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Pharmacognostical analysis of different parts of *Cyperus rotundus* L.

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Abstract

Medicinal plants have been used as chief antidotes for the cure of numerous diseases since time immemorial. To investigate the quality assurance and authentication of medicinal plants, pharmacognostical, physicochemical and preliminary phytochemical studies of the different parts (root, stem and flower) of *Cyperus rotundus* were carried out. Present study deals with WHO (World Health Organization) recommended methods, fluorescence, phytochemical characteristics for standardization of plant parts by using a soxhlet apparatus in order of increase polarity. The extracts were subjected to qualitative phytochemical screening using standard procedures. Phytochemical investigation led to the revelation of the presence of enormous kinds of primary and secondary metabolite's as protein, carbohydrate, phenole, flavonoid, alkaloid, terpenoid, and saponin, *etc*., in trace, moderate and high amount in various extracts of different parts of experimental plant. It was concluded that the plant is rich in phytochemicals with significant pharmacological applications that may supply drugs for modern medicines and can be valuable for the therapeutic index. Information obtained from these studies help in determining the antioxidant capacity and contributes to the predominant group of bioactive components which can be used as markers in the identification and standardization of this plant as a herbal remedy.

Keywords: motha; pharmacognosy; pharmacology; phytochemical screening; secondary metabolites

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Introduction

Cyperus rotundus L. (Cyperaceae) commonly known as Nagarmotha and Nut grass, and is considered to be one of the world's worst weeds. It is indigenous to India, but now distributed around the globe in tropical, subtropical and humid areas (1). It grows up to 10 cm in a small clump. The immense distribution of the nut-grass is due to its capacity to adapt divergent environmental conditions, altitudes, climates, moisture level and soil pH. Herbal medicine is humanity's earliest recognized type of medication. According to the Ayurveda, *C. rotundus* possess different pharmacological actions like anti-parasitic (2), hepato-protective (3), anticancer (4), anti-inflammatory (5), astringent (6) etc. The literature survey did not provide enough data about pharmacognostical studies of different

parts of this plant. The current work revolves on the quality evaluation, pharmacognostical parameters and preliminary phytochemical screening of different (root, stem and flower) parts of *C. rotundus.*

Materials and Methods

Procurement and authentication

Different parts of *Cyperus rotundus* were collected from Krishi Vigyan Kendra (KVK), Banasthali Vidyapith, Banasthali (Tonk), Rajasthan in the month of October 2018. The plant was morphologically recognized by the botanist from KVK, Banasthali Vidyapith, Banasthali and has been documented by accessible literature.

Preparation of extracts

Different solvent extracts (Petroleum Ether, Benzene, Chloroform, Ethyl Acetate, Ethanol and Distilled Water) ranging from non-polar to polar was prepared by placing the powdered plant material (50 g) in the soxhlet apparatus and the solvents in the attached round bottom flask (RBF). The plant material was refluxed after each cycle with solvent (250–300 ml) for 12–36 h at 60 °C to 100 °C. Extracts were collected with the help of rotary evaporator and cooled at room temperature after the cycle of each solvent. After drying extracts were kept in desiccators for one to two days and stored in air-tight containers at 5 °C (7, 8).

Pharmacognostical analysis

Pharmacognostical values such as the foreign organic matter, the percentage of total ash value, acid insoluble and water-soluble ash value, moisture content, determination of pH value and extractive values of medicinal plant materials were experimentally conducted according to the WHO guidelines on quality control methods (8), chemical test and fluorescence analysis (9) were also studied.

Fluorescence characterization

A pinch of finely ground sample was taken in test tubes of different parts of plant. Various chemical treatments were given and fluorescence was observed at the different wavelength of U.V. short (254 nm), U.V. long (366 nm) and visible (8).

