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Research Article

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PHYTOCHEMICAL CONSTITUENTS, FLUORESCENCE ANALYSIS AND PROXIMATE COMPOSITION OF *Cephalandra indica* Naud. UNRIPE FRUITS

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ABSTRACT

The aim of the present study was to investigate the presence of phytochemicals, the fluorescence characteristics of powdered drug under UV light after treating with different chemical reagents and proximate analysis of powdered drug in the unripe fruits of *Cephalandra indica* Naud. Our findings provided evidence that crude aqueous and alcoholic extracts of fruits contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

Keywords: *Cephalandra indica*; Phytochemical constituents; Fluorescence Analysis; Proximate Composition.

INTRODUCTION

Cephalandra indica, the ivy gourd, also known as baby watermelon, little gourd or gentleman's toes, Kundru, Bimbi, Lindora. is a tropical vine. It is also known as *Coccinia grandis* and *Coccinia indica*. *Cephalandra indica* (Cucurbitaceae) is found in warmer and humid part of India. It is more commonly seen in areas like Bengal, Bihar and Orissa. It is found in southern Asian islands, West Indies and Hawain islands. In traditional medicine, fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice dysentery, vomiting, mouth ulcers, asthma, diabetics, gastrointestinal disturbances and bronchitis. The fruit possesses mast cell stabilizing, anti-anaphylactic and antihistaminic potential.[1] The Phytochemicals of this plant includes saponins, flavonoid, glycosides and polysaccharides, xyloglucan, taraxerol, carotinoids, cryptoxanthinin. The fruits have compounds that inhibit the glucose-6-phosphatase, the key liver enzyme involved in regulating sugar metabolism. Therefore, ivy gourd is sometimes recommended for diabetic patients. The fruits are also rich in beta carotenes. The plant has also been used extensively in ayurvedic and unani practice in the Indian subcontinent.[2] It has long tuberous fleshy rots, smooth and green fruits. The roots, stems, leaves and fruits are used in indigenous system of medicine for treating diabetes.

MATERIAL AND METHODS

Plant material

Fresh fruits of *Cephalandra indica* were collected in the month of June 2011 from Kulamangalam village, Thanjavur, Tamilnadu, India. The plant material was taxonomically identified and shade dried until all the water molecules evaporated and fruits became well dried. After drying, the material was ground well using mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

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Preparation of fruit extracts

Aqueous and Alcoholic extracts of fruits were prepared following standard procedures. Matured unripe fruits were dried in an incubator for two days at 40°C. The powder (500g) was extracted sequentially with 2.5 liters of water and 2.5 liters of 70% Ethanol in a soxhlet apparatus at 65°C until the powder became exhausted totally. The resulting extracts were filtered, concentrated and dried in vacuo (yield). The extracts were stored in desiccators for use in subsequent and experiments.

Phytochemical analysis

Phytochemical analysis was done following the method of Pulok and Mukherjee,[3] Trease and Evans[4]; Kokate et al[5]. The various extracts of fruit samples were subjected to chemical tests for identification of its active constituents.

Tests for Alkaloids

A small portion of the extract was stirred with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloid reagents such as:

- Mayer's reagent - Cream precipitate
- Dragondroff's reagent - Orange brown precipitate
- Hager's reagent -Yellow precipitate
- Wagner's reagent - Reddish brown precipitate.

Tests for carbohydrates & glycosides

The minimum amount of extracts were dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates & glycosides.

Molisch's test: The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol, and 2 ml of concentrated sulphric acid was added along the sides of the test tube. Violet color was obtained.

Fehling's test: The filtrate was treated with 1 ml of Fehling's solution and heated. A reddish orange precipitate was obtained.

Tests for glycosides

Another portion of the extract was hydrolyzed with hydrochloric acid for a few hours on a water bath and the hydrolysate was subjected to Legal's, Borntrager's test to

detect the presence of different glycosides.

Legal's test: To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. None of the extracts produced pink to red color.

Borntragers's test: Hydrolysate was treated with chloroform and the chloroform layes was separated. To this equal quantity of dilute ammonia solution was added. No color change in Ammonical layer was observed.

Test for phytosterol

1 g of the extract was dissolved in few drops of dilute acetic acid, 3 ml of acetic anhydride was added followed by few drops of conc. sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

Test for fixed oils and fats

Small quantity of the various extracts was separately pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oil.

Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for saponins

The extract was diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 min. The presence of saponins was indicated by formation of 1cm layer of foam.

Test for tannins and phenolic compounds

Small quantities of various extracts were taken separately in water and tested for the presence of phenolic compounds and tannins with

- Dilute ferric chloride solution (5%) - violet color
- 1% solution of gelatin containing 10% NaCl - white precipitate
- 10% Lead acetate solution - white precipitate.

