



GC-MS analysis and Anti-Oxidant Activity of *Simarouba* *Glauca* Leaf Extract

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ABSTRACT

Various medicinal plants have been used for years in daily life against diseases, whole over the world. *Simarouba glauca* DC., (Family. *Simaroubaceae*) is very important medicinal plant and it is commonly known as *Simarouba* otherwise called as *paradise tree* or *Laksmi taru*, is a multi-purpose evergreen tree receiving great interest as a promising energy crop and medicinal plant for the future. *Simarouba* is a medium sized evergreen tree (height 7-15 m) with tap root system and cylindrical stem. *Simarouba glauca* can be cultivated in tropical regions all over the world. *Simarouba glauca* has a long history in herbal medicine in many countries. *Simarouba* has been used for various ailments like, anti -viral, anti -bacterial and anti-protosoal, anti-dysenteric and anti-malarial activities. It has aroused great enthusiasm as miraculous tree of solace for cancer patients. In the present study GC-MS analysis of Ethanolic extract of *Simarouba glauca* were carried out and revealed the maximum phytochemicals with their activity. Quantitative estimation of the *Simarouba glauca* leaves extracts showed the presence of Vitamin-A, Vitamin-B (B1, B2, and B3) and Vitamin-C was determined by using standard methods. Free -radical scavenging activities of *Simarouba glauca* were examined in the Ethanolic extract shows a good inhibitory activity against the DPPH radical.

Keywords: GC-MS analysis, quantitative estimation and Antioxidant activity

I. INTRODUCTION

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value ^[1]. Some of them are also used for prophylactic purposes. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice. Diseases that have been managed traditionally using medicinal plant include malaria, epilepsy, infantile convulsion, diarrhea, dysentery, fungal and bacterial infections ^[2]. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene, lactones and oils (essential



and fixed)^[3]. (Amrit pal Singh, 2005). India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants.

Simarouba glauca leaves and bark have a long history of medicinal use in the tropics, particularly in the treatment of malaria, fevers and dysentery; as an astringent to stop bleeding; and as a tonic. They are also used as a digestive, emmenagogue and to treat parasites both within and on the body. Research has discovered a range of medically active compounds in the plant. The main active compounds are a group of triterpenes called quassinoids. The anti-protozoal and anti-malarial properties of these chemicals have been documented for many years. Several of the quassinoids found in *simarouba*, such as ailanthinone, glaucarubinone, and holacanthone, are considered the plant's main therapeutic constituents and are the ones documented to be anti-protozoal, anti-amoebic, anti-malarial, and even toxic to cancer and leukaemia cells. Studies have shown that the plant is over 90% effective against amoebic dysentery. *Simarouba glauca* containing large number of such compounds as alkaloids, flavonoids, carbohydrates, glycosides, phenolic compound, tannins, triterpenoids, carotenoids, saponins, fixed oils . The chemicals present in leaf, fruit pulp and seed are known to possess the medicinal properties such as amoebicide, analgesic, anthelmintic, antibacterial, anti-dysenteric, anti-leukemic, antimalarial, antimicrobial, anti-tumorous, antiviral, astringent, cytotoxic, emmenagogue, febrifuge, skin hydrator, stomachic, sudorific, tonic, vermifuge. These are mainly involved in pharmacological activities of this plant. The present study was aimed to investigate the phytochemical constituents quantitatively and anti-oxidant activity.

II. MATERIALS AND METHODS

2.1 Plant collection and preparation of extract

Simaoruba glauca trees are cultivated in Lalbagh botanical garden Bangalore and fresh leaves were collected and authenticated at Rapinet Herbarium. Healthy plant leaves were collected, washed thoroughly in tap water and dried in room temperature for 30 days. The coarse powder (50g) of the leaves of *Simaruba glauca* was extracted successively with Ethanol, each 250 ml in a Soxhlet apparatus for 24 h. The extracts were filtered through Whatman No.41 filters paper. The extracts were concentrated in a rotary evaporator. The ethanolic extracts were analyzed by GC-MS analysis and anti-oxidant activity.

2.2 Estimation of vitamin analysis

Determination of Thiamine by the method of Okwu (2004)

5gm of *Simarouba glauca* leaves was homogenized in 50 ml ethanolic sodium hydroxide. It's 10 ml filtrate was added to 10 ml potassium dichromate and absorbance was recorded at 360 nm after development of color.

