iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 5 **Issue:** XI **Month of publication:** November 2017

DOI: http://doi.org/10.22214/ijraset.2017.11074

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor:6.887
Volume 5 Issue XI November 2017- Available at www.ijraset.com

Qualitative and Quantitative Phytochemical Analysis of Simarouba Glauca Leaf Extract.

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Abstract: ***Simarouba glauca is well known as paradise tree in India and other parts of world. It has been used for various ailments like, including gastrointestinal to complains, amoebicide, analgesic, anthelmintic, anti-bacterial, anti-dysenteric, anti-leukemic, anti-malarial, anti-microbial, anti-tumorous, anti-viral, astringent. In the present study the quantitative and qualitative analysis of the plant has been done. In qualitative analysis, the phytochemical compounds such as, reducing sugars, carbohydrates, protein, alkaloids, phenolic compounds, flavonoids, saponins, tannins, phytosterols and glycosides were screened in five solvent extracts. The Ethanolic extract from the leaf showed positive results of 7 phytochemical tests. Ethyl acetate and chloroform leaf extract exhibited positive results for 5 tests. Hexane and Dichloromethane extract of the leaf showed positive results for very less phytochemical compounds. The quantification of the compounds like alkaloids, saponins, total phenols and total flavonoids has also been done. From the results, it is evident that the plant Simarouba glauca leaves are found to have maximum amount of phytocomponents.***

Keywords: Phytochemical constituents, Simarouba glauca, qualitative and quantitative analysis

I. INTRODUCTION

India possesses a rich biodiversity of the medicinal plants that were 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 [1, 2, 3, 4]. According to WHO (World Health Organization), 80% of the people rely primarily on traditional health care system and mostly on herbal medicines [5]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosoids, and volatile oils [6, 7]. It is necessary to identify that bioactive constituent of medicinal plants usually employed by herbalists in the treatment of infectious diseases.

glauca is a species of flowering tree that is native to Florida in the United States, southern Florida, South America, and the Lesser Antilles. Common names include Paradise Tree, Aceituno, and Bitter wood. Its seeds produce edible oil. The tree is well suited for warm, humid, tropical regions. Its cultivation depends on rainfall distribution, water holding capacity of the soil and sub-soil moisture. It is suited for temperature range of 10 to 50 °C. It can grow at elevations from sea level to 1,000 m (3,300 ft). It grows 40 to 50 ft (12 to 15 m) tall and has a span of 25 to 30 ft (7.6 to 9.1 m). It bears yellow flowers and oval elongated purple colored fleshy fruits. Simarouba glauca decoction is also used in traditional medicine in the treatment of cancer and tumors. Simarouba leaf decoction when taken in limited amounts can raise the immunity, reduce appetite loss and increase quality of life in cancer patients. It can also be used to complement modern cancer treatment. Simarouba glauca has very good anti-bacterial, anti-tumorous properties. 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, quantitative determination, and the qualitative separation of primary and secondary metabolites from leaves of selected medicinal plants.

II. MATERIALS AND METHODS

A. Sample Collection:

Simarouba glauca trees are cultivated in Lalbagh botanical garden Bangalore and fresh leaves were collected and authenticated with the help of Rapinat herbarium.

B. Extraction of plant material

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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 from Dichloromethane, Chloroform, Hexane, Ethyl acetate and Ethanol, each 250 ml in a Soxhlet apparatus for 24 hs. All the extracts were filtered through What man No.41 filters paper and extracts were concentrated on a rotary evaporator. The extract was stored at the refrigerator for further studies.

C. Qualitative analysis of primary and secondary metabolites:

The leaf extracts from Simarouba glauca were analyzed for the presence of alkaloids, carbohydrates, saponin, protein, phenolic compounds, flavonoids and glycoside according to the common phytochemical methods described by Harborne (1998)

D. Quantitative Determination of Simarouba glauca:

1) *Determination of Alkaloid by the method of Harborne[8] (1973):* 5 gs of the Simarouba glauca leaves powdered was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solutions were allowed to settle and the precipitated were collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.
2) *Determination of Saponin by the method of Obadoni and Ochuko [9](2001):* 5 gs of *Simarouba glauca* leaves powder were put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hswith continuous stirring at about 55^0C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{o}C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant.
3) *Determination of total phenols by spectrophotometric method of Kim et al., [10] (2003):* A diluted Simaroubaglaucaleaves extract (1 ml) or Gallic acid standard phenolic compound was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was mixed in to the test sample solution was diluted to 25 ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. Total phenol content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The Total Phenolic content was expressed as milligrams of Gallic acid (GAE) equivalents per gram dried sample.
4) *Determination of total Flavonoids by the method of Katasani[11](2001):* Simaroubaglaucaleaves extract (0.5 ml) were mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions to concentrations 20 to 80 µg/ml in methanol**.** The Total flavonoids content was expressed as milligrams of quercetin equivalents per gram of dried sample.