Qualitative phytochemical analysis

Standard procedures (8, 10, 11) were used for qualitative phytochemical analysis of all the plant extracts of three different parts *i.e.* stem, root and flower of *Cyperus rotundus*. In primary metabolite's protein, amino acids (Xanthoproteic, ninhydrin and biuret test), carbohydrates and reducing sugar (Molisch's and fehling's test) were analyzed. In secondary metabolites, alkaloids (Hager's, tannic acid, mayer's and wagner's test), anthraquinones (Hydroxyanthraquinone test), cardiac glycosides and free sugars (Keller-Killiani test), flavonoids (Alkaline reagents and lead acetate test), phenolics (Potassium dichromate and ferric chloride test), tannins (Ferric chloride and vanillin hydrochloride test), saponins (Olive oil test), terpenoids (Salkowski test) and fixed oils and fats (Saponification and oil stain test) were analyzed.

Statistical analysis

The obtained results were expressed as Mean ± Standard deviation (SD) of three replicates.

Results

Preparation of extracts

Phyto-profile of root, stem and flower different extracts of *C. rotundus* were prepared from non-polar to polar sequence with the help of soxhlet apparatus. After sequential extraction, residue left was, 29.455±0.706 g in roots, 34.33±2.43 g in a stem and 28.87±6.32 g in a flower. The color, physical state and consistency of the extracts of different parts were analyzed as listed in Table 1. Aqueous extract of root shows yield at (6.0%) and stem (5.1%) and flower (6.53%).

Pharmacognostical analysis

Proximate analysis

Ash value estimation is also an important parameter. It usually shows the inorganic salts present in the test sample and the highest amount of total ash value is 24.325% in stem part of the plant. The highest amount of acid insoluble ash value is 79.85% in the root part of the plant. The highest amount of water-soluble ash value is 91.35% in the root part of the plant indicating availability of acids, sugar and inorganic compounds. The highest amount of moisture content is 94.95% in flower part of the plant. At 10% solution the pH is 6.8 highest in root as listed in Table 2.

Fluorescence characterization

The behavior of *C. rotundus* roots upon treatments with different chemical reagents showed light brown when powder as such; dark brown when with distilled water; pinkish light brown when glacial acetic acid is used; reddish brown with 1 N HCl; light brown with 1 N H_2SO_4; orange with conc. HNO_3; dark green with ferric chloride (1 M); bluish red with iodine solution; light orangish brown with ammonia solution; dark bluish brown with NaOH (1 N); purplish orange with potassium dichromate; bluish green with methanol; violet brown with ethanol; bluish dark green with glycerin.

C. rotundus stem showed fluorescent green colour when powder as such; light green colour when with distilled water; reddish green when glacial acetic acid is used; brown with 1 N HCl; dark brown with 1 N H_2SO_4; light brown with conc.

Table 1. Preliminary phyto-profile of root, stem and flower of *Cyperus rotundus* in different organic solvent.

	Solvents	Boiling point	Polarity index	Color of extracts	Consistency	Nature	%Yield ± S.D
Root	Petroleum ether	60° C	0.0	Fluorescent light green	Oily	Semi solid	1.55 ± 0.10
	Benzene	80° C	2.7	Green	Oily	Solid	0.78 ± 0.35
	Chloroform	61° C	4.1	Light green	Sticky	Solid	0.46 ± 0.22
	Ethyl acetate	77° C	4.4	Yellow	Sticky	Solid	0.67 ± 0.31
	Ethanol	79° C	5.2	Dark brown	Sticky	Semi solid	1.69 ± 0.50
	Water	100°C	9	Light brown	Dry	Semi solid	6.0 ± 1.6
Stem	Petroleum ether	60° C	0.0	Yellow black	Oily	Semi solid	1.10 ± 0.12
	Benzene	80° C	2.7	Dark green	Oily	Semi solid	0.86 ± 0.15
	Chloroform	61° C	4.1	Green	Sticky	Solid	0.47 ± 0.38
	Ethyl acetate	77° C	4.4	Dark brown	Sticky	Semi solid	0.70 ± 0.22
	Ethanol	79° C	5.2	Red brown	Sticky	Semi solid	1.80 ± 0.04
	Water	100°C	9	Dark brown	Dry	Semi solid	5.1 ± 0.48
Flower	Petroleum ether	60° C	0.0	Dark brown	Oily	Semi solid	1.49 ± 0.15
	Benzene	80° C	2.7	Dark green	Sticky	Solid	0.4 ± 0.25
	Chloroform	61° C	4.1	Green	Sticky	Solid	0.50 ± 0.02
	Ethyl acetate	77° C	4.4	Yellow green	Sticky	Solid	2.50 ± 2.8
	Ethanol	79° C	5.2	Dark brown	Sticky	Solid	4.59 ± 6.09
	Water	100°C	9	Dark brown	Dry	Semi solid	6.53 ± 0.21

