



Research Article

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EVALUATION OF PHYSIOCHEMICAL & PHYTOCHEMICAL STANDARDS OF *CYPERUS ROTUNDUS* LINN. AND *CYPERUS PROCERUS* ROTTB. RHIZOME WITH HPLC PROFILING

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Received on: 25/08/17 Accepted on: 02/02/18

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DOI: 10.7897/2277-4343.09387

ABSTRACT

Cyperus rotundus Linn. (Mustaka) and *Cyperus procerus* Rottb. (Nagarmustaka) are used for various conditions of ailments in traditional system of medicine since ancient times. They are often considered to be synonymous with each other in ayurvedic texts because of close similarities present between these two species. In the markets of crude drugs also, mostly Nagarmustaka is being sold under the name of Mustaka. So, there is a need to differentiate them for their authentication. This study is designed to establish the various physiochemical and phytochemical standards of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. and compare them for their correct identification and authentication. The investigations included determination of various standardization parameters such as physiochemical, phytochemical analysis with TLC and HPLC of both the drugs. Physiochemical and phytochemical standards of both the plants were showed almost similar results but some differences were also found. In TLC of both the drugs, most of the chemical constituents were have similar R_f value but some differences in R_f values were also found. Beta-sitosterol was also separated and have R_f value 0.38. In HPLC, 7.3% of Beta-sitosterol was found in the *Cyperus rotundus* Linn. while only 2.04 % of Beta-sitosterol was found in *Cyperus procerus* Rottb. Differences in the physiochemical, phytochemical, TLC and HPLC profiling will be helpful in identification and authentication of these two species of Cyperus and the parameters which are established from this study may be helpful in standardization of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb.

Keywords: *Cyperus rotundus*, *Cyperus procerus*, Physiochemical, Phytochemical, Standardization.

INTRODUCTION

The use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their health care in different national health-care settings. Long historical use of many practices of herbal medicine, including experience passed on from generation to generation, has demonstrated the safety and efficacy of traditional medicine. However, Standardization is needed to provide additional evidence of its safety and efficacy because standardization is the process of implementing and developing technical standards and is an essential measurement for ensuring the quality control of herbal drugs. Standardization of drugs means confirmation of its identity, determination of its quality, purity and safety by various parameters like morphological, microscopical, physical, chemical and biological evaluations. Most of the attention is normally paid to the quality indices such as macroscopic and microscopic examination, physiochemical analysis, phytochemical analysis and chromatographic examination.

In this study, *Cyperus rotundus* Linn. (Mustaka) and *Cyperus procerus* Rottb. (Nagarmustaka) were selected for their authentication and standardization because they are often considered to be the same plant. Mostly *Nagarmustaka* is being sold in different regions in place of Mustaka.

Cyperus rotundus Linn. and *Cyperus procerus* Rottb., both are belonging to Cyperaceae family. *Cyperus rotundus* Linn. commonly known as Nut grass in English, Mustaka in Sanskrit and Motha in Hindi, is a perennial herb which is 10-75 cm in

height and bearing hard, black, fragrant tubers. Leaves are 10-18 cm. long, narrowly linear. Inflorescence, an umbel of more or less condensed spikes and spikelets are more often red-brown in colour. It is found throughout India up to an elevation of 1800m., from Kashmir to Shimla, Garhwal, throughout the plains of almost all the states and ascending the mountains of the Central table-land from Mount Abu and Pune to the Nilghiri hills. *Cyperus procerus* Rottb. is an perennial plant grows in swamps, marshes, pools, wet rice fields, seasonally flooded places and open moist depressions. It is a stoloniferous sedge to about 120 cm. high. with elongated rhizomes, winged triangular stems, long acuminate bracts, and a small umbel of flattened pale brownish spikelets with membranous-edged. In India it occurs in Assam, Karnataka, Madhya Pradesh, Tamil Nadu, Kerala and West Bengal. *Cyperus procerus* Rottb. can be considered as Nagarmustak or Nagarmotha in Ayurveda. Presence of polyphenol, flavanol glycoside, alkaloid, saponin, sesquiterpenoids and essential oil were revealed from phytochemical investigations of *Cyperus rotundus* Linn. rhizome. Many Pharmacological and medicinal characteristics like anti-diarrhoeal, anti-inflammatory, anti-pyretic, analgesic, lipolytic, anti-diabetic, cytoprotective, antioxidant, anti-malarial are exhibited by these plants and has proved to be a multi-purpose medicinal herbs.¹⁻³²

