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In vitro Studies of Phytochemical Screening, Antioxidant Activity and Anti inflammatory Effect of *Cyperus rotundus* Rhizomes.

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ABSTRACT:

In this study *Cyperus rotundus* rhizomes extract was evaluated in a series of in vitro assay involving phytochemical screening, Antioxidant activity and anti-inflammatory effect were determined. Phytochemical screening is an important step for the isolation of biological active chemical constituents. The chloroform extract showed the presence of Tannin, Saponins, Flavonids, Quinones, Cardic Glycosides, Terpenoids, Phenol, Coumarins, Steroids, Phytosteroids and Carbohydrate. Total phenolic and Total flavonoid content was found to be 56.73 mg Tannic acid equivalent /gm and 16.43 mg Quercetin/gm respectively. The results indicate the chloroform extract of *Cyperus rotundus* rhizomes exhibited good antioxidant activity when compare to the other extracts. At a concentration of 300µg of chloroform extract produced 85.61% inhibition of RBC haemolysis. The data justify the traditional use of *Cyperus rotundus* as medicinal plant which has a potential source of bioactive molecules to treat inflammatory diseases.

KEYWORDS: *Cyperus rotundus*, Human Red Blood Cell & Anti inflammatory.

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INTRODUCTION:

India has a rich wealth of medicinal plants, most of which have been traditionally used in Ayurveda, Unani system of medicine and by tribal healers for many generation. A wide variety of active photochemical including the flavonoids, terpenoids, lignin's, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkoloid, thiophenes have been identified in the medicinal plants. There is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industry purposes. This is mainly due to their strong biological activity excluding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts[1]. A number plants have been investigated for their biological activities and antioxidant principles [2,3]. *Cyperus rotundus* Linn, sedge of the family Cyperaceae and other Cyperales, is widely distributed in the Mediterranean basin areas [4].The tuber part is one of the oldest known medicinal plants used for the treatment of dysmenorrheal and menstrual irregularities [5].The rhizomes are cooling, intellect Promoting, nervine tonic, diuretic, antiperiodic and used to treat diarrhea, dysentery, leprosy, bronchitis and blood disorder [6]. It is also traditional medicinal plant appearing among the Indian. Chinese and Japanese natural drugs. It is used in the treatment of spasms, stomach disorder and inflammatory diseases[7]. *Cyperus rotundus* extracts offer a rich potential source of novel antiplatelet agents [8]. It is increasingly being realized that many of today's diseases are due to the oxidative stress that results from an imbalance between formation and neutralization of pro–oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid per oxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases [9]. Based on traditionally uses we selected this plant for the study. The purpose of the present study was to evaluate the phytochemical screening, antioxidant activity and anti-inflammatory effect.

MATERIALS AND METHODS:

Collection of plant Material:

The rhizomes of the *Cyperus rotundus* were collected from the Salem District, Tamilnadu, India. Authenticated by ABS Botanical Conservation Research and Training centre, Kaaripatti, Salem District, Tamilnadu, India. The rhizomes were shade dried at room temperature for 15 days and make in to coarse powder.

Preparation of rhizome extract:

The coarse powder (100g) was extracted exhaustively in a Soxhlet apparatus with Hexane, chloroform and Ethanol for 72 hours. The extract was separately collected and the solvents were allowed to evaporate using rotary vacuum evaporator. The yield from 100 g of sample contains 900 mg of Hexane, 400 mg of chloroform and 970 mg of ethanol extract. These three solvent extracts were used for further analysis.

Preliminary Phytochemical analysis:

The rhizomes of the *Cyperus rotundus* extracts were screened for the presence of photochemical constituents. Photochemical test were carried out adopting standard procedure [10].

Estimation of Total Phenolic content:

Total phenol content in the rhizomes of the *Cyperus rotundus* extracts (Hexane, Chloroform and Ethanol) was determined using the Folin Ciocalteu Regent method [11]. Each extract solution (0.1 ml containing 1000 microgram) was transferred to a 100 ml Erlenmeyer flask and then the final volume was adjusted to 46 ml by the addition of distilled water. Afterward, 1 ml of Folin Ciocalteu Regent was added into this mixture, and after 3 minutes, 3 ml of sodium carbonate (2%) was added. This mixture was shaken on a shaker for 2hours at room temperature and then absorbance was measured at 760 nm. The amount of total phenolics was calculated as the tannic acid equivalents.

