



Qualitative and Quantitative Analysis of Phytochemicals of *Cyperus corymbosus* Rottb Rhizome

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ABSTRACT

The present study was aimed to analyze phytochemicals present in rhizome of *Cyperus corymbosus* Rottb. The rhizome of the plant was collected from their natural habitat from the river bank of Cauvery, Mandya and Mysore, India. Dried rhizome powder was subjected to sequential solvent extraction with increasing polarity. Various solvent extracts of rhizome found to contain different phytochemicals to various extents. Acetone and methanol extracts were rich in phenols, flavonoids, alkaloids, saponins, terpenoids, and Tannin. Cardiac glycosides recorded highest in ethyl acetate extract and water extract was rich in saponins and phlobatannins. Anthraquinones was found equally distributed in ethyl acetate, acetone, and methanol extracts. Quantitative analysis revealed that methanol extract contains the highest total phenol (0.615 mg GAE/mg extract) and flavonoid content (0.23 mg QCTE/mg extract) was highest in acetone extract, and total Tannins content (0.089 mg TAE/mg extract) was found highest in the methanol extract. Further, isolation of phytochemicals present in different solvent extracts of *C. corymbosus* rhizome will pay the way for significant application in medicinal and pharmaceutical industries.

Key words: *Cyperus corymbosus*, Rhizome, Phenol, Flavonoid, Tannin.

INTRODUCTION

Plant-derived secondary metabolites are highly diverse in their structure and function. Though these secondary metabolites are not involved in the primary function, their timely expression enhances the fitness of plants in nature. These plant metabolites provide either protection against various pest and pathogens or climatic stress or even help for pollination

or even to improve their adaptation abilities. Today, phytochemicals are widely used in medicines for their high effectiveness and low toxicity¹. The phytochemicals also have tremendous application in agriculture, cosmetics and food too, apart from medicine^{2,3,4}. The potential of these infinite pools of metabolites is yet to be explored.

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Among the several phytochemical sources, the family Cyperaceae and genus *Cyperus* was one of the vital one, which possess highly valuable phytochemicals. Among them, *C. rotundus* is a multivalent drug plant possessing phytochemicals of pharmacological properties like, analgesic, antibacterial, antidiarrheal, antidiabetic, anti-inflammatory, antioxidant, antipyretic, antisaturative, appetizer, diaphoretic, digestant, lactodepurant, thirst relieving and tranquilizing effect^{5,6,7,8,9}.

The *C. rotundus* studied for phytochemicals revealed the presence of sesquiterpenes, flavonoids, phenylpropanoids, phenolic acids, alkaloids, and saponins¹⁰. The *C. rotundus* rhizomes analysis revealed the presence of alkaloids, carbohydrates, glycosides, steroids, saponins, resins, tannins and phenols in its various solvents extracts¹⁰. The total oligomeric flavonoids endowed in rhizome extract of *C. rotundus* exhibits a broad spectrum of biological properties such as antimicrobial, antioxidant, antimutagenic, antigenotoxic, anticancer and neuroprotective properties¹¹.

One of the problems that existed with *C. rotundus* is availability of biomass for large scale extraction. On the other hand, several related species with high biomass producing capability exist. But a detailed scientific study is required to use these plants as an alternative to *C. rotundus*. One such neglected plant which produces higher biomass compared to *C. rotundus* is *C. corymbosus*. Hence, in the present study, we performed preliminary work on the phytochemicals of *C. corymbosus* rhizome.

MATERIAL AND METHODS

Collection and identification of plant materials

Cyperus corymbosus found growing naturally on the river banks of Cauvery in and around Mandya and Mysuru districts of Karnataka were collected during January 2015 and one kg of rhizome samples were collected (Fig. 1). The plant specimen was identified by Dr. Sampath kumara, K. K., taxonomist, lecturer in Biology, Govt. P. U. College, Davangere.

Further, the plant identity was authenticated by sending the herbarium to Botanical Survey of India (BSI), Howrah, West Bengal, India.

