This is to certify that the project titled "Evaluation of in vitro and in vivo anticancer activity of Cyperus rotundus [L]" by Dr. Hema N has been approved by the YU-IAEC bearing number 4/25/8/2011.
PUBLICATIONS
AATLAS OF MACRO-MICROSCOPY OF RAW DRUG SOLD AS MUSTA – CYPERUS ROTUNDUS (L.)

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Keywords:
Authentication, Macro-microscopy atlas, Cyperus rotundus, Cyperaceae

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ABSTRACT: The quest for eternal health has made mankind to intensively research the nature itself. Nature has a rich source of medicinal plants, the proper use of which is necessary to maintain health. According to WHO, almost 80% of the population of developing countries has utmost faith in traditional medicines, of which plants are the important source. WHO recommends macroscopic and microscopic studies of the herb should be the first step in authentication, which is necessary to ensure the quality of any medicinal product. Cyperus rotundus (L.), a medicinal plant belonging to the family of the Cyperaceae, grows all over India. In the present study macroscopic and microscopic characterization of the rhizomes of Musta was done to develop an atlas of diagnostic characters by the standard methodology of macro-microscopic characterization of herbal raw drugs. Microscopical and organoleptic characters as well as the macroscopic features of Musta rhizomes were documented. A self-explanatory atlas of photomicrographs was prepared to aid rapid identification of raw drug called Musta. The atlas will serve as standard reference for identification and distinguishing Musta from its substitutes and adulterants.

INTRODUCTION: The quest for eternal health has made mankind to intensively research the nature itself. Though the medicines obtained from medicinal plants are effective in various ailments the standardization of drugs remains an uphill task. Authentication should be the primary criteria of any research using plants, which will help to ensure the quality of any medicinal product 1.

Pharmacognostic characters of herbs play an important role since particular macro-microscopic features are unique for each plant. WHO recommends macroscopic and microscopic studies of the herbs should be the first step to identify the botanical source before doing any research on plants 3.

Cyperus rotundus, commonly called Musta is a medicinal plant belonging to the family of the Cyperaceae. It grows all over India up to 2000 meters altitude, especially on the banks of streams, rivers. It appears among Indian, Chinese and Japanese natural drugs used as home remedy 3.
This plant grows well in tropical, subtropical and temperate regions and it is an invasive weed which significantly reduces the crop yield.

In Indian system of medicine, its rhizomes are used in the treatment of several clinical conditions like candidiasis, diabetes, diarrhea, malaria, dysmenorrhea and menstrual irregularities.

It is also known for its cytoprotective, anti-mutagenic, antioxidant, anti-inflammatory, antipyretic and analgesic properties. Even though the rhizome of this plant has got tremendous medicinal properties, there is very minimum information on proper identification of the same. The present communication deals with the detailed macroscopic and microscopic features of the rhizome of *C. rotundus* as whole rhizomes as well as powdered form.

**MATERIALS AND METHODS:** The dried rhizomes of *C. rotundus* were collected from the local Ayurvedic pharmacy in Mangalore. The specimen (No. 11110101) is being stored in the Pharmacognosy Department of SDM Centre for Research in Ayurveda and Allied Sciences, Laxmanrao V. Nair, Kudupady, Udupi for future reference. The dried rhizomes were cleaned, coarsely powdered and preserved in air tight container for further studies. Dried whole rhizomes were used to study the external appearance, color, odor and taste. Coarse and fine powders are also prepared to record the organoleptic characters.

Few fully matured rhizomes were preserved in fixative solution FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml) for more than 48 hours. The preserved specimens were cut into thin transverse section using sharp blade. The sections were stained with safranine as per standard methodology. The selected diagnostic characters of the transverse section were photographed under suitable magnification using camera (Zeiss AxioCam ERC 5s) attached to trinocular microscope (Zeiss Axio Lab.A1) and the micro.measurements were taken using the pre-calibrated scale available in the software (AxioVision Rel. 4.8). A pinch of powder was warmed with drops of chloral hydrate on a microscope slide and mounted in glycercin. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO trinocular microscope.

