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PHYTOCHEMICAL EVALUATION OF MONOCOT GRASS KYLLINGA TRICEPS ROTTB.

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ABSTRACT

Kyllinga triceps rottb. A monocot perennial herb found in various parts of India is traditionally used in vitiated conditions pitta and vata, hyperdipsia, fever, liver disorders, verminosis, cough, splenopathy, diabetes and dermatitis. The aim of the present study was to evaluate the phytochemical and pharmacognostical study of ignored ayurvedic medicinal herb *kyllinga triceps* rottb. The plant is monocot grass belongs to the family cyperaceae, commonly used in various ayurvedic preparation's and called musta. In various ayurvedic texts it is also known as nirvishi. Many species of family cyperaceae resembles the original drug thus the present study will help in identification and collection of original plant. The study includes identification and characterization of chemical component and preliminary phytochemical screening of the plant extract. The generated information of the present study will provide data which are helpful in the correct identification and authentication of medicinal plant *kyllinga triceps* rottb. and may help in prevention of its adulteration.

Keywords: *Kyllinga triceps* rottb., phytochemical, cyperaceae, adulteration, ayurevedic.

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INTRODUCTION

Herbal medicines are the use of plants and plant extracts as medicines. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained thereof to ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance.[1, 2, 3]

Plant is a major weed of improved pastures, but also occurs in crops, gardens, plantations and roadsides. It grows best in moist fertile soil that is seldom cultivated and in full sunshine. It is present in areas up to 7000 ft. elevation. The plant is naturalized primarily in gardens and lawns. The rhizomes of plant *Kyllinga triceps* rottb are fragrant, aromatic, sweet, astringent, bitter, refrigerant, febrifuge, antidiarrhoeal, diuretic, stomachic, anthelmintic, expectorant, demulcent and tonic.[4-7] They are useful in vitiated conditions of pitta and vata, fever, cough, bronchitis, hepatopathy, splenopathy, diabetes, dermatitis, fistula and tumors[7-11]. The plant is used as an antidote in many parts of India. The root is a good refrigerant much used in fevers. Drug is also used in skin diseases and eye diseases Chinese call Kyllinga "shui wu gong" and uses it for common colds, bronchitis, malaria, arthritis and injuries[12-13]. Kyllinga is used for diarrhea in Malaysia and dysentery in china. Kyllinga is used in various places in Polynesia for joint pain and Rheumatic problems[13-15]. The spikes are applied as poultices from gathered nails. A decoction of the rhizome in used as diuretic, demulcent and tonic. It is given to relieve thirst in fevers and diabetes. Therefore, the objective of the present work is to

evaluate various pharmacognostic and phytochemical properties of the plant.

MATERIALS AND METHODS

Collection of specimen

The species for the proposed study that is *Kyllinga triceps* rottb were collected from Bhoora Khon area of Shivpuri District of Gwalior Division (M.P.) with the help of Mr. N.K. Pandey (R.O.) National Research institute for ayurvedic-siddha (CCRAS) Amkho, Gwalior.

Taxonomical Identification

The species for the proposed study was identified as *Kyllinga triceps* by Dr. (Smt.) M.D. Gupta (Asst Director) and Mr. N.K. Pandey (R.O.) National Research Institute for Ayurveda and siddha (C.C.R.A.S.) under Ministry for Health and Family Welfare, Govt. of India, Amkho Gwalior (M.P.)

Treatment

First of all the rhizomes were washed with water and dried for one hour and then it was dried in shade. By the help of grinder the dried rhizome was powdered and was passed through the sieve no. 60 for powder analysis and coarse powder was used for phytochemical work.

Preparation of plant extracts

Preparation of the extract of *Kyllinga triceps* rottb, powdered rhizome is done by using ethanol and petroleum ether solvents. For both extracts cold percolation method was used for preparation of extracts of dried *kyllinga triceps* rottb, rhizomes powder, rhizome powder were extracted with 80% ethanol and petroleum ether separately for 24 hrs, which was filtered with 80 mesh nylon cloth. Raw material and solvent ratio was 1:8, total extraction procedure was repeated for five times, clean and sterile conditions were maintained throughout the extraction process so that there should be no chance of contamination. All the filtrates obtained after extraction were combined and again subjected for filtration with 250 mesh nylon cloth, finally extract was obtained was concentrated with reduced pressure[16, 17, 18, 19]

.Thin Layer Chromatography (TLC) [18-21]

Thin layer chromatography is an important analysis tool in the separation, identification and estimation of different components. When a mixture of components is spotted on a TLC plate, the compounds which are readily soluble but not strongly adsorbed moves up along with the solvent and those not so soluble but strongly adsorbed move up less readily leading to separation of the compounds.