Extraction of phytochemicals from the rhizome

Surface of rhizomes was cleaned by washed under tap water, blot dried and used for further process. Surface dried rhizomes are cut into small pieces (4-6 mm) and dried at 45°C until a constant weight was attained. The dried samples were coarse powdered in a mechanical blender and stored at the dry and cool place until further use. The powdered samples were taken in timple and used for extraction using Soxhlet apparatus with different solvents with increasing polarity [hexane (0), benzene (3.0), ethyl acetate (4.3), chloroform (4.4), acetone (5.4), methanol (6.6) and water (9.0)]. The extracts are concentrated using Rota vapor and used for further studies.

Qualitative phytochemical analysis

The bioactive constituents present in different solvent extracts of rhizome was qualitatively analysed following standard procedures as described by Harborne¹², Trease and Evans¹³ and Sofowara¹⁴.

Alkaloids: 8 ml of HCl (1%) was added to plant extracts (100 mg), mixed, warmed and filtered. The filtrate (2 ml) was treated with Dragendorff's reagents. The solution was observed for the formation of brownish red precipitate, which specifies the presence of alkaloids.

Flavonoids: Dilute ammonia (5 ml) (1%) was added to plant extract (100 mg), mixed well and few drops of concentrated sulphuric acid was added. The solution was observed for the formation of yellow coloration which specifies the presence of flavonoids.

Saponins: 5 ml of distilled water was added to 200 mg of plant extracts, mixed and filtered. 500 µl of filtrate was withdrawn to a test tube and makeup to 5 ml with distilled water. The test tube was shaken vigorously for 2 min. the test tube was observed for the formation of stable foam which specifies the presence of saponins.

Phenols: The different solvent extracts (100 mg) were added to a test tube and mixed with

2 ml of distilled water. To this few drops of aqueous ferric chloride (10%) solution was added. The solution was observed for the formation of green, purple, deep blue or black colour shows the existence of phenols.

Terpenoids: Plant extracts (100 mg) was mixed with 2 ml chloroform. To this solution few drops of concentrated sulfuric acid was carefully added. The solution was observed for the development of a layer of the reddish-brown coloration which specifies the presence of terpenoids in extracts.

Anthraquinones: Plant extracts (500 mg) is boiled 6 ml of HCl (1%) and filtered. To the filtrate, benzene (5 ml) was added and shaken well, and benzene layer was removed. To this solution 3 ml of NH₄OH (10 %), and observed for the formation of pink or violet or red colour in alkaline phase with specifies the presence of anthraquinones.

Cardiac glycosides: Plant extracts (5 ml) was added to premixed solution containing glacial acetic acid (2 ml) and one drop of ferric chloride (FeCl₃). To this solution, 1 ml concentrated Sulphuric acid was added. The solution was observed for formation of brown ring at the interface which specifies the presence of deoxysugar of cardenoloides. Similarly, formation violet and brown ring specify the presence of cardiac glycosides.

Phlobatannins: Plant extracts (200 mg) dissolved in water (2 ml) was boiled with (few drops) 1% aqueous hydrochloric acid. The solution was observed for the formation of red precipitate, thus specifying the presence of phlobatannins.

Tannins: Plant extracts (250 mg) was dissolved in distilled water (10 ml) and filtered. To this solution few drops of aqueous Iron chloride (FeCl₃) solution (1%) (Few drops) was added. Development of intense green, purple, blue, or black colour specifies the presence of tannins.

Quantitative analysis of phytochemicals

Cyperus corymbosus rhizome extracts (RE) were dissolved in a common solvent, methanol except water extract which was dissolved in water to prepare stock solutions of 2 mg/ml. Further double dilutions were made to obtain different concentrations.

Estimation of Total Phenols

The total phenol was estimated, according to Abirami *et al.*¹⁵, with minor modification. The Gallic acid was used as standard. 10 µl of different dilutions of sample was taken in a 5 ml test tube and 50 µl of Folin-Ciocalter reagent (FC reagent) (1 mol/L) was added, mixed. To this solution, 2 ml of distilled water was added, mixed well and incubated for 3 minutes. Further, 500 µl of 20 % sodium carbonate solution was added and mixed well by vortexing. The reaction mixture was then incubated for 40 min in the dark and absorbance was read at 765 nm with UV-vis spectrophotometer. The total phenolic content of different solvent extracts of plant was expressed equivalents of Gallic acid (mg GAE/mg extract).

