eicosenoic acid	C ₁₅ H ₂₆ O	222
25	26.916	1.16	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E	C ₁₈ H ₃₆ O ₂	284
26	27.165	4.71	Icosanoic acid \$\$ Arachinsaeure	C ₂₀ H ₄₀ O ₂	321
27	29.575	1.27	2-Hydroxy-3-[(9E)-9-Octadecenoylo	C ₃₉ H ₇₂ O ₅	620
28	29.665	1.26	Solanesol	C ₄₅ H ₇₄ O	360
29	30.091	1.06	Glycidol stearate	C ₂₁ H ₄₀ O ₃	340
30	31.495	0.31	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	390

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Shorea robusta* by GC-MS

S. No	Compound name	Biological activity**
1.	9,12-Octadecadienoic acid (Z,Z)-	Antiinflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
2.	Tetra decanoic acid \$\$ myristic acid	Anti oxidant, cancer preventive, hypercholesterolemic, nematocide, lubricant, cosmetic.
3.	Nonanedioic acid , dibutyl ester \$\$ Azelaic acid	Anti microbial, anti inflammatory, anti tumor, anti hyperpigmentative, anti proliferative, anti acne, cyto toxic, Anti leukemic, oxy radical scavenging activity.
4.	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Anti oxidant, anti scorbutic, anti inflammatory, anti nociceptive, anti mutagenic, wound healing property.
5.	Heptadecanoic acid	Antioxidant, anti fungal, surfactant
6.	Phytol isomer	Anti inflammatory, anti cancer, anti microbial, diuretic.
7.	Oleic acid \$\$ 9-octadecenoic acid (Z)	5- α reductase inhibitor, allergenic, α -reductase inhibitor, anti inflammatory, anti androgenic, cancer preventive, anemiagenic, anti alopecic, anti leukotriene-D ₄ , choleric, dermatitogenic, hypocholesterolemic, insectifuge, perfumery, propepic, flavour.
8.	Octadecanoic acid \$\$ stearic acid	5- α reductase inhibitor, hypo cholesterollemic, suppository, cosmetic, lubricant, surfactant & softening agent, perfumery, propepic, flavour.
9.	Cis-11,14-Eicosadienoic acid, methyl ester	Anti inflammatory, anti oxidant, anti arthritic, anti coronary.
10.	Hexadecanoic acid	Anti oxidant, hypocholesterolemic, nematocide, pesticide, lubricant, anti androgenic, flavour, hemolytic-5- α reductase inhibitor.

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

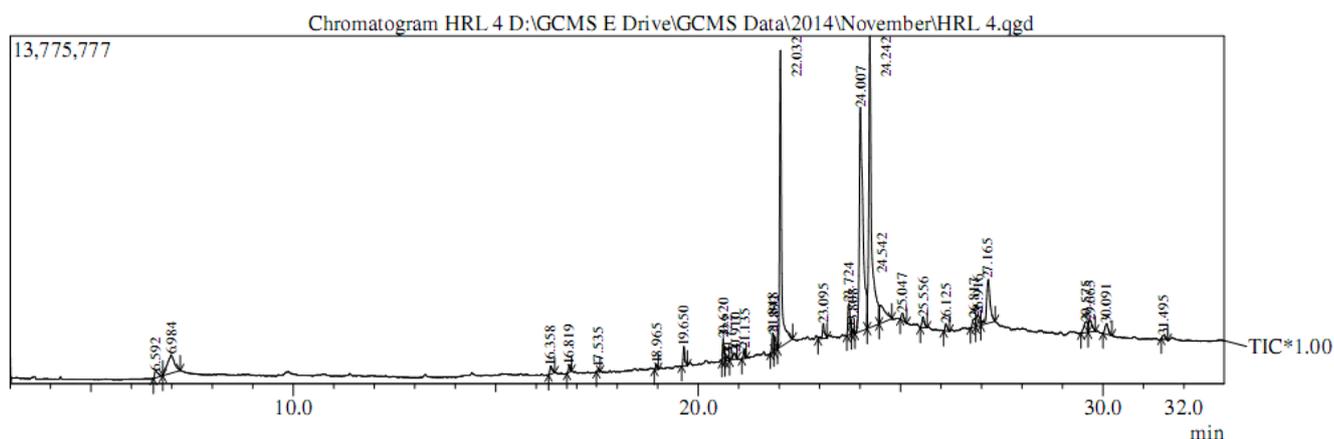


Figure 1: Chromatogram obtained from the GC/MS with the extract of *Shorea robusta*.

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