MATERIAL AND METHODS

Plant Material

The plants material which were taken for study are -

- *Cyperus rotundus* Linn.
- *Cyperus procerus* Rottb.

Cyperus rotundus Linn. is taken as a source of Mustaka and *Cyperus procerus* Rottb. is taken as a source of Nagarmustaka

Plant Collection and authentication

- The genuine samples were collected after identifying the source of plant as per standard description.
- The genuine sample of *Cyperus rotundus* Linn. rhizomes (Mustaka) were collected from Rishikul Campus,

Uttarakhand Ayurveda University, Haridwar District, State-Uttarakhand, India.

- The genuine sample of *Cyperus procerus* Rottb. rhizomes (Nagarmustaka) were collected from Muni ki reti, State-Uttarakhand, India.
- From these sources as mentioned above, samples were collected, Herbarium were made and authenticated at Botanical Survey of India (BSI), Dehradun, India.

Table 1: List of Plants with Herbarium account number

Sl.no	Plant Name	Place of Collection	Herbarium Account No.
1	<i>Cyperus rotundus</i> Linn.	Rishikul Campus, Haridwar, Uttarakhand	116043
2	<i>Cyperus procerus</i> Rottb.	Muni ki reti, Uttarakhand	116039

METHODS

PHYSIOCHEMICAL ANALYSIS

The various physiochemical parameters like foreign matter, moisture content, pH, total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value, petroleum-ether soluble extractive value, essential oil were calculated according to the standard procedures³³⁻³⁵.

Determination of pH Value

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per litre.

The pH of a given solution is measured by using digital pH meter. First Standardized the pH meter. Tablets of different pH are taken and one tablet dissolve in 100 ml of distilled water to prepare solutions of different pH 4,7 and 9 (buffer solutions). The instrument is switched on. Leaved for some time unless or on the board requirement of different pH solution appears. Buffer solution is taken in the beaker and the electrode is dipped in it. Same procedure is repeated for the other buffer solutions after washing the electrode thoroughly with distilled water. The sample is taken (10% aqueous solution) and dips the electrode in it and note the value of pH.

Determination of Moisture Content (Loss on drying)

Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). Moisture content was determined by placing weighed sample of 5gm of drug in oven at 105°C for 5 hours, and calculate weight of sample for every 30 minutes, until the weight of the sample was constant, no variation of weight is recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

Weight of the empty petridish = W1 gm

Weight of the drug sample = X gm

Weight of the petridish with drug before drying (W3) = (W1 + X)

Weight of petridish after drying = W2 gm

Loss on drying in % = $\frac{W3 - W2 \times 100}{X}$.

Determination of Alcohol Soluble Extractive

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently for six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and

weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Determination of Water Soluble Extractive

Proceed as directed for the determination of alcohol-soluble extractive, using *chloroform-water* instead of ethanol.

Determination of Ether Soluble Extractive

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air-dried, crushed drug to an extraction thimble, extract with solvent ether (or petroleum ether, B.P. 40°C to 60°C) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105°C to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

Determination of Total Ash

The total ash method is designed to measure the total amount of material remaining after ignition. Silica Crucible was cleaned, dried well, labeled with glass pencils and then weighed to constant weight. 5 gm of powdered drug sample put in the Silica crucible. The drug was spread evenly in to a thin layer. This crucible was placed in a muffle furnace and ignited at a temperature of 450°C for about 6 hrs or more until the ash was totally free from Carbon. The crucible containing the ash was allowed to be cooled in desiccators and subsequently weighed to constant weight. The percentage of ash with reference to the air-dried drug was calculated.

