Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4224-4227
Received: 07-03-2019
Accepted: 09-04-2019

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Proximate and phytochemical analysis of an Anticancerous *Simarouba glauca* leaves

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Abstract
Simarouba glauca is commonly known as '*Laxmitaru*' or 'paradise tree' and belongings to the family Simaroubaceae. Simarouba was first used as a remedy for dysentery, colitis and malaria. Infusion of the leaves or bark is considered to be astringent and used as a digestion and menstrual stimulant and an antiparasitic remedy. It is taken internally for diarrhea, dysentery, malaria and colitis. The leaves (and occasionally the bark) are boiled in water to yield a powerful astringent tonic used to wash skin sores and to treat stomach and bowel disorders, hemorrhages and internal bleeding. The *S. glauca* has promising antioxidant activity. The extract of *S. glauca* has been used in Guatemala for the treatment of gastrointestinal disorders. In the present study the proximate and phytochemical analysis of simarouba leaves have been done. Both fresh and shade dried simarouba leaves were subjected to proximate analysis such as moisture, protein, fat, ash, fibre and total carbohydrates using standard reference protocols of AOAC (2000) and phytochemical properties such as total phenols, antioxidant activity and total flavonoids were also been analysed using standard protocols. From the results, it is evident that the plant *Simarouba glauca* leaves are found to have maximum amount of phytochemicals.

Keywords: *Simarouba glauca*, proximate analysis and phytochemical constituents

Introduction
India possesses a rich biodiversity of the medicinal plants that are still not explored completely. Medicinal plants have been a valuable source of natural products of maintaining human health. Nowadays, the need for natural products of pharmaceutical purposes of the plant has attained a great interest in the present research world due to the cost and the higher side effects that is associated with the chemically manufactured drugs. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosoids, and volatile oils [Siripong *et al.*, 1992] [15]. It is necessary to identify that bioactive constituent of medicinal plants usually employed by herbalists in the treatment of infectious diseases. Simarouba (*Simarouba glauca*) is an evergreen, multi-purpose edible oil tree, also known as Aceituno, bitterwood, dysentery bark, Palo Amargo, paradise tree, pitomba, robleceillo, simaba and Shorgum Maram (Osagie-Eweka *et al*., 2016) [11]. *Simarouba glauca* is commonly known as '*Laxmitaru*' or 'paradise tree' and belongings to the family Simaroubaceae. The species name *glauca* means covered with the bloom which refers to the bluish green foliage. It is derived from Greek word '*glaukos*' (bluish). It is indigenous to Southern Florida, the West Indies and Brazil. It is native to the Bahamas, Costa Rica, Cuba, EI-Salvador, Guatemala, Haiti, Honduras, Jamaica, Mexico, Puerto Rico and United States of America but exotic to India, Myanmar, The Phillippines and Srilanka. It grows under tropical conditions in Central America spreading from Mexico to Panama, Southern Florida as well as Caribbean Islands. *S. glauca* was introduced in African countries like Burundi and Kenya. In India, it was first introduced in 1966 at Amravati Research Station, Maharashtra by National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The cultivation was extended to other parts of Maharashtra, Rajasthan, West Bengal and Orissa. Now the cultivation of *S. glauca* is also spread to semi-arid dry and saline land areas of Gujarat, Tamil Nadu, Karnataka and Andhra Pradesh. *S. glauca* tree has an ability to grow well even in marginal wastelands or dry lands with degraded soil. Simarouba cultivation came to the University of Agricultural Sciences, Bangalore during the year 1986. However, systematic research and developmental activities of *S. glauca* begun only from the year 1992 (Joshi and Hiremath, 2000) [6]. Simarouba is an evergreen tree which grows to a height of 12-15 m with a large circular crown. Leaves are pinnately compound with 3-21 leaflets oblong and often notched or smooth at apex; alternate, even, bluish oily green. Leaves and bark of simarouba have long been used as natural medicines in tropics. Simarouba was first used as a remedy for dysentery, colitis

