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Phytochemical Analysis and Anti-microbial Activity of Leaves Extract of *Simarouba gluaca* DC

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Abstract: *Simarouba gluaca* DC *has a long history in herbal medicine in many countries including India. The leaves, fruit pulp and seeds of the plant are known to possess the medicinal properties such as analgesic, antibacterial, antidysenteric, antileukemic, antimalarial, antimicrobial, antitumorous, and antiviral activities. Very less work has been done in India regarding phytochemicals present in Simarouba gluaca* DC. *So the current research work aims to analyse phytochemicals present, and to study and antimicrobial activity of the leaves extracts. Young and healthy leaves were air dried in shed and powdered. The powder was extracted using different solvent as n-hexane, water and methanol. Phytochemical analysis carried out for all three extracts had shown presence of secondary metabolites like alkaloids, flavonoids, phenolic compounds, saponin, alkaloids and tannins. Further, antimicrobial activity was carried out using bore-well method against the pathogenic bacteria like E. coli and fungi like Aspergillus niger. The results were obtained in methanolic extract against E. coli.*

Keywords: Simarouba gluaca, anti-microbial activity, phytochemical analysis

I. INTRODUCTION

India is a country having a wide herbal medicinal history as “Ayurveda”. Many plants and their parts have been explored and exploited for their medicinal importance. The belief that herbal medicines have no side effect and side effects of allopathic medicines have increased the use of herbal medicines. *Simarouba glauca* is an evergreen tree and has a long history of herbal medicine. Various secondary metabolites such as alkaloids, steroids, flavonoids, terpenoids, glycoside, saponia, tannins, phenolic compounds etc synthesized by medicinal play important role as therapeutic agent [12].

II. REVIEW OF LITERATURE

Simarouba glauca (Family: Simaroubaceae) is a medium-sized tree that grows up to 20 m high, with a trunk 50 to 80 cm in diameter. It produces bright green leaves 20 to 50 cm in length, small white flowers, and small red fruits. This tree species is a native of Central and South America and found under a wide range of conditions and at low to medium elevation from Southern Florida to Costa Rica, Caribbean islands, Bahamas, Jamaica, Cuba, Hispaniola, Puerto Rico, Nicaragua, Mexico, EI Salvador etc. It was first introduced by National Bureau of Plant Genetic Resources in the Research Station at Amravati, Maharashtra in 1960s. It is also called as Paradise Tree or Lakshmi Taru [4].

The leaves and bark of *Simarouba* have long been used as a natural medicine in tropics. It is taken internally for diarrhea, dysentery, malaria and colitis. It is used externally for wounds and sores [5]. *Simarouba gluaca* is well known for its anticancer, analgesic, anti-inflammatory, antipyretic, astringent, hemostatic, antiparasitic, antiviral, antimicrobial, and antioxidant properties [1, 7].

Traditionally for therapeutic applications of the medicinal plants normally crude extracts are in use. These extracts contain large number and variety of bio-components. Concentration of these components varies from plant part to part and changes with climatic conditions. So for effective use of medicinal plants in various applications, identification and quantification of phytochemical compounds are crucial.

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As compare to other medicinally useful plants, *Simarouba gluaca* is unexplored one. Few references are available in India based on research work done on *Simarouba gluaca*. Therefore for the current research project this plant is selected to explore its biological activities and to detect the phytochemicals present.

III. MATERIALS AND METHODS

Collection of Plant material:

Simarouba gluaca DC was obtained from Ram Biotech Lab Nashirabad Jalgoan and authenticated from experts of Botany Department of the college.

Extraction:

Young and healthy leaves were washed thoroughly 2-3 times with running water followed by distilled water and finally shed air-dried. The leaves were finely powdered and used for extraction. The powdered samples (25gm/250ml) were extracted using Soxhlet apparatus in various solvents (n-hexane, and methanol) for 8 hours. Distilled water extract was obtained by macerating the powder on shaker (25gm/250ml) in distilled water. The extracts were concentrated using rotary vacuum evaporator (Evator).These extracts were used for further analyses.

