Int J Pharm Bio Sci 2013 Jan; 4(1): (P) 45 - 52

Research Article **Pharmacognosy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

INVESTIGATION OF ANTHELMINTIC ACTIVITY OF AN IGNORED PLANT '*KYLLINGA NEMORALIS*' TUBER - A POTENTIAL HOPE

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ABSTRACT

Medicinal plants belongs to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethno medicines. The use of herbal drugs, extracts and their remedies have significantly increased throughout the world and the 'green revolution' in terms of herbal medicines has now achieved astonishing popularity. The present investigation was aimed on the Phytochemical evaluation and screening of root extract of *Kyllinga nemoralis* for its antihelmintic efficiency in animal model which has traditional claims also. The phytochemical tests revealed the presence of limited phytoconstituents like carbohydrates, alkaloids and volatile oils in ethanolic tuber extract. The anthelmintic activity of ethanolic extract was investigated by employing animal model. The results were compared with standard drugs like Piperazine citrate and Albendazole. In animal model the parameters studied include paralysis time followed by death time. In this animal model, the ethanolic tuber extracts treated animals showed significant dose dependent anthelmintic activity compared to control group and also results were comparable with standards treated animals. The ethanol tuber extracts of *K. nemoralis* promote remarkable anthelmintic activity and hence can be suggested for treating various types of worm infections in human beings too.

KEY WORDS: ***Kyllinga nemoralis,*** **Anthelmintic activity.** ***Pheritima pustoma*****.**

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INTRODUCTION

Vast majorities of the world population today refinding themselves are unable to afford the products of the western pharmaceutical industry, and so they have depend mainly upon the use of traditional medicines. This reality has been recognized, documented and complied by the WHO in an inventory of medicinal plants numbering over 20000 species[1]. In terms of world health, herbal medicines continue to play a central role in the healthcare system of large portions of the world's population[2]. This is particularly true in developing countries, where traditional system of medicine have a long history of use. A good example is traditional Chinese Medicine, an integral part of Chinese culture that has served the health needs of the Chinese population for almost 5000 years[3,4]..The world health organization (WHO) has emphasized the need for better utilization of the indigenous system of medicine, based on the locally available medicinal plants in the developing countries[1]. Anthelmintics are the drugs that either kill (vermicide) or expel (vermifuge) infesting helminthiasis. Helminthiasis is prevalent globally and almost 1/3rd of world's population harbours them but it is more common in developing countries with poorer personal and environmental hygiene. They harm the host by depriving him of food, causing blood loss, injury to organ, intestinal or lymphatic obstruction and by secreting toxins[5]. The plant *Kyllinga nemoralis* commonly known as Whitehead spike sedge or poverty grass, belongs to the family Cyperaceae, grows along the roadside and thickets throughout India. It is erect glabrous sedge with elongated creeping rhizome, 7.5 - 30 cm in height. The leaves are up to 15cm in length simple, linear, acute with strong mid-nerve and upto 3-4 mm wide. Kyllinga plant flowers are minute in spikes and solitary. The spikelets are very numerous, 3 to 3.5 millimeters long, the flowering glume distinctly winged along the keel. The fruit is an achene, approximately 1.2-1.5 mm long x 0.5-0.7 mm wide. *Kyllinga nemoralis* leaves contain many biologically active chemicals like essential oils (terpenes α-cyperone, β-selinene, and α-humulene),[6] terpenoids, saponins and phenolic compounds[8]. The rhizomes possess flavonoids, triterpenoids and glycosides[7]. The most representative compounds are α-cadinol, caryophyllene oxide, α- muurolol, α-humulene, and α-atlantone[9]. The plant leaves are traditionally used for the relief of malarial chills, pruritus of the skin, and thirst due to fever and diabetes[10]**.** In India plant leaves are used as anti-venom[11, 12]**.** The rhizomes of the plant are fragrant, sweet, refrigerant, antidiarrhoeal, diuretic, stomachic, expectorant and anthelmintic [13. 14]. The paste of rhizomes mixed with milk is used internally for worm infection[15]. It is also used in fever, hepatopathy, splenopathy, diabetes and tumours[16]. Till date, there is no scientific evidence available to assess the traditional claim about the anthelmintic property of *Kyllinga nemoralis* tuber root extracts. Hence the present study was undertaken to investigate the anthelmintic activity of *Kyllinga nemoralis* tuber root extracts using animal models and thereby to provide scientific evidence to the same.