Estimation of Total Flavonoid content:

Total flavonoids content in the rhizomes of the *Cyperus rotundus* extracts (Hexane, Chloroform and Ethanol) was determined by Saleanalea. Each extracts solution (0.1 ml containing 1000 microgram) with 0.9 ml of distilled water in test tube followed by addition of 75 micro liter of 5% sodium nitrite solution. After 6 minutes 150 micro liter of 10% aluminum chloride solution was added, then 0.5 ml of 1M sodium hydroxide was added. In this reaction mixture was brought to 2.5 ml with distilled water and mixed well. Then measured at 510 nm. The result was expressed as Quercetin equivalent.

Antioxidant Activity:

The hydrogen atom or electron donation abilities of the consequent extracts and standards were measured from the bleaching of the purple colored methanol solution of 2, 2-diphenyl-1-1-picryl hydrazyl (DPPH). 0.5mM solution of DPPH in Methanol was prepared. Each extract was taken in three concentration of 50,200,500 µg. Each extract was made up to 1.5 ml and add 1.5 ml of DPPH solution. This mixture was measured at 517 nm by using the spectrophotometer.

Anti inflammatory activity:

Membrane stabilizing method:

Membrane stabilizing activity of the extract was assessed using hypotonic solution – induced Human erythrocyte haemolysis. The test sample consist of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 ml of hypotonic solution (50 mM Nacl) in 10 Mm sodium phosphate buffered saline contain the extracts (100-300 mg/ml).The control sample consist of 0.5 mL of HRBC mixed with hypotonic- buffered saline solution alone. The mixtures were incubated for 10 minutes at room temperature and centrifuged for 10 minutes at 3000g and the absorbance of the supernatant was measured at 540 nm.

RESULTS AND DISCUSSION:

Phytochemical investigation:

The phytochemical evaluation showed the presence of Tannin, Saponins, Flavonids, Quinones, Cardic Glycosides, Terpenoids, Phenol, Coumarins, Steroids, Phytosteroids and Carbohydrate in the Chloroform extract (Table - 1)

Table1: Phytochemical Investigation

S.No	Phytochemical constituents	Hexane	Chloroform	Ethanol
1	Tannins	-	+	-
2	Saponins	+	+	-
3	Flavonids	+	+	+
4	Alkaloids	-	-	-
5	Quinones	+	+	+
6	Glycosides	-	-	-
7	Cardic Glycosides	+	+	+
8	Terpenoids	+	+	+
9	Phenol	+	+	+
10	Coumarins	+	+	-
11	Phlobatannins	-	-	-
12	Steroids	+	+	+
13	Phytosteroids	+	+	+
14	Anthraquinone	-	-	-
15	Carbohydrate	+	+	+

Presence of constituents (+) & Absence of constituents (-)

Isolation of pharmacologically active components from the medicinal plant is the long and tedious process. Therefore the phytochemical screening is necessary to eliminate unnecessary separation procedures. This method is performed to allow localization and targeted isolation of new or useful constituents with potential activities and also this procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is significant for inexpensive [12] *Cyperus rotundus* contain oils, alkaloids, glycosides, saponins, flavonoids, tannins sesquiterpenes, hydrocarbons, epoxides and ketones and also used as anti-inflammatory estrogenic, antipyretic, antiemetic, diuretic & hypertensive agent [13].

Total Phenolic content:

Phenolic compounds are a class of antioxidant agents, which act as free radical terminators. Total phenols were measured by Folin cio calteu reagent in terms of Gallic acid equivalent. The results indicates the Chloroform extract of *Cyperus rotundus* possess high phenol content when compared to Hexane and Ethanol extract as given in Table 2.The amount of total phenolic content was found to be 56.73mg/g of extract calculated as tannic acid equivalent.

