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Original Article

QUALITATIVE CHARACTERIZATION OF PHYTOCHEMICALS AND *IN VITRO* ANTIMICROBIAL EVALUATION OF LEAF EXTRACT OF *COUROUPITA GUIANENSIS* AUBL. - A THREATENED MEDICINAL TREE

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ABSTRACT

Objective: Screening of phytochemicals present in aqueous extract and evaluation of the antibacterial and antifungal activities from different organic extracts of leaf of *C. guianensis* Aubl.

Methods: Antimicrobial activity of different extracts was evaluated by using the disc diffusion assay. Methanolic, ethanolic and chloroform extracts of leaf were tested against fungus and representatives of Gram-positive and Gram-negative bacteria.

Results: Presence of alkaloids, flavonoids, glycosides, phlobatannins, steroids, tannins and terpenoids was observed in aqueous extract of leaf. Chloroform extract showed better activity against Gram-positive bacteria in comparison to Gram-negative bacteria. Methanolic extract was more effective on Gram negative bacteria. Leaf extract was also effective against *Candida species*. Minimum inhibitory concentration was 25 mg/ml for ethanolic, 50 mg/ml for methanolic and 100 mg/ml for chloroform extracts against *S. aureus.*

Conclusion: Present study of *C. guianensis* seems to be promising for pharmaceutical industries for making an antimicrobial drug or cream especially against *S. aureus* and provides details of pharmacological investigation, identification, isolation and characterization of novel bioactive compounds.

Keywords: *Couroupita guianensis*, Phytochemicals, Antibacterial activity, Antifungal activity, Leaf extract, Threatened plant, Medicinal plant.

INTRODUCTION

Bacterial and fungal infections are the most common cause for illness of humans, animals and plants. These microorganisms sometimes create very serious problem. The alarming world-wide spread of drug-resistant bacteria and limited access to anti-infective drugs emphasizes the importance of discovering new antimicrobial compounds [1]. Plants have always been an important source of medicines since ancient times and 70 % of the worldwide population still relies on one or other forms of traditional plant based medicine [2]. There is a variety of pharmaceutically important molecules, but only a small percentage of plants have been explored for their phytochemical constituents and activities [3]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. [4]. The trend of using natural products is increasing because of its negligible side effect. The principles of many products in plants are secondary metabolites such as alkaloids, flavonoids and terpenoids which are responsible for antimicrobial activity [5]. Since ancient time, active plant extracts are frequently used in traditional medicine system and screened for new drug discoveries.

Couroupita guianensis Aubl. (family-Lecythidaeae) popularly known as the cannon ball tree, is a highly medicinal tree. This tree is commonly known as Nagalingam Pushpam in Tamilnadu because its shape of flower. Almost all parts of this plant like leaf, flower, bark, stem and fruit-shell are used in the treatment of various ailments. People from an Amazonian region and other states of the north region of Brazil use infusions or tea obtained from the leaves, flowers, and barks of *Couroupita guianensis* to treat hypertension, tumours, pain and inflammatory processes [6]. Chemical studies of this species have shown the presence of α-amirin, β-amirin, β-sitosterol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, carotenoids and sterols [7-12]. In leaf, triterpenoid ester of fatty acids such as β-amirin palmitate has been reported [13]. Bark of *C. guianensis* possesing antimicrobial and antioxidant activity [14] and fruit having antibacterial activity [15] have also been reported. Immunomodulatory activity [16] and antioxidant activity [17] in flowers have been also reported.

The leaves of *C. guianensis* possess herbal hand wash formulation and yielded an aliphatic triterpene. Methanolic extract of root has been observed with an anti-depressant in mice [18]. The phenolic compounds obtained from extract of *C. guianensis* have been reported having anti-inflammatory activity and curing the kidney and stomach ailment [5].

