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Research article

Evaluation of physiochemical and phytochemical properties of kyllinga monocephala rottb rhizomes extracts

Abhishek kumar[1*], Anil Kumar Singh[1], Jalalpure S.S[2], Surendra Singh[3]

[1]Dept. of Dravyaguna, Faculty of Ayurveda, I.M.S B.H.U Varanasi, U.P, India.
[2]Dept. of Pharmacognosy, K.L.E'S University, Belgaum, Karnataka, India.
[3]Dept. of Botany, Faculty of Sciences, B.H.U Varanasi, U.P, India.

Abstract

Kyllinga monocephala (Cyperaceae) perennial herb growing one to several erect stems to heights up to about half a meter and found throughout India. It is traditionally useful in vitiated conditions *pitta* and *vata*, hyperdipsia, fever, strangury, verminosis, cough, bronchitis, hepatopathy, splenopathy, diabetes and dermatitis. Phytochemical, physico-chemical standardization of rhizome of *Kyllinga monocephala* has been carried out in the present study. The study includes organoleptic characters along with an estimation of its physicochemical parameters such as loss on drying, ash values, extractability in Petroleum ether, ethanol, water and preliminary phytochemical screening. The generated information of the present study will provide data which are helpful in the correct identification and authentication of this medicinal plant and may help in preventing its adulteration.

Key words: Rhizomes extracts, *Kyllinga monocephala* Rottb., physiochemical, phytochemical

***Corresponding Author: Abhishek kumar,** Dept. of Dravyaguna, Faculty of Ayurveda, I.M.S B.H.U Varanasi, U.P, India.

1. Introduction

An estimate of the world health organization (WHO) states that around 85-90% of the world's population consumes traditional medicine. Most of the cases of accidental herbal medicine misuse start with wrong identification of a medicinal plant prescribed. Many of the traditional systems have records where one common vernacular is supplied in place of two or more entirely different species. The second major reason for accidental herbal medicine misuse is the non-characterization of chemical constituents of the controversial plant.

The main goal of pharmacognosy is to assess the value of raw materials and to ensure that the final product is of the required standard. Strict standardization procedure and pharmacognostical studies of medicinal plant would reduce drastically much of the accidents in wrong prescription of traditional herbal medicine. WHO has developed several guideline for carrying out the standardization procedure of raw herbal product, which basically include

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pharmacognostical, physico-chemical, pharmacological and toxicological method to standardize a certain herbal material. Phytochemistry has evolved as a major branch of Pharmacognosy in developing markers for the purpose of identification and standardization [1].

Kyllinga monocephala Rottb. family Cyperaceae found throughout India. It is traditionally used to treat diabetes. The rhizome is fragrant, aromatic, sweet, astringent, bitter, diuretic, refrigerant febrifuge, sudorific, antidiarrhoeal, expectorant, and stomachic. They are also useful in vitiated conditions *pitta* and *vata*, hyperdipsia, fever, strangury, verminosis, cough, bronchitis, hepatopathy, splenopathy, diabetes and dermatitis. So that the medical benefits of the plant may be used properly and scientifically and reach to the larger populations of the world. Therefore, in the present research work was to evaluate the physicochemical parameters and phytochemical constituents of the rhizome of *Kyllinga monocephala* Rottb [2-5].

2. Materials and Methods

Collection of plant material

The rhizomes of *Kyllinga monocephala* Rottb. were collected from Himalaya region and authenticated by Prof. R.S. Goudar, Department of Botany, KLES's Raja lakhamagouda Science Institute, Belgaum, Karnataka.

Extraction of Rhizomes of *Kyllinga monocephala* rottb.

The shade dried rhizomes of *Kyllinga monocephala* family Cyperaceae were reduced to fine powder (# 40 size mesh) and around 450 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether (60-80^{0}C), ethanol and aqueous. Each time before extracting with the next solvent the powdered material will be air dried in hot air oven below 50^{0}C. After the effective extraction, the solvent was distilled off, the extract was then concentrated on a water bath and the extract obtained with each solvent will be weighed. Its percentage will be calculated in terms of air-dried weight of plant material and color and consistency of the extracts will be noted [6].

Preliminary Pharmacognostic Characteristics: [7-9]

Macroscopic Characteristic of Rhizomes of *Kyllinga monocephala* rottb.

In present, study the Rhizome of *Kyllinga monocephala* rottb. was investigated for its macroscopic characteristics.

