Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 11
ISSN: 2321–3647(online)

GC-MS Analysis of Oil Isolated and Antioxidant Activity of *Shorea robusta* Oleoresin

Shakkeela Yusuf[1]*, K K Srinivasan[2]
1. Department of Pharmaceutical Sciences, Cheruvandoor Campus, Ettumanoor, Kottayam, Kerala, India-686631.
2. Department of Chemistry, Shri Madhwa Vadiraja Institute of Technology and Management, (Affiliated toVITU, Belgavi), Bantakal Udupi, Karnataka -574115.

ABSTRACT

Shorea robusta widely distributed in moist and dry forest in the tropics, has been paradoxically described as deciduous, semi-decidous or evergreen species. The resin is astringent sweet acrid, cooling anodyne, vulnerary, antibacterial, deodorant constipating and detergent. It is useful in hyperhydrosis, vitiated conditions of pitta, wound ulcers, pruritis, fractures, fever, diarrhoea, dysentery etc. The main aim of the present study was to carry out the *in vitro* antioxidant activity of ethanolic extract *Shorea robusta* oleoresin and GC-MS analysis of the oil obtained from petroleum ether fraction by column chromatography. *In vitro* antioxidant activity screening was done by oxygen radical scavenging assay such as DPPH and Iron chelating method and the IC_{50} was found to be 357.44and 151.27µg/ml using ascorbic acid as standard with IC_{50} value of 52.28 and 17.85µg/ml respectively. Total antioxidant was 94.95µg equivalent to that of ascorbic acid. Thirty chemical constituents have been identified by GC-MS; the major chemical constituents are 7-Tetracyclo [6.2.1.0(3.8)0(3.9)] undecano (7.23%), Bis(2-ethylhexyl) phthalate (7.06%),3-Octenoic acid, methyl(tetramethylene)Si (6.25), Ursa-9(11),12-dien-3-ol (4.96%), phenol (4.68%) and 1-Fluoroforskolin (4.08%).

Keywords: *Shorea robusta*, GC-MS, antioxidant, radical scavenging, Column chromatography, chemical constituents.

*Corresponding Author Email: shakkeelayusuf@yahoo.com
Received 30 September 2015, Accepted 27 October 2015

Please cite this article as: Yusuf S *et al*., GC-MS Analysis of Oil Isolated and Antoxidant Activity of *Shorea Robusta* Oleoresin. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis[1]. Modern medicine has evolved from folk medicine and traditional system only after thorough chemical and pharmaceutical screening[2]. Dependency and sustainability of man and animal life has been revolving around plants through their uses as food, fibers and shelter; the use of plants as medicines is an ancient and reliable practice[3]. *Shorearobusta* Gaertn. f. (Dipterocarpaceae) is widely distributed 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 .It is reported to possess a number of medicinal properties[4]. It is used in the treatment of excessive perspiration, wounds, ulcers, neuralgia, burns, pruritis, fractures, fever, diarrhoea, dysentery, hiccough, asthma,haemorrhoids, gonorrhoea. menorrhagia, splenomegaly, obesity, cephalalgia. Odontalgia and burning sensation of the eyes[5]. The plant is also found to possess hypolipidemic effect. The oleoresin (gum) of the aerial parts has been reported in indigenous system of medicine as it also used as an ingredient of ointments to heal wounds, burns, pains, skin diseases and to control diarrhoea and dysentery[6]. Many chemical constituents are isolated from various parts of this plant. These include ursolic acid, α and β amyrin, mangiferonic acid, benthamic acid, asiatic acid, amyrenone and uvaol[6]. Asiatic acid, 3,25-epoxy-1,2,3,11-tetrahydroxyurs-12-en-28-oic acid,3,25-epoxy-1,2,3-trihydroxyurs-l2-en-28-oic Acid, Phayomphenol and 3,7-dihydroxy-8-methoxyflavone 7-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside(7).A literature survey revealed that no GC-MS analysis of theoleoresin of this plant has been carried out(8). We now report antioxidant activity of ethanolic extract of the oleoresin and GCMS analysis of the oil obtained by column chromatography.

MATERIALS AND METHOD

Plant material:

Shorea robusta oleoresin was collected from Idukki district, Kerala in India during March2014 and authenticated by Mr. Rogimon Joseph, Assistant Professor Department of Botany, C.M.S College, Kottayam, Kerala, India.