Determination of Acid Insoluble Ash

To the crucible containing total ash, add 25 ml of dilute hydrochloric acid. Collect the insoluble matter on an ash less filter paper and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot-plate and ignite to constant weight. Allow the residue to cool in a suitable desiccators and weigh without delay. Calculate the content of acid-insoluble ash with reference to the air-dried drug.

Determination of Water Soluble Ash

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the

water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Determination of Volatile Oil in Drugs

The determination of volatile oil in a drug is made by distilling the drug with a mixture of water and glycerin, collecting the distillate in a graduated tube in which the aqueous portion of the distillate is automatically separated and returned to the distilling flask, and measuring the volume of the oil. The content of the volatile oil is expressed as a percentage v/w. The Clevenger's apparatus is recommended but any similar apparatus may be used provided that it permits complete distillation of the volatile oil. All glass parts of the apparatus should be made of good quality resistance glass.

The apparatus is cleaned before each distillation by washing successively with acetone and water, then inverting it, filling it with chromic sulphuric acid mixture, after closing the open end, and allowing to stand and finally rinsing with water.

Method of determination

A suitable quantity of the coarsely powdered drug together with 75 ml of glycerin and 175 ml of water in the one litre distilling flask, and a few pieces of porous earthen ware and one filter paper 15 cm cut into small strips, 7 to 12 mm wide, are also put in the distilling flask, which is then connected to the still head. Before attaching the condenser, water is run into the graduated receiver, keeping the tap open until the water overflows. Any air bubbles in the rubber tubing are carefully removed by pressing the tube. The tap is then closed and the condenser attached. The contents of the flask are now heated and stirred by frequent agitation until ebullition commences. The distillation is continued at a rate, which keeps the lower end of the condenser cool. The flask is rotated occasionally to wash down any material that adheres to its sides.

At the end of the specified time (3 to 4 hours) heating is discontinued, the apparatus is allowed to cool for 10 minutes and the tap is opened and the tube lowered slowly; as soon as the layer of the oil completely enters into the graduated part of the receiver the tap is closed and the volume is read.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Freshly prepared extracts were tested for the presence of various active phytochemicals like Carbohydrate, Alkaloids, Amino acids, Proteins, Saponin, Glycosides, Phenolic compound, Steroids, and Tannins.

Test conducted to detect the presence of carbohydrates were: Molisch's test, Benedict's test, Barfoed's test and Fehling's Solution test. For the phytochemical screening of alkaloids freshly prepared Mayer's reagent, Dragendorff's reagent, Wagner's reagent and Hager's reagent were used and observed for the presence of turbidity or precipitation. Ninhydrin test was used to detect the presence of amino acids. To detect the presence of proteins, Xanthoprotic's test, Millon's test and Biuret's test were applied. To detect the saponins, Foam test was applied. Borntrager's test was applied to detect the presence of Glycosides. Ferric chloride solution was used for phenolic compound testing. To detect the presence of steroids, chloroform and concentrated sulphuric acid were used. For the screening of tannins, ferric chloride test, lead acetate test and potassium dichromate test were performed.³³⁻³⁵

THIN LAYER CHROMATOGRAPHY (TLC)

Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures. Thin-layer chromatography is performed on a sheet of glass, plastic, or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose. This layer of adsorbent is known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates, separation is achieved. The mobile phase has different properties from the stationary phase.

After the experiment, the spots are visualized. Often this can be done simply by projecting ultraviolet light onto the sheet; the sheets are treated with a phosphor, and dark spots appear on the sheet where compounds absorb the light impinging on a certain area. Chemical processes can also be used to visualize spots; anisaldehyde.

To quantify the results, the distance traveled by the substance being considered is divided by the total distance traveled by the mobile phase. (The mobile phase must not be allowed to reach the end of the stationary phase.) This ratio is called the **retention factor** or R_f . Retention factors are characteristic but will change depending on the exact condition of the mobile and stationary phase³⁶.