The biological functions of flavonoids, apart from their antioxidant properties, include protection against allergy, inflammation, platelet aggregation, infections, ulcers, heptatotoxins and tumors [19]. It is known that one of the active constituents of the medicinal plant *C. guianensis,* namely isatin**,** is known to exert cytotoxic activity against certain cancer cell lines, being a potential source of new chemotherapeutic agents [20].

Although antibacterial activity in leaf extract was studied [21] but they did not used chloroform as solvent. In the light of above observation, the present work was undertaken to study the phytochemical characterization and antibacterial and antifungal activity of leaf extracts of this plant in different solvents namely methanol, ethanol and chloroform.

MATERIAL AND METHODS

Collection of plant material

Young leaves of *Couroupita guianensis* Aubl. were collected from the campus of Banaras Hindu University (BHU), Varanasi, India in the month of April. Leaves were dried in shade condition at room temperature for 4-5 d and then dried at 40-45 °C for 2 h. Leaves were crushed to coarse powder using mechanical grinder. Powder was stored at room temperature in air tight container.

Preparation of organic solvent extract

Extracts were prepared by taking 20 g leaf powder for extraction process. Extraction was done in 200 ml of different solvents for 8 h using soxhlet apparatus. Ethanol, methanol and chloroform were used as extraction solvents. Extracts were dried in vacuum

evaporator at 40 °C. Extracts were stored at -20 °C till use. Percentage yield (w/w) of crude extract was calculated by using following formula.

$$PY = \frac{\text{Wt of crude extract recovered (g)}}{\text{Wt of powder used (g)}}$$

Where, *PY* is percentage yield of extract.

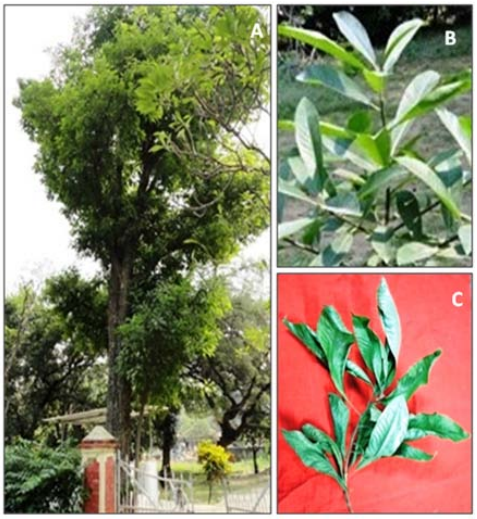

Fig. 1: Source of extract material A) Tree of *Couroupita guianensis* Aubl. B) Green leaves C) Shade dried leaves

Aqueous extraction of plant material for phytochemical screening

Aqueous extract was used for phytochemical screening. For preparation of aqueous extract, five gram of leaf powder was soaked in double distilled water for 50 h in air tight bottle and left at room temperature and filtered with eight layers of muslin cloth. Extract was stored at -20 °C till use.

Preliminary Phytochemical screening

Preliminary phytochemical screening was carried out by using aqueous extract to identify various constitutes using standard methods [22-24].

Screening of antimicrobial activity

Preparation of sample extract for microbiological assay

For screening of antimicrobial activity, sample extract was prepared in following manner. In brief**,** stock solution of extract was prepared in concentration of 100 mg/ml in dimethyl sulphoxide (DMSO). From which, about 5 µl extracts was dispensed onto sterile disc for susceptibitlity test. Standard drugs were prepared in concentration of 1 µg/µl.

Test microorganisms

Some selected Gram positive, Gram negative bacteria and fungus were used for screening anti microbial activity. Four Gram positive bacteria (*Staphylococcus aureus ATCCC 25323, Enterobacter aerogenes, E. fecalis, S. faecalis*), three Gram negative bacteria (*Salmonella Typhimurium, Klebsiella pneumoniae, Escherichia coli ATCC 35218*) and three fungal strains (*Candida albicans ATCC 90028*, *Candida tropicalis ATCC 750, Candida parapsilosis ATCC 22019*) were used for investigation. All microbial cultures were obtained from the Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The young bacterial broth cultures were prepared for screening experiments.