and malaria. Infusion of the leaves or bark is considered to be astringent and used as a digestion and menstrual stimulant and an antiparasitic remedy. It is taken internally for diarrhea, dysentery, malaria and colitis. The leaves (and occasionally the bark) are boiled in water to yield a powerful astringent tonic used to wash skin sores and to treat stomach and bowel disorders, hemorrhages and internal bleeding. It is used externally to heal wounds and sores. Simarouba leaf extract is used for reducing patchy skin pigmentation (Manasi and Gaikwad, 2011) [10]. Simarouba water extract was found to increase skin keratinocyte differentiation and to improve skin hydration and moisturization. The seeds extracted in alcohol are used against snake bites. It was reported that the leaf, fruit, pulp and seed of *S. glauca* possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent, emmenagogue, stomachic, tonic and vermifuge (Joshi and Joshi, 2002) [7]. *Simarouba glauca* leaf have 11 medicinally important quassinoids namely, glaucarubin, quassinoids, ailanthinone, benzoquinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide and sitosterol. *Simarouba glauca* extract has been reported for the presence of alkaloids, flavonoids, cardenolides, glycosides, phenolic compounds, saponins and fixed oils (Manasi and Gaikwad, 2011) [10]. The extract of *S. glauca* has been used in Guatemala for the treatment of gastrointestinal disorders. Glaucarubin, a crystalline glycoside isolated from *S. glauca* leaf have amoebicidal properties. Several quassinoids from *S. glauca* leaf and seed have exhibited cytotoxic activity *in vitro* against KB cells (human oral epidermoid carcinoma), including glaucarubin, glaucarubinone, glaucarubol and glaucarubolone. The esters of glaucarubolone, ailanthinone and glaucarubinone exhibited significant activity *in vivo* in the P388 lymphocytic leukemia model. Chloroform-soluble extract of *S. glauca* exhibited significant cytotoxicity against several human cancer cell lines (Kupchan *et al*., 1976) [9]. The quassinoid, 2-acetylglaucarubine was found significantly to inhibit growth of murine lymphocytic leukemia (Polonsky *et al*., 1975) [12]. The ethanol extracts of *Simarouba glauca* significantly ($p<0.05$) inhibited T-24 bladder cancer cell line with IC50 value of 533.55 ± 25.02 µg/ml (Puranik *et al*., 2017) [13]. Hence, Simarouba glauca is very effective in reducing the size of tumors and secondary infections in cancer patients. It is very effective in curing cancer of first/second stages, whereas in later stages it can considerably increase the quality of life. In the present study, we have concentrated on the preliminary screening of proximate compounds and secondary metabolites from *Simarouba glauca* leaves.

Materials and Methods

A. Sample Collection

Well matured *Simarouba glauca* leaves were harvested from trees of UAS, GKVK campus, Bengaluru.

B. Drying of Simarouba Leaves

Well matured leaves were harvested, washed thoroughly in water, drained, spread over trays (~2 mm bed thickness) and dried in a convective tray dryer by setting the tray dryer temperature to approximate room temperature (shade drying) i.e., 32 °C, until attainment of desirable moisture content (~10%). During drying, care was taken to avoid any contaminations. After drying, the dried leaves were packed in polyethylene bags and stored under refrigerated condition (~4 °C) for further research.

C. Proximate analysis of leaves

Both fresh and shade dried simarouba leaves were subjected to proximate analysis such as moisture, protein, fat, ash, fibre and total carbohydrates using standard reference protocols of AOAC (2000) [3] and the average values of three replications were reported in percentage wet basis (% wb). The moisture content was determined by measuring weight loss of sample in a moisture box by desiccation in an oven maintained at 105 ºC until constant weight. The nitrogen value, which is measure of crude protein content of a substance, was determined by Micro-Kjeldahl method involving digestions, distillation and finally titration of the sample. About 250 mg sample with 7 ml conc. H2SO4 and a Kjeldahl digestion mixture (1:20 of CuSO4 and K2SO4) was digested at 300-400 °C until the mixture was clear. Digested sample were brought to Kjeldahl distillation and titration unit (Make: Pelican, Model: Kel plus elite Ex) where titrations were carried out using 45% NaOH, 4% H3BO3 and 0.1 N HCl reagents. The nitrogen value was converted to protein by multiplying a factor of 6.25. Soxhlet method was used for the determination of total fat. Weighed samples were dried and treated with extraction reagent (petroleum ether) in a fat extractor (Make: Pelican, Model: Socs Plus SCS 6). The total ash was determined by measuring inorganic residue left in crucible after removing organic matter of a sample by heating on normal flame and burning at 550ºC for 5 h in a muffle furnace. Crude fibre was determined by defatting the well dried samples, separating the residue in fiber extractor (Make: Pelican, Model: Socs Plus SCS 6) by treating with H2SO4 (1.25%) and KOH (1.25%) solutions under hot condition and intermittent hot water and cold water washings. This was followed by filtration, drying and measuring the mass loss by burning in muffle furnace at 550 ºC for 5 h. Total carbohydrate was determined by difference method [100 - (moisture + protein+ crude fat + fiber + ash)]. Total solids/dry matter was calculated by deducting percentage moisture from 100.

