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Morphological, microscopical and physico-chemical investigations on the rhizomes of *Cyperus rotundus* Linn.

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ABSTRACT

Cyperus rotundus Linn. (Cyperaceae) vernacularly called 'Nagarmotha' is an Indian medicinal plant demonstrated to exert multiple health benefits. This plant grows naturally in tropical, subtropical and temperate regions. It is traditionally used for various purposes including as an antidiarrhoeal, antidiabetic, antipyretic, anti-inflammatory, antimalarial and for treatment of stomach and bowel disorders. In view of the diverse medicinal importance of selected plant and in order to ensure the quality, especially in terms of adulteration, substitution and identification of the plant material, the present investigation was carried out. The study includes pharmacognostical evaluation by macroscopy, microscopy, powder analysis, fluorescence characteristics, WHO recommended physiochemical and phytochemical procedures. Later, these characteristics could be used for rapid identification of the drugs particularly in case of powdered material and may possibly help to differentiate the drugs from its other species.

Keywords: Cyperus rotundus, Cyperaceae, physico-chemical parameters, phytochemical screening, fluorescence analysis.

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INTRODUCTION

Cyperus rotundus Linn. (Family Cyperaceae), commonly known as 'Nagarmotha' is found throughout India [1]. It is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers [2, 3]. A number of pharmacological and biological activities including anti-candida [4], anti-diabetic [5], anti-diarrhoeal [6], cytoprotective [7], anti-mutagenic [8], antioxidant [9], anti-malarial [10], anti-inflammatory, anti-pyretic and analgesic [11] activities have been reported for this plant. The rhizome part of Cyperus rotundus is one of the oldest known medicinal plants used for treatment of dysmenorrheal and menstrual irregularities [12]. The phytochemical investigation of Cyperus rotundus rhizome has revealed the presence of polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil [13, 14]. However, there are no reports on the pharmacognostical features of the plant. Hence, the present investigation is an attempt in this direction and includes morphological and anatomical evaluation, determination of physico-chemical constants and preliminary phytochemical screening of different extracts of Cyperus rotundus rhizome.

MATERIAL AND METHODS

Plant Material

The plant material was collected at its flowering stage from Hisar district, Haryana, July 2009 (29º10'12" N Latitude and 75º43'12" E Longitude). The plant was identified and authenticated by the Dr. H. B. Singh, Head, Raw Materials Herbarium & Museum, NISCAIR, New Delhi, Ref. No. NISCAIR/ RHMD/Consult/1491/89. The fresh rhizomes were taken for macroscopic and microscopic studies. The dried rhizomes were ground to a coarse powder and subjected to physico-chemical analytical parameters like ash values, extractive values and phytochemical screening.

Chemicals and Instruments

Rotary microtome was used to take sections of the tubers. Compound microscope, glass slides, cover slips, watch glass and other common glassware were used in this experiment. Photographs were taken with using Zeiss Primo Star Microscopic Unit. Various solvents used mainly ethanol (95%), petroleum ether, chloroform and reagents used for staining different sections like toluidine blue, safranin, fast-green and iodine in KI were procured from S D Fine chemicals, Mumbai, India.

Morphology

A systematic examination of the shape, size, surface, texture, taste and odour of the rhizomes of Cyperus rotundus Linn. was carried out. The external features of rhizome were observed under dissecting microscope.

Microscopy

The histological examination of the plant drug helps in identifying the shapes, size and position of different cells and tissues. The anatomical study helps in distinction of the drug from adulterants [15]. Material collected (were fixed in formalin – acetic acid – alcohol (10.5 : 50 : 35). The fixed material was preserved in 70% alcohol, washed thoroughly in 70% alcohol, dehydrated through xylene – alcohol series and embeded in paraffin [16]. Serial transverse sections were cut at 7-8 µm on a rotary microtome (Weswox Optik). Haupts adhesive was used for affixing the paraffin ribbons to the slide. The sections were stained in safranin (1%) and light green (0.2%). Slides were cleared in xylol and mounted in DPX mountant. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Cannon Zeiss Primo Star Microscopic unit [17].

