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Assessment of phytochemical constituents, trace metals and antimicrobial efficacy of holy plant *Couroupita guianensis*, Southern India

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Abstract

Photochemical constituents, trace metals concentration and antimicrobial activity of (three different extract such as petroleum ether, chloroform and ethanol) *C. guianensis* leaves were investigated. The phytochemical screening of the crude extract revealed the presence of steroids, triterpenes, alkaloids, phenols, flavonoids, saponins and tannins. The leave powder was subjected to analyse the trace metals using 797 VA Computrace voltametry, Metrohm. The average mean concentrations of Cd, Cr, Cu, Fe, Ni, Pb and Zn were BDL, 0.02, 0.32, 0.89, BDL, BDL and 0.54 mg kg^{-1}, respectively. The three different solvents extract of *C.guianensis* were analyzed for in-vitro antimicrobial activity against certain pathogens and the zone of inhibition were compared with positive control such as Methicillin – 10 mcg and Itraconazole – 10 mcg. The *Enterococcus fecalis* and *Trichophyton rubrum* were most sensitive (19 mm) while smallest inhibitions were recorded in *Micrococcus luteus* and *Cryptococcus* sp. (7 mm).

Keywords: *Couroupita guianensis*, Antimicrobial activity, Phytochemistry, Trace metals

1. Introduction

A medicinal plant represents a rich source of antibiotic, antifungal, antiseptic and analgesic qualities. The Cannonball (*C.guianensis*) tree possesses antibiotic, antiseptic and analgesic qualities and is used to cure the common cold and stomach ache. The juice from the plant leaves is used to cure skin diseases, and shamans and malaria. The fruits of the plant can disinfect wounds and young leaves cure toothache.[1] The *C.guianensis* (Aubl) belongs to the family Lecythidaceae, commonly known as cannon ball tree. A truly amazing tree does not grow branches that reach out from the straight trunk; it bears large, showy flowers, almost through the year, on the trunk and not on braches like most other trees. The trees are grown extensively in lord Shiva temples in India.

Hindus revere it as a sacred tree because the petals of the flower resemble the hood of the naga, a sacred snake, protecting a Shiva-Lingam, the stigma. The tree also produces globular brown woody, indehiscent, amphisarcun (double fleshy) fruits of an astonishing size, almost the size of a human head.[2] It is widely planted in trophical and subtrophical botanical gardens as an ornamental throughout the tropics and sub tropics, it does well under cultivations and it is used to feed animals. Chemical studies of this species showed the presence of α-amirin, β-amirin, β-sitosterol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, carotenoids and sterols.[3] In this present study to investigate the phytochemical, trace metal concentration and antimicrobial analysis of petroleum ether, chloroform and ethanolic extracts from the leaves of *C. guianensis*.

2. Materials and Methods

2.1 Plant Material

The plant materials were collected from pudukkottai district of Tamil Nadu in India during the period of January to February 2014. The shade dried *C. guianensis* powders (100 g) were successively extracted with petroleum ether, chloroform and ethanol by soxhlet apparatus and is used as test sample for antimicrobial activity.

2.2 Phytochemical screening

2.2.1 Qualitative analysis

The solvent extracts were subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in sample.[4]

Steroids: A 3 ml of test solution and minimum quantity of chloroform was added with 3-4 drops of acetic anhydride and one drop of concentrated H_2SO_4. Purple color thus formed changes into blue or green color indicating the presence of steroids.

Triterpenoids: A 3 ml of test solution was added with a piece of tin and 2 drops of thionyl chloride. Formation of violet or purple colour indicates the presence of triterpenoids.

Reducing Sugars: A 3 ml of test solution was added with a 2 ml of Fehling's reagent and 2 ml of water. Formation of reddish orange color indicates the presence of reducing sugar.

Sugars: A 3 ml of the test solution was added with very small quantity of anthrone reagent and a few drops of concentrated H_2SO_4 and heated. Formation of green or purple color indicates the presence of sugars.

Alkaloids: A 3 ml of test solution was taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent.

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Formation of white precipitate or turbidity indicates the presence of alkaloids.

Phenols: A 3 ml of test solution in alcohol was added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

Flavonoids: A 3 ml of test solution in alcohol was added with a bit of magnesium and one (or) two drops of concentrated HCl and heated. Formation of red or orange color indicates the presence of flavonoids.

Saponins: A 3 ml of test solution was added with water and shacked. Formation of foamy lather indicates the presence of Saponins. Tannins: A 3 ml of test solution was added with water and lead acetate. Formation of white precipitate indicates the presence of tannins.

