Journal of Pharmacology and Biomedicine **ISSN 2456-8244**

RESEARCH ARTICLE

An Evaluation of Phytochemicals in Maceration and Infusion Extracts of *Cyperus rotundus* Linn Leaves in Two Different Solvents

Neelam Singh and Madhulika Singh

Department of Botany and Biotechnology, Sadhu Vaswani P.G. College, Bhopal (M.P) India

Received June 2017; Revised July 2017; Accepted July 2017

* Corresponding Author

Email: nhari1965@gmail.com

ABSTRACT

The objective of the study to evaluate the hepatoprotective effect of ethanolic extract of *Curcuma caesia* rhizomes in Wistar rats on paracetamol induced liver. Albino wistar rats were separated into five groups (n=6). Ethanolic extract of *Curcuma caesia* rhizomes was prepared and evaluated for its hepatoprotective efficacy against paracetamol (PCM) (2g/kg p.o.) induced hepatotoxicity in rats. Silymarin (100mg/kg p.o.) was used as standard. Levels of Serum glutamic pyruvate transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), and total protein was evaluated along with histopathological investigation in various experimental groups of rats. Present study suggested thatthe protective effect of ethanolic extract of *Curcuma caesia* against paracetamol induced hepatic damage.

Keywords: *Cyperus rotundus*, Phytochemicals, Infusion, Maceration

Introduction

Cyperus rotundus Linn., also known as purple nutsedge or nutgrass is a common perennial plant is widely distributed in Mediterranean basin naturally grows in tropical, subtropical and temperate regions with extensive distribution in northeast part and plant is used in India, Srilanka, China and Japan to obtain natural drugs for many ailments (Anonymous, 2001; Vershaeve, *et al*., 2004; Rai, *et al*., 2010; Sivapalan and Jeyadevan2012).

The systematic scientific classification of *C. rotundus* as per the IUCN Red List of Threatened Species 2015-3 with the reference of Lansdown and JuffeBignoli (2013) are as follows;

Kingdom:	Plantae
Division:	Tracheophyta
Class:	Liliopsida
Order:	Cyperales
Family:	Cyperaceae
Genus:	Cyperus
Species:	*C. rotundus* (Linn)

C. rotundus is a versetile medicinal plant being used as sedative, carminative, stimulant, tonic, aphrodisiac, diuretic, stomachic, anthelmintic, colic remedy and to remove renal calculi (Boulos and El-Hadidi 1984). Several scientific studies reported the analgestic, anticancer, antiviral, antimalarial, antibacterial and antifungal, antifeedant and antifertility activity of some plants (Vishnukanta, 2008). This plant is also used as hypertensive, estrogenic, antiemetic, remedy for women's diseases, fever, pain, diarrhea & dysentery, vomiting and other intestinal problems (Muthu, *et al.,* 2014).This plant is reported to have the of analgesic, alternative, astringent, antispasmodic, antibacterial, carminative, contraceptive, demulcent, emmenagogue, emollient, febrifuge, immunostimulant, laxative, stimulative, tonic, and vermifuge properties (Sivapalan and Jeyadevan2012). The aromatic oils of this plant are used in perfumes and splash (Yeung, 1985).

A large number of phytochemicals and essential oils have been reported from rhizome this plant including polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil (Sharma and Singh 2011; Khan, *et al*., 2011; Sivapalan, 2013). The characteristic aroma and flavor of *C. rotundus* is due to its e essential oil sesquiterpene hydrocarbons, epoxides, ketones, monoterpenes and aliphatic alcohols (Sivapalan, 2013). Selinene, isocurcumenol, nootkatone, aristolone, isorotundene, cypera-2,4 (15)-diene, and norrotundene, as well as the sesquiterpene alkaloids rotundines A-C are the sesquiterpenes present in this plant (Jeong, *et al*., 2000; Khan, *et al*., 2011). To add the piece of information the present work is aimed at extraction and analysis of phytochemicals from the leaves of *Cyperus rotundus* Linn by two different methods of

phytochemical extraction name maceration and infusion

Materials and Methods

Sampling & Processing:

Leaves of *Cyperus rotundus* L. were collected from plants samples obtained from areas around Hoshangabad District (M.P) India. The identification of the whole plant and its parts were done by Dr. Madhulika Singh, Department of Botany and Biotechnology, Sadhu Vaswani P.G. College, Bhopal (M.P) India. After washing, cleaning and drying leaves were grounded to fine powder and stored in a container.