**RESULTS:**

**Macroscopy:** *C. rotundus* rhizomes are bluntly conical with number of wiry and tough slender roots, often attached to one another by a thin and tough connective. Each rhizome is tuberous, varies in size and thickness, crowned with remains of stem and leaves forming a hairy to scaly covering.

The length of the rhizome is 1.5 to 3cms and diameter is 0.8 to 1.6 cms. Its stolons are elongated and about 1.5 to 3.5cms long.Externally the rhizome is dark brown or black in color and internally creamish yellow; odor– fragrant, taste– slightly pungent (**Figure 1A**). Fracture rough, mealy, shows dots of stellar vascular bundles and a distinct endodermal margin (**Figure 1B**). Coarse and fine powders are coffee brown in color, slightly pungent in taste, odor fragrant (**Figure 1C-D**).

**Microscopy:** Transversely cut surface of rhizome shows a line of demarcation of endodermis separating cortical portion from the central ground tissue. Few remnants of scale leaves are seen at the few places of the periphery (**Fig. 1B**). Detailed transverse section of rhizome shows single layered epidermis with brown colored pigment, often peeling off during maturity, leaving behind 2-6 layers of...
suberized sclerenchymatous cap-like patches filled with brown pigment; epidermis and outer sclerenchymatous layers are followed by 4 to 6 layers of radially elongated thick-walled cork-like cells which ends in compact outermost cortical parenchyma cells with no intercellular spaces; ground tissue of cortex consists of circular to oval, thin-walled, parenchymatous cells with small intercellular spaces; endoderm is distinct surrounding the wide central zone beneath endodermis, the stelar region is composed of circular to oval, thin-walled, parenchymatous cells with intercellular spaces, numerous collateral, closed, vascular bundles surrounded by bundle sheath of fibres, scattered in this region; vessels spiral to simple pitted; simple round, oval to elongated starch grains, a number of pigmented cells filled with reddish-brown oleo-resin content, present throughout the cortex and stele (Figure 2).

**Powder microscopy:** Creamish-brown; shows reddish-brown cells, spiral and simple pitted vessels; fibre-like, closely packed sclerified cells, narrow tracheids with pitted thickness and thick-walled trichomes from the remnants of leaves simple, thick-walled rounded to elongated parenchyma with brown content of tannin or pale oleo-resin, fragment of endodermis with pitted wall in surface view; round, oval to elongated starch grains and few rosette crystals of calcium oxalate (Figure 3).

![Figure 2: Microscopy of Rhizome of Cyp...](image)

![Figure 3: Powder Microscopy of Cyp...](image)
CONCLUSION: In the present study, we analyzed the macroscopic and microscopic characters of the dried rhizomes of C. rotundus. The results obtained will help to identify the correct species since no such detailed photographic atlas is available for exact picture of the characteristics.

This study will serve as a reference for identification and distinguishing rhizomes of Cyperus sp commercially available and to differentiate them from their substitute and adulterants. Detailed study on chemical analysis will be essential to acquire significant results.

ACKNOWLEDGEMENT: The authors are grateful to Yenepoya University for giving permission to carry out this study. Authors thank Dr B. Ravishankar, SDM Centre for Research in Ayurveda and Allied Sciences for providing the facilities, Miss Sangeetha and Miss Rajalekshmi for help in carrying out the studies.

REFERENCES:

18. O'Brian TP, Fox S, McCue M. Polychromatic staining of plant cell walls by inohome micro-CCIF. Phytochemistry 1964.
EVALUATION OF PHYSICOCHEMICAL STANDARDS OF CYPERUS ROTUNDUS RHIZOME WITH PHYTOCHEMICAL AND HPTLC PROFILING OF ITS EXTRACTS
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ABSTRACT
Cyperus rotundus (Muta), is a medicinal plant growing in tropical, sub-tropical and temperate regions having many pharmacological and medicinal characteristics. The current study attempts to standardize the extracts of raw drug Muta sold in the markets of Mangalore were collected. Test sample was analysed for ash and extractive values. Tests for phytochemicals like alkaloids, flavonoids, steroids, phlobatins, carbohydrates, saponins, tannins and coumarins were performed in both ethanolic and aqueous extracts. Total phenolic content of the extracts were done as per standard protocol. Fingerprint profile of ethanolic and aqueous extract has been derived by photodensitometry and HPTLC densitometric scans. Ash values, extractive values, phytochemical tests, total phenol content and the HPTLC fingerprint of the Muta has been derived from the current study. The set of values obtained from the studies can be used as standards for testing, standardization and quality control of medicinal materials sold as Muta.