Steps involved in TLC

(a) Plate preparation

(b) Sample application as spots or bands over the chromatographic plate

(c) Solvent selection

(d) Adsorbent selection

(e) Detecting agent

(f) Qualitative/Quantitative analysis

Silica gel G was weighed in required quantity homogenous slurry was made with sufficient distilled water the slurry was poured on TLC glass plates by spreading technique and the uniform silica gel layer was adjusted to 0.25 mm thickness.

The coated plates were allowed for dry in air and activated by heating in hot air oven at 100-105°C for 1 hour and then used for TLC. The extracts were prepared with the respective solvent like ethanol and Petroleum ether and made up to 10m1 in different test tubes.

Then with the help of capillary tube, extracts were spotted .on TLC plate, which was developed in TLC chamber, previously saturated with different solvent systems. By try and error, method, ethanol and Petroleum Ether extracts showed isolation and resolution of spots with following solvent systems, various solvent systems were developed according to fact that extract may contain terpenes and terpenoides, as main constituents which was previously suggested by literature survey and are already present in other species of cyperaceae.

Column Chromatography

Each compound in a mixture will have a particular solubility in the solvent and a particular tendency to be absorbed by the solid adsorbent, No two compounds mostly behave exactly, alike in these respects. This principle is utilized in column chromatography.

Details of Column chromatography

Adsorbent: Silica gel (for column chromatography 60-120 #)
Fluent: Petroleum ether to water in gradation.
Length of Column: 60 cm
Diameter of Column: 3.5 cm
Amount of Ethanolic Extract used: 5 gm
Length of Column: 40 cm Packed
Rate of Elution: 30 drops per minute.
Fractions collected: Each of 100 ml

Procedure

First of all the column was filled with the sufficient silica gel 120 #) was filled up to 40 cm in the given column having height of 60 cm end 3.5 cm width. Then the column with cotton plug was carefully packed and uniformly filled with silica gel, by tapping the side of the column. Then the ethanolic extract of powdered Rhizome of *Kyllinga triceps* rottb was charged on column and eluted with solvents ranging from ˉnonpolar to polar at the rate of 30 drops per minute. Each fraction was collected in the volume of 100 ml with different solvent ratio is given in table-8.

Spectral data of isolated compound

Compound isolated by reverse TLC of isolated fraction were subjected to Mass, ^{1}HNMR, ^{13}CNMR Spectral analysis for identification and structure establishment.

RESULT AND DISCUSSION

Table 1: TLC of Ethanolic Extract and Petroleum Ether Extract of Powdered Rhizome of Kyllinga Triceps Rottb.

S.No	Extract	Solvent System	No of Spots	Color of Spots	RF Value
1	Ethanolic Extract	Chloroform Ethyl Acetate (60:40)	3	Dark Blue Greenish Blue Black	0.60 0.48 0.40
2	Petroleum ether extract	Ethyl Acetate: Hexane (30:70)	3	Dark Blue Greenish Blue Black	0.52 0.45 0.50

Column Chromatography

Table 2: Column Chromatography Of petroleum ether Extract of Powdered Rhizome of Kyllinga Triceps Rottb.