Table 2. Proximate analysis of stem, root and flower of *Cyperus rotundus*.

Plant parts	Total ash Value	Water soluble ash Value	Moisture content	pH	
				1%	10%
Root	19.025	91.35	93.65	7.9	6.8
Stem	24.325	68.6	92.85	8	6.7
Flower	19.75	77.15	94.95	7.5	6.6

dark green with ferric chloride (1 M); fluorescent dark red with iodine solution; bluish green with ammonia solution; dark greenish blue with NaOH (1 N); reddish brown with potassium dichromate; dark fluorescent green with methanol; fluorescent green with ethanol; bluish green with glycerin while treating with different chemical reagents.

C. rotundus flower showed green colour when powder as such; greenish brown colour when with distilled water; light brown when glacial acetic acid is used; reddish brown with 1 N HCl; violet green with 1 N H_2SO_4; transparent brown with conc. HNO_3; dark green with ferric chloride (1 M); fluorescent dark blue with iodine solution; fluorescent greenish blue with ammonia solution; light bluish brown with NaOH (1 N); violet blue with potassium dichromate; bluish green with methanol; reddish blue with ethanol; fluorescent red with glycerin, as listed in Table 3. Colours are mentioned by using colour identification chart, Royal Botanical Garden, Edinburg, 1969. The fluorescence characterization of powdered root, stem and flower after treating with various reagents emitted different colour radiation after observing under different ultraviolet light and invisible light.

Evaluation of preliminary phytochemical screening

Qualitative screening for various biochemicals revealed the presence of primary metabolites as well as secondary metabolites in different extracts of root, stem and flower of *C. rotundus*. Qualitative assay for protein, carbohydrate, flavonoid, phenolics and terpenoid appeared as positive. However, the level of these biochemicals varied according to different constituents of extracts. Stem showed remarkable presence of terpenoids, flavonoids, polyphenols, alkaloids, protein, tannins, saponins, cardiac glycosides and phenolic compounds. In some extracts carbohydrates and fixed oils were found but in less amount. In flower extracts, the protein, carbohydrates, cardiac glycosides, flavonoids and terpenoids were found in appreciable amount whereas, fixed oils, phenolics and anthroquinones were found in a negligible amount as listed in Table 4.

Table 3. Fluorescence characteristics of powder of different (root, stem and flower) parts of *Cyperus rotundus*.

Reagents used	Low U.V. (254 nm)	High U.V. (366 nm)	Visible
Root			
Powder as such	Light brown	Light brown	Brown
Distilled water	Dark brown	Dark brown	Dark brown
Glacial acetic acid	Pinkish light brown	Pinkish light brown	Reddish brown
1 N HCl	Reddish brown	Light reddish brown	Reddish brown
1 N H_2SO_4	Light brown	Light brown	Yellowish brown
Concentrated HNO_3	Orange	Orange	Orangish brown
Ferric chloride (1 M)	Dark green	Dark green	May Green