III. RESULTS AND DISCUSSION

A. Qualitative phytochemical analysis

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 **[12].** Steroids and triterpenoids show the analgesic properties. The Steroids and saponins were responsible for central nervous system activities **[13]**, 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 **[14].**

The present study carried out the Simarouba glauca leaf extract revealed the presence of medicinal active constituents. The phytochemical active compounds of Simarouba glauca were qualitatively analyzed for leaf separately and the results are presented

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in Table 1. In these screening process alkaloids, tannins, saponins, flavonoids and carbohydrate, glycosides, phenols shows different types of results in different solvents.

Dichloromethane extractsis found to have minimum amount of phytocomponents like alkaloids, carbohydrate and reducingsugar. Ethanolic extracts was found to have a wide range of bioactive compounds like alkaloids, flavonoids, carbohydrates, reducing sugar, saponins, protein, and glycoside. Chloroform extracts was positive about reducing sugars, carbohydrate, protein, glycoside, phenolic compoundsand tannins. Ethyl acetate extract was positive about carbohydrate, reducing sugar, saponin and protein.The hexane extract being highly non-polar in nature was able to extract very less compound characterized like alkaloids, carbohydrate, reducing sugar, phenolic compounds and tannins. The presence of bioactive constituents indicates that the *Simarouba glauca*can be used in a multitude of ways for the beneficiary of population.

B. Quantitative phytochemical analysis

The leaf extract has been further subjected to quantification of major phytocompounds like alkaloids, total flavonoids, phenols and saponin was determined by using standard methods. Secondary metabolites analysis is necessary for extraction, purification, separation, crystallization, identification of various phytocompounds. Alkaloids protected against chronic diseases [15] and earlier recorded that bitter leaf contains an alkaloid that is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [16]. The level of alkaloids was 94.5 ±0.1024mg/g in the leaf extracts as shown in the Table-2. Saponins were responsible for central nervous system activities [12]**.** The level of saponin was 61.1 ±0.0823 mg/g in the leaf extracts.

The amount of total phenol was determined with Folin-Ciocalteu reagent. The calibration curve obtained for gallic acid is shown in figure 1. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: y = 0.016x+0.255, R^2= 0.997 (Figure: 1) where y is absorbance at 750 nm and x is total phenolic content in the extracts of Simarouba glauca expressed in mg/gm. The total phenolic content was 102.3±0.0027 mg/g in the leaf extracts, respectively shown in Table-2. The higher amount of phenol is important to the regulation of plant growth, development and disease resistance. Consumption of diets rich in plant polyphenols offers protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases.

The amount of total flavonoid was determined with aluminum chloride reagent. The calibration curve for Quercetin is shown in figure2. Quercetin was used as a standard compound and the total flavonoid were expressed as mg/g Quercetin equivalent using the standard curve equation: y = 0.022 xs+0.192. R^2 = 0.996 (Figure 2). Where y is absorbance at 510 nm and x is total flavonoid content in the leaf extracts Simarouba glauca expressed in mg/gm. The total flavonoid content was 86.3±0.2937mg/g in the leaf extracts, respectively (Table- 2). Flavonoids had been reported to exert wide range of biological activities. These include: Anti-inflammatory, antibacterial, antiviral, anti-allergic [17-19], cytotoxic anti-tumour, treatment of neurodegenerative diseases, vasodilatory action [20-22]. The phytochemical analyses of the medicinal plants is important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. Thus, we hope that the important phytochemical properties identified in the present study ofSimarouba glaucaleaves will be helpful in the treatment of various ailments.