Keywords: Physico-chemical, phytochemical, total phenol, HPTLC profiling

INTRODUCTION
In this era where modern medicines are front runners in treating diseases of all kinds, Indian system of medicine has been able to gain a foothold and is becoming popular. The Ayurvedic system of medicine remedies are basically formulated using various medicinal plants. For many centuries, plants have provided a rich source of therapeutic agents and bases for synthetic drugs. Despite the great development of organic synthesis, currently 25% of prescribed drugs worldwide are still derived from plant sources. Showing that plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer7. Although herbs have proved to be highly curative, their standardization is a must before formulating medicines using them, which will help to ensure the quality, safety and efficacy of these herbal medicines. Even if the morphologic and microscopic characters are helpful to establish the identity of any herb, finding their physical and chemical constituents will provide thorough knowledge for authentication of the chemical characteristics. For the identification of herbs and their constituents, WHO guidelines recommend the fingerprinting methods to meet the universal standards of quality control of the herbal formulations8. Along with other physico-chemical characterization, High performance thin layer chromatography (HPTLC) is more innovative, consistent and efficient method used for identification and separation of constituents in the herb or any herbal formulations9. Cyperus rotundus, a member of Cyparissacae and vernacularly called as Nagarmotha is medicinal herb and has gained its own recognition in principles of Ayurveda. It grows in tropical, sub-tropical and temperate regions. Based on different parts it has vital uses. Many pharmacological and medicinal characteristics like anti-diabetic10, anti-inflammatory11, antimicrobial12, anti-malarial13, anti-inflammatory, anti-glycemic and analgesic16, are exhibited by this plant and has proved to be a multi-purpose medicinal herb. Rhizomes of Cyperus rotundus is one of the oldest medicinal plants used to treat dysmenorrhea and menstrual irregularities17. Presence of polyphenols, flavonoid glycosides, alkaloids, saponins, sesquiterpenoids and essential oil were revealed from phytochemical investigations of Cyperus rotundus rhizome18,19. The present study is an attempt to investigate the physico-chemical, preliminary phytochemical, total phenol and HPTLC fingerprint profiling of aqueous and ethanol extracts of rhizomes of Cyperus rotundus.

MATERIALS AND METHODS
Collection and Identification of the herb
Rhizomes of Cyperus rotundus were collected from local Ayurvedic pharmacy in Mangalore. The plant material was examined at Pharmacognosy department of SDM Centre for Research in Ayurveda and Allied Sciences, Udipi and sample specimen (No.11110101) was maintained for further references. The dried rhizomes were coarsely powdered and preserved in air tight container for further studies.

Preparation of the extracts
Ethanolic and aqueous extracts were prepared as per the standard procedures explained by Raman11.

Physico-chemical characteristics
The various physico-chemical parameters like loss on drying at 105°C, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined according to the standard procedure prescribed in Ayurvedic Pharmacopoeia of India20.
Table 1: Physicochemical parameters of C. anisata

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result (%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss on drying at 105°C</td>
<td>12.65</td>
</tr>
<tr>
<td>Total ash</td>
<td>3.72</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.31</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>1.54</td>
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<tr>
<td>Water soluble extractive</td>
<td>5.2</td>
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<tr>
<td>Ethanol soluble extractive</td>
<td>1.9</td>
</tr>
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Table 3: Total phenolic content

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result (%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude drug</td>
<td>8.025</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>0.144</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>74.63</td>
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</tbody>
</table>

Table 2: Results of Preliminary Physicochemical Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Resin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 4: Rf values of Ethanol Extract of C. rotundata