MATERIALS AND METHODS

Collection and Authentication of Kyllinga nemoralis plant.

Tubers of *Kyllinga nemoralis* were collected from the road sides of Trikaripur forest area, Kasaragod district of Kerala, India, in the month of September 2011 in a quantity sufficient for all the experiments in a single batch and the plant material was authenticated. The tuber roots were washed under running tap water, cut into small pieces of 2-3cm and shade dried (30^0C, 50 ± 5% relative humidity) for 15days. The shade dried plant material was powdered using a dry grinder to get the coarse powder (sieve no.

10/44). The powder was stored in air tight container for further use.

Preparation of extract[17]

The shade dried tuber root powder of *Kyllinga nemoralis* was subjected solvent (ethanol) extraction. The powder material was refluxed with ethanol (90%) in a Soxhlet extractor for 18 hrs in batches of 50g each cycle. The marc was gently pressed and dried before completing the extracting. The extracts obtained by the above techniques were concentrated in vacuum under reduced pressure using a rotary flash evaporator.

Preliminary phytochemical screening

The Successive extract was subjected to preliminary phytochemical testing for the detection of major chemical groups. The preliminary phytochemical screening was carried out according to the recommended standard procedures[17,18,19] as follows:

TEST FOR CARBOHYDRATES

Molisch's test: To the extract added few drops of Molisch's reagent and 1-2 ml conc. sulphuric acid slowly through the sides of the test tube. Development of a violet ring at the junction, indicate the presence of carbohydrates.

Fehling's test: Extract on boiling with equal proportions of Fehling's A and Fehling's B solution gives yellow to brick red coloured precipitate.

Benedict's test: Extract on boiling with Benedict's reagent gives green, yellow or red colour.

Barfoed's test: Extract when boiled with few ml of Barfoed's reagent shows brick red precipitate

TEST FOR PROTEINS

Biuret test: Extract on treatment with 4% Sodium hydroxide and 1% Copper sulphate solution gives violet or pink colour.

Ninhydrin test: Appearance of blue colour when the extract was treated with Ninhydrin reagent indicates the presence of proteins.

Millon's test: Extract on heating with Millon's reagent on a water bath gives white/yellow precipitate.

Xanthoproteic test: Extract treated with concentrated sulphuric acid gives white precipitate. After boiling, precipitate shows yellow and turns orange when Ammonium hydroxide was added.

TEST FOR ALKALOIDS

Mayer's test: Extract treated with Meyer's reagent gives cream coloured precipitate.

Dragendorff's test: Addition of Dragendorff's reagent to the extract gives reddish brown coloured precipitate.

Hager's test: Extract with Hager's reagent gives yellow precipitate.

Wagner's test: Extract with Wagner's reagents gives reddish/brown coloured precipitate.

TEST FOR GLYCOSIDES

Borntrager's test: Extract when shaken with Benzene gently for few minutes. The organic layer on treatment with Ammonia, give rose pink color in Ammoniacal layer.

Keller-killiani test: Extract was treated with water and Lead sub-acetate solution, filtered, and evaporated to dryness. The residue dissolved in Glacial acetic acid and Ferric chloride solution give reddish colour ring at the junction of two layers.

Raymond's test: Extract when treated with dinitrobenzene in hot methanolic alkali shows violet colour.

TEST FOR SAPONINS

***Foam test*:** Specified quantity of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 min. formation of 1cm foamy layer indicate the presence of saponins.

Libermann Buchard Test: Addition of Acetic anhydride and concentrated sulphuric acid (19:1) to the extract gives violet purple colour.

TEST FOR TANNINS

Frric chloride test: Extract on treatment with Ferric chloride solution give bluish colour.

Lead acetate test*:* Extract on treatment with Lead acetate solution gives white precipitate.

Gelatin test: Addition of gelatin solution to the extract gives white precipitate

TEST FOR FALVONOIDS

Shinoda test: Extract on boiling with few pieces of Magnesium ribbon and few drops of concentrated hydrochloric acid give pink colour.

Ferric chloride test: Extract treated with Ferric chloride solution gives green to black colour.

Mineral Acid test*:* Extract on treatment with Sulphuric acid gives yellow orange colour.

Lead acetate test**:** Extract treated with Lead acetate solution gives yellow precipitate.

