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GC–MS analysis and antimicrobial activity of various solvent extracts from *Simarouba glauca* leaves

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Abstract
Phytochemical profile of leaf extracts of *Simarouba glauca* in different solvents were analyzed using Gas Chromatography – Mass Spectrometry method. The dried leaves were powdered and soaked in different solvents in increasing polarity namely Chloroform, Ethyl acetate and Methanol. The extracts were then subjected to GC-MS analysis which was carried out using Clarus 680 Gas chromatography. The spectrum of unknown compounds was compared to that of known using National Institutes of Standards and Technology database. The analysis revealed the existence of different high and low molecular weight chemical entities present in each of the extracts. The compounds were considered to be pharmacologically and biologically significant. The extracts when tested against the growth of selected bacteria and fungi exhibited good antimicrobial activity. From this preliminary study it is understandable that the plant contains many bioactive compounds and thus suggested as a phyto pharmaceutically important plant.

Keywords: *Simarouba glauca*, chloroform, ethyl acetate and methanol extracts, bioactive compounds

1. Introduction
Plants prevent and decrease the adverse effects of conventional treatments and also play a noteworthy role in the prevention and treatment of many diseases [1]. History discloses that plants are sources of many efficient drugs and also will continue to be important for screening of new lead compounds of biological and pharmacological importance [2]. Nowadays due to less side effects and easy availability of medicinal plants, herbal remedies have become more popular [3, 4]. Several diseases in mankind are cured because of the presence of bioactive secondary metabolites in the medicinal plants [5]. *In vitro* screening methods could provide the required initial observations necessary to elect crude plant extracts with potentially useful properties for further pharmacological investigations [6]. The metabolites possess interesting biological activities and applications such as pharmaceuticals, insecticides, dyes, flavors, fragrances etc. Many chronic and infectious diseases can be treated using traditional medicine which contains a wide range of substances [7].
Simarouba glauca (Figure 1), a plant very commonly known as Paradise tree, belongs to the family Simaroubaceae and is reported for the presence of alkaloids, flavonoids, cardinolides, glycosides, phenolic compounds, saponins and oils [8]. The main constituents of this plant are quassinoids which are bitter substances and possess various pharmacological properties including antiviral, antimalarial, antinflammatory and anticancer activities. The *in silico* studies of the quassinoids showed favorable results as an inhibitor of Phosphoinositide 3-kinases (PI3Ks) [9].

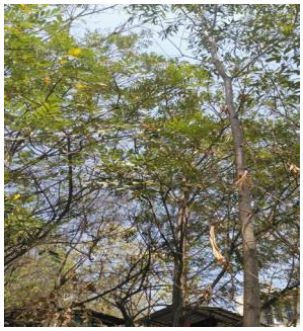


Fig 1: *Simarouba glauca*

In India, healthy growth of the plant is seen in the waste lands of Orissa, Karnataka and Gujarat. Apart from this, the growth rate is successfully larger in Andhra Pradesh, Tamil Nadu, Maharashtra and Bihar. The significance of this plant is that it will give secured returns even with erratic and low rain fall conditions [10]. This plant is a rich source of fat with melting point of 290˚C. The green energy components and their sources were biodiesel from seeds, ethanol from fruit pulps, biogas from fruit pulp, oil cake and thermal power from leaf litters, shells and unwanted branches. Biodiesel is a renewable home of energy unlike fossil fuels as it is from biological sources like plants and microbes [11]. The deoiled meal of *S. glauca* is a rich source of protein (~48 g/100g) with high solubility (~94%); *in vitro* protein digestibility and amino acid based calculated nutritional indices.

Published literature regarding the presence of bioactive compounds in different extracts of *S. glauca* leaves by Gas chromatography – Mass spectrometry analysis is not available. Keeping this in view, the present study was initiated to investigate the antimicrobial effects and identify the bioactive compounds in the chloroform, ethyl acetate and methanol extracts of *S. glauca* leaves using GC-MS analysis.