In this study, alcoholic extract of the samples was applied with the help of capillary 1(one) cm above the base of T.L.C. plate coated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator, (Merck) (Each plate dimension is 10 cm long and 2 cm width). Then it was dipped in mobile solution of Toluene - ethyl acetate - glacial acetic acid 8:2:0.2. (v/v). Then T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1(one) cm below the top of the T.L.C. plate. The developed plate was visualized under Anisaldehyde sulphuric acid reagent. Measure and record the distance of each spot from the point of its application and calculate the R_f (Retention factor) value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High-performance liquid chromatography is a technique in analytical chemistry used to separate, identify and quantify each component in a mixture. It is a separation technique that can be used for the analysis of organic molecules and ions. HPLC is based on mechanisms of adsorption, partition and ion exchange, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from the difference in the relative distribution ratios of the solutes between the two phases. HPLC can be used to assess the purity and/or determine the content of many pharmaceutical substances.³⁷⁻³⁹

In this study, Sample chosen for HPLC were the rhizome of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. Beta-sitosterol % in all samples were calculated with the help of Standard Beta-sitosterol.

Standard

1mg of Beta-sitosterol dissolve 1ml HPLC grade Chloroform

Sample Preparation

1 mg Chloroform Extract of test sample dissolve in 1 ml of HPLC grade Chloroform.

Preparation of Mobile Phase

Mobile Solution: 95% Acetonitrile and 5% ethanol

Procedure

Inject equal volume (20µl) of standard and sample preparation. Record the chromatograms, calculate the average area and finally calculate the percentage of Beta-sitosterol.

Instrument setup conditions

High Performance Liquid Chromatography equipped with UV-Visible Detector.

Column: Shim-pack XR ODS (75mmL x 33mm, ID., 22microM)

Flow rate: 1.5 ml per minute

Run Time: 15 min

Injection Volume: 20 µl

Detector: UV-Visible 280 nm

RESULTS**Table 2: Physiochemical Analysis**

Sl. No.	Test	Genuine sample of <i>Cyperus rotundus</i> Linn.	Genuine sample of <i>Cyperus procerus</i> Rottb.
1.	Moisture Content	8.67 %	7.98 %
2.	pH	5.2	5.9
3.	Alcohol Extractive Value	18.43 %	19.56 %
4.	Aqueous Extractive Value	26.64%	24.54%
5.	Petroleum Ether Extractive Value	7.64 %	6.85 %
6.	Total Ash	5.7 %	5.3 %
7.	Acid Insoluble Ash	1.5 %	1.6 %
8.	Water Soluble Ash	4.65 %	4.85 %
9.	Essential oil	0.78%	0.63%

Table 3: Phytochemical Analysis

Sl. No	Tests	Genuine sample of <i>Cyperus rotundus</i> Linn.			Genuine sample of <i>Cyperus procerus</i> Rottb.		
		W.E	A.E	P.E.E	W.E	A.E	P.E.E
Carbohydrate							
A.	Molisch test	+ve	-ve	-ve	+ve	-ve	-ve
B.	Benedict test	+ve	+ve	-ve	-ve	+ve	-ve
C.	Barfoed's test	+ve	+ve	-ve	+ve	+ve	-ve
D.	Fehling test	-ve	-ve	-ve	-ve	-ve	-ve
Alkaloids							
A.	Dragendorff test	-ve	+ve	-ve	-ve	+ve	-ve
B.	Wagner's test	-ve	-ve	-ve	+ve	-ve	-ve
C.	Hager's test	+ve	-ve	-ve	+ve	-ve	-ve
Amino Acids							
A.	Ninhydrin test	+ve	+ve	+ve	+ve	+ve	+ve
Protein							
A.	Biuret test	+ve	-ve	-ve	+ve	-ve	-ve
B.	Xanthoprotic test	-ve	-ve	+ve	-ve	-ve	+ve
C.	Millon's test	-ve	+ve	-ve	+ve	+ve	-ve
Saponin							
A.	Foam test	+ve	-ve	-ve	+ve	-ve	-ve
Glycosides							
A.	Borntagor's test	+ve	+ve	-ve	+ve	+ve	-ve
Phenolic Compounds							
A.	Phenolic test	+ve	-ve	-ve	+ve	-ve	-ve
Steroids							
A.	Salkowaski reaction	+ve	-ve	-ve	+ve	-ve	-ve
Tannins							
A.	FeCl ₃ test	+ve	-ve	-ve	+ve	-ve	-ve
B.	Lead acetate test	+ve	-ve	-ve	-ve	-ve	-ve
C.	Potassium dichromate test	-ve	-ve	-ve	+ve	-ve	-ve

