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Physicochemical and phytochemical studies on leaves of *Couroupita guianensis* Aubl

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Abstract
The present study comprises physicochemical and phytochemical analysis of leaves of *Couroupita guianensis* Aubl by using standard protocols. The Pharmacognostic study of plant material is an important tool for detecting any adulterations present in it. In the present study, physicochemical parameters such as ash values, foreign matter, loss on drying, moisture content, fluorescence analysis and extractive values in four different solvents (methanol, ethanol, ethyl acetate and petroleum ether) were analysed. Fluorescence analysis of leaf powder showed the presence of different fluorescence with different reagent treatments. The qualitative phytochemical screening was carried out in four different extracts. The results revealed the presence of secondary metabolites such as alkaloids, flavonoids, steroids, carbohydrates, tannins and proteins.

Keywords: *Couroupita guianensis*, physicochemical, fluorescence, phytochemical

Introduction
Herbal medicines play a significant role in therapy throughout the world [16]. India has a long history of using several forms of traditional medicine, including Siddha, Unani and Ayurveda [11, 14]. The natural medicines can be readily adulterated with or replaced entirely with low-quality components in order to satisfy the increasing demand. Therefore, it is critical to set guidelines for the authentication of potent medicinal plants that are utilised to treat diseases. Pharmacognostic studies will provide standard guidelines and confirm plant identity, hence contributing to the prevention of adulteration [2, 7].
Couroupita guianensis Aubl commonly called a Cannonball tree belonging to family Lecythidaceae family [12]. It is also known as Naglingam in Tamil and Kailashpati in Hindi. It often seen in Siva temples in South India and is considered a sacred tree. Hindus believes that the staminal sheath resembles the hood of the sacred snake Naga which is meant to protect a shivalingam and is symbolised by reduced stigma. Therefore the name is Naglingam tree. It is also planted as a decorative plant throughout the world. It is a huge tree with vibrant and strongly perfumed flowers. The fruits are large and rounded in shape. Due to shape and size of fruits, the plant is commonly called as 'Cannon ball tree'. The fruits have acidic pulp inside and hard shell around it. The fruits are edible and have properties like neuropharmacological, wound-healing, hepatoprotective, antinociceptive, immunomodulatory, antiulcer, antioxidant, antimicrobial, cytoprotective, anti-inflammatory, antihyperglycemic and antidiabetic [18].
Traditionally the plant is used in the treatment of stomach ache, skin diseases, malaria, cold, headache, asthma, arthritis, scorpion bite and dysentery [21]. Bark is used in the treatment of hypertension, inflammatory diseases, tumours [10]. Leaves are used as an analgesic [4] and leaf juice used in treatment of skin diseases in South America [21]. Fresh fruit pulp used for making cooling medicinal drink [4], pulp can disinfectant wounds as it has antibacterial properties [18].

Materials and Methods
Collection of samples: The healthy and fresh leaves and fruits were collected from LIC colony, Borivali (west), Mumbai. The samples were carefully washed under tap water to remove dust and dried under shade. The dried material coarsely powdered and kept in air-tight containers for further study.

Preparation of extracts
10 gm of leaf powder was macerated with 100 ml solvents such as methanol, ethanol, ethyl acetate and petroleum ether for 48 hours. Then the extracts were filtered and concentrated in sand bath. These extracts were used further for phytochemical screening.

Physicochemical analysis
The various physicochemical parameters such as foreign matter, ash values, extractive values, foaming index, swelling index, moisture content, pH and fluorescence analysis were studied according to standard methods [1, 22].

Foreign matter
The 100 g sample was weighed and spread out evenly. Foreign matter was divided into groups by visual inspection, using a magnifying glass, or using an appropriate sieve. The remaining material was sieved using a sieve number 250. The separated foreign matter components were weighed.

Ash values
Ash is the remaining matter after the ignition of the plant material. It is composed of inorganic components after incineration of organic components. This gives the total mineral contents within it. There are 4 different methods of calculating ash values such as total ash, water-soluble ash, acid-insoluble ash and sulphated ash.