Determination of Niacin by the method of AOAC (1987)

5 gm of *Simarouba glauca* leaves was homogenized in sodium hydroxide and distilled water. The mixture was heated for 1 hour over a boiling water bath, cooled and pH was adjusted to 4.5. 17g of ammonium sulphate was added. Color development achieved by reaction of the extract with few drops of cyanogen bromide was observed and then was measured at 450nm in spectrophotometer.



Determination of Riboflavin by the method of Okwu (2004)

5 gm of the *Simarouba glauca* leaves was extracted with 100 ml of 50% ethanol solution and shaken for 1 hr. This was filtered into a 100 ml flask; 10 ml of the extract was pipetted into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% H₂O₂ were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% Sodium sulphate was added. This was made up to 50 ml and the absorbance was measured at 510 nm in a spectrophotometer.

Determination of Ascorbic acid by the method of Barkat et al., 1973.

5gm of *Simarouba glauca* leaves was taken into 100 ml EDTA/ TCA (2:1) and mixed well. This mixture was centrifuge at 3000 rpm for 20 min. It was transferred to 100ml volumetric flask and volume was made up. 20ml of this mixture with 1% starch solution was titrated with 20% CuSO₄ till the appearance of dark end point.

Determination of vitamin A by the method of (Bayfield and Cole, 1980).

Grind 1 to 5 gm of the *Simarouba glauca* leaves material to a fine paste and add 1.0ml of saponification mixture. Reflex the tubes gently for 20minutes at 600C and cool the tubes at room temperature added 20ml water and mix well. Extract vitamin with 10ml of petroleum ether in a separating funnel twice. Pool the extract and added sodium sulphate to remove the moisture for 30-60minutes evaporate 5ml aliquot of the ether extract to dryness at 600 °C dissolve the dried residue in 1.0ml of chloroform. Make up the volume in each test tube to 1.0ml with chloroform. Added 2.0ml of TCA solution from a fast delivery pipette, rapidly mixing the contents of the tube. Read at 620 nm immediately in a spectrophotometer.

2.3 Determination of anti-oxidant activity

The ability of the ethanolic extract of *Simarouba glauca* leaves to scavenge DPPH radicals was determined according to the method described by Shimada *et al.* (2001). Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_c - A_s}{A_c} \right) \times 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

2.4 GC-MS analysis.

The ethanolic leaves extract of *Simarouba glauca* was analyzed for its chemical constituents by GC-MS analysis. The GC-MS analysis was performed on a combined GC-MS instrument (ITQ 900 Model of Thermo Fisher Scientific make) using a HP-5 fused silica gel capillary column. The method to perform the analysis was designed for both GC and MS. 1 µL aliquot of sample was injected into the column using a PTV injector whose

temperature was set at 275°C. The GC program was initiated by a column temperature set at 60°C for 5 min, increased to 300°C at a rate of 8 C/min, held for 10 min. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode with mass source was set at 200°C. The chromatogram and spectrum of the peaks were visualized. The particular compounds present in the ethanolic extract of the leaves were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library.

III. RESULTS AND DISCUSSION

3.1 Quantitative analysis of *Simarouba glauca* leaves

Simarouba glauca leaves showed the presence of ascorbic acid, vitamin -A, riboflavin, Niacin and Thiamine. (Table 1) shows the different Vitamin concentration contained in *simarouba glauca* leaves. It was observed that the Vitamin-B complex family were found to be minimum concentration with Niacin (vitamin B3) having the maximum concentration of 1.65 ± 0.08 mg/g. Thiamine (Vitamin B1) also having the minimum concentration of 0.64 ± 0.05 mg/g. and contains significant amounts of Riboflavin (vitamin B2) contain 0.38 ± 0.03 mg/g respectively while vitamin A content had the moderate value 4.42 ± 0.15 mg/g and Ascorbic acid (vitamin C) having the highest concentration of 22.94 ± 0.07 mg/g.

Natural ascorbic acid is very good for body performance. Lack of ascorbic acid impairs the normal formation of intercellular substances in the body viz., collagen, bone matrix and tooth dentine. The striking patho-physiological change resulting from this defect includes the weakening of the endothelial walls of the capillaries due to reduction in the amount of intercellular substances. Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid. This function of ascorbic acid can also be accounts for its requirement in normal wound healing^[8]. So it has been proved that the availability of ascorbic acid in *Simarouba glauca* can be used as herbal medicine in future for the treatment of various ailments.