TEST FOR STEROIDS

Libermann-Burchard Sterol Reaction test: Addition of few drops of acetic anhydride and concentrated sulphuric acid through the sides of test tube shows formation of a brown ring at the junction of two liquids.

Salkowski Reaction: Extract when treated with concentrated sulphuric acid gives red colour.

TEST FOR TRITERPENOIDS

Salkowaski test: Extract was shaken with a few drops of concentrated sulphuric acid and allowed to stand for a few minutes. Development of yellow colour in the lower layer, indicate the presence of triterpenoids.

Liebermann-Burchard test: Extract was treated with a few drops of acetic anhydride. Addition of concentrated sulphuric through the sides of the test tube shows the formation of deep red colour.

Anthelmintic Bioassay

Animal Used

Healthy adult Indian earthworms, *Pheretima postuma* (Annelida, Megescolecidae) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[37, 38, 39], were used in the present study. All earthworms were of approximately equal size. They were collected from local places of Trikaripur, washed and kept in water.

Assessment of Anthelmintic Activity

Drugs Used

The ethanolic extract of *Kyllinga nemoralis* was tested in various doses in each group. Normal saline water was used as control. Piperazine citrate and Albendazole were used as the standard drugs for this study with ethanolic extract.

Experimental method

The method of Nargund[20] was followed with modification for the screening of anthelmintic activity which was evaluated on adult Indian earthworm, *Pheretima postuma*. Earthworms were divided into seven groups (6 each). The first group (I) served as normal control which received saline water only. The second (II) and third (III) groups received the standard drugs, such as Piperazine citrate and Albendazole at a dose level of 10 mg/ml. Groups (IV) to (VII) received doses of ethanol extracts of 10 mg/ml, 20 mg/ml, 30 mg/ml and 50 mg/ml respectively. Observations were made for the time taken to cause paralysis and death of individual worms for two hours. Paralysis was said to occur when the worms do not revive even in normal saline water. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Statistical analysis

The data on biological studies were reported as mean ± Standard deviation (n = 3). For determining the statistical significance, standard error mean and analysis of variance (ANOVA) at 5 % level significance was employed. $P < 0.05$ were considered significant[41].

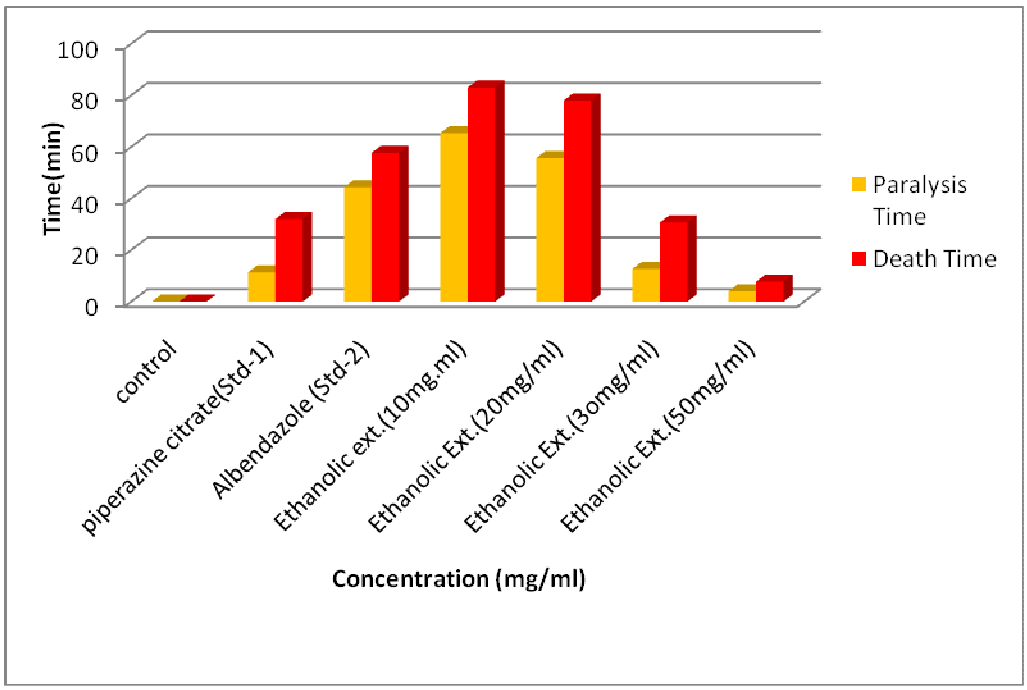
RESULT AND DISCUSSION

The morphological characters like color, odour, taste, size, shape, were identified with the help of sense organs and the observations are depicted in Table 1. Tuber roots were branched, long, thin and blackish brown surface and fibrous.

