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In vitro Antimicrobial Activity and Phytochemical Analysis of Indian Medicinal Plant *Couroupita guianensis* Aubl.

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ABSTRACT

Phytochemical analysis and fluorescence characters of leaf of Couroupita guianensis were investigated. The leaf extracts showed the presence of alkaloids, phenolics, flavonoids, saponins and tannins in the medicinal plant. The antimicrobial effect of selected Indian medicinal plant was evaluated on bacterial strains like Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Leuconostoc lactis and the fungal strains such as Aspergillus niger, Aspergillus flavus, Rhizopus indicus and Mucor indicus. The solvents used for the extraction of plant were water and methanol. The in vitro antimicrobial activity was performed by well diffusion method. The antibacterial activities of leaf of Couroupita guianensis in successive different solvent were tested against gram +ve and gram -ve organisms as well as the fungus. The methanol extract of C. guianensis showed the broader spectrum of antibacterial and antifungal activity when compared with aqueous extract.

Key Words: phytochemical, antimicrobial, *Couroupita guianensis*, Alkaloids, Flavonoids.

INTRODUCTION

Plants have formed the basis of traditional systems of medicine that have been in existence for thousands of years and continue to provide mankind with new remedies [1]. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main source of drugs [2]. For centuries, people have used plants for healing. Plant products as part of food or botanical portions and powder have been used with varying success to cure and prevent diseases throughout history [3]. Ethnomedicinal plants are used both for primary health care and for treating chronic diseases

such as AIDS, cancer, hepatitis disorders, heart and old age related diseases like memory loss, osteoporosis and diabetic wound. In the Indian coded system (Ayurveda, Unani, Siddha, Amchi), Ayurveda currently utilizes as many as 1000 single drugs and over 8000 compound formulations of recognized merit .Similarly, 600-700 plants are utilized by other systems like Unani, Siddha and Amchi [4].

The increase of microbial resistance to antibiotics threatens public health on a global scale as it reduces the effectiveness of treatments and increases morbidity, mortality and health care costs. Evolution of highly resistant bacterial strains has compromised the use of newer generations of antibiotics [5]. Food antioxidants such as a-tocopherol, ascorbic acid, carotenoids, amino acids, peptides, proteins, flavonoids and other phenolic compounds might also play a significant role as physiological and dietary antioxidants, thereby augmenting the body's natural resistance to oxidative damage. Development of safer natural antioxidants from the extracts of oilseeds, spices and other plant materials that can replace synthetic antioxidants is of interest today. Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, antiinflammatory, antiallergic, antithrombotic and vasodilatory activities [6].

One important plant that is used in traditional medicine is *Couroupita guianensis* is a tree belonging to the family Lecythidaceae. It is native to South India and Malaysia and is commonly known as Nagalinga pushpam in Tamil. Various part of the tree have been reported to contain oils, keto steroids, glycosides, couroupitine, indirubin, isatin and phenolic substances [7].

The tree grows 30-35m tall, with leaves in whorls on the ends of the shoots. The flowers, which are borne only on special stems on the main trunk, are orange, scarlet or pink, forming racemes up to 3m long. They mature into large fruits, from which the common name (cannon ball tree) is derived. They are spherical woody fruits, 15-24cm diameter, containing numerous (200-300) seeds. The pulp of the fruits oxidizes bluish-green when exposed to air and is extremely malodorous probably because of sulphur compounds in the fruits. The fruit contains small seeds in a white, unpleasant smelling edible jelly [8]. This plant is used for treating mange and other skin conditions. The pulp of the fruit of the cannon ball tree is rubbed on the infected skin of mange dog. It is claimed that when the dog licks its skin, this medicine will also work internally [9]. The flowers are used to cure cold, intestinal gas formation and stomachache[10].

EXPERIMENTAL SECTION

Preparation of plant extracts

Fresh Plant leaf of *Couroupita guianensis* was collected from Chengam, Thiruvannamalai district, Tamil Nadu, India; they were identified with the help of Gamble's flora.

Preparation of powder

The leaves of plants were collected and dried under shade. These dried materials were mechanically powdered sheaved using 80 meshes and stored in an airtight container. These powdered materials were used for further physiochemical, phytochemical and fluorescent analysis [11].