Preparation of Extract

The dried sample *Shorea robusta* oleoresin was powdered and soaked with ethanol until the powder was fully immersed overnight and refluxed for 3 hours ; the sol. The ethanolic extract was dried and subjected to fractionation using petroleum ether(PE)(9).PE was chromatographed

over silica gel using graded mixtures of different solvents according increasing polarity. Methanol: chloroform (1: 1) fractions (59 to 66) were pooled to get a red dish brown oil. The oil was subjected to GC-MS analysis. The ethanolic extract was directly used for the antioxidant assays.

Preliminary Phytochemicals Screening:

Chemical tests were carried out in the alcoholic extract using standard procedures to identify the various phytochemicals[10].

ANTIOXIDANT ACTIVITY

DPPH Radical Scavenging Assay

The hydrogen atom or electron donor capacity of the extracts was tested by its ability to bleach DPPH radical various concentration of the extract (20-100µg/mL) were added to 1 ml of 025 mm DPPH solution in ethanol[11]. The tubes were incubated in rooms for thirty minutes and the absorbance was read at 517 nm. Assay was carried out in triplicate. The percentage scavenging was calculated as the ratio of absorption of the sample relative to the control without the extract. DPPH radical scavenging activity (%) = (1-antioxidant OD / control OD) X100. The concentration of the extract necessary to decrease the initial concentration of DPPH by 50 % was calculated.

Total Antioxidant Capacity

The antioxidant activity of the ethanolic extracts of *shorea robusta* was evaluated by the phosphomolybdenum method[12]. The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Iron Chelating Activity Assay

In the assay, 2mL of various concentrations of extracts and ascorbic acid solutions were incubated with 1mL *O*-phenanthroline solution (0.005gm in 10 ml methanol) and 2ml 200µM ferric chloride solution at ambient temperature for 10 minutes. After incubation, the absorbance of solutions was measured at 510nm against the corresponding blank solution. The blank used

here was a mixture of methanol and distilled water. A control was also prepared omitting the sample. The experiments were performed in triplicate[13].

GC-MS ANALYSIS

Working procedure

One microliter sample was subjected for the study. The Instrument used, Varian CP-3800 Saturn 2200 GC/MS/MS with factor four VF-5MFcolumn.Oven temperature maintained at 100^{o}C for 1.5 minutes and temperature gradually increased to270^{o}c at 5^{o}c per minute and 1µlitre sample was injected for analysis. Helium gas 99.9 % was used as the carrier gas, the flow rate of carrier gas was1ml per minute sample injected temperature was maintained at 250^{o}c and split ratio is 20 throughout the experiment period. The ionization mass was done with 70 eV. The mass spectra were recorded for the mass range 40-600 m/z for 60 minute. Identification of compound was based on comparison of their mass spectra. As the compound separated, on elution through column were detected in electronic signals. The m/z obtained was calibrated through graph obtained which was called as the mass spectrum graph, which is the finger print of the molecule. The identification of the compound was based on the comparisons of their mass spectra with Nist library.

Identification of the Compounds

Identification of the compounds were done by studying the peaks obtained in the total ion current chromatogram, separately from the mass fragmentation pattern and then comparing the same with the standard library present in the data station.

RESULTS AND DISCUSSION

Pharmaceutical research conducted over the past three decades has shown natural products are a potential source of novel molecules for drug development. *Shorea robusta*, the selected plant had a higher degree of positive results.

Phytochemical screening of the *shorea robusta* oleoresin revealed the presence of various chemical constituents like alkaloids, tannins, carbohydrates, flavonoids triterpenoids, proteins, resins, saponins and steroids etc. The antioxidant property of the compounds may be due to these constituents. DPPH radical scavenging activity of plant extract of *Shorea robusta* and standard as ascorbic acid are presented in the Table 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterized as a stable free radical by virtue of

the delocalization of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 357.44µg mL^{-1} and 52.28 µg mL^{-1} respectively. The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration.