Table- 1: Preliminary phytochemical analysis of *simarouba glauca* leaf extract

Phytochemical Tests	Ethanol	Ethyl acetate	Dichloromethane	Chloroform	Hexane
Alkaloids (Mayer's reagent)	+	-	+	_	+
Carbohydrates (Molisch's test)	+	+	+	+	+
Sugar (Benedict's reagent)	+	+	+	+	+

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Saponin (foam test)	+	+	_	_	_
Protein (Millon's test)	+	+	_	+	_
Phenolic compounds and tannin (Ferric chloride test)	_	_	-	+	+
Flavonoid (Alkaline reagent test)	+	_	_	_	_
Glycoside (Legal's test)	+	+	_	+	_

(Presence of Phytoconstituents = +) (Absence of phytoconstituent = -)

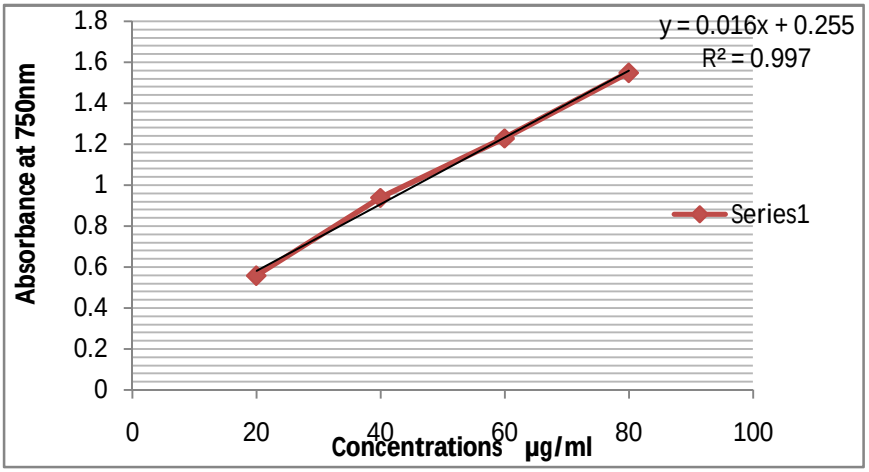


Fig 1: Standard Curve for Total Phenol using Gallic Acid

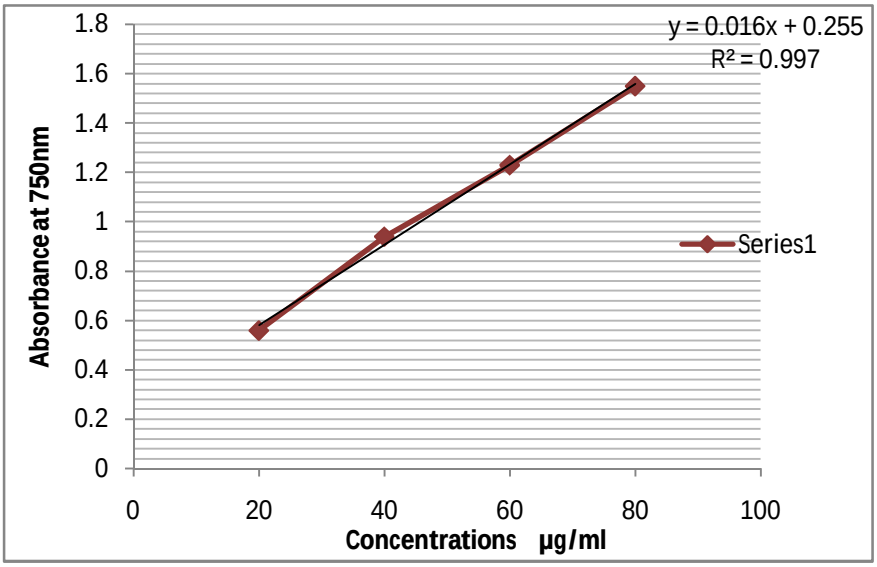


Fig 2: Standard Curve for Flavonoids using Quercetin

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Table - 2: Quantitative determination of *Simaroubaglauca* leaves

S.No.	Name of the phytochemicalconstituents	Results(mg/gm)
1	Alkaloids	94.5±0.1024
2	Saponin	61.1±0.0823
3	Total Phenols	102.3±0.0027
4	Total Flavonoids	86.3±0.2937