Phytochemical Extraction

The powdered leaves of *C. rotundus* were subjected to maceration with pure acetone and also leaves were subjected to process of infusion with 80% ethanol for short time separately. The marc is removed by filtration and the liquid extract so obtained is then concentrated in hot water bath at 50°C till the complete evaporation of the solvents from both extracts separately.

Test of Phytochemicals

Chemical tests were carried out for different extract to detect the presence of bioactive components in them by using standard methods described by Harbourne (1983) and Sofowora, (1993). A small portion of the dry extracts were subjected to test the presence of carbohydrates, proteins, alkaloids, tannins, terpenoids, saponins, flavonoids and glycosides(Khandelwal, 1997; Sreenu, *et al*., 2014).

a. *Test for Carbohydrates:*

Dissolved small quantities of extracts in distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

b. *Test for Alkaloid:*

3 ml each extract was stirred with 3 ml of 1% HCl on steam bath. After that the extracts were cooled to room temperature and Mayer and Wagner's reagent was added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

c. *Test for Tannins:*

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of 1% $FeCl_3$ Solution were added. Formation of blue, green or brownish green colour indicated the presence of tannins.

d. *Test for Saponins:*

A small amount of extract was shaken with 4ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

e. *Test for Flavonoids:*

To 3ml of test sample, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

f. *Test for Terpenoids:*

2 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

Tests for glycosides:

Sodium hydroxide reagent: Dissolved a small amount of extract in 1ml water and added sodium hydroxide solution. Development of yellow colour indicates the presence of glycosides.

Keller-Kiliani's test: dissolved the extract in water followed by glacial acetic acid. Than one drop of 5%$FeCl_3$ and conc. H_2SO_4 was added. Formation of reddish brown colour at junction of two liquid layers indicates the presence of Glycosides.

Test for Phenols:

The dried plant extracts about 100mg was dissolve separately in double distilled water; few crystals of ferric sulfate were added. Formation of dark violet colour indicates the presence of phenolic compound.

Results and Discussions

It can be clearly inferred from the results obtained that there is difference in the yield of extraction in the two different methods of extraction. The yield of extraction for acetonic maceration is 0.83%, while that of ethanolic infusion is reported to be 3.27%. Rai*et al*., (2010) reported 1.2%, 2.52%, 2.92%, 1.75%, and 6.88% as yield of extraction for petroleum ether, benzene, chloroform, ethyl acetate and methanolic extracts respectively obtained from rhizome of *C. rotundus* in addition to the presence of various phytochemical groups. Though the plant material for extraction was leaf powder in both methods but the organoleptic properties are slightly different which might also be due to the used of two different solvents for extraction. The acetonic maceration extracts was waxy dark brown in color while that of ethanolic infusion extract was chocolaty powder. Both the extracts were pleasant in odor.

Table 1: Organoleptic properties of phytochemical extracts of *Cyperus rotundus* Leaves

S. N	Extraction process	Plant part used	Yield of Extraction	Color	Odor	Consistency
1.	Acetonic Maceration	Leaf powder	0.83%	Dark Brown	Pleasant	Waxy
2.	Ethanolic Infusion	Leaf powder	3.27%	Chocolaty	Pleasant	Powder

Extracts obtained in present study were subjected to test for presence of carbohydrates, alkaloids, glycosides, tannins, phenols, terpenoids, flavonoids and saponins. There is huge difference observe for the present of different phytochemical groups in both the extracts which could seen as reflection in yield of extraction. The

acetonic extract obtained from maceration whose yield of extraction was 0.83% was found to be positive for the presence of alkaloids only out of all the phytochemical tests conducted. Contrary to this, the ethanolic extract obtained from infusion, whose yield of extraction was 3.27%, is found to be rich in carbohydrates, alkaloids, tannins, phenols, flavonoids and saponins.