Phenolics are the most wide spread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to acts as both efficient radical scavengers and metal chelators. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [14]

Table 2: Total Phenolic content

S. No	Sample	Total Phenolic content
1.	Hexane Extract	42.06mg TAE/g
2.	Chloroform Extract	56.73mg TAE/g
3.	Ethanol Extract	32.59mg TAE/g

TAE –Tannic Acid Equivalent

Total flavonoid content:

Total flavonoid content in the *Cyperus rotundus* rhizomes extract was measured by aluminium chloride reagent in terms of quercitin equivalence were tabulated in table 3 respectively. The results indicate the Chloroform extract of Cyperus *rotundus* posses high flavonoid content when compared to other extracts. Total flavonoid content of samples was obtained in comparison with quercetin standard. Flavonoids are known to inhibit lipid peroxidation and exert these effects as antioxidant, free radical scavengers and chelators of divalent cations. Flavonoids have antioxidant activites.[15] The flavonoid and phenolic compounds have been known to exhibit anti-inflammatory, antioxidant and metal chelating properties [16, 17]

Table 3: Total Flavonoid Content

S. No	Sample	Total Flavonoid Content
1.	Hexane Extract	10.83mg QUE/g
2.	Chloroform Extract	16.43mg QUE/g
3.	Ethanol Extract	9.07mg QUE/g

QUE – Quercetin Equivalent

DPPH Assay:

Free radical scavenging activity of *Cyperus rotundus* rhizomes extracts at different concentrations 50,200,500µg was evaluated by the DPPH method. Chloroform extract of *Cyperus rotundus* showed highest scavenging activity.

Table 4: Anti oxidant activity

S. No	Sample	Concentration	% of Inhibition
1	Hexane Extract	50µg	26.42
		200µg	32.71
		500µg	35.56
2	Chloroform Extract	50µg	37.24
		200µg	48.83
		500µg	61.37
3	Ethanol Extract	50µg	25.32
		200µg	30.21
		500µg	33.18

Free radical is the major cause of various chronic and degenerative diseases in the living systems. The vast amounts of synthetic molecules are available for free radical scavenging antioxidants, but adverse side effects are associated with these compounds. An alternative solution for this problem is to consume the naturally available antioxidants from the medicinal plants because they are having lower side effects and comparatively safe [18]

Membrane stabilizing:

Anti inflammatory study was performed with different extract at a concentration range of 100, 200 and 300 (µg/ml) protects the HRBC membrane against lysis induced by hypotonic solution. It showed the maximum stabilization 85.61% at 300ug/ml of chloroform extract. The presence study indicates the chloroform extract has the highest anti-inflammatory activity when compared to the other extract.

Table 5: Anti inflammatory activity of *Cyperus rotundus* rhizomes

S. No	Sample	Concentration (µg/ml)	% of inhibition
1	Hexane Extract	100	67.56
		200	75.68
		300	81.32
2.	Chloroform Extract	100	68.69
		200	78.67
		300	85.61
3	Ethanol Extract	100	67.89
		200	76.65
		300	83.34

Anti inflammatory activity has been observed by the extract of *Cyperus rotundus* without producing any toxic symptoms. The extract may have such type of active principles that produces the anti-inflammatory effect more than aspirin. [19] It also reported the anti-inflammatory response in

chloroform extract. The presence of active principles such as flavonoids and triterpenoids and related poly phenols may responsible for Anti inflammatory activity *C.asiatica* can be used as a potent Anti inflammatory agent [20.]

CONCLUSION:

The present study has demonstrated that rhizomes part of *Cyperus rotundus* exhibits biological active phytoconsitutants. This plant also contained the phenolic and flavonoids compounds which showed antioxidant activity. The rhizome could therefore serve as a cheap source of raw material for chemical industries. Bioactive substances from this plant can therefore, employed to develop drugs for the treatment of various inflammatory diseases. The *Cyperus rotundus* will also serve as source of non protein nitrogenous phytocompounds whose allelopathic properties will be of great economic value in general agriculture.

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