Total Ash
2 grams of air-dried leaf powder taken in a pre-weighed silica crucible. Spread the powder evenly in a crucible. Then the crucibles were placed in a muffle furnace for igniting at a temperature of 600 ºC until the powder turns completely white. After getting white ash, the crucibles kept in desiccator for cooling and weighing the final matter. The percentage of total ash is calculated by using the following formula-

$$\text{Percentage of Total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Water-soluble Ash
Collect total ash in a beaker and then add 25 ml of water. Boil it for 5 minutes. Filter this matter using ash-less filter paper. After filtration wash the insoluble matter with hot water. Ignite this filter paper in a crucible for approximately 15-20 minutes at a temperature 400 ºC. For cooling, crucibles kept in desiccators. Weigh this ash residue. To calculate the water-soluble ash, subtract the weight of ash residue from the weight of total ash.

$$\text{Percentage of Water soluble ash} = \frac{\text{Weight of water soluble ash}}{\text{Weight of sample}} \times 100$$

Acid-insoluble Ash
About 25 ml of 6N hydrochloric acid was added to the total amount of ash in a silica crucible, and the mixture was heated for five minutes. After cooling, filter with ash-free filter paper, and then the filtrate was washed in hot water until it was acid-free. After filtration, the filter paper containing the insoluble material was put into the same precisely weighed crucible and ignited in a muffle furnace to maintain a constant weight. The percentage of the acid-insoluble ash is calculated by using the following formula-

$$\text{Percentage of Acid insoluble ash} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of sample}} \times 100$$

Sulphated ash value
A silica crucible was heated for 10 minutes until it turned red, and then it cooled in a desiccator before being weighed. A precisely measured 2 g of the chemical added to the crucible, gently ignited at first, and then thoroughly burned. The residue then cool, moistened with 1 ml of conc. sulphuric acid, heated gradually until no longer giving white fumes, and then burn once more at 800 °C until no longer releasing any black particles. The entire procedure repeated until two successive weighs do not differ by more than 0.5 mg. The percentage of the sulphated ash is calculated by using the following formula-

$$\text{Percentage of Sulphated ash} = \frac{\text{Weight of sulphated residue}}{\text{Weight of sample}} \times 100$$

Extractive values
10 gm of leaf powder was macerated with 100 ml solvents such as methanol, ethanol, ethyl acetate and petroleum ether for 48 hours. Then the extracts were filtered and evaporated in sand bath. Their constant extractive values were recorded.

Foaming index
1 gm of crude drug was taken in a beaker. Then 100 ml of distilled water was added to it. The beaker kept in a hot water bath for 30 minutes. Cool the beaker and filtered by using filter paper. Add enough distilled water to make a 100 ml volume. Prepared 10 test tubes and labelled as 1 ml, 2 ml, 3 ml, 4 ml up to 10 ml. Then respective volume of filtrate to respective test tubes was added. Each test tube is diluted with distilled water and the volume is up to 10 ml. Each test tube shake for approximately 15 seconds. After shaking test tubes are allowed to stand for 15 minutes. Measure the height of the foam.

Swelling Index
Take the appropriate quantity of the leaf powder which has already been precisely weighed and pour into a 25-ml glass stopper measuring cylinder. The cylinder's internal diameter should be around 16 mm. Add 25 ml of water and give the mixture a good shake every ten minutes for an hour. At room temperature, let stand for three hours. Calculate the volume in millilitres that the leaf powder including any sticky mucilage.

Moisture content
Empty silica crucibles and lids dried in the oven at a temperature 105 °C for approximately 3 hours. After removing from the oven, crucibles and lids kept in a desiccators for colling. Pre-weigh the empty crucibles and lids. 3 grams of fresh sample of leaves taken in the crucibles and spread evenly in the crucibles. For drying crucibles were kept in the oven at a temperature 105 °C for 3 hours. After drying, crucibles kept in desiccators for cooling. Reweighed the crucibles with the samples.

pH
The pH of 1% and 10% solutions was measured using pH meter.