Iodine solution	Bluish red	Bluish red	Reddish black
Ammonia solution	Light orangish brown	Light brown	Dark brown
1 N NaOH	Dark steel blue	Steel blue	Blackish brown
Potassium dichromate	Purplish orange	Purplish orange	Reddish brown
Methanol	Pastel turquoise	Pastel turquoise	Pale brown
Ethanol	Violet brown	Light violet brown	Pale brown
Glycerin	Bluish dark green	Bluish light green	Dark brown
Stem			
Powder as such	Fluorescent green	Light green	Light Brown
Distilled water	Light green	Light green	Green brown
Glacial acetic acid	Reddish green	Pinkish green	Dark brown
1 N HCl	Brown	Light brown	Light brown
1 N H_2SO_4	Dark brown	Light brown	Light brown
Concentrated HNO_3	Light brown	Light brown	Translucent brown
Ferric chloride (1 M)	Dark green	Dark green	Reddish green
Iodine solution	Dark red	Dark red	Reddish black
Ammonia solution	Pastel turquoise	Pastel turquoise	Yellowish brown
1 N NaOH	Dark water blue	Yellow grey	Reddish green
Potassium dichromate	Reddish brown	Reddish light brown	Yellowish red
Methanol	Dark Fluorescent green	Light fluorescent green	Light green
Ethanol	Fluorescent green	Fluorescent green	Light green
Glycerin	Pastel turquoise	Pastel turquoise	Brownish green
Flower			
Powder as such	May Green	May Green	Pastel green
Distilled water	Tarpaulin grey	Reed green	Ocher Brown
Glacial acetic acid	Clay brown	Brown red	Green brown
1 N HCl	Red brown	Red brown	Red brown
1 N H_2SO_4	Ocean blue	Turquoise blue	Red brown
Concentrated HNO_3	Transparent beige brown	Translucent blue green	Transparent sand yellow
Ferric chloride (1 M)	Leaf green	Leaf green	Leaf green
Iodine solution	Night blue	Night blue	Black red
Ammonia solution	Water blue	Water blue	Red brown
1 N NaOH	Light steel blue	Dark steel blue	Brown red
Potassium dichromate	Brilliant blue	Squirrel grey	Bright Red orange
Methanol	Pastel turquoise	Dark steel blue	May Green
Ethanol	Reddish blue	Reddish blue	Pastel green
Glycerin	Pure orange	Green red	Dark brown

Discussion

To detect the nature of the material, determination of ash values are valuable parameters to investigate adulteration, impurities, authenticity, quality and purity in the sample of crude drugs. The soxhlet extracts have different polarity hence difference observed in the yield of various extracts of different parts of plant, indicating the presence of diverse kinds of bio-constituents in all extracts of experimental plant (12). Acid insoluble ash value was comparatively higher indicating the high content of silica, carbonates, phosphates and silicates. The water-soluble extract in a solvent of drug is usually estimated to be the amount of bio-constituents that the drug contains and that values indicate the presence of polar constituents such as steroids, flavonoids, phenols and glycosides (12, 13). High and low percentage of moister content indicates the use of in in moist, dry and humid conditions. The lower moisture level can prevent the growth in bacteria, yeast and fungi (14). The pH of crude drugs prevents the microbial growth and also indicates its quick absorption by the stomach.

Fluorescence characterization plays an important role by finding different chemical constituents which is present in plant material. If the substances are not fluorescent themselves, they can often become fluorescent derivatives using different reagents, so that some crude products are frequently qualitatively assessed (15). It is very helpful distinguishing features for determining the drug. The analysis of powdered drug under ultraviolet light establishes with different reagents (16).

In the study, the plant has been revealed to be rich in phenolic compounds like flavonoids, alkaloids, saponins and other secondary metabolites like terpenoids. Phenolic compounds are shown to poses different pharmacological significance and are revealed to have an important role in cancer prevention and treatment (17). Properties like anti-carcinogenic, antioxidant, anti-inflammatory effects, induction of apoptosis and blocking signaling pathways, etc. are associated with phenols (18). Hence, the presence of phenols in the plant suggests to its antioxidant and many other pharmacological properties. The products of its anion radicals are flavonoids that promote health (19) and could prevent the accumulation of DNA damage induced by UV radiation and by other environmental factors. Secondary metabolites observed in this plant might be responsible for various pharmacological effects (20) and provide help to combat with different human ailments (21–23).