2. Materials and Methods

2.1 Plant Sample and extraction of crude extracts

Fresh leaves of the prevailing plant *S. glauca* were collected in and around Chennai. The leaves of the plant were washed thoroughly in tap water, rinsed with distilled water and air dried in shade for about 30 days as the leaves are rich in oil content. Dried leaves were powdered and fifty grams of it was subjected to selective sequential extraction using solvents in increasing polarity namely, Chloroform, Ethyl acetate and Methanol. The extracts were filtered through what man filter paper 40. The extracts were concentrated using rotary evaporator. The extracts were analyzed for GC-MS analysis and antimicrobial activity.

2.2 The GC-MS analysis

The Clarus 680 Gas chromatography used in the analysis employed a fused silica column, packed with Elite – 5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m x 0.25 mm ID x 250 µm df) and the components were separated using Helium as carrier gas at a constant flow rate of 1 ml/min. The injector temperature was set at 260˚C during the chromatographic run. The extract sample (1µl) was injected into the instrument. The oven temperature was as follows: 60˚C (2 min); followed by 300˚C at the rate of 10˚C/min; and 300˚C, where it was held for 6 minutes.

The mass detector conditions were: Transfer line temperature 240 ˚C; ion source temperature 240 ˚C and ionization mode electron impact at 70 eV, a scan time 0.2 seconds and scan interval of 0.1 seconds. Total running time was 32 minutes. The scanned fragments ranged from 40 to 600 Da. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Perkin Elmer and the software used to handle mass spectra and chromatograms was a Turbo-Mass version 5.4.2.

2.3 Identification of chemical constituents

The bioactive compounds extracted from different extracts of *S. glauca* were identified based on the GC retention time. The spectrum of the components was compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

2.4 Antimicrobial activity

Based on the clinical and pharmacological importance three bacterial strains namely *Pseudomonas aeroginosa*, *Salmonella typhi*, *Salmonella paratyphi* and fungal strains namely *Fusarium oxysporum* and *Sclerotium rolfsii* were selected for screening. The microbial cultures were obtained from Tamil Nadu Agricultural University, Coimbatore. The bacteria were grown in nutrient broth at 37˚C and the slants were prepared on nutrient agar and maintained at 4˚C.

2.4.1 Antibacterial screening

The antibacterial activity of chloroform, ethyl acetate and methanol extracts was studied by well diffusion method [14, 15]. Agar plates were inoculated with 50µl of homogeneous inoculums (1.75 x 10^6 CFU/ml) of each bacterium and were spread using sterile swabs. Wells of 6 mm were made using sterile borer into agar plates and swabbed with bacterial inoculum. Five concentrations (50µl, 100µl, 150µl, 200µl and 250µl) of solvent extracts dissolved in DMSO (Dimethyl Sulphoxide) were transferred into each well. Ampicillin was used as positive control and the negative control used was DMSO. The experiment was carried out in triplicates.

2.4.2 Antifungal screening

The antifungal activity of the three extracts was determined by testing its efficiency on the growth inhibition of selected fungi. Two different plant pathogenic fungal strains namely *Fusarium oxysporum* and *Sclerotium rolfsii* were tested. These fungi sometimes even act as allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes leads to asthma [16]. *In vitro* biological activity of the extracts was assessed based on radial hyphal growth rate in the presence and absence of the extract. Percentage growth inhibition was calculated as follows:

% growth inhibition = (Mycelial growth control (MGC) – Mycelial growth sample)/MGC * 100

2.4.3 Statistical analysis

All the experiments were carried out in triplicates. The results are expressed as mean ± standard errors and the comparison of the antibacterial activity of the samples with standard antibiotic was evaluated by applying one way analysis of variants.

3. Results and Discussion

3.1 Percentage yield

From approximately 50gms of powdered sample, yield of 0.38% of chloroform extract, 0.32% of ethyl acetate extract and a high yield of 4.01% of methanol extract were obtained.