<table>
<thead>
<tr>
<th>At UV 254 nm</th>
<th>At UV 366 nm</th>
<th>Post - derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03 L Green</td>
<td>0.03 Blue</td>
<td>0.09 L Phenol</td>
</tr>
<tr>
<td>0.03 Green</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.04 Green</td>
<td>0.04 Blue</td>
<td>0.08 L Phenol</td>
</tr>
<tr>
<td>0.05 Green</td>
<td>0.05 Blue</td>
<td>0.12 L Phenol</td>
</tr>
<tr>
<td>0.06 Green</td>
<td>0.06 Blue</td>
<td>0.16 L Phenol</td>
</tr>
<tr>
<td>0.07 Green</td>
<td>0.07 Blue</td>
<td>0.20 L Phenol</td>
</tr>
<tr>
<td>0.08 Green</td>
<td>0.08 Blue</td>
<td>0.24 L Phenol</td>
</tr>
<tr>
<td>0.09 Green</td>
<td>0.09 Blue</td>
<td>0.28 L Phenol</td>
</tr>
<tr>
<td>0.10 Green</td>
<td>0.10 Blue</td>
<td>0.32 L Phenol</td>
</tr>
<tr>
<td>0.11 Green</td>
<td>0.11 Blue</td>
<td>0.36 L Phenol</td>
</tr>
<tr>
<td>0.12 Green</td>
<td>0.12 Blue</td>
<td>0.40 L Phenol</td>
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<td>0.13 Green</td>
<td>0.13 Blue</td>
<td>0.44 L Phenol</td>
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<td>0.14 Green</td>
<td>0.14 Blue</td>
<td>0.48 L Phenol</td>
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<td>0.15 Green</td>
<td>0.15 Blue</td>
<td>0.52 L Phenol</td>
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<td>0.16 Green</td>
<td>0.16 Blue</td>
<td>0.56 L Phenol</td>
</tr>
<tr>
<td>0.17 Green</td>
<td>0.17 Blue</td>
<td>0.60 L Phenol</td>
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<td>0.18 Green</td>
<td>0.18 Blue</td>
<td>0.64 L Phenol</td>
</tr>
<tr>
<td>0.19 Green</td>
<td>0.19 Blue</td>
<td>0.68 L Phenol</td>
</tr>
<tr>
<td>0.20 Green</td>
<td>0.20 Blue</td>
<td>0.72 L Phenol</td>
</tr>
<tr>
<td>0.21 Green</td>
<td>0.21 Blue</td>
<td>0.76 L Phenol</td>
</tr>
<tr>
<td>0.22 Green</td>
<td>0.22 Blue</td>
<td>0.80 L Phenol</td>
</tr>
<tr>
<td>0.23 Green</td>
<td>0.23 Blue</td>
<td>0.84 L Phenol</td>
</tr>
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<td>0.24 Green</td>
<td>0.24 Blue</td>
<td>0.88 L Phenol</td>
</tr>
<tr>
<td>0.25 Green</td>
<td>0.25 Blue</td>
<td>0.92 L Phenol</td>
</tr>
<tr>
<td>0.26 Green</td>
<td>0.26 Blue</td>
<td>0.96 L Phenol</td>
</tr>
</tbody>
</table>

L - Light, M - Medium, D - Dark

Preliminary phytochemical analysis

This analysis is to detect the presence of various organic functional groups, which is the indicative of type of phytochemicals present in the plant. These tests indicate the presence different classes of constituents present in the extract. The tests for alkaloids, carbohydrates, steroids, saponins, tannins, flavonoids, phenols, coumarins, terpenoids and carboxylic acid were performed as per the methodology mentioned by Harborne.16

Estimation of total phenolic content

Total phenolic content of the crude drug, aqueous extract and ethanol extract were done according to standard procedure explained by Ayurvedic pharmacopoeia of India.1 A ml of Polia's reagent diluted with 1 ml of water 20 kg of NaClO3 dissolved in 100 ml of water at 70-80°C C and cooled it overnight. After adding the reagents, the mixture was incubated at room temperature for 40 min in dark and absorbance of the sample was measured at 725 nm in a UV spectrophotometer. Tannic acid was used as standard (1 mg/ml). All the tests were performed in triplicates. The phenol content was estimated from the calibration curve of standard tannic acid obtained by plotting concentration vs. absorbance.