Anthroquinones: A 3 ml of test solution was added with magnesium acetate. Formation of pink color indicates the presence of anthroquinones.

Amino Acids: A 3 ml of test solution was added with 1% ninhydrin in alcohol. Formation of blue or violet color indicates the presence of amino acids.

Catechins: A 3 ml of test solution in alcohol was added with Ehrlich reagent and a few drops of concentrated HCl. Formation of pink color indicate the presence of catechins.

2.2.2 Quantitative analysis of phytoconstituents

The chlorophyll pigments in the leaves were estimated following the method of Arnon.[5] After pre-cleaning; weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated and is expressed in mg/g fresh weight. Amino acids were estimated by ninhydrin method which is calorimetrically measured at 570nm.[6] Proteins were estimated by bradford method and the absorbance was measured at 595nm against blank/ sample.[7] Carbohydrates were estimated by anthrone method which can be measured by using colorimetrically at 620 nm (or) by using a red filter.[8] All the trials were performed thrice and the mean values were presented.

2.2.4 Trace metal analysis

The plant leaves of *C. guianensis* were carefully removed and washed with sterile distilled water. The cleaned leaves were dried in shadow area and were grinned with agate mortar and pestle. The powered plant samples were stored in sterile plastic container. The 1 g of powdered plant sample was treated with aqua-regia mixture in Teflon bomb and was incubated at 140 °C for 2-3 days. After incubation, the reaction mixture was filtered with whatman No.1 filter paper. Then, the extraction was tested for trace metals (Fe, Cu, Zn, Pd, Cd, Cr and Ni) analysis by the 797 VA Computrace voltametry, Metrohm. To avoid the contamination, the devices were rinsed with acidified water (10% HNO_3) and weighted to dissolve metals before analysis. And, all the equipments and containers were soaked in 10% HNO_3 for 24 h then rinsed thoroughly in de-ionized water before use. Also find the below detectable limit of the instruments.

Table 1: Qualitative phytochemical constituent of *C. guianensis*

Phytochemical Constituents	Petroleum ether	Chloroform	Ethanol
Steroids	-	-	+
Triterpenes	+	-	-
Reducing sugars	-	-	-
Sugars	-	-	-
Alkaloids	-	+	-
Phenolics	-	+	-
Catechins	-	-	-
Flavonoids	-	+	-
Saponins	-	-	+
Tannins	-	-	+
Anthraquinones	-	-	-
Amino acids	-	-	-

+ = Present; - =Absent

Table 2: Quantitative phytochemical constituent of *C. guianensis*

Biochemical constituents	*C. guianensis* (mg/g)
Chlorophyll A	0.119
Chlorophyll B	0.952
Total Chlorophyll	1.071
Amino acid	160.0
Protein	2.110
Carbohydrate	1.009
Phenol	0.026

Table 3: Concentration of trace metals in *Couroupita guianensis*

Sampling Site Name	Sampling Site No.	Sample Name/ Family	Sample No.	S. Code	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Pudhukottai, Tamil Nadu	**S1**	*Couroupita guianensis*	P1	Cg1	BDL	0.02	0.32	0.89	BDL	BDL	0.54

BDL – Below detectable limit

2.3 Testing of antimicrobial activity

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the well diffusion method.[9-12] The antibacterial and antifungal activities of test samples were analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively.[13-14] The solvent extracted samples were dissolved in concentrated DMSO. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The three different concentrations (2.5, 5 & 10 mg/ml) of sample were poured into well (1 cm in diameter and 4 mm in depth) of the agar plates, separately. For negative control study, the DMSO was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungi). After incubation, the zone of inhibition was measured with ruler.[15] The assays were performed in triplicate and the average values are presented. Methicillin – 10mcg (for bacteria) and Itraconazole – 10mcg (for fungus) was used as positive control. All the media, standard discs and sterile disc were purchased from Hi-Media (Mumbai, India).