Test for proteins and free amino acids

Dissolved small quantities of various extracts in a few ml of water and treated with

- Million's reagent - Red color
- Ninhydrin reagent - Purple color
- Biuret Test - Equal volume of 5% solution and 1% copper sulphate solution were added. Pink or Purple color.

Test for gums and mucilages

About 10ml of the extracts was added to 25ml of absolute alcohol with stirring and filtered. The precipate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for flavonoids- Shioda's test

To the extract, Magnesium turnings and dew drops of concentrated hydrochloric acid were added and boiled for 5 minutes. Red color was obtained.

Test for lignin

With alcoholic solution of phloroglucinol and hydrochloric acid - Red color was observed.

Detection of volatile oils

About 50 g of powered material was taken in a distillation apparatus and subjected to hydrodistillation for the detection of volatile oil.

Extraction procedure

Soxhlet extraction (Alcohol Extract) method was used. The coarse powder (100gm) of the given sample was extracted with 500 ml of Ethanol 95% by continuous hot percolation using Soxhlet apparatus until the extraction was completed. After the completion of the extraction, the extract was filtered and the solvent was removed by distillation under reduced pressure. Greenish black colored residue was obtained.

Extraction procedure –Cold Maceration

The shade dried coarsely powdered drug was extracted with distilled water by cold maceration in shaker for two days. After completion of extraction it was filtered and evaporated. The extract was then stored in desiccators. Greenish brown colored residue was obtained.

Proximate analysis

Total Ash: Weigh accurately 2g of the air dried crude drug in the tarred silica crucible and incinerate at a temperature not exceeding 450°C until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air-dried drug.

Acid Insoluble Ash: To the crucible containing the total ash, add 25ml of hydrochloric acid, cover with a watch glass and boil gently for 5 minutes. Rinse the watch glass with 5ml of hot water and add this liquid to the crucible. Collect the insoluble matter on an ash less filter paper and wash with hot water until filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weigh. Allow the residue to cool in suitable desiccators for 30 minutes, and then weigh without delay. Calculate the content of the acid insoluble ash in mg/g of the air dried material.

Water soluble Ash: To the crucible containing the total ash, add 25ml water and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or on an ash less filter paper. Wash with hot water and ignite in a crucible for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of this residue in mg from the weight of total ash. Calculate the content of water soluble ash in mg/g of the air dried material.

Sulphated Ash: About 2 gm of the ground drug was taken in a silica crucible. Ignited gently at first until the substance was thoroughly charred. Cooled, moistened the residue with 1 ml of sulphuric acid, heated gently until white fumes were no longer evolved and ignited at 800°C±25°C until all black particles disappeared.

Alcohol Soluble Extractive

About 5 gm of the air dried coarse powder macerated with 100 ml of ethanol in a closed flask for 24 hours. The flask was shaken frequently during the first 6 hours, and was allowed to stand for 18 hours. There after it was filtered rapidly, taking precautions against loss of the solvent. About 25 ml of the filtrate was evaporated to dryness at 105°C in a tarred flat-bottomed shallow dish and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air-dried drug.

Water Soluble extractive

About 5 gm of the drug was added to 50 ml of water at 80°C in a stoppered flask. It was shaken well, allowed to stand for 10 minutes, cooled at 15°C and to it 2 gm of Kieselguhr was added and filtered. 5 ml of the filtrate was transferred to a tarred evaporating basin, 7.5cm in diameter, the solvent was evaporated on a water bath, drying was continued for half an hour. Finally it wad dried in a steam oven for 2 hours and weighed. The percentage

of water soluble extractive was calculated with reference to the air-dried drug and the values are recorded.

Loss on Drying

About 2 gm powder drug was accurately weighed in a tarred dish and dried in an oven at 105°C for one hour. It was cooled in a dessicator and again weighed. The loss on drying was calculated with reference to the amount of air dried drug and the values are recorded.

Crude Fiber Content

About 2g of the drug sample, accurately weighed, was extracted with ether. Then 200ml of 1.25% sulphuric acid was added to the extracted drug and the whole mixture boiled for 30 minutes under reflux in a 500ml flask. The mixture was then filtered through a hardened filter and the residue washed with boiling water until free of acid. The entire residue was rinsed back into the flask with 200ml of boiling 1.25 % sodium hydroxide solution and again boiled under reflux for 30 minutes. The liquid was then quickly filtered through a tarred filter and the residue on the filter is washed with boiling water until neutral, dried at 110°C to constant weight, and incinerated, likewise to constant weight. The difference between the weight of the dried residue and that of the incinerated residue represents the weight of the crude fiber. It is expressed as percentage of the original weight of the material.