Qualitative phytochemical analysis:

The various phytochemical tests were performed in triplicates for establishing a profile of the extracts for their secondary metabolite composition.

- **Alkaloids:** Alkaloids were detected by Dragendorff's test by adding 1ml sample to 1 to 2ml of Dragendorff's reagent. A prominent yellow precipitate indicated positive test.
- **Saponin:** For saponin detection, extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. A layer of foam was observed for presence of saponins.
- **Phenolic compounds:** Phenolic compounds were detected by lead acetate test by adding 3ml sample to 3ml 10% lead acetate solution. A bulky white precipitate indicated the presence of phenolic compounds.
- **Flavonoids:** Alkaline reagent test was used to detect flavonoids. Five ml filtrate was heated with 1ml NH4OH solution. Yellow fluorescence indicated the presence of flavonoids [2, 8].
- **Tannins:** Tannins were detected by adding 1ml sample, 1ml Folin-Denis reagent and 2ml 35% sodium carbonate solution. Blue colored indicated presence of tannin [9].

Estimation of secondary metabolites:

Estimation of all secondary metabolites mentioned below was done in triplicates.

- **Alkaloids:** Alkaloids were estimated [3] by adding 1ml sample, 1ml 0.025M FeCl3 in 0.5M HCl and 1ml 0.05M 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath maintained at 70ºC ± 2ºC temperature. Absorbance was read at 510nm. Colchicine was used as standard. The values were expressed as colchicine equivalent (CE) in mg/g of the extract.
- **Tannins:** Tannins were estimated [9] by adding 1ml sample, 75ml water, 5ml Folin-Denis reagent and 10ml 35% sodium carbonate solution. Volume was made to 100ml using distilled water. After shaking well it was kept for incubation at room temperature for 30min in dark. Absorbance was read at 700nm. Tannic acid (5mg%) was used as standard and values expressed as tannic acid equivalent (TAE) in mg/g of the extract.
- **Phenolic compounds:** Total phenolic content was estimated [3] by adding 1ml sample, 5ml of Folin-Ciocalteu reagent, 4ml of 7.5% sodium carbonate solution and 1ml methanol. The test tubes were incubated for 30min at room temperature to complete the reaction. Absorbance was read at 765nm. Gallic acid (1mg%) was used as standard and values were expressed as gallic acid equivalent (GAE) in mg/g of the extract.
- **Flavonoids:** Flavonoids were estimated [3] by adding 1 ml sample, 0.1ml 10% aluminum chloride, 0.1ml 1M potassium acetate and 2.8ml distilled water. Test tubes were incubated at room temperature for 30min.

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Absorbance was read at 415nm. Rutin (1mg%) was used as standard and values were expressed rutin equivalents (RE) in mg/g of the extract.

Determination of antimicrobial activity:

The antibacterial activity of the extracts (5µg/ml) was carried out against *Escherichia coli* (NCIM 2931) and *Aspergillus niger* by the bore well method. Streptomycin (5µg/ml) was used as standard [6].

IV. RESULTS

Yield of extracts:

Yield and changes in colour of the extracts after completion of reaction with the suitable reagents were recorded. All the extracts were found to be sticky. The color of the extract varied from green to brownish green with respect to the solvent used. Percentage yield of methanolic extract was highest followed by water, and hexane. The crude extract quantity, purity, and quality greatly depend on the plant part used and the solvent used for the extraction [13]. Moreover, the polarities of the solvents, the chemical compositions of the sample also determine the yield of extraction [10]. The results also showed that the extraction yields obtained was affected by the solvent used. High yield of extract was obtained in polar solvents as compared to non-polar solvents.

Table No. 1: Yield percentage of different solvent extracts of *Simarouba gluaca* DC.