Estimation of Total Flavonoids

The flavonoid content was estimated, according to Abirami *et al.*¹⁵ with minor modification. The rhizome extracts was mixed with AlCl₃ (2 %) in methanol in 1:1 ratio. The reaction mixture was incubated for 15 min at 30°C. The absorbance of the samples were read at 415 nm. The total flavonoid content was expressed as equivalents of Quercetin (mg QCTEs/mg extract).

Estimation of Tannins

The tannin content was determined according to Muthukumar *et al.*¹⁶. 100 µl of each solvent extract of rhizome were taken and made up to 7 ml with distilled water. To this solution, potassium ferric cyanide (8 mM) and ferric chloride (20 mM) prepared in 0.1 M hydrochloric acid was added. The contents were mixed and absorbance was measured at 700 nm. The Tannic acid (TA) was used as standard. The Tannin content was expressed equivalents of TA (mg TAE/mg extract).

Statistics

The data obtained from the experiments were statistically analyzed by subjecting data's to Analysis of Variance (ANOVA) using SPSS V21 software (SPSS Inc., Chicago IL). The significant difference between the means was compared using the highest significant difference (HSD) as obtained by Tukey's-b test at $p < 0.05$ level.

RESULTS AND DISCUSSION

The herbal traditional medicine is the oldest medicinal practice of the world. The WHO in 1985 estimated that 80% of the world inhabitants were dependent primarily on traditional medicines for their primary health care facilities¹⁷. The crude drug was prepared from the rhizomes of *C. corymbosus* used for birth control processes in indigenous medicines¹⁸.

Identification of plant materials and its solvent extractions

One Kg of plant specimens was collected from the river bank of Cauvery in Mandya and Mysore districts of Karnataka. Upon processing, it yielded 400 gm dried coarse powder. The rhizomes powder was subjected to phytochemical extractions using different solvents of increasing polarity (Fig. 2). The different solvent extracts were then subjected to further analysis.

Qualitative phytochemical analysis

Presence of alkaloids, carbohydrates, phenols, glycosides, phytosterols, etc. in ethanolic extracts of leaves of *C. rotundus* was reported by Elezabeth and Arumugam¹⁹. Several early researchers recorded the presence of flavonoids, tannins, glycosides, monoterpenes, sesquiterpenes, sitosterol, saponins, terpenoids, starch and proteins in *C. rotundus* rhizome^{20,21,22,23,24}. In our study, Different solvent extracts of rhizome found to contain different phytochemicals to various extents. Acetone and methanol extracts were found positive for all the metabolites. They were rich in alkaloids, flavonoids, saponins, phenols, terpenoids, and Tannin. Cardiac glycosides recorded highest in ethyl acetate extract, and water extract was rich in saponins and

phlobatannins. Anthraquinones was found equally distributed in ethyl acetate, acetone, and methanol extracts (Table 1).

Quantitative analysis of phytochemicals

Among the different extracts, Methanol extract was found rich in total phenol content (0.615mg GAE/mg extract) followed by Acetone extract (0.577mg GAE/mg extract). Water extract was found to contain least total phenol content of 0.127 mg GAE/mg extract (Fig. 3). Similarly, Acetone and Methanol extracts were also found to contain the highest flavonoid content of 0.23mg QCTE/mg extract and 0.17 mg QCTE/mg extract, respectively (Fig. 4). Highest of 0.089 mg TAE/mg extract of tannin was recorded in Methanol extract followed by Acetone extract (0.0689 mg TAE/mg extract) (Fig 5). In all the three cases, the benzene extract was found least in metabolites.

Bashir *et al.*²⁵ reported antioxidant activity from flavonoids in different parts of *C. rotundus* L. by using methanol and ethanol solvents extraction. The total flavonoids content of *C. rotundus* rhizome solvents extracts varied from 7.196 to 200.654 µg Quercetin (QE)/mg, where acetone extract showed the higher value of total flavonoids content¹⁰. Flavonoids, tannins, and polyphenols were identified in these extracts. Further, the extracts were found to decrease the ear oedema in mouse, abdominal contractions in mice, the peripheral analgesic activity of extracts significantly enhance lymphocyte proliferation. It also gives clues that analgesic, anti-inflammatory, antioxidant, and immunomodulatory effects of the extracts may be attributed to the presence of flavonoid, tannin, and polyphenol contents²⁶.

