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Evaluation of *In Vitro* anthelmintic activity of *Cymbopogon citratus* (lemon grass) extract

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Abstract

Lemon grass a popular aromatic plant which is commonly used as a substitute of green tea has a tremendous phytomedicinal potential. Like other tea plants it also having antimicrobial as well as antiprotozoal potential. Main objective of the present study was to find out the anthelmintic potential of aqueous extract of *Cymbopogon citratus* (lemon grass) by using a model worm i.e. *Pheretima posthuma* (earthworm). In this bioassay, three different concentrations i.e. 25, 50 and 1000mg/ml of above mentioned extract were tested in bioassay by noting time of paralysis and time of death of worms in minutes. Piperazine citrate was used as a standard reference compound along with normal saline as control in the same concentrations. The results indicated that lemon grass crude extract possessed anthelmintic activity in dose dependent manner.

Key-Words: Helminthes, *Cymbopogon citratus*, Lemon grass, Pheretima posthuma, Anthelmintic

Introduction

Since the beginning of human history, plants have been in practice as folk medicine. All natural plants are rich source of medicinal agents, commonly in traditional medicine (Bonjar *et al*., 2004). One or more parts of medicinal plants having substances that can be useful for the therapeutic purpose (Rios, 2005). *Cymbopogon citratus* belongs to the Poaceae family which is monocotyledonous aromatic perennial having slender sharp edged leaves and pointed apex commonly inhabitant to tropical Asia. In west India and Pakistan, *C. citratus* is also known as Guatemala, lemongrass (Ernst, 2008). The extract of *C. citratus* having different compounds where as the major component is citral approximately 65-85%. Additionally, lemongrass extracts having small quantity of geranylacetate, monoterpene olefins and geraniol (Fair & Kormas, 2008). Lemon grass are being used as the therapeutic agent for the treatment of gastrointestinal disturbances, nervous, hypertension and fever were used.

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The extracts of *C. citratus* are also used in elephantiasis, coughs, flu, headache, gingivitis, malaria, leprosy, ophthalmia, vascular disorders and pneumonia. Several studies of lemongrass extracts have been reported on its antifungal and antibacterial activities (Onawunmi et *al*., 1989; Wannissorn *et al*., 1996; Schuck., 2001; Paranagama; 2003; Wannissorn *et al*., 2005). Moreover, antiviral potential especially against Herpes simplex virus were also reported (Minami *et al*., 2003).Gore et al in 2010 reported the anti helminthes activities of lemongrass (Gore *et al*., 2010) Helminths are most common agents spread human infections frequently in developing countries and and contribute by increasing the global burden of diseases actively (Hotez *et al*., 2008). World Health Organization (WHO) estimated 2 billion people with helmenthic infection and this population was suggested to get medicine to expel and kill the helminthes from human body (WHO, 2012). The purpose of present study is to investigate the anthelminthic activity of lemongrass against earthworm.

Material and Methods

Preparation of extract

The lemon grass tea was purchased locally from the retail market in Karachi-Pakistan in reasonable price and brought to the laboratory of Federal Urdu University of Arts, science and Technology (FUUAST)-Karachi-Pakistan. The extract was prepared in 5% concentration (5 grams of weighted lemon grass tea added in 100 ml of distilled water). The aqueous extract was prepared by boiling method of tea in water bath by constant agitation of for 15 minutes. After extract preparation, coarse suspended particles of tea were removed by passing through strainer and later by passing via 0.22um filter (Sherwani *et al*., 2013). The extract was stored in refrigerator in small vials as aliquots for further use.

Selection of worms

The anthelmintic activity was done by following the method of (Ajaiyeoba *et al*., 2001) with slight modifications. The assay was carried out on adult earthworm (*Pheretima posthuma*) owing to its resemblance in terms of anatomy and physiology with the intestinal roundworm parasite of human beings (Vigar, 1984; Sherwani *et al*., 2013; Thorn, 1977; Chaterjee 1967; Vidyarthi 1967). This worm has been a choice as a model for researchers to investigate anthelminthic activity of natural plant product is its easy availability (Szewezuk *et al*., 2003; Deore *et al*., 2009).Because of this reason; earthworms have been used commonly for in vitro evaluation at initial level of anthelmintic bearing natural and synthetic substances (Sollmann, 1918; Jain and Jain., 1972; Shikvar and Kumar., 2003).

Worm Collection and authentician

Earthworms of approximately within the size range of 8 cm (Vidyadhar *et al*., 2010) were collected from moist garden soil of Federal Urdu University of Arts, science and Technology-Karachi Pakistan with the help of a Zoologist from MRCC-Department-University of Karachi-Pakistan and authenticate the *Pheretima posthuma*. Later, all the worms were washed with saline to remove the soil particles, debris and fecal material (Satish and Ravandra, 2009) and kept them in phosphate buffer saline (PBS) (Eugale and Giday, 2009) till further work.

Anthelminthic assay

The earthworms were divided into three groups having six earthworms. Group first serve as control, receive only normal saline; Group second serve as standard, receive standard piperazine citrate and Group third serve as aqueous extract of different concentration (Dwivedi et al., 2011). All the extracts and the standard solution in autoclaved distilled water were freshly prepared before conducting the assay (Rajesh et al., 2010). The concentration of control, standard and extract that were used in this assay were 25 mg/ml, 50mg/ml and 100mg/ml. Observations were made for the time taken until the paralysis as well as death of an individual worm occurred (Trapti *et al*., 2009; Grime et al., 2006). The mean time of paralysis and death was recorded in minutes. The paralysis was declared when the worms were not able to move even in normal saline (Zafar et al., 2001). Death was considered when the worms lost their motility followed with fading away of their body colors (Mali and Mahale, 2008).