3.2 Bioactive compounds present in the extracts

The bioactive compounds present in the three extracts from *S. glauca* leaves are recorded (Table 1, 2 and 3). Based on the elution order in the Elite-5MS column, the compounds were identified and characterized. The retention time, molecular formula and the percentage area of the bioactive compounds are also presented. Based on the abundance, the top three compounds present in the chloroform extract were Oxalic acid hexyl neopentyl ester, Myristic acid vinyl ester, Dodecane 4,9-dipropyl. The ethyl acetate extract contained Sulfurous acid heptadecyl 2-pentyl ester, Dodecane, 4, 9-dipropyl, Heptadecane, 4-propyl. The methanol extract contained Cyclopropane, 1-(1, 2-dimethylpropyl)-1-methyl-2-nonyl, followed by Octatriacontane 1, 38-dibromo, Palmitic acid vinyl ester.

Table 1: Bioactive compounds of Chloroform extract of *S. glauca* leaves

Retention time	Area %	Name of the compound	Molecular weight
24.302	4.161	Tetradecane, 6,9-dimethyl	$C_{16}H_{34}$
24.908	23.557	Myristic acid vinyl ester	$C_{16}H_{30}O_2$
25.888	1.49	Hexadecanoic acid, (3-bromoprop-2-ynyl) ester	$C_{19}H_{33}O_2Br$
27.139	1.512	Hexadecanoic acid, 2-oxo-, methyl ester	$C_{17}H_{32}O_3$
28.799	3.176	Triarachine	$C_{63}H_{122}O_6$
29.284	10.72	Docosane, 6-methyl	$C_{23}H_{48}$
30.035	19.977	Dodecane, 4,9-dipropyl	$C_{18}H_{38}$
30.305	33.545	Oxalic acid, hexyl neopentyl ester	$C_{13}H_{24}O_4$
31.125	1.861	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]meth	$C_{55}H_{106}O_6$

Table 2: Bioactive compounds of Ethyl acetate extract of *S.glauca* leaves

Retention time	Area %	Name of the compound	Molecular weight
25.092	2.344	Hexadecanoic acid, 2-oxo-, methyl ester	$C_{17}H_{32}O_3$
27.623	3.019	Docosane, 7-butyl	$C_{26}H_{54}$
28.489	17.284	Dodecane, 4,9-dipropyl	$C_{18}H_{38}$
28.814	10.258	Octatriacontane, 3,5-dimethyl	$C_{40}H_{82}$
29.969	36.583	Sulfurous acid, heptadecyl 2-pentyl ester	$C_{22}H_{46}O_3S$
30.365	10.876	Heptadecane, 4-propyl	$C_{20}H_{42}$

Table 3: Bioactive compounds of Methanol extract of *S.glauca* leaves

Retention time	Area %	Name of the compound	Molecular weight
27.428	78.587	Cyclopropane, 1-(1,2-dimethylpropyl)-1-methyl-2-nonyl	$C_{18}H_{36}$
29.394	16.845	Octatriacontane, 1,38-dibromo	$C_{38}H_{76}Br_2$
31.07	11.649	Palmitic acid vinyl ester	$C_{18}H_{34}O_2$
31.29	9.764	3-heptadecenal	$C_{17}H_{32}O$

Figures 2, 3 and 4 depict the GC chromatograms of different solvent extracts of leaves of *S. glauca.*