HPTLC

1 g of the plant powder was extracted with 10 ml of solvent 2 times by cold percolation for 48 hrs. 25 µl of the ethanol extract was applied on a pre-coated silica gel F254 on aluminum plates to a bain width of 8 mm using Linomat 5 TLC applicator. The applied plate was developed in suitable solvent system and the developed plates were visualized and scanned under UV 254, 366 and after derivatization in vanillin-sulphuric acid spray reagent. Rf, colour of the spots and densitometric scan were recorded. Toluene - ethyl acetate (8:2) and ethyl acetate: formic acid:water (8:0:0.6:0.6) were used as solvent system for development of ethanolic and aqueous extracts respectively.18

RESULTS AND DISCUSSION

The phytochemical results are depicted in Table 1. The results of phytochemical investigation of aqueous and ethanolic extracts were given in Table 2. The extract was tested positive for coumarins, carbohydrates, steroids, phenols and saponins. In ethanolic extract, in addition to above constituents, terpenoids and tannins are also seen. Alkaloids, flavonoids and resins are absent in both the extracts. The results of total phenolic content of crude drug, aqueous extract and ethanol extract is given in Table 3. Water soluble extract is found to contain more of phenol content than ethanolic extract. Figure 1 shows the TLC photo documentation of ethanolic extracts of Cypresses rotundata at UV 254 nm and UV 366 nm using toluene: ethyl acetate solvent at 8:2 ratio and Table 4 gives the Rf values of the same. 12 spots were seen at UV 254 nm, 5 spots at UV 366 nm and 11 spots at post-derivatization. In HPTLC densitometric scan of same extract at UV 254 nm, 15 peaks were obtained (Figure 2). Here at 12th peak with Rf value 0.67 has maximum area percentage i.e.34.11%. At UV 366 nm only 3 peaks were observed with maximum height at 2nd
peak having 0.60 Rf value and 83.34% of area percentage (Figure 3). Figure 4 shows the TLC photo documentation of aqueous extract of C. rotundus at UV 254 nm and UV 366 nm using solvent system - Ethyl acetate: Formic acid: Water (8:0.6:0.6). Table 5 gives the Rf values for the same. 5 spots were seen at UV 254 nm, 12 spots at UV 366 nm and 11 spots at post-derivatisation. In HPTLC densitometric scan of this extract at UV 254 nm, 9 peaks were obtained (Figure 5). Here at 9th peak with Rf value 0.93 has maximum area percentage i.e. 44.94%. At UV 366 nm only 11 peaks were observed with maximum height at 1st peak having 0.02 Rf value and 43.38% of area percentage (Figure 6).

CONCLUSION
The physio-chemical, phytochemical, total phenolic content and HPTLC fingerprint reported in the current study could be used as the analytical tool for the standardization of the rhizome of Cypara rotundus since these features are distinctive for the identification of Musta. It could help in laying down standards as per WHO guidelines for authentication of the drug and also may aid to distinguish the above drug from its substitute or adulterants if any.
ACKNOWLEDGEMENT

The authors are thankful to Yenepoya University for giving permission to conduct the above study. Authors gratefully acknowledge Dr. B. Ravishankar, Director, IDM Centre for Research in Ayurveda and Allied Sciences, Udupi for his kind suggestion, B Sangeetha and M Rajalakshmi for their help in analysis.

REFERENCES

A Comparative Analysis of Antioxidant Potentials of Aqueous and Ethanol Extracts of 
*Cyperus Rotundus* (L.)

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**ABSTRACT**

This study deals with the evaluation of free radical scavenging capacity of aqueous and ethanol extracts of rhizome of *Cyperus rotundus* by their ability to scavenge DPPH, nitric oxide, hydroxyl radical and exhibit reducing power. Both extracts showed effective free radical scavenging activity. Compared to aqueous extract, ethanolic extract has shown better activity. The results support its extensive usage in various diseases. The main basis for this use seems to be its role as a rich source of natural antioxidant which protects the body from various oxidative damage.

**Keywords:** *Cyperus rotundus*, DPPH assay, Nitric oxide scavenging assay, hydroxyl radical scavenging assay, reducing power assay.