+ve (present), -ve (absent)

Thin Layer Chromatography (TLC)

Table 4: R_f Value of Genuine Sample of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb.

Test	<i>Cyperus rotundus</i> Linn.	<i>Cyperus procerus</i> Rottb.
R _f Value	0.12, 0.16, 0.22, 0.38, 0.42, 0.51, 0.58, 0.62, 0.73, 0.86	0.12, 0.38, 0.46, 0.49, 0.51, 0.58, 0.62, 0.73, 0.86

High Performance Liquid Chromatography (HPLC)

Table 5: Peak Table of Genuine Sample of *Cyperus rotundus* Linn.

Name of Sample	Peak	Ret. Time	Peak Area	Beta-sitosterol %
<i>Cyperus rotundus</i> Linn. (S1)	1	2.564	563421	7.3%

Table 6: Peak Table of Genuine Sample of *Cyperus procerus* Rottb.

Name of Sample	Peak	Ret. Time	Peak Area	Beta-sitosterol %
<i>Cyperus procerus</i> Rottb. (S2)	1	2.564	157224	2.04%

DISCUSSION

Physiochemical Analysis

Various physiochemical parameters are important in determination of adulterants and improper handling of drugs. Table No.2 shows the result of various physiochemical parameters of powdered drugs carried out using standard methods. Ash value used to determine quality and purity of crude drugs. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in particular solvent. Moisture content determines the amount of volatile matter in the drug.

Alcohol soluble extractive value of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. samples were found within the standard of API and it is higher in *Cyperus procerus* Rottb. than the *Cyperus rotundus* Linn. Water soluble extractive value of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. were found within the standard of API and it is higher in *Cyperus rotundus* Linn. than the *Cyperus procerus* Rottb. Essential oil (main constituent) of genuine sample of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. were 0.78% and 0.63%. Essential oil percentage of *Cyperus rotundus* Linn. is higher than the *Cyperus procerus* Rottb. [Table 2]

Phytochemical Analysis

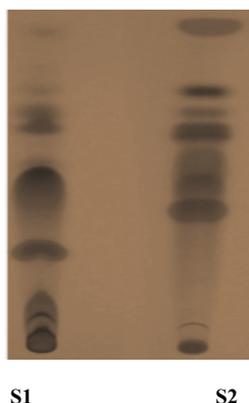
In Phytochemical analysis of all samples, Carbohydrate, Alkaloids, Amino acids, Proteins, Saponin, Glycosides, Phenolic compound, Steroids, Tannin were present in genuine sample of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. both. Some Differences in the results of carbohydrates, alkaloids, protein and

tannin were found in the genuine sample of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. In Benedict's test, *Cyperus rotundus* Linn. shows presence of carbohydrates in aqueous extract but carbohydrates were absent in aqueous extract of *Cyperus procerus* Rottb. In the Wagner's test, *Cyperus rotundus* Linn. shows absence of alkaloids in aqueous extract while this test was positive in aqueous extract of *Cyperus procerus* Rottb. Protein was found in genuine samples of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. both but in Millon's test, *Cyperus rotundus* Linn. shows absence of proteins in aqueous extract but aqueous extract of *Cyperus procerus* Rottb. shows the presence of protein. *Cyperus rotundus* Linn. have given positive Lead acetate test for tannin while this test was negative in *Cyperus procerus* Rottb. in aqueous extract and *Cyperus rotundus* Linn. have given negative potassium dichromate test for tannin while this test was positive in aqueous extract of *Cyperus procerus* Rottb. [Table 3]