No.of fraction.	Solvents	Colour of fractions
F1-F2	Hexane (100)	Green
F3-F10	Hexane: Chloroform(80:20)	Light Green
F 1 1-F12	Hexane: Chloroform(60:40)	Blackish green
F13 -F14	Hexane: Chloroform(40:60)	Dark green
F15 - F16	Hexane: Chloroform(20:80)	Green
F17 - F18	Chloroform (100)	Greenish Blue
F19 - F20	Chloroform : Ethyl Acetate (80 : 20)	Blue
F21- F22	Chloroform : Ethyl Acetate (60:40)	Dark Blue
F23 - F24	Chloroform: Ethyl Acetate(40:60)	Dark Blue
F25 - F26	Chloroform: Ethyl Acetate(20:80)	Blackish Blue
F27 - F28	Ethyl Acetate (100)	Brown
F29 - F30	Ethyl Acetate : Ethanol (80:20)	Yellowish Brown
F31- F32	Ethyl Acetate : Ethanol (60:40)	Reddish Brown
F33 - F40	Ethyl Acetate : Ethanol (40:60)	Green
F41- F42	Ethyl Acetate : Ethanol (20:80)	Brownish green
F43 - F44	Ethanol (100)	Red
F45 - F46	Ethanol: Water (80:20)	Dark Red
F47 -F48	Ethanol: Water (60:40)	Brown
F49 - F50	Ethanol: Water (40:60)	Reddish Brown
F51-F52	Ethanol: Water (20:80)	Brown
F53-F54	Water (100)	Light Brown

TLC of Isolated Fractions

Fraction F_{21} to F_{24} is considered as single fraction as reverse TLC of these fractions have given spots of same Rf values, which may be due to the presence of same compound.

Table 3: TLC of Isolated Fractions

Sl. No,.	Isolated Fractions	Solvents	Colour of Spot	RF Value
1	F21	chloroform Ethyl Acetate: (60:40)	Blue	0.6
2	F22	chloroform Ethyl Acetate: (60:40)	Blue	0.61
3	F23	chloroform Ethyl Acetate: (40:60)	Blue	0.61
4	F24	chloroform Ethyl Acetate: (60:40)	Blue	0.61

Spectral data of isolated compound

Compound: Colorless liquid

MS m/z (%): 286, 271, 253, 187, 145, 117 and 91

^{1}HNMR (δppm) Table: 10, Fig: 15

^{13}CNMR (δppm) Table: 11, Fig: 16

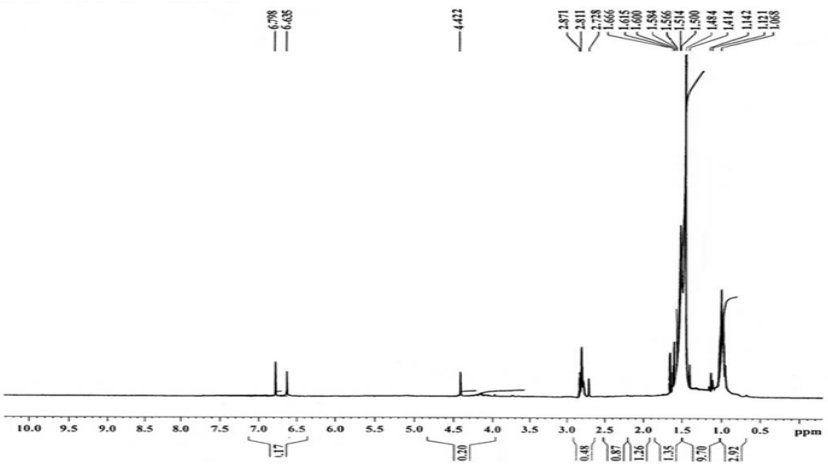


Figure 1: 1 HNMR spectra of isolated compound of Kyllinga triceps rottb petroleum ether extract

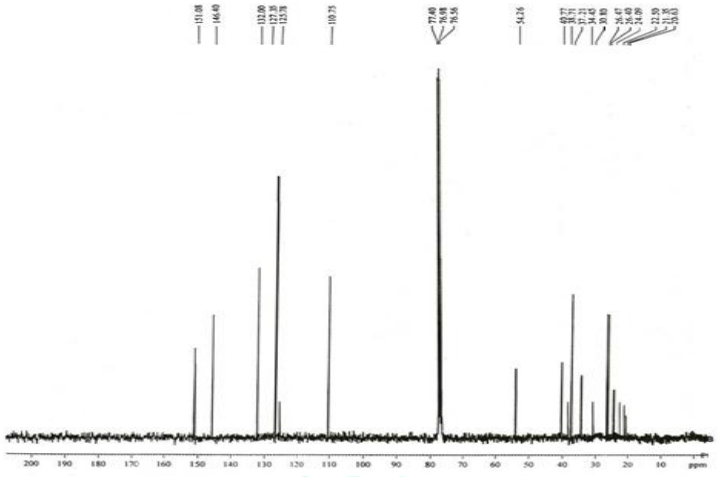


Figure 2: ^{13}CNMR spectra of isolated compound of Kyllinga triceps rottb Ethanolic extract