Preliminary phytochemical analysis
Phytochemical analysis of different extracts such as methanol, ethanol, ethyl acetate and petroleum ether were carried out by qualitative test according to standard methods [20, 5, 8]. The extracts were screened for alkaloids, flavonoids, steroids, carbohydrates, saponins, tannins, proteins and Cardiac glycosides.

Test for alkaloids
Dragendroff's test: 1 ml Dragendroff's reagent added to 2 ml extract. Formation of white precipitation indicated the presence of alkaloids.

Mayer's test: To the 1 ml test solution, Mayer's reagent (Mercuric-potassium iodide) was added. Creamy precipitation showed the presence of alkaloids.

Wagner's test: 2 ml of Wagner's reagent was added to a diluted extract solution. Formation of reddish brown precipitate indicated the presence of alkaloids.

Test for flavonoids

Shinoda test: Magnesium chips were added to the extract followed by addition of conc. HCl. Reddish pink showed the presence of flavonoids.

Test for steroids

Libermann-Buchard test: Few drops of acetic anhydride, 2 ml chloroform and few drops of conc. H_2SO_4 was added to the test solution. Formation of green colour indicated the presence of steroids.

Salkowski test: To the 1 ml extracts, 0.5 ml chloroform and 1 ml conc. H_2SO_4 was added. A reddish brown colour at the interface indicated the presence of terpenoids.

Test for carbohydrates

Barfoed test: In 1 ml of extract, 2 ml barfoed reagent was added. Red precipitate indicated the presence of carbohydrates.

Benedict's test: To the 2 ml test solution, 0.5 ml Benedict's reagent was added. Mixed it well and boiled for 2 minutes. A green colour showed the presence of carbohydrates (reducing sugar).

Test for saponins

Foam test: 5 ml distilled water added to the 3 ml of extract. Foam formation indicated the presence of saponins.

Test for tannins

Lead acetate test: 1% lead acetate was added to 2 ml of extracts. A yellow precipitation indicated the presence of tannins.

$FeCl_3$ test: Few drops of 5% $FeCl_3$ were added to 2 ml of extracts. A grey or black colour indicated the presence of tannins.

Test for proteins

Biuret test: To the 1 ml test solution, 2 ml of biuret reagent (2 drops of 1% $CuSO_4$ + 1 ml of 40% NaOH) was added. The violet colour indicated the presence of proteins.

Ninhydrin test: About 2 ml of extract was treated with ninhydrin reagent. Purple colour indicated the presence of proteins.

Test for cardiac glycosides

Keller-Kiliani Test: To the 2 ml of extract, 2 ml glacial acetic acid added. Mixed it well. After mixing, 2 drops of ferric chloride were added followed by conc. H_2SO_4 along the side wall of the test tube. A reddish-brown coloured ring at the interface indicated the presence of cardiac glycosides.

Results and Discussion

Physicochemical analysis

The physicochemical parameters of powdered crude drug were studied and the results were calculated shown in Table 1. The powdered crude drug was treated with different reagents and the results were recorded under daylight, short UV light (254 nm) and long UV light (365 nm) for fluorescence analysis. This helps to detect the phytoconstituents along with colour variations. The detailed results tabulated are shown in Table 2.

Table 1: Physicochemical analysis of *Couroupita guianensis* Aubl leaf

Sr. No.	Physicochemical constants	Leaf%(w/w)
1.	Foreign matter	0.01
Ash values		
2.	Total ash	15.01±0.02
	Water soluble ash	1.02±0.05
	Acid insoluble ash	1.21±0.08
	Sulphated ash	1.18±0.04
Extractive values		
3.	Methanol soluble	41.02±0.14
	Ethanol soluble	34.65±0.11
	Ethyl acetate soluble	33.64±0.09
	Petroleum ether	29.19±0.03
4.	Foaming Index	Less than 100
5.	Swelling Index	3.1±0.05
6.	Moisture content	0.12±0.04
pH		
7.	1% solution	6.67
	10% solution	6.09