Conclusion

Different parts of *Cyperus rotundus* were selected for this study to explore the various pharmacognostical analyses and to provide an appropriate data for the identification and adulteration prevention. Out of three parts of *C. rotundus*, various extracts of flower consists wide range of primary and secondary metabolites in appropriate amount. It is concluded that the study

Table 4. Preliminary phytochemical screening of different parts (root, stem and flower) of *Cyperus rotundus*.

Plant constituents	Test performed	*C. rotundus* root						*C. rotundus* stem						*C. rotundus* flower					
		PE	B	C	EA	E	AQ	PE	B	C	EA	E	AQ	PE	B	C	EA	E	AQ
Primary metabolites																			
Protein and Amino acids	Biuret test	-	-	-	+	+++	-	-	-	-	-	+	-	-	-	-	+	++	-
	Ninhydrin test	-	-	-	-	+	-	-	-	-	-	++	-	-	-	-	-	++	-
	Xanthoproteic test	++	+	+	+	-	-	++	++	+++	+	-	+	+	++	++	+++	-	+
Carbohydrates	Molisch's test	+++	-	+	-	+	-	-	-	+	-	+	-	+	+	++	+++	+	-
	Fehling's test	-	-	+++	-	-	-	-	-	+++	-	-	-	-	-	+++	-	-	-
Fixed oils and fat	Oil Stain test	+	-	-	+	+	+	+	+	-	+	++	+	+	-	+	+	-	-
	Saponification test	-	+	++	-	+	-	-	+	++	-	-	-	-	-	+++	-	-	-
Secondary metabolites																			
Alkaloids	Mayer's test	-	+	-	-	-	-	-	++	++	-	-	-	-	+	-	-	-	-
	Hager's test	++	-	-	-	-	-	++	-	-	-	-	-	++	-	-	-	-	-
	Wagner's test	+	-	-	++	-	-	+	-	-	-	-	-	++	-	+	-	-	-
	Tannic acid test	+	-	-	-	+	+	+	-	+	-	-	-	-	-	+	-	+	+
Anthraquinone	Hydroxyanthraquinone test	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Glycosides	Keller killiani test	+	+	+	-	+	-	+++	++	++	+	+	-	+	++	++	+	+	-
Flavonoids	Alkaline reagents test	-	-	-	-	++	+	-	-	-	-	++	+	-	-	-	-	++	+
	Lead acetate test	-	+	+	+	+	+	-	+	-	-	+++	+	+	-	-	+	++	++
Phenolics	Ferric chloride test	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	+	-
	Potassium dichromate test	-	-	-	++	++	-	-	-	-	++	++	-	-	-	-	++	++	-
Tannin	Ferric chloride test	-	-	-	-	+	-	+	-	-	-	+	-	+	+	-	-	+	++
	Vanillin HCL test	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-
Saponin	Olive oil test	-	-	-	+	+++	+++	-	-	+	-	++	+++	-	-	+	+++	+	++
	Froth test	+	+	-	+	+	++	+	-	+	-	+	+	+	+	+	+	+	-
Steroids	Salkowski test	+++	-	-	++	+	-	-	-	+	+	++	-	-	-	-	+	+	-
Phlobatanin	Hydrochloride test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Polyphenol	Folin-ciocalteau test	++	++	-	++	++	++	+	+	+	++	+++	+++	-	-	+	++	+	++

Symbols : - not present, + present in trace amount, ++ present in moderate amount, +++ present in high amount
Abbreviations : PE – Petroleum ether, B - Benzene, C- Chloroform, EA - Ethyl acetate, E - Ethanol, AQ - Aqueous

is quite helpful to define, standardize, improve and prepare the formulation of crude drugs by including various pharmacopoeias for treating different distinct illnesses. The current observation will also be helpful in differentiating plant parts of the species from closely linked species of same genus and family.

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Author's contributions

All the authors contributed equally to the work presented in this paper.

Conflicts of interest

Authors do not have any conflicts of interest to declare.

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