Table1. Antioxidant activity of ethanolic extract of oleoresin of *Shorea robusta* by DPPH Method

Sl No	*Shorea robusta*				Standard Ascorbic acid			
	Conc. (µg/ml)	% Inhibition	SEM	IC_{50} (µg/ml)	Conc. (µg/ml)	SEM	% Inhibition	IC_{50} (µg/ml)
1	100	20.08	0.786±0.0006	357.44	20	0.716± 0.001	27.20	52.28
2	200	29.64	0.692±0.0008		40	0.609±0.007	38.11	
3	300	40.24	0.587±0.0005		60	0.517± 0.0015	47.39	
4	400	55.35	0.474±0.0173		80	0.465±0.018	54.87	
5	500	69.14	0.283±0.0104		100	0.387±0.003	61	

Table 2: Antioxiodant Activity of Ethanolic Extract of Oleoresin of *Shorea robusta* by Total Antioxidant Method

Sl. No	Shorea robusta		Ascorbic acid equivalent mg/gm
	Concentration(µg/ml)	SEM	
1	20	0.095±0.002	
2	40	0.223±0.001	
3	60	0.343±0.002	94.95
4	80	0.452±0.002	
5	100	0.562±0.008	
6	120	0.693±0.013	
7	140	0.764±0.031	
8	160	0.809±0.002	
9	180	1.014±0.006	
10	200	1.196±0.005	

The yield of the ethanol extract of the plant extract and its total antioxidant capacity are given in Table 2. Total antioxidant capacity of *Shorea robusta* is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate Mo (V) complex with a maximal absorption at 695 nm. The assay is being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract.

Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid and was found to be 94.95.

Ortho substituted phenolic compounds may exert pro -oxidant effects by interacting with iron. Ortho phenanthroline quantitatively forms complexes with Fe^{2+}, which get disrupted in the presence of chelating agents. The ethanolic extract interfered with the formation of ferrous-o-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. Iron stimulates lipid peroxidation by Fenton reaction and accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals which themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The metal chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation. It has been reported that the chelating agents, that form bonds with a metal are effective as secondary antioxidant because they reduce the redoxpotential, thereby stimulating the oxidized form of the metal ion. The observed results151.28 µg mL^{-1} for *shorea robusta* and 52.28µg mL^{-1} for the standard ascorbic acid, demonstrate a marked capacity of the extract for iron binding, suggesting that their action as a peroxidation protector may be related to its iron binding capacity. The good antioxidant property of the plant may be due the various phyto constituents present in the extract. Phytochemicals isolated from the *Shorea robusta* oleoresin are11-oxoasiatic acid, dryobalanolide, mono-, sesqui- and triterpenoids including ursolic acid, tri and tetrahydroxy ursenoic acid, asiatic acid, α and β-amyrin, α –amyrenone, mangiferonic acid, benthamic acid and uvaol (Liu., 1995). In the present study the GC/MS of the oil sample obtained from petroleum ether fraction by column chromatography showed five major peaks at RT 18.992, 39.921, 4.099, 56.164, 2.185 min and the corresponding compounds were found to be7-Tetracyclo [6.2.1.0(3.8)0(3.9)] undecano, Bis (2-ethylhexyl) phthalate,3-Octenoic acid, methyl (tetramethylene)Si, Ursa-9(11),12-dien-3-ol, phenol and 1-Fluoroforskolin and Percentage compositions are 10.240, 9.996,8.857,7.033,6.627 respectively (Table 4)

Table 3: Antioxidant Activity of Ethanolic Extract of Oleoresin of *Shorea robusta* by Iron Chelating Method

Sl No	*Shorea robusta*				Standard Ascorbic acid			
	Conc. (µg/ml)	% Inhibition	SEM	IC_{50} (µg/ml)	Conc. (µg/ml)	SEM	% Inhibition	IC_{50} (µg/ml)
1	100	45.30	0.060±0.034	151.28	20	0.716± 0.001	27.20	52.28
2	200	54.17	0.072±0.001		40	0.609±0.007	38.11	
3	300	62.21	0.087±0.003		60	0.517± 0.001	47.39	
4	400	68.17	0.103±0.002		80	0.465±0.018	54.87	
5	500	75.16	0.135±0.003		100	0.387±0.003	61	