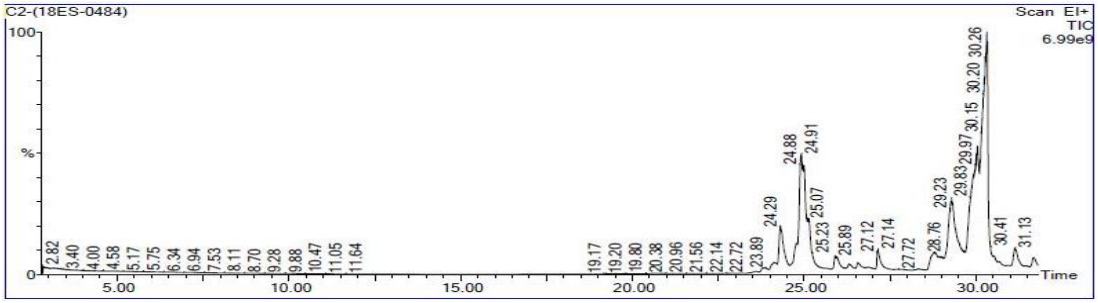


Fig 2: Gas Chromatogram of Chloroform extract

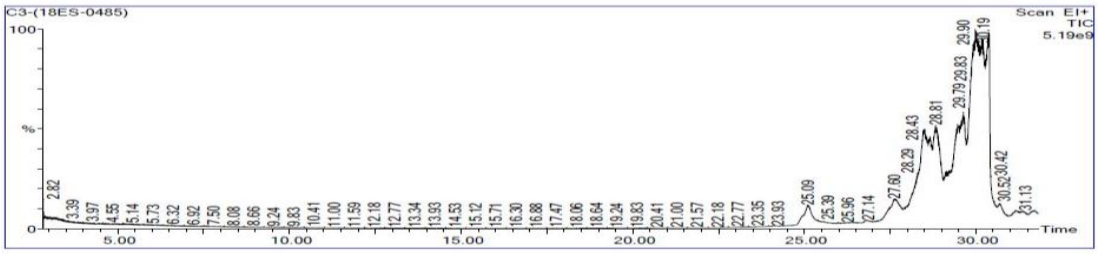


Fig 3: Gas Chromatogram of Ethyl acetate extract

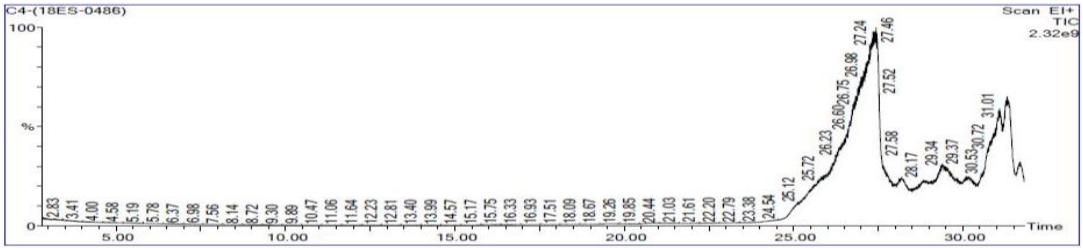
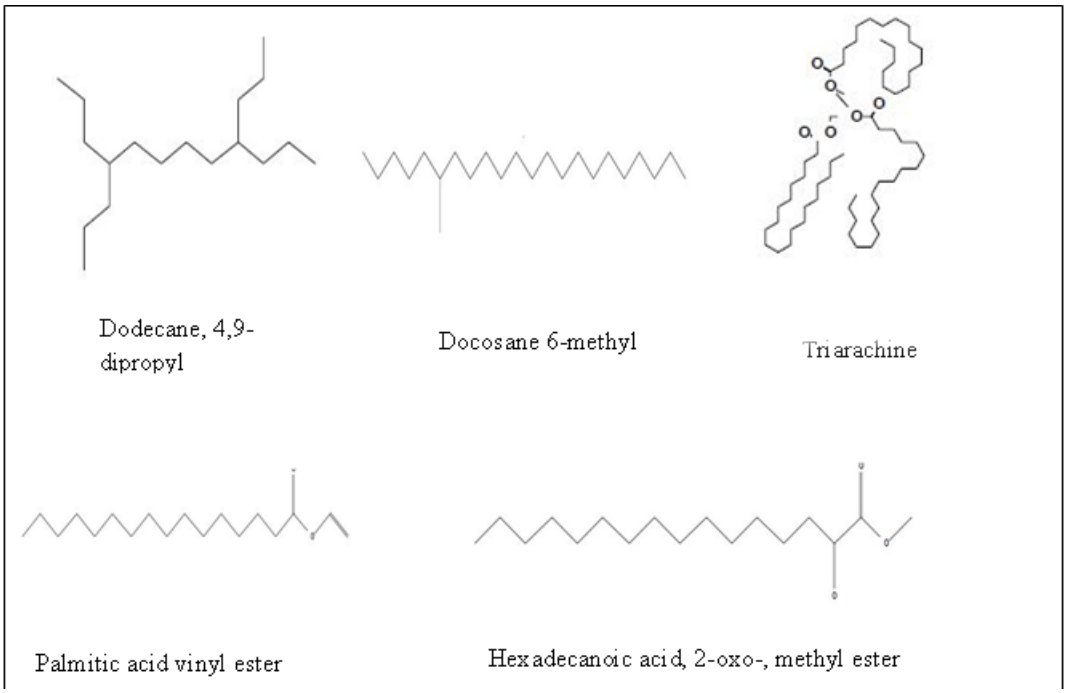