1. **INTRODUCTION:**

Oxygen is necessary for living beings for various metabolic activities and the reactive oxygen species generated in the cells are essential in aerobic life and metabolism but excess of it can cause damage to the cell (1). The free radicals when formed in excess during the process of oxidation cause damage to the cell membrane, DNA, RNA and different enzymes of the cell. Environmental agents like toxicity of lead, pesticides, cadmium, ionizing radiation, alcohol, cigarette smoke, UV light and pollution may also initiate free radical generation (2-4). Antioxidants produced in the body neutralize the free radicals and protects the body from their harmful effects. This natural production of antioxidants is not sufficient always as in case of exposure to environmental hazards and in increasing age (5-6). The synthetic antioxidants may substitute the natural antioxidants, but their use is attendant with severe side effects (7). So there is a great need to identify and use natural antioxidants. Various fruits and vegetables were already proven for their various antioxidant contents (8). A large number of Indian medicinal plants have recently received a great attention in their antioxidant properties (9). Antioxidant-based drug formulations are used for the prevention and treatment of complex degenerative diseases like atherosclerosis, stroke, diabetes, Alzheimer’s disease and cancer (10).

*Cyperus rotundus* (L), commonly called “Musta” is a medicinal plant belonging to the Cyperaceae family grows all over India up to 2000 meters altitude, especially on the banks of streams and rivers. It commonly appears among Indian, Chinese, Japanese natural drugs used as home remedy (11). It is said to possess antidiarrhoeal, anti-inflammatory and antipyretic activities (12). As per Ayurveda the tubers have carminative and demulcent property, they are used to treat the abdominal disorders particularly diarrhea, indigestion and flatulence. They are used as analgesic, diuretic and for the treatment of cold and congestion, inflammation, wounds and sores, amenorrhoea and dysmenorrhoea (13-14). *C. rotundus* was found to produce protective effect in inflammatory bowel disease (15). The oil of *C. rotundus* showed a remarkable antibacterial activity and antimutagenic activity (16). In many of the above therapeutic effects free radical scavenging can play major role, hence

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in the present study a comparative analysis of free radical scavenging potential of both aqueous and ethanolic extracts of *C. rotundus* rhizome was carried out.

2. MATERIALS AND METHODS

2.1. Collection and identification of the herb: Dried rhizomes of *C. rotundus* were collected from local Ayurvedic pharmacy in Mangalore. The plant material was authenticated by Dr. Sunilkumar, Senior Research officer, Department of Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi and sample voucher specimen (No. 11110101) has been deposited in the above laboratory's plant depository. The shade dried rhizomes of the test plant were coarsely powdered and preserved in freezer for further studies.

2.2. Preparation of the extracts: Extracts were prepared according to the procedure explained by Reemam [17]. Aqueous extract was prepared by using 10 g of coarse powder of rhizomes by refluxing the powder in 100 ml of distilled water for 24 h and filtered. Filtrate was further diluted to suitable concentration (mg/ml solution). Working solutions were diluted to get 10-100 μg/ml concentration for antioxidant evaluation activity. For ethanolic extract, 10 g of coarse powder of rhizomes of *C. rotundus* were similarly processed as described above to obtain the test extract. These two extracts were subjected for following tests to assess the antioxidant capacity.

2.3. DPPH SCAVENGING ASSAY [18]

2.3.1. Reagents: DPPH (1, 1-Diphenyl-2-Picryl-Hydrayl) was purchased from Sigma, USA. All the other chemicals used were of analytical grade.

2.3.2. Procedure: DPPH (1, 1-Diphenyl-2-Picryl-Hydrayl) is a stable free radical with purple color. If free radicals have been scavenged, DPPH will degenerate to yellow color. This assay uses this character to show free radical scavenging activity. The change in color from purple to yellow was measured at 517 nm in a spectrophotometer (SYSTRONICS 2201). A 0.002% of DPPH in methanol was prepared and 1 ml of this solution was added to 1 ml of different concentrations of extracts. Methanol with extract served as blank and DPPH in methanol without the extracts served as positive control. The percentage inhibition of DPPH radical by the sample was calculated using the following formula:

\[
\% \text{ inhibition} = \frac{(A_0 - A_s) \times 100}{A_0}
\]

Where, 
\( A_0 = \text{Absorbance of control} \)
\( A_s = \text{Absorbance of sample} \)

The activity was compared with ascorbic acid, which was used as a standard antioxidant.