D. Phyto-Chemical Analysis of Simarouba Leaves

1. Total phenols (mg Gallic Acid Equivalents /100 g)

Total phenols of fresh and dry simarouba leaves were estimated by spectrophotometric method at 700 nm absorbance (Singleton and Rossi, 1965) [14] using Folin Ciocalteu Reagent (FCR). About 1 to 2 g of fresh or 0.5 g of dried leaf sample was ground in a pestle and mortar and extracted with 20 ml of methanol (80%) for 2-3 times. The extracts were pooled and the volume was made up to 50 ml. One ml of extract was taken and diluted with 80% methanol. 0.5 ml of diluted extract was taken in test tube, 0.2 ml of Folin-Ciocalteau's Phenol Reagent was added followed by 3.3 ml of distilled water and mixed well. After 2 min, 1 ml of sodium carbonate solution was added and thoroughly mixed. The mixture was incubated at room temperature for 30 minutes and the intensity of blue colour was measured in a spectrophotometer (Make: Systronics, Model: UV-VIS 118) at 700 nm. Preparation of standard curve for phenols was done using gallic acid (GA) as standard and a total phenols was calculated using below equation.

$$\text{Total flavonoid content (mg Quercetin equivalents/100g)} = \frac{\text{OD510nm} \times \text{Standard value (mg/OD)} \times \text{Total Volume of extract} \times 100 \times \text{Dilution factor}}{\text{Assay volume} \times \text{Weight of sample (g)}}$$

2. Total flavonoids (mg Quercetin equivalents /100 g)

Total flavonoids of fresh and dry simarouba leaves were estimated by spectrophotometric method (Chun *et al*., 2003)[5]. One to two g of fresh or 0.5 g of dried leaf sample was ground in a pestle and mortar and extracted with 20 ml of methanol (80%) for 2-3 times. The extracts were pooled and the volume was made up to 50 ml. One ml of extract was taken and diluted with 80% methanol. One ml of extract was taken in test tube, 0.3 ml of 5% $NaNO_2$ was added and 0.3 ml of 10% $AlCl_3$ was added for 2 minutes. After another 2 min, 3.4 ml of 4N NaOH was added. The mixture was incubated at room temperature for 10 minutes. The intensity of brick red colour was measured at 510 nm against blank. The standard curve for flavonoids was prepared by using quercetin as standard and total flavonoids was calculated using below equation.

$$\text{Total flavonoid content (mg Quercetin equivalents/100g)} = \frac{\text{OD510nm} \times \text{Standard value (mg/OD)} \times \text{Total Volume of extract} \times 100 \times \text{Dilution factor}}{\text{Assay volume} \times \text{Weight of sample (g)}}$$

3. Antioxidant capacity (mg AEAC equivalents/ 100 g)

Antioxidant capacity of a sample was expressed in terms of DPPH (2, 2-diphenyl-1-picrylhydrazyl) activity. Antioxidant capacity of fresh and dried simarouba leaf was estimated by spectrophotometric method (Kang and Saltveit, 2002)[8]. One to two g of fresh or 0.5 g of dried leaf sample was homogenized with 50 ml of 80% methanol. One ml of extract was taken and diluted with 80% methanol. 0.5 ml of diluted extract was taken in test tube and 0.3 ml of acetate buffer was added followed by 2.5 ml of DPPH solution and mixed well. The absorbance of the solution was read spectrophotometrically at 517 nm after 30 minutes of incubation (A1). The absorbance of DPPH solution without sample (A2) was also determined. The difference in the absorbance of DPPH solution with and without sample (A2–A1) was calculated. The decrease in absorbance with sample addition was used for calculation of antioxidant activity. Standard curve was prepared using different concentrations of ascorbic acid (20-100 µg/ml). The results were expressed as ascorbic acid equivalent antioxidant capacity and antioxidant capacity was calculated using below equation.