Powder Microscopic Study

The shade dried plant materials were ground with a wood grinder and sifted through 40 mesh sieves. To study the ingredients of powder, a pinch of powder was bleached with 5% chloral hydrate and taken on slide and mounted with phloroglucinol, HCl and glycerine. The slide was observed under microscope.

Colour Reactions

The powdered drug materials were treated with different chemical reagents so as to aid in detection of chemical constituents under ordinary day light by standard methods [18]. To study the behaviour of drugs a pinch of each drug was treated with different chemical reagents viz 1N HCl, 1N NaOH, acetic acid, 5% ferric chloride, picric acid, 1N HNO_3, 5% iodine and 1N HNO_3 followed by ammonia solution and colours were observed [19].

Fluorescence Behaviour

To study the fluorescence nature of powder, a pinch of powder after bleaching with 5% chloral hydrate was treated with different chemical reagents viz. 1N HCl, 1N NaOH, 50% HNO_3, 50% H_2SO_4, methanol, acetic acid, picric acid, 1N NaOH in methanol, nitro-cellulose in amyl acetate, 1N NaOH in methanol and nitrocellulose in amyl acetate, 1N HCl followed by nitrocellulose in amyl acetate and 1N NaOH followed by nitrocellulose in amyl acetate and observed under UV light [19, 20].

Physico-chemical Parameters

The determination of various physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive and alcohol soluble extractive were determined and estimated in percentage by using method as recommended by

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Indian Pharmacopoeia [21]. Successive soxhlet extractives of the drug were carried out with various solvents and weight, color/consistency of extractives were observed [20]. Loss on drying for drug was also determined [22, 23].

Preliminary Phytochemical Screening

The powdered rhizomes extracts were subjected to qualitative phytochemical tests for alkaloids, glycosides, carbohydrates, sterols, phenolic compounds and tannins, flavonoids, saponins, proteins and free amino acids [24, 25].

RESULTS AND DICUSSION

Macroscopic Characters

The organoleptic characters of Cyperus rotundus rhizome are shown in (Table1). The rhizomes are dark brown, ovoid and covered with fibers (Figure 1).

Table 1: Macroscopical characters of Cyperus rotundus rhizome

S. No	Rhizome	Characters
1	Shape	Ovoid, tunicate
2	Size	0.8 to 2.5 cm
3	Color	Brownish black externally and white internally
4	Fracture	Mealy
5	Surface	Rough with striations
6	Odour	Fragrant
7	Taste	Starchy

Figure 1: Cyperus rotundus rhizome

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Microscopic characters

Transverse section of the rhizomes shows the following characteristics:

Epidermis consists of typical parenchymatous cells with brownish pigments. Hypodermis consists of 2-3 layers of thick walled cells. Cortex is composed of parenchymatous cells. Outer part is compact and inner part arenchymatous with large intercellular spaces. Some cells in cortex region contain brownish oleoresinous matter and other starch grains. Vascular bundles are loosely distributed around the perimeter of a central pith. The xylem vessels possess ligneous secondary wall thickenings. The remainder of the rhizome vascular system is scattered in small bundles throughout the cortex. Pith is composed of parenchymatous cells containing starch grains and few filled with oleoresinous contents (Figure2)

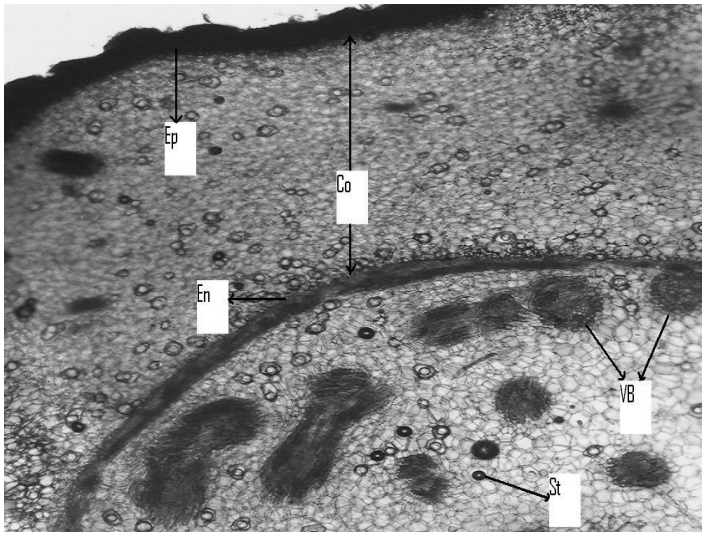

Figure 2: Transverse section of Cyperus rotundus rhizome (Ep-Epidermis; En-Endodermis; Co-Cortex; VB-Vascular bundles; St-Starch).