Table 2: Fluorescence analysis of leaf Powdered of *Couroupita guianensis* Aubl

Sr. No.	Powder + Reagent	Fluorescence in daylight	Fluorescence (254 nm) Short UV light	Fluorescence (365 nm) Long UV light
1.	Powder only	Green	Dark green	Dark green
2.	Powder + conc. HNO_3	Green	Dark green	Dark green
3.	Powder + conc. HCl	Dark green	Dark green	Blackish green
4.	Powder + conc.H_2SO_4	Dark green	Blackish brown	Blackish brown
5.	Powder + Chloroform	Light green	Light green	Pinkish orange
6.	Powder + Picric acid	Green	Dark green	Orange
7.	Powder + Acetic acid	Pink	Pinkish orange	Dark pink
8.	Powder + Iodine	Green	Dark green	Dark green
9.	Powder + Ethyl acetate	Light green	Light green	Pinkish red
10.	Powder + 1N NaOH	Green	Dark green	Dark green
11.	Powder + Acetone	Green	Dark green	Dark green
12.	Powder + $FeCl_3$	Green	Yellowish green	Dark green
13.	Powder + Ethanol	Green	Dark green	Dark green

Preliminary Phytochemical analysis: A preliminary phytochemical analysis of *Couroupita guianensis* Aubl leaf were performed in different extracts by using various qualitative tests. The results of the tests are shown in Table 3.

Table 3: Preliminary phytochemical analysis of *Couroupita guianensis* Aubl leaf

Sr. No.	Plant constituents	Test	Methanol	Ethanol	Ethyl acetate	Petroleum ether
1.	Alkaloids	Dragendroff's test	+	+	+	+
		Mayer's test	+	+	+	+
		Wagner's test	+	+	+	+
2.	Flavonoids	Shinoda test	+	+	+	-
3.	Steroids	Salkowski test	+	+	+	+
		Libermann Burchard test	+	+	+	+
4.	Carbohydrates	Barfoed test	+	+	-	-
		Benedict's test	+	+	+	-
5.	Saponin	Foam test	+	+	-	-
6.	Tannins	Lead acetate	+	+	+	-
		5% $FeCl_3$	+	+	+	-
7.	Proteins	Biuret test	+	+	-	-
		Ninhydrin test	+	-	-	-
8.	Cardiac glycosides	Keller kiliani test	-	-	-	-

Discussion

Physicochemical constituents determine the quality of the drugs and help in setting the standards of the drugs. The ash values give an idea about impurities and inorganic compositions in a crude drug. The amount of silica present, particularly in the form of sand and siliceous earth is measured by acid insoluble ash which is a part of total ash. The amount of total ash that is soluble in water is known as water soluble ash. The total ash content in *C. guianensis* is 15.01±0.02% whereas water soluble ash and acid insoluble ash is 1.02±0.05% and 1.21±0.08% respectively.

Extractive values are beneficial for estimating the chemical constituents that are soluble in the specific extraction solvent and for evaluating crude drugs since it provides information about the types of chemical constituents contained in the drug [6]. The extractive values in methanol, ethanol, ethyl acetate and petroleum ether are 41.02±0.14%, 34.65±0.11%, 33.64±0.09% and 29.19±0.03%. Methanol showed high extractive values because methanol penetrated deep into the cells of leaves as compared to other three solvents.

The accurate identification and quality validation of the raw material is essential for ensuring the consistent quality of the herbal drugs because safety and efficacy are the most important objectives [15].

Moisture content may affect the crude drugs by infecting microbial growth. Therefore it is necessary that the crude drug should have less or negligible moisture content to increase the preservability. About 0.12±0.04% moisture content was recorded.

Phytochemical analysis is one of the most crucial factors which indicates the presence of pharmacologically active components in the plant [9]. The present phytochemical analysis showed the presence of alkaloids, flavonoids, steroids, proteins, carbohydrates and tannins.

Conclusion

The present physicochemical and phytochemical screening of leaf Powdered of *Couroupita guianensis* Aubl provides valuable information about their identification and it may also help to prevent adulteration. The standardisation of the chemical components of this plant could be done using phytochemical analysis. Further analysis on the extracts is in progress in order to identify, describe and characterise the structure of the bioactive substances present in plants which could be responsible for pharmacological activity.

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