2.4. Nitric oxide radical scavenging assay [19]

2 ml of sodium nitroprusside (10 μM) in 0.5 ml of standard phosphate buffer solution was incubated with 0.5 ml of different concentration of the test extracts (10μg/ml to 100μg/ml) dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25°C for 2½ h. To 0.5 ml of incubated mixture 1 ml of 0.33% sulfanilic acid was added and allowed to stand at room temperature for 5 min. After incubation 1 ml of 0.1% of naphthylethenediamide dichloride was added, mixed the content and incubated at room temperature for 30 minutes. All tests were performed in triplicate. The absorbance of the mixture at 540 nm was measured with a Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). Distilled water served as the blank. Nitric oxide radical scavenging activity was calculated according to the following formula:

\[
\% \text{ inhibition} = \frac{(A_0 - A_s) \times 100}{A_0}
\]

Where, 
\( A_0 = \text{Absorbance of control} \)
\( A_s = \text{Absorbance of sample} \)

2.5. Reducing power assay [20]

A 0.75 ml of various concentrations of the extracts (10 μg/ml to 100 μg/ml) was mixed with 0.75 ml of phosphate buffer (0.2 M pH 6.6) and 0.75 ml of potassium ferriyannide (3% v/v) and incubated at 50°C for 20 min. The reaction was stopped by adding 0.75 ml of 10% trichloroacetic acid, centrifuged at 800 rpm for 10 minutes. 1.5 ml of supernatant was mixed with 1.5 ml distilled water and 0.1 ml ferric chloride (0.1%). Incubated at room temperature for 10 minutes and the absorbances at 700 nm were measured with Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). All tests were performed in triplicate. Higher absorbance of reaction mixture indicates the greater reducing power as compared to ascorbic acid (Standard). The percentage of radical scavenging activity was calculated using the following formula:

\[
\% \text{ inhibition} = \frac{(A_0 - A_s) \times 100}{A_0}
\]

Where, 
\( A_0 = \text{Absorbance of control} \)
\( A_s = \text{Absorbance of samples} \)

2.6. Hydroxyl radical scavenging activity [21]

Two series of tubes were taken. In the first set 60 μl of 1 mM ferrous chloride, 90 μl of 1 mM 1, 10 phenanthroline, 2.4 ml of 0.2 M phosphate buffer saline (pH 7.4) were taken and 150 μl of 0.17 M hydrogen peroxide was added to initiate the reaction. This set was labeled as blank. In the second set before adding hydrogen peroxide, 1.5 ml of either the extracts or Vitamin C in varying concentrations such as 10, 20, 40, 60, 80 and 100 μg/ml were added. After incubation at room temperature for 5 min the absorbance of the mixture at 560 nm was measured with Double beam UV-visible Spectrophotometer (SYSTRONICS 2201). The hydroxyl
radical scavenging activity was calculated according to the following formula:

\[
\%\text{ Inhibition} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

Where, \(A_0\) = Absorbance of control, \(A_1\) = Absorbance of samples.

3. STATISTICAL ANALYSIS

The data obtained have been presented as Mean ± SE. The difference between the control group and test extracts treated group was analyzed by employing one way ANOVA (Analysis of Variance) followed by Bonferroni’s multiple t-test as post hoc test. P<0.05 was considered as statistically significant.

4. RESULTS

Yield of Aqueous extract of \(C.\) rotundus was 5.2% and that of ethanolic extract was 1.9%.

4.1. DPPH Assay:

Fig.No.1 shows the effect of \(C.\) rotundus related to the DPPH scavenging activity of aqueous and ethanolic extracts. Aqueous extract of \(C.\) rotundus showed moderate to good activity to scavenge free radicals and this activity was found to be dose dependent up to 80\(\mu\)g/ml. The difference between the control group and test drug group was found to be highly significant with respect to all the doses (p<0.001) except 10\(\mu\)g/ml group in which the difference was found to be statistically non-significant (p>0.05). DPPH assay of ethanolic extract of \(C.\) rotundus also shows significantly high activity in a dose independent manner, but at very high concentrations of the extract the effect was found to be decreased. The highest effect is found in lowest dosage level (10 \(\mu\)g/ml). The difference between the control group and test drug group was found to be very highly significant in all the test doses (p<0.001).