Extraction of plant material

Various extracts of the study plant was prepared according to the methodology of Indian Pharmacopoeia [12]. The leaves were dried in shade and the dried leaves were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Both the extracts were analyzed for phytochemical screening of compounds, antimicrobial and pharmacological activity.

Qualitative phytochemical studies

Qualitative phytochemical analyses were done by using the procedures of Kokate *et al.* (1995). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, proteins, amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test organisms

The stored culture of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Leuconostoc lactis* and *Salmonella typhi* were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India.

The pathogenic fungal strains *Aspergillus niger*, *Aspergillus flavus***,** *Rhizopus indicus* and *Mucor indicus* were collected from the Microbiological Lab, Christian Medical College, Vellore, Tamil Nadu, India.

Antibacterial Studies

Bacterial Media (Muller Hindon Media)

Thirty Six grams of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into petridishes. The solidified plates were bored with 6mm dia cork porer. The plates with wells were used for the antibacterial studies.

Antifungal studies

Fungal media (PDA)

Two Hundred gram of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20g of dextrose was mixed with potato infusion. 20g of agar was added as a solidifying agent. These constituents were mixed and autoclaved. The solidified plates were bored with 6mm dia cork porer.

Well diffusion method

Antibacterial and Antifungal activity of the plant extract was tested using well diffusion method [14]. The prepared culture plates were inoculated with different bacteria and fungus by using plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2^{o}C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

RESULTS

Fluorescence analysis

The results of Fluorescence analysis of the powder in visible range has been shown in Table1.

Phytochemical analysis

Phytochemical investigation of the crude extracts revealed the presence of carbohydrates, tannins, phenols, flavonoids, gums and mucilages, proteins, amino acids and saponins in the crude extracts of *C. guianensis* (Table 2).

Antimicrobial activity

The methanol and aqueous extract of the *C. guianensis* leaf were screened against six human pathogenic bacteria and four fungal pathogens to check antibacterial and antifungal activities by well diffusion method which showed valuable zone of inhibition. The specific zone of inhibition against various types of pathogenic bacteria and fungus was shown in table 3 and 4. Methanol extract was better than the aqueous extract against bacteria as well as fungal pathogens.

The zone of inhibition against bacterial pathogens ranged between 31 – 12mm in methanol extract and 30 – 12mm in aqueous extract. The maximum activity (31mm) was recorded from 200mg of methanol extract of *C. guianensis* against *Salmonella typhi* followed by 29mm against *E. coli* and minimum (12mm) against *Streptococcus aureus* at 50mg level whereas, the aqueous extract showed the maximum activity (30mm) was recorded from 200mg of leaf extract against *E. coli* and minimum (12mm) by 50mg of extract against the above bacteria. *Leuconostoc lactis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* did not showed the any activity against both the extract. The zone of inhibition against fungal pathogens ranged between 19 - 8mm in methanol extract and their was no activity in aqueous extract. The maximum activity (19mm) was recorded from 200mg of methanol extract against *Aspergillus niger* and minimum (8mm) by *Rhizopus indicus* at 50 mg level.

DISCUSSION

One such medicinal plant is *Couroupita guianensis,* commonly called as the cannon ball tree. The medicinal uses of the parts of the cannon ball tree are strongly implicated in traditional medical practices. The leaves, bark and fruit flesh is used to treat various ailments. The tree parts are used to cure colds and stomach aches. The juice made from the leaves is used to cure skin diseases, and the Shamans of South America have even used the tree parts for treating malaria. The fruit pulp can disinfect wounds and young leaves ease toothache [15].

Albeit the known uses of the plant parts in various disorders, especially those against microbial infections, no systematic study on the nature of the antimicrobial action and the phytochemical responsible for this action has been reported. Hence, the present study was formulated to analyze these aspects. The antibacterial and antifungal activity of the leaf of the *Couroupita guianensis,*

phytochemical and fluorescence characters was evaluated. The leaves of *Couroupita guianensis* exhibited considerable antibacterial activity. The methanolic extract exhibited a better antibacterial activity as well as antifungal activity than the aqueous extract. The solvents used were methanol, ethanol, ethyl acetate and chloroform. Among all the extracts, maximum in vitro inhibition was scored in methanol extracts [16]. This reveals that the active components have been extracted in methanol, which is similar to our results.