TLC

Cyperus rotundus Linn. and *Cyperus procerus* Rottb. have found lot of unknown chemical constituents were separate in mobile solution toluene - ethyl acetate - glacial acetic acid 8:2:0.2. Most of the chemical constituents were have similar R_f value but some R_f values differentiate the chemical constituents of both plants. 0.16, 0.22, 0.42 R_f value were found in the genuine sample of *Cyperus rotundus* Linn. only and these values were absent in *Cyperus procerus* Rottb. and 0.46 and 0.49 R_f values were present only in *Cyperus procerus* Rottb. [Table 4 & Figure 2]

Beta-sitosterol is a phytochemical that have anti-inflammatory activity had separate in mobile solution and have R_f value 0.38.⁴⁰

Figure 1: TLC Analysis of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb.

HPLC

In HPLC, percentage of Beta-sitosterol in both genuine sample of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. were calculated with the help of standard Beta-sitosterol. Beta-sitosterol was present in higher concentration in *Cyperus rotundus* Linn. than the *Cyperus procerus* Rottb. 7.3% of Beta-sitosterol was found in the genuine sample of *Cyperus rotundus* Linn. while only 2.04 % of Beta-sitosterol was found in the genuine sample of *Cyperus procerus* Rottb. [Table 5 & 6 and Figure 3, 4 & 5].

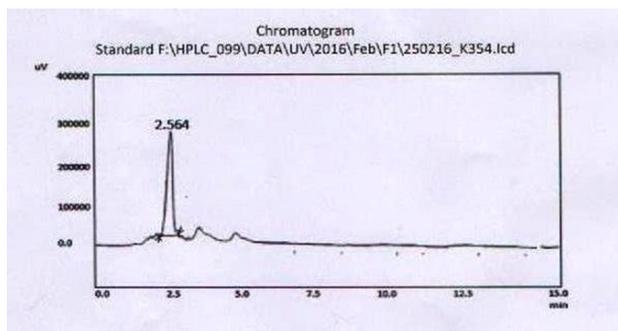


Figure 2: Standard of Beta-sitosterol

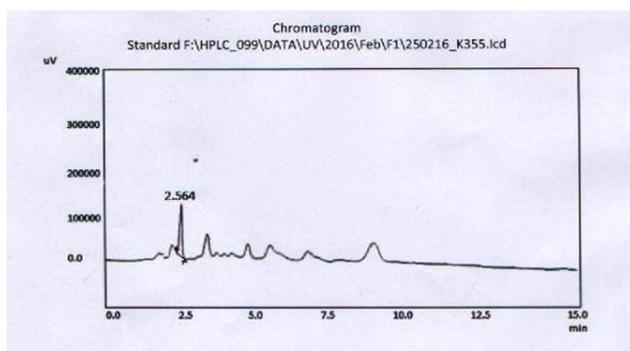


Figure 3: HPLC of Genuine Sample of *Cyperus rotundus* Linn.

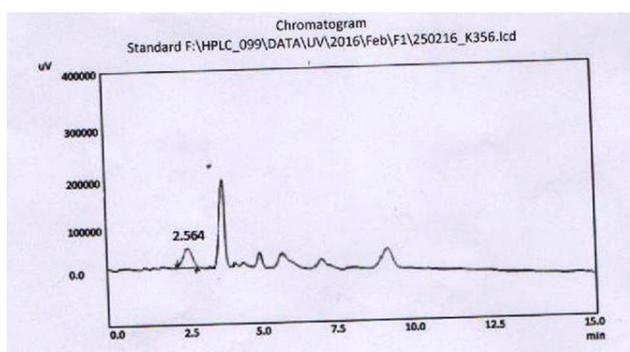


Figure 4: HPLC of Genuine Sample of *Cyperus procerus* Rottb.