Microscopic Characteristics of rhizomes of *Kyllinga monocephala* Rottb.

In the present, study the dried rhizomes of *Kyllinga monocephala* rottb. were pulverized into fine powder separately The pulverized powder of the root was boiled separately with chloral hydrate solution in small quantity. Remove cleaved powder in three separate watch glass respectively and stain with one drop each of phloroglucinol and concentrated hydrochloric acid. Mount a little of the treated powder in dilute glycerine and observed the slide under microscope at low power.

Standardization of rhizomes of *Kyllinga monocephala* Rottb [10-12]

Rhizomes were subjected to size reduction to get coarse powder and then passed through sieve no. 40 to get uniform powder. Then the uniform powder was subjected to standardization with different parameters.

Extractive Values

Determination of Petroleum ether-soluble Extractive

Macerate 5 gm of the air dried rhizomes coarsely powdered, with 100 ml of pet. ether(60-80^{0}C) of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for 18 hrs. Filter rapidly taking precautions against loss of Pet. ether. Evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105^0, and weigh. Calculate the percentage of Pet. ether-soluble extractive with reference to the air-dried drug.

Determination of Ethanol-soluble Extractive

Macerate 5 gm of the air dried rhizomes coarsely powdered, with 100 ml of ethanol of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for 18 hrs. Filter rapidly taking precautions against loss of ethanol. Evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105^0, and weigh. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

Determination of Water-soluble Extractive

Macerate 5 gm of the air dried rhizomes coarsely powdered, with 100 ml of distilled water of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for 18 hrs. Filter rapidly taking precautions against loss of water. Evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 105^0, and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Loss on Drying (LOD)

About 2-3 gm of powder is accurately weighed in a petridish and kept in a hot-air oven maintained at 110^{0}C for four hours. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

Loss on drying (%) (LOD) =

$$\frac{\text{Loss in weight} \times 100}{\text{Weight of the drug in gms}}$$

Ash Value

Determination of Total Ash

Weigh accurately 2 to 3 gm of air–dried rhizomes of *Kyllinga monocephala*, separately in a tared platinum or silica dish and incinerate at a temperature not exceeding 450^{0}C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way exhaust the charred mass with hot water, collect the residue on an ashless filter paper, incinerate the residue and filter paper until the ash is white or nearly so, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450^{0}C. Calculate the percentage of ash with reference to the air – dried drug.

$$\text{Total ash value of the sample} = 100 \frac{(z - x)}{Y} \%$$

z = weight of the dish + ash (after complete incineration)

x = weight of the empty dish

y = weight of the drug taken

Determination of Acid-insoluble Ash

Boil the ash for 5-10 minutes with 25 ml of 2M hydrochloric acid, collect the insoluble matter in a Gooch crucible or on an ashless filter paper, wash with hot water, ignite and weigh. Calculate the percentage of acid

insoluble ash with reference to the air-dried drug.

Determination of Water-soluble Ash

Boil the ash for 5-10 min with 25 ml of water collect the insoluble matter in a Gooch crucible or on ashless filter paper, wash with hot water and ignite to constant weight at a low temperature. Subtract the weight of insoluble matter from the weight of the total ash and the difference in weight-represents the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

Fluorescence Analysis of the Drug

Many crude drugs show the fluorescence when the sample is exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of the fluorescent effect. Fluorescence lamps are fitted with suitable filters which eliminate visible radiation from the lamp and transmit UV radiation of definite wavelength. Several crude drugs show characteristic fluorescence useful for their evaluation.

Determination of Foreign matter

Weigh a 50 gm of plant material, spread it in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6X or 10X), or with the help of a suitable sieve, according to the requirements for the specific plant material. Sift the remainder of the sample through a No. 250 sieve; dust is regarded as mineral admixture. Weigh the portions of this shorted foreign matter to within 0.05 gm. calculate the content of each group in grams per 100 g of air dried sample.

Phytochemical Investigation [13]

Qualitative chemical tests were conducted for all the extracts of rhizomes of *Kyllinga monocephala* rottb. to identify the various phyto-constituents.

Tests for Carbohydrates

Molisch's test (General test)

To 2-3 ml aqueous extract, added a few drops of α-naphthol solution in alcohol, shaken and added concentrated H_2SO_4 from the sides of the test tube was observed for violet ring at the junction of two liquids.