From this current investigation, the maximum activity (31mm) was recorded from 200mg of methanol extract of *C. guianensis* against *Salmonella typhi* followed by 29mm against *E. coli* and minimum (12mm) against *Streptococcus aureus* at 50mg level whereas, the aqueous extract showed the maximum activity (30mm) was recorded from 200mg of leaf extract against *E. coli* and minimum (12mm) by 50mg of extract against the above bacteria. The maximum activity (19mm) was recorded from 200mg of methanol extract against *Aspergillus niger* and minimum (8mm) by *Rhizopus indicus* at 50 mg concentration. The antimicrobial activities of *Cynara scolymus* L. leaf, head and stem extracts were tested against 15 microbial species and the leaf extract was found to be the most effective, followed by head and stem extracts [17].

Table 1: Analysis of fluorescence characters of leaf powder of *Couroupita guianensis* in different chemical reagents

Sl. No	Chemical reagent	Appearance
1	Powder colour	Green
2	5% NaOH	Green
3	10% NaOH	Dark green
4	Con. H_2SO_4	Green
5	Acetic Acid	Green
6	1N NaOH in H_2O	Green
7	5% KOH	Dark green
8	50% HNO_3	Light brown
9	5% $FeCl_2$	Dark green
10	1N HCl	Light green
11	Con.HNO_3	Light brown
12	1N NaOH in Ethanol	Light green
13	50% H_2SO_4	Green
14	50% HCl	Green
15	Con. HCl	Green

Table 2: Results of phytochemical screening of aqueous leaf extracts of *Couroupita guianensis*

S. No.	Name of the compounds	Name of the test	Status of the substances	
			Aqueous extract	Methanolic extract
1	Carbohydrates	Fehling's	+	+
		Benedict's	+	+
2	Alkaloids	Mayer's	-	-
		Hager's	-	-
		Wagner's	-	-
		Dragen Dorfff's	-	-
3	Steroids	Chloroform + Acetic acid + H_2SO_4	-	-
4	Tannins & Phenols	10% Lead acetate	-	+
		5% Ferric chloride	+	+
		1% gelatin	+	-

5	Saponins	Foam test	+++	+++
6	Fixed oils & Fats	Spot test	-	-
7	Gums & Mucilage	Alcoholic precipitation	+	++
8	Proteins	Biuret test	+	-
9	Flavonoids	NaOH / HCl	+	+
10	Volatile oils	Hydro distillation method	-	-

++++ - *High rich amount*
+++ - *Rich amount*
++ - *Moderate amount*
+ - *Minimum amount*
- - *Absent*

Table 3: Inhibition zone of Aqueous and Methanol extracts of *Couroupita guianensis* against bacterial pathogens

Sl. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Staphylococcus aureus*	-	-	-	12±2.4	21±2.8	26±3.7
2	*Escherichia coli*	12±2.8	24±3.7	30±2.8	18±2.8	21±1.4	29±1.4
3	*Leuconostoc lactis*	-	-	-	-	-	-
4	*Salmonella typhi*	-	-	-	16±2.8	21±2.4	31±2.8
5	*Pseudomonas aeruginosa*	-	-	-		-	-
6	*Streptococcus pyogenes*	-	-	-	-	-	-

Table 4: Inhibition zone of Aqueous and Methanol extracts of *Couroupita guianensis* against fungal pathogens

Sl. No.	Name of the organisms	Zone of inhibition					
		Aqueous extract			Methanol extract		
		50mg	100mg	200mg	50mg	100mg	200mg
1	*Aspergillus flavus*	-	-	-	-	-	13±2.4
2	*Mucor indicus*	-	-	-	-	-	-
3	*Aspergillus niger*	-	-	-	12±2.8	16±2.8	19±5.1
4	*Rhizopus indicus*	-	-	-	08±2.8	10±2.8	16±3.7

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