Table.1: Qualitative phytochemical analysis of different solvent extracts of *Cyperus corymbosus* rhizome

Name of Test	Observation	Hex	Ben	EA	Chl	Ace	Met	Wat
Alkaloids	Orange color/ Yellow precipitate	-	-	-	-	+++	+++	-
Flavonoids	Yellow coloration	-	-	++	-	+++	+++	+
Saponins	Formation of honeycomb like froth	-	-	-	-	+++	+++	+++
Phenols	Formation of deep blue or black color	-	-	++	-	+++	+++	+
Terpenoids	Reddish brown coloration at the interface	+	-	+	+	+++	+++	NC
Anthraquinones	Presence of pink, violet, or red color in the ammoniacal phase	-	-	+	-	+	+	-
Cardiac glycosides	Presence of brown ring at the interface	+	-	+++	-	+	+	NC
Phlobatannins	Red precipitate	-	-	+	-	+	+	+++
Tannins	appearance of intense green, purple, blue or black color	-	-	+	+	+	+++	+

‘+’ positive, ‘+++’ Strongly positive, ‘-’ negative, ‘NC’ Not Clear

The *Cyperus corymbosus* rhizome solvent extracts - Hex-Hexane extract, Ben-Benzene extract, EA-Ethyl Acetate extract, Chl-Chloroform extract, Ace-Acetone extract, Met-Methanol extract, and Wat-Water extract.