Fig 4: Gas Chromatogram of Methanol extract

Different crude extracts were obtained from the leaves of *S. glauca* through sequential solvent extraction in the increasing polarity namely Chloroform, Ethyl acetate and Methanol. GC-MS analysis revealed the presence of bioactive compounds in

all the three extracts. Dodecane, 4,9-dipropyl and Hexadecanoic acid, 2-oxo-, methyl ester were present in both chloroform (19.97%, 1.51%) and ethyl acetate (17.28%, 2.34%) extracts but in different quantities. The three extracts had no common key compound in them. The compounds revealed by GC-MS are biologically active and they possess pharmacological activities which also may be useful in self-healing of the plant. The Hexadecanoic acid, 2-oxo-, methyl ester, a fatty acid ester, has antioxidant, hypocholesterolemic, pesticide and 5 Alpha Reductase inhibitor activities [17]. The Palmitic acid vinyl ester, a fatty acid present in the methanolic extract possesses many therapeutic activities wherein it is used in throat disorders, as anti-asthmatics, anti-pruritic, anti-psoriatic, anti-epileptic, anti-convulsant and anti-migraine [18]. Docosane 6-methyl and Dodecane 4, 9-dipropyl act as Ubiquinol-cytochrome-c Reductase inhibitor which helps as a fungicide and anti-malarial agent [19]. Triarachine, a triglyceride, is considered to be safe and is used in cosmetics [20] and also plays an important role in metabolism as energy source and transporters of dietary fat [21]. The structures of some of the compounds analyzed by GC-MS are as follows:



3.3 Antimicrobial activities

3.3.1 Antibacterial activity

The antibacterial activity of different leaf extracts of the plant *S. glauca* was carried out by well diffusion method with ampicillin as positive control. All the three extracts exhibited good antibacterial activity and the measured zone of inhibition are recorded (Tables 4, 5 and 6).

Table 4: Antibacterial activity of Chloroform extract of *S. glauca* leaves

Pathogen	Concentrations (µl)					Ampicillin 5mg/ml
	Zone of inhibition in mm					
	50	100	150	200	250	
Pseudomonas aeroginosa	3±0.5	5±1.5	13±1.5	15±1.1	18±2.1	16
Salmonella typhi	5±1.1	10	14±1.1	16±2	18±2	16
Salmonella paratyphi	6±0.5	10±0.5	13±0.5	16±2.1	19±0.5	16

Data given are mean of three replicates ± Standard error

Table 5: Antibacterial activity of Ethyl acetate extract of *S. glauca* leaves

Pathogen	Concentrations(µl)					Ampicillin 5mg/ml
	Zone of inhibition in mm					
	50	100	150	200	250	
Pseudomonas aeroginosa	-	-	10±0.5	12±0.6	17±1.5	16
Salmonella typhi	.	-	9±0.5	14±0.5	18±2	16
Salmonella paratyphi	-	-	10±1.3	11±1.8	14±1.5	16

