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

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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 Rf value but some differences in Rf values were also found. Beta-sitosterol was also separated and have Rf 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, microscopic, physical, chemical and biological evaluations. Most of the attention is normally paid to the quality indices such as macroscopic and microscopic examination, physicochemical 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 Nilgiri 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, cytotoxic, 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

Plan 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-Uttarkahand, India.
- From these sources as mentioned above, samples were collected, Herbarium were made and authenticated at Botanical Survey of India (BSI), Dehradun, India.

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Plant Name</th>
<th>Place of Collection</th>
<th>Herbarium Account No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Cyperus rotundus</em> Linn.</td>
<td>Rishikul Campus, Haridwar, Uttarakhand</td>
<td>116043</td>
</tr>
<tr>
<td>2</td>
<td><em>Cyperus procerus</em> Rottb.</td>
<td>Muni ki reti, Uttarakahand</td>
<td>116039</td>
</tr>
</tbody>
</table>

**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 procedures 33-35.

**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 4,7and 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 petridish with drug before drying (W3) = (W1 + X) gm
Weight of petridish after drying = W2 gm
Loss on drying in % = (W3-W2)/X*100.

**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 thimble, dry on a hot-plate and ignite to constant weight. The residue of insoluble ash with reference to the air-dried drug was calculated.

**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 phycompounds 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, Xanthoproteic’s test, Million’s test and Biuret’s test were applied. To detect the saponins, Foam test was applied. Bontragar’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.

**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 the spots ion excited with ultraviolet light onto 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 phase.

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.

In this study, Sample chosen for HPLC were the rhizome of *Cyperus rotundus* Linn. and *Cyperus procerus* Roth. Beta-sitosterol % in all samples were calculated with the help of Standard Beta-sitosterol.
Standard
1 mg of Beta-sitosterol dissolve 1 ml 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 (20 µl) 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

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

Table 3: Phytochemical Analysis

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Tests</th>
<th>Genuine sample of <em>Cyperus rotundus</em> Linn.</th>
<th>Genuine sample of <em>Cyperus procerus</em> Rottb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td>Molisch test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>B.</td>
<td>Benedict test</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>C.</td>
<td>Barfoed’s test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>D.</td>
<td>Fehling test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>A.</td>
<td>Dragefoff test</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>B.</td>
<td>Wagner’s test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>C.</td>
<td>Hager’s test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>A.</td>
<td>Ninhydrin test</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>A.</td>
<td>Biuret test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>B.</td>
<td>Xanthoproteic test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>C.</td>
<td>Millon’s test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>A.</td>
<td>Foam test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>A.</td>
<td>Borntrager’s test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>A.</td>
<td>Phenolic test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>A.</td>
<td>Salkowski reaction</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>A.</td>
<td>FeCl3 test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>B.</td>
<td>Lead acetate test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>C.</td>
<td>Potassium dichromate test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve (present), -ve (absent)
Thin Layer Chromatography (TLC)

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Cyperus rotundus Linn.</th>
<th>Cyperus procerus Rottb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rf Value</td>
<td>0.12, 0.16, 0.22, 0.38, 0.42, 0.51, 0.58, 0.62, 0.73, 0.86</td>
<td>0.12, 0.38, 0.46, 0.49, 0.51, 0.58, 0.62, 0.73, 0.86</td>
</tr>
</tbody>
</table>

High Performance Liquid Chromatography (HPLC)

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

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

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 proteins 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 Rf value but some Rf values differentiate the chemical constituents of both plants. 0.16, 0.22, 0.42 Rf 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 Rf 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 Rf value 0.38. 

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

**REFERENCES**


CONCLUSION

In this study, different quality control parameters (Physiochemical, Phytochemical, TLC and HPLC) have been established for *Cyperus rotundus* Linn. and *Cyperus procerus* Rothb. These parameters would be useful as an analytical tool for the standardization of the rhizome of *Cyperus rotundus* Linn. and *Cyperus procerus* Rothb. since these features are distinctive for the identification of *Cyperus rotundus* Linn. (Mustaka) and *Cyperus procerus* Rothb. (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* Rothb. which indicate high therapeutic value of *Cyperus rotundus* Linn. (Mustaka) than the *Cyperus procerus* Rothb. (Nagarmustaka).


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