Table 1
Organoleptic evaluation of Kyllinga nemoralis tuber.

Sl. no.	Character	When fresh	After drying	Powder
01.	Colour	Dark brown	Blackish brown	Dark brown
02.	Odour	Characteristic	Characteristic	Characteristic
03.	Taste	Bland	Bland	Bland
04.	Texture	Fibrous	Fibrous	Fibrous

Figure 1
Anthelmintic activities of ethanolic extracts of roots of plant Kyllinga nemoralis on indian earthworm Pheretima postuma.



Each bar is represented as mean ± standard deviation (n = 6). Group I – Control (Normal saline water), group II – standard – 1 (Piperazine citrate), group III – standard – 2 (Albendazole), group IV to VII – Ethanolic extract of dose 10, 20, 30 and 50 mg/ml respectively.

The Ethanolic extract demonstrated paralysis as well as death of worms in a less time as compared to piperazine citrate and Albendazole in case of *Pheretima posthuma* Fig-1. The results are shown in Table -3. Phytochemical analysis of the ethanolic extracts of plant tuber root material of *kyllinga nemoralis* revealed presence of carbohydrate, alkaloids and volatile oil as chemical constituents. Results are shown in Table.-2.

Table 2
Preliminary phytochemical analysis of ethanolic extracts of kyllinga nemoralis tuber root.

Sl no.	Chemical Constituents	Ethanolic extract
01.	Carbohydrates	+
02.	Proteins	-
03.	Alkaloids	+
04.	Glycosides	-
05.	Saponins	-
06.	Tannins	-
07.	Flavonoids	-
08.	Steroids	-
09.	Triterpenoids	-
10.	Volatile oil	+

+ = Present, - = Absent.

Table 3
Anthelmintic activity of Ethanolic extracts of kyllinga nemoralis.

Groups	Treatment	Dose (mg/ml)	Time taken for paralysis (min) (X± S.D)	Time takenfor death (min) (X± S.D)
I.	Control	-	-	-
II.	Standard-1 (Piperazine citrate)	10	11.33±0.33	32.00±1.15
III.	Standard- 2 (Albendazole)	10	44.33±1.86	57.67±1.67
IV.	Ethanolic extract	10	65.33±1.45	83.00±2.52
V.	Ethanolic extract	20	55.67±1.76	78.00±1.73
VI.	Ethanolic extract	30	12.67±0.88	30.67±1.45
VII.	Ethanolic extract	50	4.00±0.58	7.67±0.88

Each values is represented as mean ± standard deviation (n = 6).

Alkaloids may act on central nervous system and caused paralysis of the earthworm [21]. The effect would be due to presence of the steroidal alkaloid oligoglycosides which may suppress the transfer of sucrose from the stomach to the small intestine together with its antioxidant effect which is capable of reducing the nitrate generation which could interfere in local homeostasis which is essential for the development of helminthes[22]. The vetnary used imidazothiazole derivative, Levamisole are nicotinic receptor agonists and elicit spastic muscle paralysis due to prolonged activation of the excitatory nicotinic acetylcholine (nACh) receptors on body wall muscle.[23]. Other phytochemical reported to have an anthelmintic effect include essential oils [24]. The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis those results in expulsion of the worm by peristalsis. Piperazine citrate act by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle to relaxation and flaccid pralalysis[25]. Even the function of the anthelmintic drugs like Albendazole is to cause paralysis of worms so that they are expelled in the feaces of man and animals. The

extracts not only demonstrated this property, they also caused death of the worms, especially at 30 mg/ml and 50mg/ml as compared with the Albendazole and piperazine citrate.

CONCLUSION

The wormicidal activity of alcoholic (ethanolic) extracts suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstitutent or constituents which are exactly responsible for the anthelmintic action and study its other pharmacological properties for futuristic and cost effective pharmaceutical formulations.

ACKNOWLEDGEMENT

The author is thankful to the principal, Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kerala for providing the necessary arrangements and constant encouragement for successful completion of this research work.

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