Table 2: Phytochemical Analysis of *Cyperus rotundus* extracts from Leaves.

S.N	Constituents	Extracts of Leaves of *C. rotundus*	
		Acetonic Maceration	Ethanolic Infusion
1	Yield (%)	0.83%	3.27%
2	Carbohydrates	–	+
3	Alkaloids	+	+2
4	Glycosides	–	–
5	Tannins	–	+
6	Phenols	–	+2
7	Terpenoids	–	–
8	Flavonoids	–	+2
9	Saponins,	–	+

(+) deontes present, (+2) denotes Prominent, (+3) denotes highly prominent and (–) denotes absent

C. rotundus Lin has been described to have wide range of medicinal and pharmacological application in traditional system of medicine and exhibits anti-inflammatory, anti-arthritic, antipyretic, analgesic, antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antimicrobial, antioxidant, cytotoxic, apoptotic etc. (Singh *et al*., 2012).

Most of the phytochemical studies for *C. rotundus* in previous studies were confined to the phytochemical investigation of rhizomes only. Only few articles say 1 or 2 are reported investigation for leaves of this important plant. Sivapalan and Jeyadevan (2012) reported the presence of phenolic compounds, steroids, flavonoids, glycosides alkaloids and reducing sugars in acetonic extract of *C. rotundus* rhizomes. Sharma and Singh (2011) also investigated the rhizome of *C. rotundus* successively extracting with petroleum ether, chloroform, ethanol and water. Their phytochemical anaylsis describes the present of alkaloids, glycosides, proteins, carbohydrates, tannins, phenols and terpenoids which is almost similar to the present investigation. Rai *et al.,* (2010) also performed successive soxhlet extraction of *C. rotundus*rhizomes with petroleum ether, benzene, chloroform, ethyle acetate and methanol. Elezabeth,. and Arumugam, (2013; 2014) are the only researchers who recently worked out on the phytochemical of leaves of *C. rotundus*. Elizabeth and Arumugam, (2013) reported the presence of alkaloids, glycosides, Carbohydrates, tannins, phenolic compounds, and phytosterols in ethanolic extracts *Cyperus rotundus* leaves while the presence of glycosides, carbohydrates, tannins, and phenolic compounds in its aqueous extract. Elizabeth and Arumugam (2014), also performed the successive soxhlet extraction of *C. rotundus*

leaves with acetone, chloroform, cyclohexane, ethylacetate, aqueous and methanol. The present investigation also coincides with the uniqueness in terms of choice of plant part for investigation but the present work was more focused and to the point on the choice of solvents and methods for extraction of phytochemicals which may fall for method of selective isolation of phytochemical groups.

Conclusions

Among Indian, Chinese and Japanese natural drugs *C. rotundus* is a traditional medicinal plant popularly known as nagarmotha in India used against spasms, stomach disorders, and inflammatory bowel diseases and it is widely studied by several authors (S. Kilani *et al*., 2011).Since there has been little work was reported in the phytochemical of leave or areal parts of *C. rotundus* L., so the present investigation on phytochemicals using two different solvents and two different but simple methods is an important step in reporting the further new information in phytochemical investigation of this plant which fulfills the objective. Present study reports the present of various phytochemical carbohydrates, alkaloids, tannins, phenols, and flavonoids in the leaves of this plant. This information could be explored further into development of new therapeutics upon extensive studies in future.

Acknowledgement

The authors are grateful to the Department of Botany and Biotechnology of Sadhu Vaswani P.G. College, Bhopal to allow us the academic research work and provided the necessary facilities in conducting the work. The author also would like to pay their sincere thanks to colleagues and friends for their constant moral support and scientific advices during the whole work.

References

Anonymous (2001). The Wealth of India: A Dictionary of Indian Raw Materials

Boulos L and El-Hadidi MN (1984). The Weed Flora of Egypt. Cairo: (1984). The American University in Cairo Press. p.58.