Media used

Media was prepared by dissolving Mueller Hinton agar 38 g/l and 10 g/l in double distilled water and saline was prepared by dissolving 8.5 g/l in double distilled water. LB broth (25 g/l) media was prepared in double distilled water and autoclaved for 15 min at 15 psi and 121 °C. The plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA).

Preparation of inoculums

Bacterial and fungal inocula were prepared by growing cells in Mueller Hinton Agar (MHA) (Himedia, Mumbai) for 24 h at 37 °C. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland turbidity standards (~1 x 10^7 CFU/ml).

Antibacterial and antifungal sensitivity test

Antibacterial activity was tested using disc diffusion method [25]. The test cultures were swabbed on the top of the solidified media and allowed to dry for 5 min. About 5 µl of extract was loaded to each disc. The loaded discs were placed on the surface of the medium. Negative control was prepared using the respective solvents. The plates were incubated for 24 h at 37 °C for bacteria and for 48 h at 28 °C for fungi. Zones of inhibition were recorded in millimeters.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by using 200 mg/ml extract on 96 well U bottom microtitre plates (Tarson, Mumbai, India). LB broth medium in the volume of 100 µl was added to each well and testing extract was added in serial dilution manner. Finally, inoculum of *S. aureus* bacterium was added to each well for determination of MIC. Plate was incubated at 37 °C for 24 h.

Statistical analysis

All experiments were done in triplicate and repeated thrice in independent manner. Data was analysed using SPSS software (version 16, Chikago, USA). Analysed data was represented as mean±SE.

RESULTS AND DISCUSSION

After extraction, extraction yield was calculated. In different solvents, maximum percentage (18.04 %) was found in methanol followed by ethanol (16.02 %) and minimum in chloroform (12.03 %). Percentage yield depends on the nature of solvent used for the extraction and the temperature of extraction. Extraction yield was also calculated in leaf and fruit extract of *Sapindus mukorossi* by other author [26].

Phytochemical screening

The phytochemical screening results showed the presence of medicinally active constituents like alkaloids, flavonoids, tannins, phlobatannins, steroids, terpenoids and absence of saponins in the aqueous extract of leaf (table 1). There are other reports of phytochemical screening of *C. guianensis* in leaf [27] and stem [19]. Similar to our results, absence of saponin was reported in aqueous extract of stem of *C. guianensis* [19]. However, presence of saponin and absence of alkaloids was reported from acetonic and methanolic extract of leaf of *C. guianensis* [27]. The distribution of bioactive compounds differs from plant to plant and extraction solvents of these compounds may be different for different plants. Phytochemical compounds found in plants may not be necessarily required for normal functioning of the plant, but have a beneficiary effect on health or play an active role in amelioration of disease [28].

Antibacterial and antifungal activity

All extracts of *C. guianensis* have shown antibacterial and antifungal activity. Leaf extract of *C. guianensis* exhibited promising activity against bacteria and fungi using disc diffusion method. The activity of all extracts against bacteria and fungi are given in table 2. Among all extracts of leaf chloroform extract showed maximum inhibition zone on *S. aureus* (Fig.2 A). Leaf extract of *C. guianensis* was more effective on Gram positive bacteria than Gram negative bacteria. Similar observation have been made by many researchers that Gram positive bacteria are more susceptible to plant's extracts as compared to Gram negative bacteria [29-30]. Chloroform extract showed higher antibacterial activity, it could be due to nature of extraction solvent.

Antimicrobial activity of leaf extract was also observed by [21] and reported significant activity on different bacteria. Antibacterial activity of chloroform extract of *C. guianensis* fruits was also reported [31].

The leaf extract of *C. guianensis* showed antimicrobial activity against *S. aureus, S. Typhimurium and S. faecalis*. These bacteria are known pathogen.