Powder Characters

Microscopic examination of powder shows the presence of cork cells, starch, vessels and fibres (Figure 3). The behavior of rhizomes powder with different chemical reagents, are shown in (Table 2).

The fluorescence analysis of powdered drug in day light, short UV and long UV were examined by reported method. The observations are given in (Table 3).

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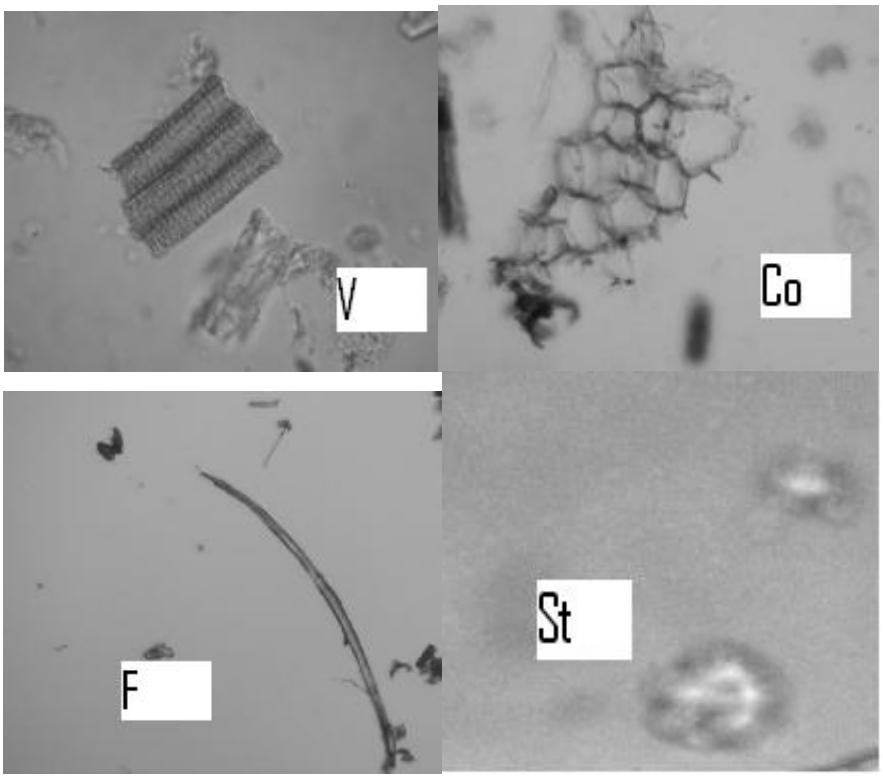

Figure 3: Microscopic characteristics of powder of Cyperus rotundus rhizome V-Vessel, Co- Cork, F- Fibre and St-Starch

Table 2: Behavior of rhizome powder with different chemical reagents

S. No.	Treatment	Colour
1.	Powder	Brown
2.	Powder + 1 NHCl	Yellowish brown
3.	Powder + 1N NaOH	Brownish black
4.	Powder + Acetic Acid	Brownish black
5.	Powder + 5% Ferric chloride	Yellowish brown
6.	Powder + Picric acid	Greenish brown
7.	Powder + HNO_3 + Ammonia solution	Yellowish brown
8.	Powder + 5% Iodine	Brown
9.	Powder + 1N HNO_3	Reddish brown

Table 3: Fluorescent nature of Cyperus rotundus rhizome powder

S. No.	Treatment	Observations		
		Visible	Short UV (254 nm)	Long UV(366nm)
1.	Powder as such	Brown	Green	Dark brown
2.	Powder + 1N HCl	Yellowish brown	Greenish black	Light brown
3.	Powder + 1N NaOH	Brownish black	Green	Dark brown
4.	Powder + 50% HNO_3	Reddish brown	Greenish black	Greenish black