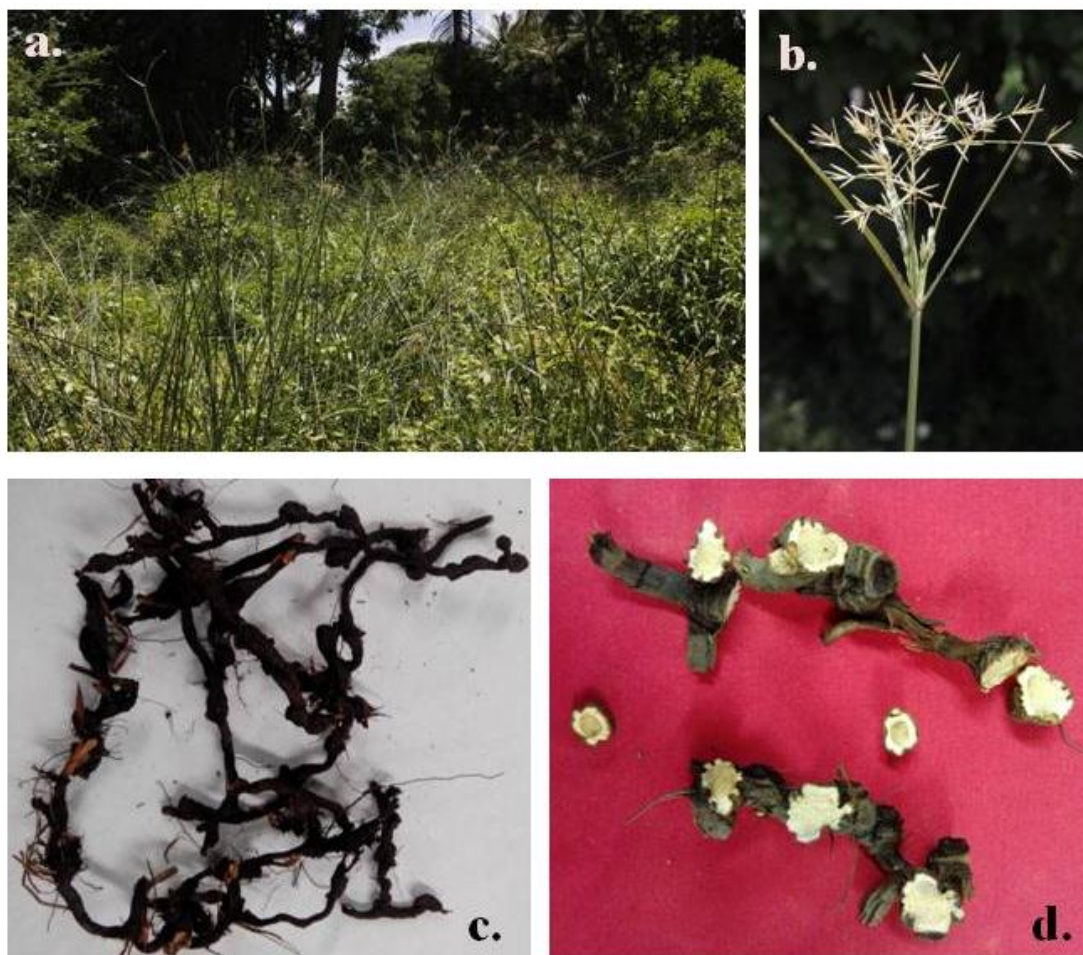


Figure1. a. The natural habitat of *Cyperus corymbosus*, b. Close up of stem and inflorescence, c & d. Intact and cut rhizome.



Figure2. The *Cyperus corymbosus* rhizome solvent extracts - Hex-Hexane extract, Ben-Benzene extract, EA-Ethyl Acetate extract, Chl-Chloroform extract, Ace-Acetone extract, Met-Methanol extract, and Wat-Water extract.

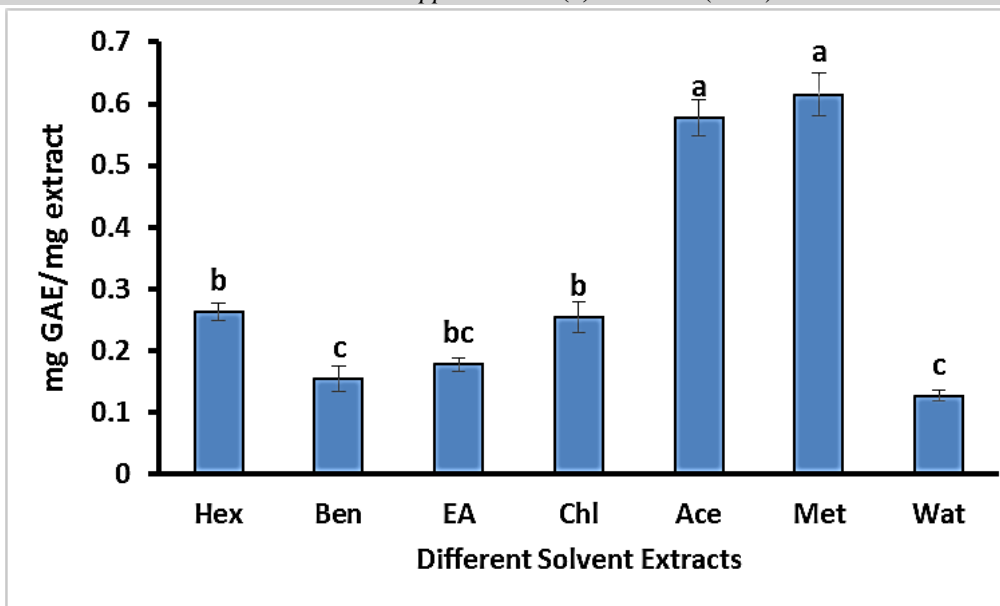


Figure3. Quantitative estimation of Total Phenols of *Cyperus corymbosus* rhizome
 Each value is expressed as mean ± standard error (SE) (n=3). Different letters in letters in each column denotes statistically significant difference compared to the positive control at (p<0.05).