Table 1: Phytochemical screening from leaf extract of *Couroupita guianensis*

Chemical constituent	Tests	Aqueous extract of leaf
Alkaloids	Mayer's reagent	Strongly present
	Wagner's reagent	Strongly present
Flavonoids	Lead acetate solution	Moderately Present
Glycosides	Liebermann's test	Present
Phlobatannins	Hydrochloric acid	Present
Saponins	Foam test	Absent
Steroids and Terpenoids	Chloroform and sulphuric acid	Present
Tannins	Ferric chloride	Present

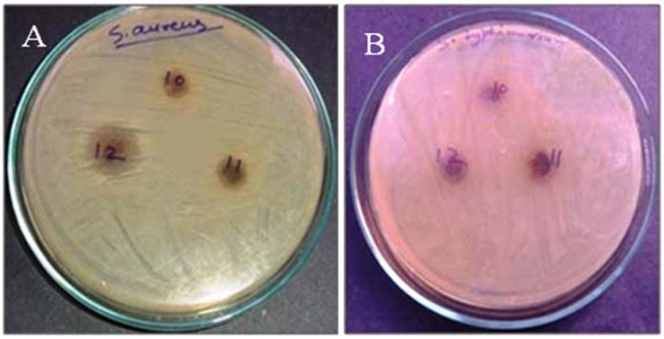

Fig. 2: Antibacterial activity of leaf extract of *C. guianensis:* 10, 11, 12 represent methanolic, ethanolic and chloroform extract respectively. A) Inhibition zone in *S. aureus* B) *S. Typhimurium*

Table 2: Antibacterial and antifungal activity of different extract of *C. guianensis* leaf (5 µl of 100 mg/ml extract)

Test organisms	Inhibition zone (mm)			
	Methanolic extract	Ethanolic extract	Chloroform extract	Standard drugs (5 µl/disc)
Gram positive				
S. aureus	09.00±0.57	10.00±0.06	16.00±0.57	22.66±0.88 (Ampicilin)
E. aerogens	00.00±0.00	00.00±0.00	00.00±0.00	25.00±0.58(Ampicilin)
S. faecalis	07.00±0.46	07.00±0.26	07.00±0.40	19.50±0.29 (Ampicilin)
E. faecalis	07.00±0.52	07.00±0.34	08.00±0.61	21.23±0.67 (Ampicilin)
Gram negative				
S. Typhimurium	09.00±0.17	11.00±0.57	10.00±0.50	26.93±0.58 (Ciprofloxacin)
E. coli	00.00±0.00	00.00±0.00	00.00±0.00	26.33±0.33 (Ciprofloxacin)
K. pneumoniae	00.00±0.00	00.00±0.00	00.00±0.00	24.86±0.13 (Ciprofloxacin)
Fungus				
C. albicans,	00.00±0.00	00.00±0.00	00.00±0.00	24.63±0.41(Fluconazole)
C. tropicalis,	00.00±0.00	00.00±0.00	00.00±0.00	21.00±0.53(Fluconazole)
C. parapsilosis	08.00±0.43	07.00±0.25	08.00±0.60	25.80±0.41 (Fluconazole)

All data represented mean±SE of three independent experiments. 00.00±0.00 showed that no inhibition zone for respective microorganisms

Leaf extract of *C. guianensis* also showed antifungal activity against *Candida parapsilosis*. Methanolic extract was slightly more effective in comparison to the other extract (table 2). *C. parapsilosis* shows significant drug resistance against azole family drugs such as fluconazole and voriconezole. The plant extract of this plant can be used against *C. parapsilosis*. However, leaf extract was ineffective on *Candida albicans* and *Candida tropicalis.*

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was found different for different leaf extract for *S. aureus*. Ethanolic extract showed higher activity than methanolic and chloroform extract. MIC was 25 mg/ml for ethanolic, 50 mg/ml for methanolic and 100 mg/ml for chloroform extract.

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CONFLICT OF INTERESTS

The authors have no conflict of interest for publication of this research article

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