Table 4: List of Compounds Identified with Retention Time and Peak Area

Sl.No	Retention time	Name of the compound	Peak Area (%)
1	2.185	Phenol	6.627
2	2.292	3-(prop-2-en-1-yl)cyclohexene	3.847
3	3.880	Pentanoic acid, 4-oxo-, ethyl ester	1.739
4	4.099	3-Octenoic acid,methyl(tetramethylene)si	8.857
5	5.908	Butanedioic acid, diethyl ester	4.044
6	8.200	3Beta-acetoxy-4,4,8,10,14-pentamethyl-17	3.773
7	8.200	3Beta-acetoxy-4,4,8,10,14-pentamethyl-17	2.709
8	11.013	Naphthalene, hexahydro-1,6-dimethyl-4-(1	1.361
9	17.013	1H-Cycloprop[e]azulen-7-ol, decahydro-1	1.416
10	18.992	7-Tetracyclo[6.2.1.0(3.8)0(3.9)]undecano	10.240
13	20.693	1R,4S,7S,11R-2,2,4,8-Tetramethyltricyclo	2.802
14	20.855	Ledene alcohol	3.037
16	21.422	Benzene, 1,3-bis(1,1-dimethylethyl)-2-me	2.338
17	22.017	Ledene alcohol	3.268
18	22.246	1-Fluoroforskolin	5.781
19	23.627	5,9-Methanobenzocycloocten-1(2H)-one, 3,	3.679
23	29.244	Tris(2,6-dimethylphenyl)borane	1.715
25	39.921	Bis(2-ethylhexyl) phthalate	9.996
28	56.164	Ursa-9(11),12-dien-3-ol	7.033
29	56.811	Ursa-9(11),12-dien-3-one	1.861
30	58.577	Tetracosanoic acid, tert-butyldimethyls	1.616

CONCLUSION

The antioxidant activity studies of the oleoresin of the plant was done using DPPH, Total antioxidant assay and iron chelating method. The results showed significant activity when compared with that of standard ascorbic acid. The major constituents of this oil are 7-Tetracyclo [6.2.1.0(3.8)0(3.9)] undecano (7.23%), Bis(2-ethylhexyl) phthalate (7.06%) and 3-Octenoic acid, methyl (tetramethylene)Si (6.25) which are chemically acyclic monotepernoids. The Oleoresin of *Shorea robusta* is a good source of antioxidants. These compounds could be isolated from the oil by suitable methods and tested for various biological activities.

REFERENCES

1. Balunas MJ, Kinghorn AD. Drug discovery from medicinal plants. 2005;78:431–41.
2. Kalimuthu K, Prabakaran R. Preliminary Phytochemical Screening And Gc-Ms Analysis Of Methanol Extract Of Ceropegia Pusilla. 2013;1(3):49–58.
3. Ebrahim Azarpour, Maral Moraditochaee HRB. Study Medicinal Plants In Holy Quran. 2014;529–36.
4. KK. Sri Rama Murthy, Lakshmi N, Ramulu. Biological activity and phytochemical screening of the. 2011;14:787–91.

5. Adlakha MK, Bhargava AK, Kapoor R, Sharma LN, Singh C, Prof A, et al. Ayurvedic Medicinal Plant - Shala (Shorea Robusta) (A BIRD ' S EYE VIEW). 2014;2(4):2–5.
6. Misra LN. Triterpenoids From Shorea Robusta Resin . 1997;45(3).
7. Sharma TC, Sharma AK, Sharma KK, Payal P, Sharma M, Dobhal MP. Phytoconstituents : Isolation and Characterization from Root Bark of. 2014;6(4):4–8.
8. Sahil K, Prashant B, Akanksha M, Premjeet S, Devashish R. Gas Chromatography-Mass Spectrometry : Applications. 2011;2(6):1544–60.
9. Satyajit D. Sarkaer ZL, Gray AI. Natural Products Isolation.
10. Kandelwal K.R. Practical Pharmacognosy Techniques and Experiments ,Tenth edition,Nirali Prakashan Pune,page 149-156
11. Sivaraman A, Johnson M, Parimelazhagan T, Irudayaraj V. Evaluation of antioxidant potential of ethanolic extracts of selected species of Selaginella. 2013;4(September):238–44.
12. Mathavi P, Nethaji S. GC-MS Analysis of Phytocomponents in the Methanolic Extract of Shorea Robusta. 2015;4(6):2013–6.
13. Nuutila AM, Pimia RP, Aarni M, Caldenty KM. O Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry 2003; 81, 485–493.