4.2. Nitric oxide scavenging activity:

Fig.No.2 depicts nitric oxide radical scavenging activity of aqueous and ethanolic extracts of \(C.\) rotundus. The observed effect is not dose dependent in case of aqueous extract. Two different dosage levels i.e 40 and 80\(\mu\)g/ml have produced similar effect. It has been observed that lower concentrations i.e 10 and 20 \(\mu\)g/ml was found be more effective compared to other dosage levels. Ethanol extract produced a moderate inhibition of nitric oxide formation at the lower dose level tested but tended to decrease at higher dose level at which only weak effect was observed. The observed activity was not concentration dependent. Moderate inhibition of nitric oxide formation was observed at 10 and 20 \(\mu\)g/ml. At higher concentration level the observed inhibition was less in comparison to the effect observed at lower concentration level. The difference between the control group and test drug group was found to be very highly significant in all the test doses (p<0.001).

4.3. Reducing power assay:

Fig.No.3 depicts data related to reducing power assay of aqueous and ethanolic extracts of \(C.\) rotundus. The aqueous extract produced moderate and dose dependent reducing activity till 60\(\mu\)g/ml. The test drug has showed greater activity of reducing power in lower dose (10\(\mu\)g/ml). The difference between the control group and test drug group (10\(\mu\)g/ml and 20\(\mu\)g/ml) was found to be highly significant (p<0.01). The effect observed at higher concentrations of the test extract (40-100 \(\mu\)g/ml) was found to be non-significant in comparison to control (p>0.05). The ethanolic extract produced marked and dose independent increase in the reducing power. The test drug has showed significant activity against reducing power in all the six different dose levels tried. Higher absorbance of reaction mixture indicates the greater reducing power. Percentage of inhibition of the sample extract is comparatively higher in lower concentrations tried for the present study. The difference between the control group and test drug group was found to be very highly significant in 10-60\(\mu\)g/ml group (p<0.001) and significant in 80\(\mu\)g/ml and 100\(\mu\)g/ml test doses (p<0.01).
4.4. Hydroxyl radical scavenging activity:

Fig. No. 4 depicts the hydroxyl radical scavenging activity of aqueous and ethanolic extracts of Crotanudus. The aqueous extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. As observed with other tests, the higher concentrations were found to be less effective. The difference between the control group and test drug group was found to be highly significant in 10μg/ml group (p<0.01) and significant in remaining test doses (p<0.05). The ethanolic extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. At the above dosage levels, the higher concentration was found to be less effective. Increased effect was observed from 10μg/ml to 20μg/ml concentration and then again activity is found to be higher at 50μg/ml. The difference between the control group and test drug group was found to be significant only in 20μg/ml group (p<0.05).

Aqueous extract of Crotanudus was found to be more effective at lower concentrations, i.e. 10 and 20μg/ml compared to other dosage levels. Ethanolic extract produced a moderate inhibition of nitric oxide formation at the lower dose level but its effectiveness decreased at higher dose.

The reducing power assay indicates the extracts ability to donate electron to react with free radicals and convert them into more stable metabolites and terminate the radical chain reaction (27). The aqueous extract produced moderate and dose dependent reducing activity till 60μg/ml. The ethanolic extract produced marked and dose independent increase in the reducing power.

Hydroxyl radical are the major active oxygen causing lipid peroxidation and enormous potential for biological damage. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein (28). The aqueous and ethanolic extract exhibited moderate hydroxyl scavenging activity which was found to be dose independent. The dose independent activity is quite common with plant extracts (29). This is likely due to presence of multiple phytochemical constituents in which some may have opposite effects.

6. CONCLUSION

The present investigation shows that both aqueous and ethanolic extracts of rhizomes of Crotanudus exhibits antioxidant and free radical scavenging activity which is more apparent in ethanolic extract. These differences in activities may be due to dissimilarity in the phytoconstituents present in each extracts. The present study is in agreement with the earlier investigations done by few researchers (16, 30). Based on the results obtained it can be suggested that this herb can be used as potent natural antioxidant which may be helpful to prevent various degenerative diseases. Detailed in vivo experiments may help to prove above results, which are in progress.

7. REFERENCES


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Conflict of Interest. None Declared