3.2 GC-MS analysis of *Simarouba glauca*

The components present in the ethanol extract of the leaf of *simarouba glauca* leaves identified by the GC-MS chromatogram are shown in Figure 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area as a percentage are presented in Table 2. The major phytochemicals and their biological activities obtained through the GC-MS analysis of the leaf of *simarouba glauca* have been tabulated Table 2.

3.3 Anti-oxidant activity of *Simarouba glauca*

The ethanolic extract of *simarouba glauca* leaves showed potent DPPH radical scavenging activity as shown in the Figure 2. The ethanolic extract of the leaves was found to have an IC₅₀ value of 51.50(µg/ml). The IC₅₀ value of the standard ascorbic acid was 46.01(µg/ml) (Table 3). From this study it was proved that the antioxidant



activity of *simarouba glauca* was a very efficient free radical scavenger due to the lowest IC₅₀ value. The activity of the reference antioxidant (Ascorbic acid) was much higher when compare with the ethanolic extract.

Table1: Vitamin analysis of *simarouba glauca* leaves

S.No	Vitamins	<i>simarouba glauca</i> (leaf)(mg/100gm)
1	Riboflavin	0.38 ± 0.03
2	Niacin	1.65 ± 0.08
3	Thiamine	0.64 ± 0.05
4	Vitamin -A	4.42 ± 0.15
5	Ascorbic acid	22.94 ± 0.07

Values are expressed Mean ± SD for triplicates

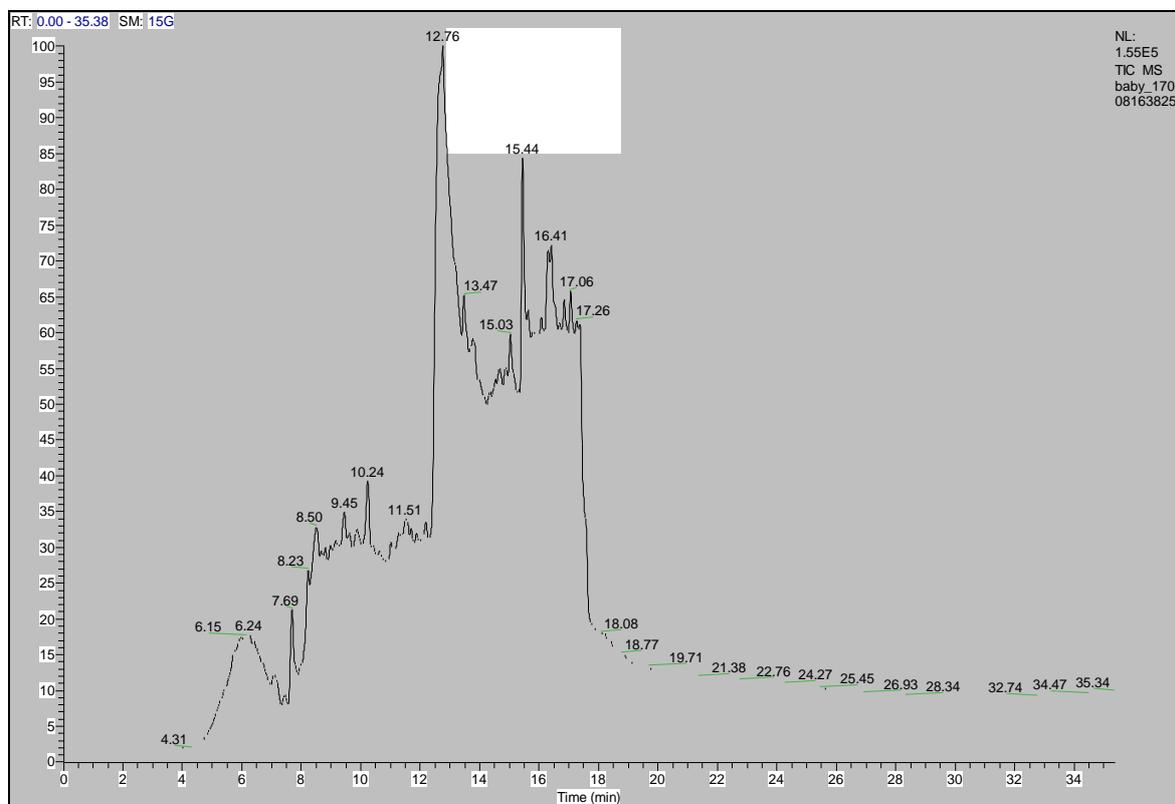


Figure 2: GC-MS chromatogram of *simarouba glauca* ethanolic leaf extract.