Results and Discussion

Helminthes infection like other infections are also the most wide spread infections in humans, affecting seriously a huge population of the world (Kosalge and Frusule, 2009). Moreover, there is a need to pay serious attention to the existing helminthic infections as the majority of infections owing to helminthes are not only injurious to health but also play a role somehow in the development of anemia, pneumonia, undernourishment, eosinophilia and some other secondary complications (Bundy, 1994). Anthelminthic or wormicidal agents are the drugs that expel out parasitic worms (helminthes) from the body by causing paralysis or killing them but these anthelminthic drugs are also now adopting resistance and therefore, no more effective in management of infections (Chartier *et al*., 2001). The plants are very well known to provide a rich and diverse source of botanical anthelmintic (Coles, 1997). In this study, in earthworms, the aqueous extract of *Cymbopogon citratus* (lemon grass) showed anthelmintic activity. A number of previously conducted report indicated that that *Cymbopogon citratus* has been used against gastrointestinal disturbances and complications (Nakamura *et al*., 2003). Earthworms belonging to control group showed paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e. 96.46 $\pm$ 0.46 mins, 78.23 $\pm$ 0.25 mins and 62.43$\pm$ 0.11 mins while; death time 160.06$\pm$ 0.11mins, 142.2$\pm$ 0.34 mins and 111.7$\pm$ 0.26 mins respectively. On the other hand, the standard reference compound piperazine citrate showed the time of paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e 18.36 $\pm$ 0.15 mins, 12$\pm$ 0.00 mins and 08.84 $\pm$ 0.03mins respectively while; death time at 54.36 $\pm$ 0.40 mins, 46.46 $\pm$0.40 mins, 13.6$\pm$ 0.17 mins respectively as indicated in Table 1.In case of aqueous extract of *Cymbopogon. citratus* (lemon grass) group, its paralysis time at the concentration of 25mg/ml, 50mg/ml and 100mg/ml i.e. 44.86$\pm$0.41mins, 22.16$\pm$1.06 mins and 10.7$\pm$0.43mins respectively death time at 120.46 $\pm$0.25mins, 92$\pm$0.51

mins and 80.7+ 0.51 mins respectively as indicated in Table 2. The activity was observed in dose dependent way in all groups including control, standard drug ad extract. From the observations achieved, higher concentration of crude extract of lemon grass showed paralytic effect much earlier and the time to death was shorter for almost all the worms. The results of similar study but conducted on green tea extract at (50mg/ml) showed paralysis within 27.02 min & time of death 47.07 mins while, aqueous extract (100mg/ml) showed paralysis within 21.19 min & time of Death 37.02 mins (Dwivedi *et al*., 2010). According to mode of action, piperazine citrate causes flaccid paralysis and expel out the worm by peristalsis (Sherwani *et al*., 2013). Piperazine citrate generates paralysis by increasing the chloride ion conductance of worm muscle membrane resulting in the hyper polarization and reduced excitability that ultimately leads to muscle relaxation thus a worm expels out by the action of peristalsis (Goshwami *et al*., 2013). Phytochemical analysis of *Cymbopogon citratus* revealed presence tannins (Ewansiha *et al*., 2012) and considered to inhibit the infective agents (Kolodziej & Kiderlen, 2005). Some other polyphenolic compounds also known to have anthelmintic activity (Bate Smith, 1962).

Conclusion

From the results of this preliminary work, it is concluded that the crude aqueous extract of *Cymbopogon citratus (*lemon grass) showed anthelmintic activity and could be apply as an effective agent in future after further exploration. Studies should be needed in next steps of the undertaken work for understanding the mechanism of action by using in vivo models to figure out the effectiveness and pharmacological rationale of using lemon grass as an anthelmintic drug.

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Table 1: Anthelminthic activity of control and standard

Concentration mg/ml	Control		Standard	
	Paralysis time (Mins)	Death time (Mins)	Paralysis time (Mins)	Death time (Mins)
25mg/ml	96.46 ± 0.46	160.06± 0.11	18.36 ± 0.15	54.36 ± 0.40
50mg/ml	78.23 ± 0.25	142.2± 0.34	12± 0.00	46.46 ±0.40
100mg/ml	62.43± 0.11	111.7± 0.26	08.84 ± 0.03	13.6± 0.17

Values are the mean ± S.E.M. of control and standard drug on three earthworms. Control is normal saline while standard drug is piperzine citrate

Table 2: Anthelminthic activity of crude extract of *Cymbopogon citratus* (Lemon grass)

Concentration mg/ml	Crude extract of *Cymbopogo citratus* (lemon grass)	
	Paralysis time (mins)	Death time (mins)
25mg/ml	44.86± 0.41	120.46 ±0.25
50mg/ml	22.16± 1.06	92±0.51
100mg/ml	10.7 ± 0.43	80.7± 0.51

Values are the mean ± S.E.M. of aqueous extract of *Cymbopogon citratus (Lemon grass)* on three earthworms