Extracts	Yield Percentage
n-Hexane	2.824%
Methanol	4.04%
Water	3.932%

Qualitative phytochemical analysis:

To detect presence or absence of secondary metabolites like alkaloids, saponins, tannins, falvonoids and phenolic compounds in the extracts qualitative phytochemical analysis was carried out. Alkaloids, phenolic compounds, tannins and flavonoids were present in all extracts while saponin was present in n-hexane and water extract only. It was not detected in methanolic extract.

Table No. 2: Qualitative phytochemical analysis of *Simarouba gluaca* DC leaves extracts

Secondary metabolite	n-Hexane	Methanol	Water
Alkaloids	+	+	+
Saponins	+	–	+
Phenolic Compounds	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+

+ indicates presence and – indicates absence

Estimation of secondary metabolites:

Secondary metabolites play important role in various applications. So estimation of secondary metabolites was carried out. Maximum concentration of tannins, flavonoids and phenolic compounds were observed in methanolic extract whereas maximum concentration of alkaloids was found in water extract of *Simarouba gluaca* DC leaves.

A good solvent is characterized by optimal extraction and its capacity in conserving the stability of chemical structure of desired compound [11]. So quantitative estimation was done for alkaloids, tannins, total phenols, and flavonoids. The type of solvent and its polarity have a significant impact on levels of poly phenols, flavonoids, tannins and alkaloids. It was found that water extract had highest alkaloid content amongst the 3 extracts whereas tannins, total phenolic content and flavonoids were found to be maximum in methanol extract. It was elucidated that the water, and methanolic (all proton donars) extracts showed higher phenolic content than n-hexane (proton acceptor) extracts, because of their lower efficiency of solubility [11].

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Table No. 3: Estimation of secondary metabolites from *Simarouba gluaca* DC leaves extracts

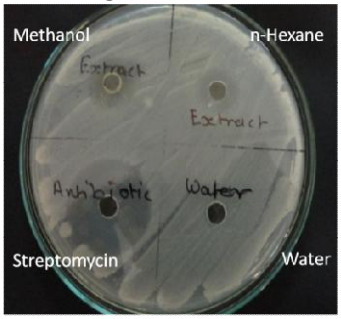
Secondary metabolites	n-Hexane	Methanol	Water
Total alkaloids (mg CE/g)	1.21±0.13	1.83±0.10	2.22±0.08
Tannins (mg TAE/g)	0.03±0.003	0.12±0.002	0.09±0.003
Total phenolic compounds (mg GAE/g)	1.46± 0.05	3.12±0.04	2.58± 0.01
Total Flavonoids (mg RE/g)	1.80±0.04	3.00±0.03	1.29±0.05

Determination of antimicrobial activity:

Antimicrobial activity was observed against *E. coli* in methanolic extract but in other extract antimicrobial activity not seen which might be due to low concentration of the components required to kill the *E. coli* in the n-Hexane and water extract. The zone of inhibition observed was 0.5 mm in diameter. All three extracts might contained low concentration of the components required to kill *Aspergillus niger*, so zone of inhibition was not observed.

The antimicrobial activity is higher in methanol extracts when compared with other extracts. This may be because some active compounds being polar, readily dissolve in methanol. The methanol extract of *S. glauca* might have caused significant membrane damage to the cell wall of the cocci, which would have resulted in the leakage of cytoplasmic materials such as nucleic acids. Moreover, *S. glauca* plant extract is known to possess quassinoids, a terpenoid that could plausibly be one of the main reasons for its antibacterial action [6].

Figure No. 1: Antimicrobial activity of *Simarouba gluaca* DC leaves extracts



V. CONCLUSION

Based on the data of the current investigation, it is strongly evident that *Simarouba gluaca* DC leaves extracts are good source of bioactive components like flavonoids, tannins, saponins, phenolic compounds, and alkaloids. Antimicrobial activity against only E. coli suggest that extract concentration used in the current study had low concentration of the bioactive components required to microorganisms. So for antimicrobial activity extract concentration should be increased. Future studies should focus on isolation and purification of these compounds for therapeutic efficacy.

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