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Phytochemical Screening of the Various Extracts of *Cyperus Rotundus*. L

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ABSTRACT

Cyperus rotundus Linn. (Family: Cyperaceae), commonly known as Mothais a multivalent plant widely used in traditional medicine around the world for treatment of various diseases (fever, dysentery, malaria, skin disease, diarrhoea etc.). The present study was carried out to investigate the phytochemical profile of leaves of *Cyperus rotundus*. The powder of the leaves of *Cyperus rotundus* was successively extracted with acetone, aqueous, benzene, chloroform, cyclohexane, ethylacetate and methanol. A number of biologically active compounds have been isolated from the plant. Phytochemical analysis shows the presence of carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, flavonoid, saponins and alkaloid. Our findings provided evidence that crude aqueous and organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases. The result of the study could be useful to description and foundation of monograph of the plant.

Key words: Phytochemical analysis, Saponins, Flavonoids and *Cyperus rotundus*.

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INTRODUCTION

Traditional use of medicine is recognized as a way to learn about potential future medicines. Researchers have identified number of compounds used in mainstream medicine which were derived from "ethnomedical" plant sources¹. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis². Plants are used medicinally in different countries and are a source of many potent and powerful drugs.

Cyperus rotundus is a hairy under-sharp with yellow flowers, found in hotter parts of India. The leaves are used as adjunct to medicines used for pile complaints. They are used in treatment of Nausea and vomiting, dyspepsia, colic, flatulence, diarrhoea, dysentery, intestinal parasites, fever, malaria, cough, bronchitis, renal and vesical calculi, urinary tenesmus, skin diseases, wounds, amenorrhoea, dysmenorrhoea, deficient lactation, loss of memory, insect bites, food poisoning, indigestion, nausea, dysuria, bronchitis, infertility, cervical cancer and menstrual disorders, and the aromatic oils are used for perfumes and splash³. It is a multipurpose plant, widely used in traditional medicine around the world to treat various diseases⁴⁻⁶. Phytochemicals play an important role in biological activities⁷, the chief of which include alkaloids, tannins, flavonoids, phenolic compounds saponins etc⁸ and hence the aim of the present study was to carry out preliminary phytochemical screening of the various extracts of *Cyperus rotundus* leaves.

MATERIALS AND METHODS

Collection of Plant Material:

The leaves of *Cyperus rotundus* were collected from Kumarai Village in Cuddalore District of Tamil nadu, India during January to December 2012 and authenticated by the Director of the Rapinat Herbarium and Centre for Molecular Systematic, St. Joseph's college (campus), Trichirappalli, Tamilnadu, India. Fresh leaves were cleaned with running tap water and dried under the shade. Then the dried plant leaves were ground to fine powder mechanically and preserved in airtight containers for further analysis.

PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical evaluation of *Cyperus rotundus* was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered leaves of plant (100g) was extracted in a Soxhlet apparatus with acetone, chloroform, cyclohexane, ethylacetate, aqueous and methanol successively. The resultant extracts were subjected to chemical test for

different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins and steroids etc.

The percentage extractive yield was calculated by formula as mentioned below:

$$\% \text{ Extractive yield (w/w)} = \text{Weight of dried extract} / \text{Weight of dried plant powder} \times 100$$

Test for Alkaloids

Dragendroff's test:-

Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each extract and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids⁷.

Mayer's test:-

Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to each extract and observed for the formation of white or cream color precipitate which may indicate the presence of alkaloids⁷.

Hager's test:-

Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each extract and observed for the formation of yellow precipitate which may indicate the presence of alkaloids⁷.

Wagner's test:-

Few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each extract and observed for the formation of reddish brown precipitate which may indicate the presence of alkaloids⁷.

Test for flavonoids

Shinoda test (Magnesium Hydrochloride reduction test):-

To *Cyperus rotundus* extract, 5ml. 95% ethanol was added. The mixture was treated with 0.5gmagnesium turnings and few drops of conc.HCL. Pink color, if produced, may confirm the presence of flavonoids⁷.

Alkaline reagent test: -

Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes appearance of yellow color precipitates which indicated the presence of flavonoids⁷.

Test for Glycosides

Keller kelliiani test:-

To each of the extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to

a small test tube added with carefully 0.5 ml of conc. H₂SO₄ acid by the side of the test tube; blue color appears in the acetic acid layer indicating the presence of glycosides⁷.

Borntrager's test:-

To each of the extract were boiled with 1 ml of dilute H₂SO₄ in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volume of dichloromethane. The lower levels of dichloromethane separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides⁷.

Froth test:-

To each of the extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides⁷.

Test for Phenolics and Tannins

Ferric chloride test:-

Small amount of *Cyperus rotundus* extract were shaken with water separately and warmed. Then about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue color which may indicate the presence of phenols⁷.

Gelatin test:-

1% gelatin solution containing 10% sodium chloride was added to each extract. Formation of precipitate indicated the presence of tannins and phenolic compounds⁷.

Iodine test:-

Cyperus rotundus extract were treated with diluted iodine solution separately. Appearance of transient red color indicated the presence of tannins and phenolic compounds⁷.

Nitric acid test:-

Cyperus rotundus extract were treated with dilute nitric acid separately. Formation of reddish to yellowish color indicated the presence of tannins and phenolic compounds⁷.

Test for Amino acid and Proteins

Biuret test:-

To each of the extract were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation of purplish violet color may indicate the presence of proteins⁷.