CONCLUSION

In this study, different quality control parameters (Physiochemical, Phytochemical, TLC and HPLC) have been established for *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. These parameters would be useful as an analytical tool for the standardization of the rhizome of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. since these features are distinctive for

the identification of *Cyperus rotundus* Linn. (Mustaka) and *Cyperus procerus* Rottb. (Nagarmustaka). This study would be helpful in distinguishing and authentication of both the drugs. Percentage of Essential Oil and Beta-sitosterol were found more in *Cyperus rotundus* Linn. than the *Cyperus procerus* Rottb. which indicate high therapeutic value of *Cyperus rotundus* Linn. (Mustaka) than the *Cyperus procerus* Rottb. (Nagarmustaka).

REFERENCES

- WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. Geneva. World Health Organisation; 2004.
- General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva. World Health Organisation; 2000.
- <https://en.wikipedia.org/wiki/Standardization> [cited on 2016 Aug 16].
- Joshi Devendra, Joshi Geeta. Quality Control & Standardization of Ayurvedic Medicines. First Edition. Varanasi. Chaukhamba Orientalia; 2011. p. 110-140.
- <http://www.pharmafans.com/pharmacognosy/standardization-of-crude-drug-and-herbal-formulation.html>. [cited on 2016 Aug 16].
- Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal Plants Used in Ayurveda. Vol.3. New Delhi. CCRAS; 2005. p.404-405.
- <http://plants.jstor.org/stable/10.5555/al.ap.flora.fwta8477>. [cited on 2016 Aug 17].
- Kumar, B. 2011. *Cyperus procerus*. The IUCN Red List of Threatened Species 2011:e. T177173A738351 5. <http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T177173A7383515.en>. [Downloaded on 17 August 2016].
- Nagulendran KR, Velavan S, Mahesh R, Hazeena begum V. In vitro antioxidant activity and total polyphenolic content of *Cyperus rotundus* rhizomes. E-Journal of chemistry 2007; 4(3): 440-49.
- Venkatsubramanian P, Kumar S, Nair SNV. Morphological, microscopical and physico-chemical investigations on the rhizomes of *Cyperus rotundus* Linn. J Ayulnt Med 2010; (1): 33-39.
- N Hema, A Ramakrishna, KN Sunil Kumar, N Anupama. Evaluation of Physicochemical Standards of *Cyperus rotundus* rhizome with phytochemical and HPTLC profiling of its extracts. Int. Res. J. Pharm. 2013; 4(6): 133-37. DOI: 10.7897/2230-8407.04630.
- Shastri JLN. Forworded by Chunekar KC. Dravayaguna Vijnana. Vol. II. 2nd Ed. Varanasi. Chaukhamba Orientalia; 2005. p. 551.
- Sharma Ram Karan, Dash Vaidya Bhagwan. Charak Samhita. Vol. I – III. Varanasi. Chowkhamba Sanskrit Series Office; Reprint 2014.
- Murthy KR Srikantha. Sushruta Samhita. Vol. I-III. 2nd Ed. Varanasi. Chaukhamba Orientalia; 2004.2005. 2005.
- Murthy KR Srikantha. Astanga Hridayam. Vol. I-9th. Vol. II. Vol. III. 7th Ed. Varanasi. Chowkhamba Krishnadas Academy; 2013. Reprint 2013, 2014.
- Murthy KR Srikantha. Astanga Sangraha of Vagbhata. Vol. I-9th. Vol.II-5th. Vol.III-4th Ed. Varanasi, Chaukhamba Orientalia; 2005.
- Tewari PV. Kasyapa Samhita or Vriddhajivakiya Tantra. Varanasi. Chaukhamba Visvabharati; Reprint 2002.
- Sharma Priya Vrat. Cakradatta. 3rd Ed. Varanasi. Chaukhamba Publishers; 2002.