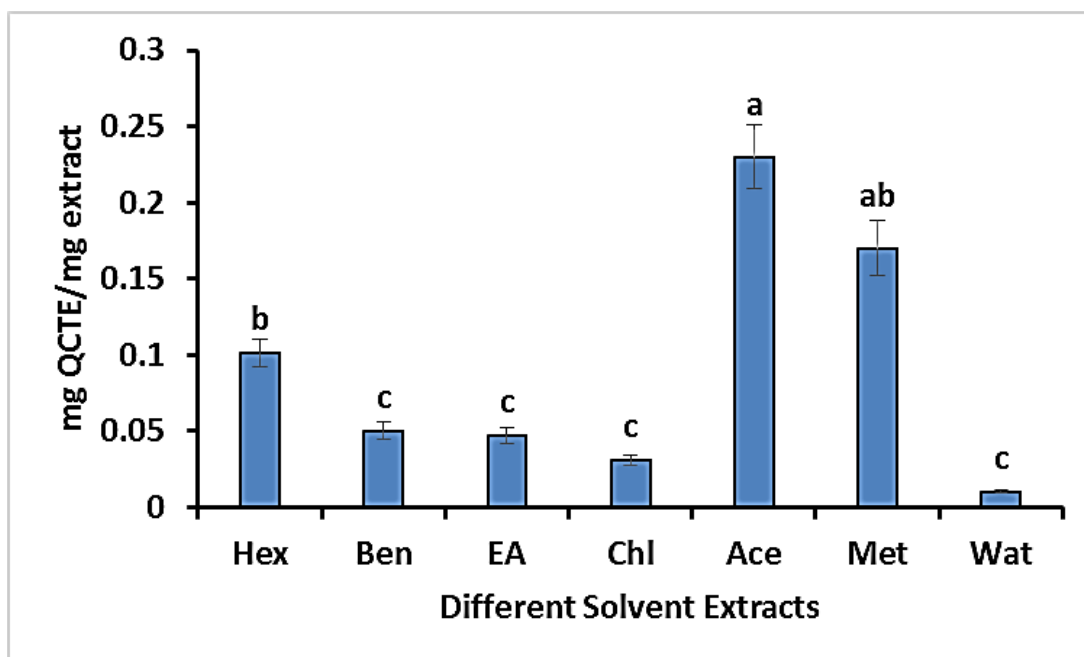


Figure4. Quantitative estimation of Total Flavonoids of *Cyperus corymbosus* rhizome
 Each value is expressed as mean ± standard error (SE) (n=3). Different letters in letters in each column denotes statistically significant difference compared to the positive control at (p<0.05).

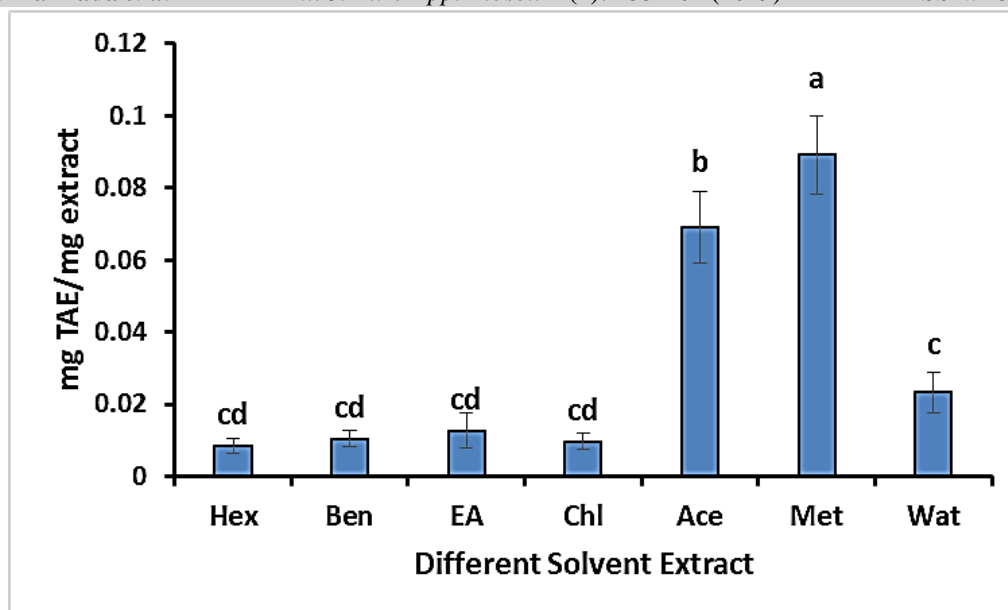


Figure5. Quantitative estimation of Tannins of *Cyperus corymbosus* rhizome

Each value is expressed as mean \pm standard error (SE) (n=3). Different letters in letters in each column denotes statistically significant difference compared to the positive control at ($p < 0.05$).

CONCLUSION

Our study of *C. corymbosus* rhizome, which was least, studied revealed the presence of significant phytochemicals that have better biological properties. Thus, the study will be helpful in the exploration of phytochemicals of *C. corymbosus* rhizome which may be helpful in the field of medicine and pharmacy.

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