$$\text{Antioxidant capacity (mg AEAC equivalents/ 100g)} = \frac{\text{OD517nm} \times \text{Standard value (mg/OD)} \times \text{Total volume of extract} \times 100 \times \text{Dilution factor}}{\text{Assay volume} \times \text{Weight of sample (g)}}$$

Results and Discussion

Recent years have seen an increase in epidemiological evidence of close relationship between several active biomolecules of plant origin and human health, particularly in treating cancer with minimal side effects. This has led to exploration of plants for their active biomolecules for development of new plant derived pharmaceuticals and herbal formulations. The present investigation aims at the evaluation of proximate compounds and secondary metabolites of *Simarouba glauca* using multiple complementary tests, where the previous studies are sparse.

The health benefit results from the secondary metabolites synthesized in the plant that includes the bioactive constituents like phenols, flavonoids and antioxidants.

Simarouba glauca leaves, both fresh and shade dried, were subjected to proximate analysis. Moisture content, protein, fat, fibre, ash, carbohydrates and dry matter of simarouba leaves were studied and the results are presented in Table1. The fresh leaves contained 65.35% moisture while shade dried leaves contained 9.95% moisture. The total dry matter content of fresh and dry leaves was 34.65 and 90.0%, respectively. The proximate composition of shade dried simarouba leaves was: protein: 12.6%, fat: 4.53%, fibre: 28.49%, ash: 3.86% and carbohydrates: 40.52%.

Table 1: Proximate analysis of *Simarouba glauca* leaves

Particulars	Fresh Leaves	Shade Dried Leaves
Moisture content (%)	65.35 ± 3.83	9.95 ± 0.28
Protein (%)	6.07 ± 0.53	12.6 ± 0.35
Fat (%)	ND	4.53 ± 0.096
Fibre (%)	ND	28.49 ± 0.54
Ash (%)	ND	3.86 ± 0.69
Carbohydrates (%)	NE	40.52
Dry matter (%)	34.65 ± 3.83	90 ± 0.28
ND = Not determined		
NE = Not estimated		

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, Alkaloids protected against chronic disease. Saponins protect against hypercholesterolemia and antibiotic properties (Aiyer *et al.*, 1962)[1]Steroids and triterpenoids show the analgesic properties. The Steroids and saponins were responsible for central nervous system activities (Akindele*et al.,* 2007)[2], flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities **(**Argal and Pathak, 2006)[4]Phenols and Antioxidants present in leaf serves as free radical scavengers.The present study carried out the Simarouba glauca leaf extract revealed the presence of medicinal active constituents. It could be seen that the leaves contained good amounts of phytochemicals.The phytochemical active compounds of Simarouba glauca were qualitatively analyzed for leaf separately and the results are presented in table 2.

i. Total phenols

In the present study, the content of the total phenolics in fresh and dried leaves were determined using Folin–Ciocalteau method and expressed as gallic acid equivalents. Total phenolics content of different *S. glauca* extracts were solvent dependent. The shade dried leaves contained higher phenolic content of 288.99±27.08 µg/mg GAE compared to fresh leaves of 151.07±11.05 µg/mg GAE.

ii. Total flavonoids

Flavonoids, the secondary plant phenolics considered to be contributing factors for antioxidant and chelating properties. Total flavonoids were expressed as quercetin equivalents. The total flavonoid content were found to be 88.03 ± 12.00 mg Quercetin/g and 102.84 ± 4.00 mg Quercetin/g for freah and shade dried leaves respectively.

iii. Antioxidant activity

Antioxidant activity is expressed as mg Ascorbic acid equivalent antioxidant capacity/g. Antioxidant Radical scavenging activities of *S. glauca* leaf extracts was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antioxidant activity of fresh leaves was found to be 128.75 ± 58.68 mg AEAC/g and 205.08 ± 41.02 mg AEAC/g for shad e dried leaves.