Table: 2 Phytoconstituents present in ethanolic leaf extract of simarouba glauca

S.No	RT	Name of a compound	Molecular Formula	MW	Peak Area %	Uses
1.	7.62	Benzenamine,N-ethyl Aniline	C ₈ H ₁₁ N	121	7.69	Anti –oxidant Activity
2.	8.40	Ethylenimine,Aziridine	C ₂ H ₅ N	43	8.50	Not reported
3.	9.37	2,6,6-Trimethyl – bicyclo (3,1,1)hept-3 ylamine,3-pinanylamine	C ₁₀ H ₁₉ N	153	9.45	pheromonal activity.
4.	10.14	2,2-Bis[4- trimethylsiloxy) phenyl propane],2,2-Bis[4-trimethylsilyloxy)phenyl]propane	C ₂₁ H ₃₂ O ₂ Si ₂	372	10.24	Anti-bacterial and anti-cancer.
5.	12.74	4-(3-pentyl)pyridine,pyridine,4-(1-ethylpropyl)	C ₁₀ H ₁₅ N	149	12.76	pheromonal activity.
6.	13.41	Benzyl-[1-methyl-(4methyl-cyclohex-3-enyl)-ethyl]-amine,N-Benzyl-2-(4-methyl-3-cyclohexan-1-yl)-2-propanamine	C ₁₇ H ₂₅ N	243	13.47	Not reported
7.	14.99	Dihydrocodeine,Morphinan-6-ol,4,5-epoxy-3-methoxy-17-methyl	C ₁₈ H ₂₃ NO ₃	301	15.03	Not reported
8.	15.40	Acetic acid,2-(2-bromoacetyl)-5-diethylaminophenyl ester,2-(Bromoacetyl)-5-(diethylamino) phenyl acetate	C ₁₄ H ₁₈ BrNO ₃	327	15.44	Not reported
9.	17.01	5Alpha-androatan-3,17-dione 17-monooxime,Androstane-3,17-dione 17-oxime	C ₁₉ H ₂₉ NO ₂	303	17.06	Neurological disease treatment

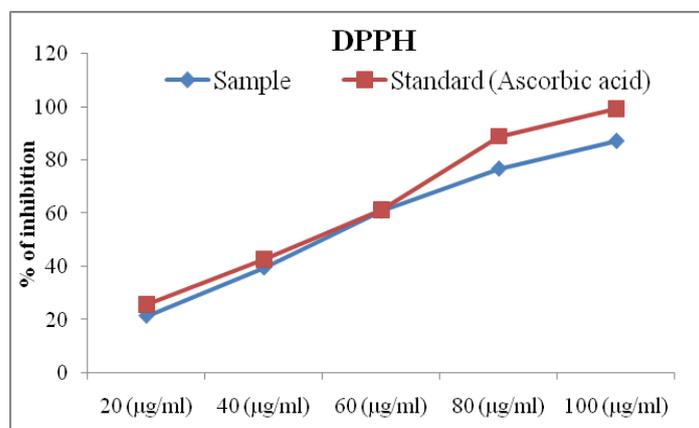


Figure 2: DPPH radical scavenging activity



Table: 3 Percentage of DPPH Radical scavenging activity of ethanolic extract of *simarouba glauca* leaves.

Parameters	20 ($\mu\text{g/ml}$)	40 ($\mu\text{g/ml}$)	60 ($\mu\text{g/ml}$)	80 ($\mu\text{g/ml}$)	100 ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
<i>simarouba glauca</i> leaf extract	21.37 \pm 1.49	39.55 \pm 2.76	60.91 \pm 4.26	76.82 \pm 5.37	87.28 \pm 6.10	51.50
Standard (Ascorbic acid)	25.6 \pm 2.04	42.73 \pm	61.26 \pm 4.90	88.98 \pm 7.11	99.34 \pm 7.94	46.01

Values were expressed as Mean \pm SD for triplicates

IV. CONCLUSION

In this study, the presence of various soluble bioactive compounds in *simarouba glauca* leaf extracts greatly contributed to antioxidant activity. In the ethanolic extract, more soluble phytochemicals were noticed. The results clearly support the use of *simarouba glauca* in traditional medicinal practices for the treatment of various diseases. The GC-MS analysis exhibit that the leaves contain secondary metabolites with anti-microbial, anti-viral, anti-oxidant, anti-cancer activity and thus are sources of natural bioactive molecules to control pathogens that cause diseases in humans.

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