19. Srivastava Shailaja. Sharangadhar Samhita of Acharaya Sharangadhar. 4th Ed. Varanasi. Chaukhamba Orientalia; 2005.
20. Krishnamurthy KH. Edited by Sharma Priya Vrat. Bhel Samhita. Varanasi. Chaukhambha Visvavharati; Reprint: 2008
21. Bhavamishra. Bhavaprakasha. Part – II. 9th Ed. Varanasi. Chaukhambha Sanskrit Sansthan; 2005.
22. Shastri Lakshmiapati. Yogratnakara. Chaukhamba Prakashan; Reprint: 2010.
23. Sen Govind Das. Bhaishajya Ratnavali. 'Siddhiprada' Hindi Commentary. By Mishra Siddhi Nandan. Varanasi. Chaukhambha Surbharati Prakashan.
24. Sitaram Bulusu. Bhavprakash of Bhavmishra. Foreword by Chunekar KC. First Ed. Varanasi. Chaukhambha orientalia; 2006. p. 217.
25. Sharma Priya Vrat. Translated by Sharma Guruprasad. Dhanwantari Nighantu. 4th Ed. Varanasi. Chaukhamba Orientalia; 2005. p. 23.
26. Chunekar KC. Bhavprakash Nighantu of Shri Bhavmishra. edited by Late Pandey GS. Varanasi. Chaukhamba Bharati Academy; Reprint 2013. p. 234.
27. Tripathi Indradeo. Rajnighantu of Pandit Narhari. Varanasi. Chaukhamba Krishnadas Academy. p. 163.
28. Shaligramvaishya. Shaligram Nighantu Bhushanam i.e. Brihat Nighanturatanakar antargat Part 7-8. Bombay-4. Khemraj Shrikrishnadas Prakashan; January 2011. p. 55-56.
29. Pandey Gyanendra. Edited by Dwivedi RR. Forword by Baghel MS. Sodhala Nighantu. Varanasi. Chaukhamba Krishnadas Academy; 2009. p. 27.
30. Pandey Gyanendra. Madanpal Nighantu. 1st Ed. Varanasi. Chaukhamba Orientalia; 2012. p. 136.
31. Sharma Priya Vrat. Priya Nighantu. 2nd Ed. Varanasi. Chaukhamba Surbharti Prakashan; 1995. p. 82.
32. Srishti Dhyani, Morphological & Microscopical features of leaf and stem of *Cyperus rotundus* Linn. And *Cyperus procerus* Rottb. A Comparative Analysis. Int. J. Res. Ayurveda. Pharm. 8(1); Jan-Feb 2017. p. 46-51.
33. The Ayurvedic Pharmacopoeia of India. Part-I. Vol. VI. First Ed. New Delhi. Govt. of India. Ministry of Health & Family Welfare. Dept. of AYUSH. 2008.
34. Khandelwal KR. Practical pharmacognosy techniques and experiments. New Delhi: Nirali Prakashan; 2002. p. 15-163.
35. Brain KR, Turner TD. The practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientecnica; 1975. p. 152.
36. https://en.wikipedia.org/wiki/Thin-layer_chromatography [cited on 24 Oct 2017].
37. Harborne JB. Phytochemical Methods. London: Chapman and Hall Ltd.; 1973. p. 49-188.
38. https://en.wikipedia.org/wiki/High-performance_liquid_chromatography [cited on 21 Aug 2016].
39. High-performance liquid chromatography. The International Pharmacopoeia. 5th Ed.; 2015. [http://apps.who.int/phint/pdf/b/Jb.7.1.14.4.pdf].
40. Sutar, Ravindra C, Kasture, Sanjay B, Kalaichelvan VK. Identification, Quantification and Validation of Beta sitosterol from *Holoptelea integrifolia* (Roxb.) planch using high performance thin layer chromatography method. Int. J. Pharm. Pharm. Sci 2014; 6(5):249-252. [http://www.ijppsjournal.com/Vol6Issue5/9259.pdf].

Cite this article as:

Srishti Dhyani & Hemant Kharkwal. Evaluation of physiochemical & phytochemical standards of *Cyperus rotundus* Linn. and *Cyperus procerus* Rottb. rhizome with HPLC profiling. Int. J. Res. Ayurveda Pharm. 2018;9(3):174-180 <http://dx.doi.org/10.7897/2277-4343.09387>

Source of support: Nil, Conflict of interest: None Declared

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