Foaming Index

One gram of the coarsely powdered leaf was weighed and transferred to 500ml conical flask containing 100ml of boiling water. The flask was maintained at moderate boiling, at 80-90°C for about 30 minutes. Then it was cooled, filtered into a volumetric flask and sufficient water was added through the filter to make up the volume to 100ml (V1). Ten stoppered test tubes were cleaned (height 16 cm, diameter 1.6cm) and marked from 1 to 10. Measured and transferred the successive portions of 1, 2, 3 upto 10ml and adjusted the volume of the liquid in each tube with water to 10ml. Then the tubes were stoppered and shaken lengthwise for 15 seconds, uniformly and allowed to stand for 15 minutes and the length of the foam in every tube was measured.

RESULTS AND DISCUSSION

Percentage yield of extract of the given powdered drug sample by alcohol extraction process - 5.5% w/w and aqueous extraction process - 12.73% w/w. The phytochemical characteristics were summarized in the Table **1**. The results revealed the presence of medically active compounds and it could be seen that, proteins, carbohydrates, flavonoids and saponins were present in all the fruits. The presence of alkaloids, glycosides, phytosterols, fixed oils and fats were revealed in both alcoholic and aqueous extracts. Tannins, phenolic compounds, gums, mucilage, flavonoids and lignin were present only in alcoholic extracts while saponins, proteins and free amino acids were present in aqueous extract. Volatile oil was absent in both extracts (Table **1**).

Table 1. Quantitative analysis of phytochemicals in *Cephalandra indica* fruits in alcoholic and aqueous extracts. The results are the based on two different screening of phytoconstituents

S No	Phytoconstituents	Alcoholic Extracts	Aqueous Extracts
1	Alkaloids	+	+
2	Carbohydrates	+	(-)
3	Glycosides	+	+
4	Phytosterols	+	+
5	Saponins	(-)	+
6	Fixed oils & Fats	+	+
7	Tannin& Phenolic compounds	+	(-)
8	Proteins & Free amino acids	(-)	+
9	Gums & Mucilage	+	(-)
10	Flavonoids	+	(-)
11	Lignin	+	(-)
12	Volatile oil	(-)	(-)

(+) indicates presence; (-) indicates absence

The fluorescence characteristics of powdered drug under UV light after treating with different chemical reagents and proximate analysis of the powdered drug were given in Table **2** and Table **3**. Many drugs fluorescence when their powder is exposed to ultra violet radiation. It is important to observe all materials on reaction with different chemical reagents under UV light. The fluorescence characteristics of powdered drug were studied under UV light after treating with different chemical reagents is reported.

Table 2. The fluorescence characteristics of powdered drug under UV light after treating with different chemical reagents.

S No	Chemical Test	Ethanolic Extract		Aqueous Extract	
		Day light	UV light	Day light	UV light
1	Sample as such	Yellowish brown	Light green	Light Brown	Pale green
2	Extract with aq. NaOH	Yellow	Dark green	Brown	Light green
3	With alc. NaOH	Dark yellow	Yellowish green	Light Brown	Dark Brown
4	With Hcl	Yellowish brown	Light green	Light Brown	Brown
5	With 50% HNO_3	Brownish yellow	Yellowish green	Light Brown	Brownish green
6	With 50% H_2SO_4	Brownish yellow	Greenish yellow	Yellowish brown	Dark green
7	Methnol	Yellow	Green	Light Brown	Light green
8	With Ammonia	Light yellow	Green	Brown	Green
9	With I_2 solution	Dark brown	Flouresent green	Brown	Brownish green
10	With $FeCl_3$	Yellowish brown	Flouresent green	Yellowish brown	Green

Table 3. Proximate analysis of powdered drug.

S No	Parameters determined	Values in (%)W/W
1	Total ash	4.2
2	Water soluble ash	1.26
3	Acid- Insoluble ash	1.8
4	Sulphated ash	3.72
5	Alcohol Soluble extractive	11.3
6	Water soluble Extract	15.16
7	Loss on drying	Not more than 3.6
8	Crude fiber Content	1.6
9	Foaming index	Less than 100

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities.[6] Analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, saponins, fixed oils, fats, tannin, phenolic compounds, proteins, free amino acids, gums, mucilage, flavonoids, lignin and volatile oil. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites.[7] They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell

proliferation activities.[8] Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds.[9,10]

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids and tocopherols.[11] Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall.[12] They also are effective antioxidant and show strong anticancer activities.[13-15]

The aqueous fruit extract revealed to contain saponins which are known to produce inhibitory effect on inflammation.[16] Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness.[15,17] Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity.[18] Several workers have reported the analgesic[19,20], antispasmodic and antibacterial properties of alkaloids[21,22]. Glycosides are known to lower the blood pressure according to many reports.[23]

CONCLUSION

The results obtained in this study suggest the identified phytochemical compounds may be the bioactive constituents which are medicinally valuable. Therefore, extracts from these fruits could be seen as a good source for useful drugs and it is suggested that further work is in progress to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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