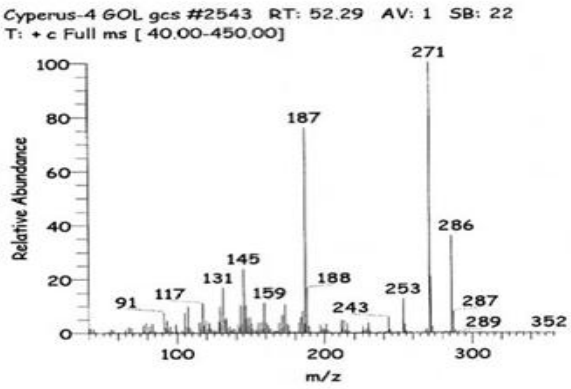


Figure 3: Mass spectra of isolated compound of *Kyllinga triceps rottb* petroleum ether extract

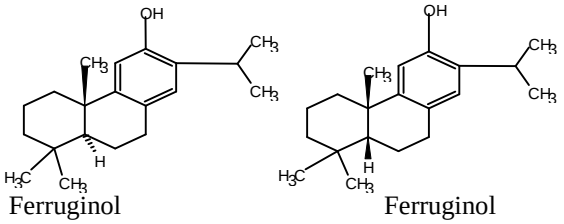
Establishment of Structure of isolated Compound

Constituent separated from petroleum ether extract of rhizomes of *kyllinga triceps* rottb. Has the molecular formula $C_{20}H_{30}O$, was established by its mass spectrum data as its showed molecular ion-peak at m/z (%) 286 (35) where the base peak is at 271. The fragment ion peaks are at 253(12), 187(78), 145(30), 117(14) and 91(9).

The ^{1}HNMR spectral data confirm the structure of the compound the presence of phenolic OH which was observed at δ 4.62 ppm. The aromatic protons were reported at δ 6.79 and 6.63 ppm. An angular methyl of *trans* configuration were recorded at δ 1.06 ppm. The geminal methyl of isopropyl moiety was observed at δ 1.12 ppm.

The structure was finally confirmed by its ^{13}CNMR spectra. A shift at δ 151.0 ppm revealed the presence of a phenolic OH at C-13. The aromatic carbons were found at δ 132.0, 127.3, 125.7, and 110.7 ppm. The geminal dimethyl carbons of isopropyl moiety were at δ 22.5 ppm.

The trans configuration of the compound was finally confirmed by the comparison of ^{13}CNMR data with its *cis*-isomers, where in the ring carbon C-3 and C-2 of *cis*-isomer were reported at δ 37.6 and 50.1 ppm, which are more shielded than the ring carbons of *trans*-isomer, Present at δ 40.7 and 54.2 ppm of carbons C-1 and C-2.



Ferruginol (Trans-form) Ferruginol (Cis-form)

Figure 4: Structure of isolated compound of petroleum ether extract of *Kyllinga triceps* rottb.

Similarly two carbons being shared by the cyclohexane and aromatic moiety of both the isomers possess different values. The carbons of *cis*-isomers at C-7 and C-9 are found at δ 126.0 and 145.3 ppm, whereas in *trans*-isomer they were found at δ 127.0 and 146.4 ppm.

CONCLUSION

Phytochemical examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies. Botanical identity of the plants is an essential prerequisite for undertaking the analysis of medicinal properties of any plant. A researcher may succeed in getting a new compound or may find many useful pharmacological active properties in the plant. If the botanical identity of the plant happens to be dubious or erratic, the entire work on the plant becomes invalid. Thus it is needless to stress the botanical identity of the crude drug is the threshold in the processes of pharmacological investigations. The researchers should be equipped with all possible diagnostic parameters of the plant on which the researchers plan to work. Present study may help in identification and future research on the plant kyllinga triceps rottb.

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