Table 2: Phytochemical analysis of *Simarouba glauca* leaves

Particulars	Fresh Leaves	Shade Dried Leaves
Total phenols (mg GAE/g)	151.07 ± 11.05	288.99 ± 27.08
Total flavonoids (mg Quercetin/g)	88.03 ± 12.00	102.84 ± 4.00
Antioxidant activity (mg AEAC/g)	128.75 ± 58.68	205.08 ± 41.02
GAE = Gallic acid equivalents		
AEAC = Ascorbic acid equivalent antioxidant capacity		

Conclusion

Simarouba glauca also known as 'paradise tree' is a multi-purpose evergreen tree receiving great interest as a promising energy crop as well as medicinal plant. Simarouba leaves and bark have been used as a natural medicine. Leaves of simarouba contain good amount of proximate compounds and phytochemicals which possess anticancer, antioxidant and thrombolytic activities and therefore, it has aroused great enthusiasm as miraculous tree of solace for cancer patients. Simarouba glauca are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs for treatment of various ailments and diseases.

References

1. Aiyer KN, Kolammal M. Pharmacognosy of Ayurvedic Drugs, Dept of Pharcognosy, Uty. of Kerala, Trivandrum, 1962.
2. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus*. Fitoterapia. 2007; 78:25-28.
3. AOAC. Official Methods of Analysis, 18th edn, Association of Official Analytical Chemists, Gaithersburg, MD, USA, 2000.
4. Argal A, Pathak AK. CNS activity of *Calotropis gigantean* roots. J Ethno pharmacoogyl. 2006; 106:142-145.
5. Chun OK, Kim DO, Moon HY, Kang HG, Lee CY. Contribution of individual polyphenolics to total antioxidant capacity of plums. Journal of Agricultural and Food Chemistry. 2003; 51(25):7240-7245.
6. Joshi S, Hiremath S. Simarouba - A potential oilseed tree. Current Science. 2000; 78(6):694-697.
7. Joshi S, Joshi S. Oil Tree – *Laxmitaru glauca*. University of Agricultural Sciences, Bangalore and Indian Council of Agricultural Research, New Delhi, India, 2002, 86p.
8. Kang HM, Saltveit ME. Antioxidant capacity of lettuce leaf tissue increases after wounding. Journal of Agricultural and Food Chemistry. 2002; 50(26):7536-7541.
9. Kupchan SM, Lacadie JA, Howie GA, Sickles BR. Structural requirements for biological activity among antileukemic glaucarubolone ester quassinoids. Journal of Medicinal Chemistry. 1976; 19(9):1130-1133.
10. Manasi PS, Gaikwad DK. A Critical review on medicinally important oil yielding plant Laxmitaru (*Simarouba glauca* DC.). Journal of Pharmaceutical Sciences. 2011; 3(4):1195-1213.
11. Osagie-Eweka SDE, Orhue NJ, Ekhaguosa DO. Comparative phytochemical analysis and *in-vitro* antioxidant activity of aqueous and ethanol extracts of *Simarouba glauca* (paradise tree). European Journal of Medicinal Plants. 2016; 13(3):1-11.
12. Polonsky J, Baskevitch Z, Gottlieb HE, Hagaman EW, Wenkert E. Carbon-13 nuclear magnetic resonance spectroscopy of naturally occurring substances. XXXI. Carbon-13 nuclear magnetic resonance spectral analysis of quassinoid bitter principles. The Journal of Organic Chemistry. 1975; 40(17):2499-2504.
13. Puranik SI, Ghagane SC, Nerli RB, Jalalpure SS, Hiremath MB. Evaluation of *in vitro* antioxidant and anticancer activity of *Simarouba glauca* leaf extracts on T-24 bladder cancer cell line. Pharmacognosy Journal. 2017; 9(6):906-912.
14. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16(3):144-158.
15. Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor WC. Cytotoxic diterpenoid constituents from A. Paniculata Nees leaves. J. Sci. Soc. Thailand, 1992; 18:187-194.