5.	Powder + 50% H_2SO_4	Brown	Greenish brown	Black
6.	Powder + Methanol	Creamish brown	Brown	Dark brown
7.	Powder + Acetic acid	Brownish black	Greenish brown	Black
8.	Powder + Picric acid	Yellowish brown	Brownish green	Black
9.	5% Iodine	Brown	Dark brown	Greenish black
10.	5% Ferric chloride	Yellowish brown	Yellowish black	Black
11.	Ammonia	Brown	Greenish brown	Black

Physico-chemical Parameters

The physico-chemical parameters are important for identifying adulterants and improper handling of drugs. The (Table 4) reveals the result of physico-chemical parameters of powdered drug, carried out by using standard procedures. viz. Ash values used to determine quality and purity of drug; the extractive values are useful to evaluate the chemical constituents present in crude drugs and also help in estimation of specific constituents soluble in particular solvent. The result of extractive values of powdered drug in different solvent obtained by successive extraction is given in (Table 5). Loss on drying is shown in (Table 6). The (Table 7) reveals the behavior analysis of different solvent extract of Cyperus rotundus rhizome, under visible light, short and long UV.

Table 4: Physico-chemical parameters of rhizomes powder of Cyperus rotundus

Physico-chemical parameters	Values [%(WW) $\pm$ SD]
Ash values	
Total ash	8.06 $\pm$ 0.002
Acid insoluble ash	2.23 $\pm$ 0.005
Water soluble ash	5.11 $\pm$ 0.007
Sulphated ash	9.56 $\pm$ 0.003
Extractive values	
Water soluble extractive	9.01 $\pm$ 0.011
Alcohol soluble extractive	7.36 $\pm$ 0.008

Data are mean ± SD values (n = 3)

Table 5: Yield of extract by successive solvent extraction

S. No.	Extract*	Values [%(W/W) $\pm$ SD]
1.	Petroleum Ether	01.53 $\pm$ 0.009
2.	Chloroform	02.52 $\pm$ 0.007
3.	Methanol	11.23 $\pm$ 0.012
4.	Water	08.76 $\pm$ 0.017

*Extraction period is 24 hrs, Data are mean ± SD values (n = 3)

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Table 6: Loss on drying

Wt. Of the crucible	Wt. of crucible+ Drug	Final wt.	%w/w
36.87gm	38.21gm	38.12gm	6.72%
		38.11gm	7.46%
		38.11gm	7.46%

Table 7: Fluorescence nature of different extracts of Cyperus rotundus by visible and ultra-violet (UV) radiations

S. No.	Extract	Observations		
		Visible	Short UV (254 nm)	Long UV (366nm)
1.	Petroleum ether	Yellow Brown	Brown	Black
2.	Chloroform	Brown	Greenish Brown	Black
3.	Ethanol	Red Brown	Dark Brown	Dark Brown
4.	Water	Black Brown	Brown	Black

Table 8: Preliminary phytochemical screening of rhizomes extract of Cyperus rotundus

S. No.	Plant Constituents	Petroleum Ether Extract	Chloroform Extract	Ethanol Extract	Water Extract
1.	Alkaloids	-	-	+	+
2.	Glycosides	+	-	+	-
3.	Protein & Amino acids	-	+	+	+
4.	Carbohydrates	-	-	+	+
5.	Tannins & Phenolics	-	+	+	+
6.	Flavonoids	-	+	+	+
7.	Steroids	+	+	-	-
8.	Saponins	-	-	-	-
9.	Fixed oil	+	-	-	-

+: Positive; -: Negative

All the extracts obtained by successive extraction were subjected to qualitative chemical tests and the results are shown in (Table 8). Such preliminary phytochemical screening is helpful in prediction of the nature of drugs and also useful for detection of different constituents in different polarity solvent.

CONCLUSION

The present investigation including the morphoanatomical characters, physico-chemical values and pharmacognostical studies will serve as standard reference for identification and distinguishing Cyperus rotundus rhizome from its substitute and adulterants. This report would assist in the identification of the crude drug in future.

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