Data given are mean of three replicates ± Standard error

Table 6: Antibacterial activity of Methanol extract of *S.glauca* leaves

Pathogen	Concentrations(µl)					Ampicillin 5mg/ml
	Zone of inhibition in mm					
	50	100	150	200	250	
Pseudomonas aeroginosa	4±1	7±0.5	13±1.2	19±0.6	22±2.1	14
Salmonella typhi	5±0.6	12±2	16±1.5	17±1.5	19±1.5	14
Salmonella paratyphi	4±2.1	6±2.1	9±0.6	11±2.3	16±3.7	14

Data given are mean of three replicates ± Standard error

3.3.2 Antifungal activity

The antifungal activity of different extracts from the plant *S. glauca* was studied by radial hyphal growth method. All the three extracts showed good antifungal activity and the percentage growth of inhibition is shown in table 7, 8 and 9.

Table 7: Antifungal activity of Chloroform extract of *S. glauca* leaves

S. No	Pathogen	Concentration (250µl) % growth inhibition	
		Extract	Fungicide (Mancozeb 75% WP)
1	*Fusarium oxysporum*	71.8	74.1
2	*Sclerotium rolfsii*	77.0	78.2

Table 8: Antifungal activity of Ethyl acetate extract of *S. glauca* leaves

S. No	Pathogen	Concentration (250µl) % growth inhibition	
		Extract	Fungicide (Mancozeb 75% WP)
1	*Fusarium oxysporum*	68.2	74.1
2	*Sclerotium rolfsii*	83.9	78.2

Table 9: Antifungal activity of Methanol extract of *S. glauca* leaves

S.No	Pathogen	Concentration (250µl) % growth inhibition	
		Extract	Fungicide (Mancozeb 75% WP)
1	*Fusarium oxysporum*	70.6	74.1
2	*Sclerotium rolfsii*	81.6	78.2

The results revealed that all the three extracts were potentially effective in suppressing microbial growth with flexible potency. The chloroform and methanol extracts were effective against tested bacteria from a minimum concentration of 50µl whereas ethyl acetate extract was effective from 150µl. With the increase in the concentration of extracts the antibacterial activity also increased linearly. Ethyl acetate extract exhibited good antifungal activity when compared with the other extracts.

Ethyl acetate and methanol extracts showed more percentage growth inhibition of *Sclerotium rolfsii* when compared with the standard fungicide, which indicates that the extracts can be used as a fungicide causing less effect to the environment when compared to that of the chemical fungicide. The remaining extracts showed almost same percentage growth inhibition when compared to that of chemical fungicide. Comparatively the growth of *Sclerotium rolfsii* was inhibited at a higher rate than *Fusarium oxysporum*. The results suggest that *S. glauca* leaf extract can be helpful in treating diseases in plants caused by the above mentioned fungal pathogens.

4. Conclusion

Plant extracts and their active constituents were used as folk medicines in traditional therapies by 80% of the world's population which was reported by World Health Organization (WHO). In the present work, the medicinal application of this plant is supported by the above mentioned biological activities. From the results obtained, it can be concluded that the extracts of *S. glauca* leaves exhibited potential antimicrobial activities which may be justified due to the presence of the bioactive compounds that are analyzed through GC-MS analysis. Till date, very few reports are available on the chromatographic analysis of different solvent extracts of this plant. Some of the important compounds isolated by GC-MS analysis explain the correlation between the phytochemical constituents with their biological activities. The present study justifies the claimed uses of leaves of this plant in the traditional system of medicine to treat various infectious diseases caused by microbes and its use as an antioxidant, hypocholesterolemic, antiasthmatic, antipruritic, antipsoriatic, antiepileptic, anticonvulsant, antimigraine and as a pesticide. Further purification and structure elucidation of these compounds assists the basis in determining probable health benefits of the plant prominent to further biological and pharmacological studies along with the clinical trials of the effective compound.

Conflict of interest

The authors declare no conflict of interest.

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