Table 4: Antimicrobial activity of the different solvent extracts of *Couroupita guianensis* leaves

S.No	Test Microorganisms	Well diffusion method - Zone of inhibition (mm) Sample (2.5, 5 & 10 mg/ml)										Diseases	Route of Transmission
		Petroleum ether extract			Chloroform extract			Ethanol extract			PC		
Bacteria		2.5	5.0	10.0	2.5	5.0	10.0	2.5	5.0	10.0	10 mcg		
1.	*Aeromonas liquefaciens* B1	8	10	12	10	12	12	7	10	11	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus fecalis* B2	12	15	19	13	16	18	13	14	15	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* B3	7	10	13	8	12	13	10	12	13	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* B4	7	9	11	7	10	12	8	13	17	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium* B5	11	12	13	7	11	13	11	14	16	0	Typhoid	Water / Food
6.	*Vibrio cholarae* B6	10	12	14	10	13	15	10	13	17	16	Cholera	Water / Food
Fungi													
7.	*Candida albicans* F1	11	13	16	11	14	16	9	13	16	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. F2	7	9	13	7	10	11	12	13	15	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* F3	12	16	17	13	15	17	13	14	16	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* F4	9	15	19	12	14	19	13	16	19	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control (Using antibiotic disc; **Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc)**
Samples – 2.5, 5, 10 mg/ml (well)

3. Results and Discussion

3.1 Phytochemical constituents of Secondary metabolites

The present study revealed that the medicinal plant such as *C.guianensis* contains bioactive compounds. The phytochemical constituents were screened by qualitative and quantitatively methods and the results are presented in Table 1 and 2. In steroids analysis, purple color thus formed changes into blue or green color indicating the presence of steroids. Similarly, based on the presence or absence of colour change indicate positive and negative results. In these screening, the positive results were obtained from three different extracts petroleum ether, chloroform and ethanol.

The low polar solvent petroleum ether used to screen the triterpenoids from *C. guianensis* likewise medium and high polar solvents chloroform and ethanol extracts were contain steroids, alkaloids, phenols, flavonoids, saponins and tannins. Commonly, secondary metabolites play an important role in plant defence against herbivory. Both lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene.

Steroids along with phospholipids function as components of cell membranes. Steroids such as cholesterol decrease membrane fluidity. Terpenes are released by trees more actively in warmer weather, acting as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature. Alkaloids are antibacterial berberine, the anticancer compound vincristine, the antihypertensionagen treserpine, the cholinomimeric galantamine, the spasmolysis agent atropine, the vasodilator vincamine, the anti-arrhythmia compound quinidine, the anti-asthma therapeutic ephedrine, and the antimalarial drug quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.[16] Flavonoids are one class of secondary plant metabolites that are also known as Vitamin P or citrin.[17] These metabolites are mostly used in plants to produce yellow and other pigments which play a big role in colouring the plants.[18-19]

3.2 Trace metals analysis

Some of the trace metals are essential for plant growth whereas many of them affect the plant physiology. Especially, the role of trace metal pollutants causing injury to plants either by direct toxic effect or modifying the host physiology rendering it more susceptible to infection.[20] which leads to affects the photosynthesis process, growth and their efficiency.[21] The mean concentrations of metals such as Cd, Cr, Cu, Fe, Ni, Pb and Zn in plant sample were BDL, 0.02, 0.32, 0.89, BDL, BDL and 0.54 mg kg^{-1}, respectively (Table 3).

3.3 Antibacterial and Antifungal screening

The antimicrobial activity of *C. guianensis* was examined with various microorganisms using the well diffusion test. The results of the antimicrobial activities are summarized in Table 4. The three tested concentrations such as 2.5, 5 & 10 mg/ml produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (10 mg/ml) concentration of sample got greater sensitivity than (2.5 & 5 mg/ml) lower concentration in most of the microorganisms. In bacteria, the petroleum ether and chloroform extract samples were most effective against *Enterococcus fecalis* (B2) while the smaller effect was noticed from *Micrococcus luteus* (B4). In fungi, the test sample was effective against *Trichophyton rubrum* (F4) in all the extracts where as the smaller effect was observed in *Cryptococcus Sp* (F2). The ethanol plant extracts were most effective against *Aeromonas liquefaciens* (B1). All the microbial strains depict higher sensitivity to the higher concentration (10 mg/ml) for the test sample when compared to the positive control except B1, B3 and B4. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (concentrated DMSO), reflecting that antimicrobial activity was directly related to the sample.

4. Conclusion

This study concluded that the presence of steroids, triterpenes, alkaloids, phenols, flavonoids, saponins and tannins are helps to the antimicrobial activity and is effective than positive control except B1, B3 and B4. The presence of heavy metals indicated that the plant was resistant to the trace metal and their secondary metabolites were not seriously affected by these metals and it may enter into the human food chain. In this endeavor, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs. The *C.guianensis* may act as an alternative antibiotic in near future.

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