For Reducing Sugars:-

a) **Fehling's test:** 1 ml Fehling's A and 1ml Fehling's B solutions was mixed and boiled for one minute. Added equal volume of test solution. Heated in boiling water bath for 5-10 min was observed for a yellow, then brick red precipitate.
b) **Benedict's test:** Equal volume of Benedict's reagent and test solution in test tube were mixed. Heated in boiling water bath for 5 min. Solution may appear green, yellow or red depending on amount of reducing sugar present in the test solution.

Tests for Monosaccharides

Barfoed's test: Equal volume of Barfoed's reagent and test solution were added. Heated for 1-2 min, in boiling water bath and cooled. Red precipitates were observed.

Tests for Hexose Sugars

Cobalt-chloride test: 3 ml of test solution was mixed with 2 ml cobalt chloride, boiled and cooled. Added $FeCl_3$ drops on NaOH solution. Solution observed for greenish blue (glucose), purplish (Fructose) or upper layer greenish blue and lower layer purplish (Mixture of glucose and fructose).

Tests for Non-Reducing Sugars

a) Test solution does not give response to Fehling's and Benedict's test.
b) Tannic acid test for starch: With 20% tannic acid, test solution was observed for precipitate.

Tests for Proteins

a) **Biuret test (General test):** To 3 ml T.S added 4% NaOH and few drops of 1% $CuSO_4$ solution observed for violet or pink colour.
b) **Million's test (for proteins):** Mixed 3 ml T.S. with 5 ml Million's reagent, white precipitate. Precipitate warmed turns brick red or precipitate dissolves giving red colour was observed.
c) **Xanthoprotein test (For protein containing tyrosine or tryptophan):** Mixed 3ml T.S. with 1 ml concentrated H_2SO_4 observed for white precipitate.
d) **Test for protein containing sulphur:** Mixed 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Solution was boiled it turned black or brownish due to PbS formation was observed.
e) **Precipitation test:** The test solution gave white colloidal precipitate with following reagents:

i) Absolute alcohol
ii) 5% $HgCl_2$ solution
iii) 5% $CuSO_4$ solution
iv) 5% lead acetate
v) 5% ammonium sulphate

Tests for Steroid

a) **Salkowski Reaction:** To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H_2SO_4 was added. Shaked well, whether the chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.
b) **Liebermann-Burchard Reaction:** Mixed 2ml extract with chloroform. Add 1-2 ml acetic anhydride and 2 drops concentration H_2SO_4 from the side of test tube observed for first red, the blue and finally green colour.
c) **Libermann's reaction**: Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added few drops concentrated H_2SO_4 observed for blue colour.

Tests for Amino Acids

a) **Ninhydrin test (General test):-** 3 ml T.S. and 3 drops 5% Ninhydrin solution were heated in boiling water bath for 10 min. observed for purple or bluish colour.
b) **Test for Tyrosine:** Heated 3 ml T.S. and 3 drops Million's reagent. Solution observed for dark red colour.
c) **Test for tryptophan:** To 3 ml T.S. added few drops glyoxylic acid and concentrated H_2SO_4 observed for reddish violet ring at the junction of the two layers.

Tests for Glycosides

Tests for Cardiac Glycosides:-

a) Baljet's test: - A test solution observed for yellow to orange colour with sodium picrate.
b) Legal's test (For cardenoloids):- To aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.
c) Test for deoxysugars (Keller Killani test):- To 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated H_2SO_4 observed for reddish brown colour at the junction of the two liquid and upper layers bluish green.
d) Libermann's test (For bufadenolids) :- Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled. Added

few drops concentrated H_2SO_4 observed for blue colour.

Tests for Saponin Glycosides:-

a) Foam test: The drug extract or dry powder was shaked vigorously with water. Persistent foam was observed.
b) Haemolytic test: Added test solution to one drop of blood placed on glass slide. Haemolytic zone whether appeared was observed.

Tests for Coumarin Glycosides:-

Test solution when made alkaline, observed for blue or green fluorescence.

Tests for Flavonoids

a) Shinoda test: - To dried powder or extract, added 5 ml 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings. Pink colour was observed.
b) To small quantity of residue, added lead acetate solution observed for Yellow coloured precipitate.
c) Addition of increasing amount of sodium hydroxide to the residue whether showed yellow colouration, which was decolourised after addition of acid was observed.
d) Ferric chloride test: - Test solution, added few drops of ferric chloride solution observed for intense green colour.