Million's test:-

3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicate the presence of amino acids⁷.

Test for Carbohydrates**Molish's test:-**

To each of the extract were treated with 2 drops of alcoholic α -naphthol solution in a test tube separately and 2 ml of conc.H₂SO₄ was added carefully along the sides of the test tubes. Formation of violet ring at the junction may indicate the presence of carbohydrates⁷.

Fehling's test:-

To each of the extract were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown color was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar⁷.

Benedict's test: -

To each of the extract were treated with equal volumes of Benedict's reagent in test tubes separately. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate⁷.

Test for Steroids**Salkowsti's Test:-**

To each of the extract was dissolved in 2ml of chloroform. Conc.H₂SO₄ was carefully added to form a low layer. A reddish brown color in the interface is indicative of steroidal ring⁷.

Test for Anthraquinone**Borntrager's test**

To each of the extract was put in a test tube and 5ml of chloroform added and shaken for 5 minutes. This was filtered and shaken with equal volume of 10% Ammonium solution. A pink violet or red color in the ammonical layer (lower layer) is indicative of the presence of anthraquinone⁷.

Test for Anthocyanin**Sodium Hydroxide Test:-**

Weigh about 0.2gm of plant each extract in separate test tube, 1ml of 2N Sodium hydroxide was added, and heated for 5 minutes at $100^0 \pm 2^0C$. A bluish green color is indicative of the presence of anthocyanin⁷.

Test for saponins**Froth Test:**

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins⁷.

Foam Test:

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins⁷.

RESULTS AND DISCUSSION

The phytochemical screening in the present study has revealed the presence of carbohydrates, glycosides, proteins, amino acids, phenolic compounds, tannins, steroids, anthraquinone, anthocyanin, saponins flavonoids and alkaloids. Most of the phytochemical constituents were present in the extracts of methanol, aqueous, ethylacetate and cyclohexane. Similarly, the acetone and chloroform extract of the plant revealed the presence of carbohydrates, glycosides, proteins, saponins, amino acids, phenolic compounds and tannins, but in the benzene extract of plant were found in flavonoids, glycosides, phenolic compounds and tannins. Table 2 showed the results of phytochemical screening of various extracts of leaves of *Cyperus rotundus*L. Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities⁷. The phytochemical constituents are very important and beneficial in industrial and medicinal sciences. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites⁹. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds^{10, 11}. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity¹². Anthraquinones are used as dyes and antibacterial agents. Tannins are known to form irreversible complexes with proline-rich protein¹³ resulting in inhibition of microbial cell protein synthesis. Tannins also have anticancer activity and can be used in cancer prevention¹⁴. Glycosides and tannins are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia¹⁵.

Table 1: Physical Characteristics and % Yield of Various Extracts of *Cyperus rotundus*. L

Solvent	Color of the extract	Odour	Consistency	Sense of touch	Amount of extract (gm)	% of yield (w/w)
Acetone	Brown	Characteristic	Semisolid	Sticky	2.10	2.1
Benzene	Greenish yellow	Characteristic	Semisolid	Sticky	2.60	2.6
Chloroform	Brownish black	Characteristic	Semisolid	Sticky	3.00	3
Cyclohexane	Yellowish green	Characteristic	Semisolid	Sticky	3.30	3.3
Ethylacetate	Greenish yellow	Characteristic	Semisolid	Sticky	4.10	4.1
Methanol	Light yellow	Characteristic	Semisolid	Sticky	6.00	6
Aqueous	Light Brown	Characteristic	Semisolid	Sticky	4.50	4.5

The % yield was maximum (6.00%) obtained with ethanol and least (2.1%) with acetone.

Table 2: Phytochemical Screening of Various Extracts of *Cyperus rotundus*L.

Chemical test	Acetone extract	Benzene extract	Chloroform extract	Cyclohexane extract	Ethylacetate extract	Methanol extract	Aqueous extract
Flavonoids							
Shinoda test	+	+	+	-	+	+	+
Alkaline reagent	+	-	++	+	+	++	++
Phenolic compounds & Tannins							
Ferric chloride test	++	+	++	++	++	+	++
Gelatintest	+	-	-	-	-	+	+
Iodinetest	+	+	+	-	+	+	++
Nitric acidtest	+	-	+	+	+	-	-
Alkaloids							
Dragendroff's reagent	-	-	-	+	+	+	+
Mayer's reagent	-	-	-	-	-	+	-
Hager's reagent	-	-	-	-	+	+	+
Wagner's reagent	-	-	-	+	-	+	+
Amino acids & Proteins							
Biuret test	+	-	-	+	+	+	+
Million's reagent	+	-	+	++	++	++	++
Glycosides							
Molish's test	-	+	+	+	++	+	+
Fehiling's test	+	+	-	+	+	++	++
Benedict's test	+	+	+	-	+	+	+
Steroids							
Salkowsti's Test	-	-	-	+	-	+	+
Anthraquinone							
Borntrager's test	-	-	-	-	+	-	+
Anthocyanin							
Sodium Hydroxide Test	-	-	-	+	-	+	-
Saponins							
Froth Test	+	-	+	++	+	++	++
Foam Test	+	-	+	+	+	++	+

++ : Present in high concentration;

+ : Present in trace concentration;

- : Constituents not detectable using the specified assay method.

CONCLUSION

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work for future

studies could include the isolation, characterization studies and various other biological activity studies in this plant using various solvents.

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