Elezabeth DV and Arumugam S (2013). Study of the Phytochemical Analysis and Antimicrobial Activity of *Cyperus rotundus* Leaves. *International J Current Biotechnol* **1(8):** 5-8.

Elezabeth DV and Arumugam S (2014). Phytochemical Screening of the Various Extracts of *Cyperus rotundus*. L. *American J Pharmtech* **4(3):** 545-553.

Harborne JB (1983). Phytochemical methods; A guide to modern Techniques of plant analysis. 3rd edition Chapman and Hall; New York.

Jeong SJ, Miyamoto T, Inagaki M, Kim YC, and Higuchi R (2000). Rotundines A-C, Three Novel Sesquiterpene Alkaloids from *Cyperus rotundus*. *J Nat Prod* **63:** 673-675.

Khan S, Choi RJ, Lee DU and Kim YS (2011). Sesquiterpene Derivatives isolated from *Cyperus rotundus* L. inflammatory signaling mediated by NFxB. *Natural Product Sciences* **17(3):** 250-255.

Khandelwal KR (1997). Practical Pharmacognosy; Techniques and experiments, first Edition, Nirali Publication, Pune; p 146

Lansdown RV and JuffeBignoli D (2013). *Cyperus rodundus*. The IUCN Red List of Threatened Species 2013: e.T158183A68258847. www.iucnredlist.org/details/158183/0

Muthu K, Hema M, Nagaraj S and Rengasamy R (2014). *In-vitro* antibacterial potential, Phytochemical characterization of *Cyperus rotundus* flower extract. *Internat J Nat Prod Res* **4(1):** 6-8.

Rai PK, Kumar R, Malhotra Y, Sharma D and Karthiyagini T (2010). Standardization and Preliminary Phytochemical Investigation on *Cyperus rotundus* Linn Rhizome. *Internat J Res Ayurveda and Pharmacy* **1(2):** 536-542.

Kilani-Jaziri S, Bhouri W, Skandrani I, Limem I, Chekir-Ghedira L and Ghedira K (2011). Phytochemical, Antimicrobial, Antioxidant and Antigenotoxic Potentials of *Cyperus rotundus* Extracts. *South African J Botany* **77:** 767–776.

Sharma SK and Singh AP (2011). Morphological, Microscopical and Physico-Chemical Investigations on the Rhizomes of *Cyperus rotundus* Linn. *Res J Pharmaceutical, Biological Chem Sci* **2(3):** 798-806.

Singh N, Pandey BR, Verma P, Bhalla M and Gilca M (2012). Phyto-Pharmacotherapeutics of *Cyperus rotundus Linn.(Motha)*: An Overview. *Indian J Natural Products Resources* **3(4):** 467-476.

Sivapalan SR (2013). Medicinal Uses and Pharmacological Activities of *Cyperus rotundus* Linn. – A Review. *Internat J Scientific Research Publication* **3(5):** 01-08.

Sivapalan SR and Jeyadevan P. (2012). Physico-Chemical AndPhyto-Chemical Study of Rhizome of *Cyperus rotundus*Linn. Internat *J Pharmacol Pharmaceutical Technol* **2(1):** 42-46.

Sofowora A (1993). Medicinal Plants and Traditional medicines in Africa. John Wily and Sons New York; p 6

Sreenu P, Samatha T, Gandhi N and Naik AS (2014). Phytochemical Analysis of Root, Stem and Leaf Extracts in *Rubia cordifolia* L. an Important Medicinal Plant. *World J Pharm and Pharmaceutical Sci* **3 (10):** 826-838.

Vershaeve L, Kestens J, Tylor E and Maes A (2004). Investigation of the Antimutagenic Effects of Selected Northeast Medicinal Plants Extracts. *J Essential Oil Res* **18:** 29-35.

Vishnukanta AC and Rana (2008). *Meliaazedarach*: A phytopharmacological review. *J Pharmacog Rev* **2:** 173-179.

Yeung HimChe (1985). Handbook book of Chines Herbs and Formulas. Institute of Chinese Medicine, Los Angeles.