Tests for Alkaloids

a) Dragendorff's test: To 2-3 ml filtrate added few drops Dragendorff's reagent observed for orange brown precipitate.
b) Mayer's test:- 2-3 ml filtrate with few drops Mayer's reagent observed for precipitate.
c) Hager's test:- 2-3 ml filtrate with Hager's reagent observed for yellow precipitate.
d) Wagner's test:- 2-3 ml filtrate with a few drops of Wagner's reagent observed reddish brown precipitate.

Tests for Tannins and Phenolic Compounds

To 2-3 ml test solution, added a few drops of whether to show following was observed:-

a) 5% $FeCl_3$ solution: Deep blue-black coloured.
b) Lead acetate solution: White precipitate.
c) Gelatin solution: White precipitate.
d) Bromine water: Decoloration of bromine water.
e) Acetic acid solution: Red colour solution.
f) Potassium dichromate: Red precipitate.
g) Dilute iodine solution: Transient red colour.
h) Dilute HNO_3 :- Reddish to yellow colour.

3. Results and Discussion

Table 1. Macroscopic characteristic of rhizomes of *Kyllinga monocephala* Rottb.

S. No	Parameters	Observation of Roots
1.	Nature	Coarse powder
2.	Color	Dark red
3.	Odour	Characteristic
4.	Taste	Characteristic
5.	Texture	Rough & fibrous
6.	Size	Uneven sized coarse particles

The morphological studies and the microscopic analysis of the rhizome powder of the reveled that, it is dark red in colour with characteristic odor and test, and having parenchymatous cells, xylem, phloem, fibers, calcium oxalate crystals, and starch grain the transverse section of the rhizome showed the presence of epidermis, cortex, endodermis, pericycles, xylem and phloem.

Table 2. Certificate of Analysis of rhizome of *Kyllinga monocephala* Rottb.

S. NO.	Parameters	Observation
		Rhizomes
I.	**Extractive Value (%w/w)**	
	Pet.ether	1.4
	Ethanol	1.6
	Aqueous	5.12
II.	**Loss on Drying (%w/w)**	6.93
III.	**Ash values (%w/w)**	
	Total ash	7.99
	Acid insoluble ash	2.03
	Water soluble ash	8.2
IV.	**Fluorescence Analysis**	NO FLUORESCENCE
V.	**Foreign matter**	NIL
VI.	**Heavy metal**	
	Arsenic	BDL
	Mercury	BDL
	Cadmium	0.06 ppm
	Lead	0.05 ppm

Table 3. Phytochemical Investigation of rhizome of *Kyllinga monocephala* Rottb.

S. No.	Chemical Tests	Rhizome Extracts		
		Pet. Ether	Ethanol	Aqueous
1.	Test For Carbohydrates	-	+	+
2.	Tests for Reducing sugars	-	+	+
3.	Test for Monosaccharides	-	-	-
4.	Test for non-reducing polysaccharide	-	-	-
5.	Tests for Alkaloids	-	-	-
6.	Tests for Volatile Oils	+	-	-
7.	Test for Proteins	-	-	-
8.	Test for Amino acids	-	-	-
9.	Tests for Fats & Oils	+	-	-
10.	Test for Steroids	+	+	-
11.	Test for Cardiac Glycosides	-	+	-
12.	Test for Anthraquinone Glycosides	-	+	+
13.	Test for Saponin Glycosides	-	-	-
14.	Test for Cyanogenic Glycosides	-	-	-
15.	Test for Coumarin Glycosides	-	-	-
16.	Test for Flavonoids	-	+	+
17.	Test for Tannins & Phenolic Compounds	-	+	+

Conclusions

In the present study, the rhizomes of *Kyllinga monocephala* Rottb. belongs to the family cyperaceae was collected and authenticated. The authenticated root was subjected to physicochemical evaluation. The root was subjected to size reduction to get a coarse powder (40#) and subjected to quality control tests with various

parameters such as physical tests, extractive values, ash values, fluorescence analysis, identification of major chemical constituents and its estimation was carried out as per pharmacopoeia/literature.

The standardized root was subjected to extraction with various solvents such as petroleum ether (60^0-80^0C), ethanol and aqueous by successive hot continuous soxhlet method. Each extract was concentrated by distillating the solvent and then evaporated to dryness on Rota vapour. The concentrated extracts stored carefully for standardization and phytochemically investigation.

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