Values are expressed Mean ± SD for triplicates

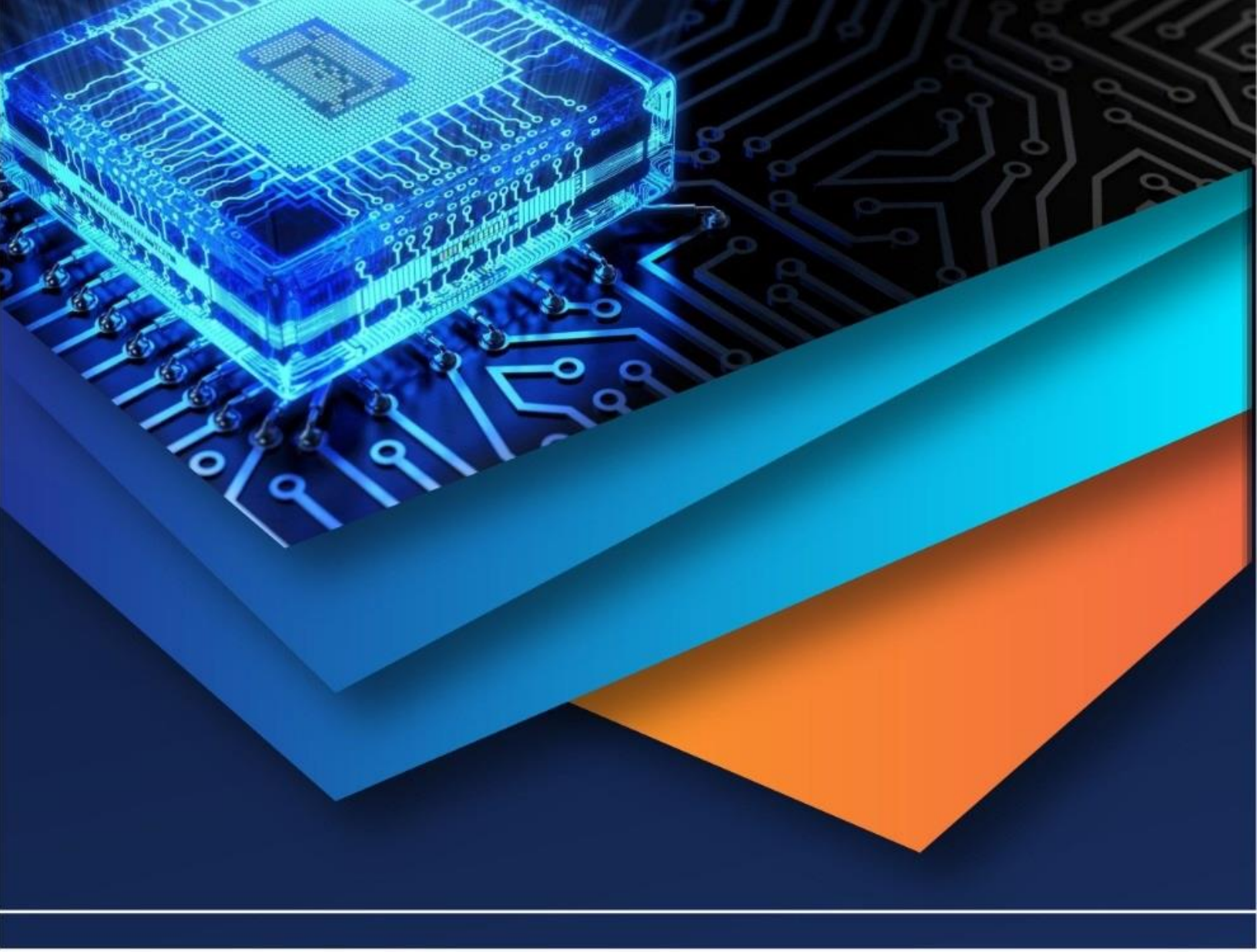
IV. CONCLUSION

The qualitative analysis of the five different leaf extract of Simarouba glaucareveals the presence of medicinally valued bio active components like, flavonoids, tannins, alkaloids, saponin, reducing sugar, phenolic compounds and glycosides. Among the five different extracts of Simarouba glauca, the ethanolic extract of the leaf showed maximum amount phytochemicals was screened in Simarouba glauca. Quantitative estimation Simarouba glauca leaf contained higher content of phenols and alkaloids with lesser amount of saponins and total flavonoids. 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 diseases. The research is in progress to discover its biological activity and enhance the pharmacological profile of it in the area of traditional medicine.

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