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EVALUATION OF ANTHELMINTIC ACTIVITY OF CURCUMA AROMATICA SALISB. TUBER AQUEOUS EXTRACT

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ABSTRACT

The aim of the present study is to evaluate the anthelmintic activity of aqueous extract of rhizomes of Curcuma aromatic Salisb in various concentration (25% to 100%) by using earth worms (Lumbricus rubellus). Albandezole 20mg/mL was used as the standard drug where distilled water as control. A strong association was seen between the concentration gradient and the results. The minimum time for paralysis (15.5 min) and death (34.7 min) was observed in 100% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb. Maximum time taken for paralysis (46.2 min) and death (105.0) was recorded in the 25% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb. Time taken for paralysis recorded in 50% and 75% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb were 34.2 min and 26.9 min accordingly. Time taken for death recorded in 50% and 75% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb were 67.6 min and 57.6 min accordingly. The results obtained were statistically analyzed by SPSS, IBM, Version 23. Using one way-ANOVA method and paired sample t-test analysis method. The standard used was albendazole at the concentration of 20mg/ml showed a significant value of 0.049 which is $P<0.05$ which indicates that the standard drug used for the research is an efficient and effective drug which elicited anthelmintic activity. Where as aqueous extract of Rhizomes of Curcuma aromatica Salisb was used as the test drug at the different concentration gradients of 25%, 50%, 75% and 100% against the Earth worms belongs to the species of Lumbricus rubellus which indicates the P values as for 25% ($p=0.033$, $p<0.05$), for 50% ($p=0.022$, $p<0.05$), for 75% ($p=0.014$, $p<0.05$) and 100% ($p=0.009$, $p<0.05$). When compared to the standard drug the test drug in all percentage of concentrations indicates effective anthelmintic activity. Among the different concentration gradients with the increase in concentrations, the anthelmintic activity also increased. Therefore, the results of this study conclude that the aqueous extract of rhizomes of Curcuma aromatica Salisb showed anthelmintic activity.

Key words: Curcuma aromatica Salisb, Anthelmintic, Earth worms, Albendazole

INTRODUCTION

Helminthiasis or infection with parasitic worms affects over two billion people of people of world population, which more often physically impair the host rather than killing them. It affects the poor people lives in developing country. The infection with helminthes are common in tropical regions and the people can spread these pathogens to previously non-exposed population by traveling, migration and military operations. (1) Helminthiasis becomes as a global burden of disease compared to malaria and tuberculosis. Albendazole is considered as the commonly used drugs for helminthes infestation but it is recorded with some potential activity exerted in the consumers upon usage. Therefore, there

is a need to look for a substitute medicine. Since Siddha medicine, become popular universally due to its high curative value with minimum toxicity. Siddha medicine like traditional medicinal systems are known for clinically effective against helminthes infections and free of adverse event. (2) Treating intestinal worms are easier than those infections occurs in other parts of the body, because the worms need to be killed by the drug where the drug need not be absorbed when administered orally. There is an urge to look for alternative approaches against the gastrointestinal nematodes because of increasing resistance to conventional anthelmintic on the environment, one of the major options is use of herbs. (3) Traditional medical systems are making use of natural products and gives more importance to them. According to the fossils, usage of plants as medicine by humans may be traced back at least 60,000 years. The era of "modern" drug began at the era of nineteenth century; But for the development of new drugs the existence of natural products are very important. (4) In developed countries, about 80% of individuals use traditional medicine, which is derived from medicinal plants. Therefore, Investigation of such valuable plants should be done to get better understanding about their properties, safety and efficiency. Plants are good sources for new safe, biodegradable, cheap and renewable drugs. (5) Herbs are being used for treating diseases pharmacologically for a long period of time and the herbal medicine is very popular because it is out of harm's way, handy and with less concomitants. Around a quarter of all pharmaceutical drugs currently on the market are derived from plants. (6) The anthelmintic drugs in the market are outrageous, beyond reach or niggardly available to the resource poor farmers of the developing countries. Therefore, these factors created a need for herbal remedies as alternate anthelmintic and evaluation of medicinal plants with anthelmintic activity is obtaining attention at present. (7) Curcuma aromatica Salisb. called as wild turmeric or wild zedory is the second highest used aromatic medicinal plant in curcumin species. Curcuma aromatica Salisb. that belongs to zingiberaceae, is a biennial/ annual herb widely distributed in tropical and subtropical regions. It grows in India, Sri Lanka, China and Japan and some other South Asian Countries.(8) In Sri Lanka it is found in shady and damp places in Lower Island and cultivated in some areas. (9) It is an erect rhizomatous herb, has green oblong simple leaves which are similar to turmeric, above the ground. The rhizomes are less pigmented aromatic and light yellow in colour and orange/ red inside. When matured it gives high fragrant.(10)

The flowers of the plant are red/ pink with orange lip, somewhat irregular, shorter than bracts and has fragrant odor. The plant grows fast during monsoon and rhizome remains dormant during winter season. (8) It has been used for long period in traditional medical system as cosmetic and for therapeutic uses. It poses the pharmacological actions like anti-inflammatory, wound healing, anti-melanogenic, antioxidant and free radicals scavenging activity, anti-tumor, anti- cancerous, anti- repellent, antitussive, anti- platelet activity and anti- nephrotoxic activity. (11) Nowadays the traditional uses of the Curcuma aromatica Salisb. are explored under the modern scientific researches. The rhizome/ tuber of Curcuma aromatica Salisb. is widely used to treat the conditions like blood stasis, pain, liver diseases, and it is internally used as carminative, tonic, and externally for

sprain, skin conditions, and antidote for snake venom.

(12) Paste of the rhizome is also used to treat gastric ailments and dysentery with milk. It is used to evacuate stillborn baby from the uterus. Villagers from Khasi and Himalayan region of India, intake the paste of the rhizome with water/ milk to treat helminthes infection .(11) Powder of rhizome is administered in the dose of 325mg- 500mg to cure abdominal pain and peptic ulcer. (13) Therefore the Plants and their extracts have capability in the management and eradication of helminthes. However, there is a necessity for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for treating helminthes.

Objectives of the study

General objective

• To evaluate the anthelmintic activity of Curcuma aromatica Salisb. tuber.

Specific objectives

• To compare the efficacy of anthelmintic activity of aqueous extract of Curcuma aromatica Salisb. tuber.

• To evaluate the anthelmintic activity of aqueous extract of Curcuma aromatica Salisb. tuber in different concentration.

MATERIALS AND METHODS

Introduction of study

Study design

In-vitro Evaluation of anthelmintic activity of aqueous extract of Curcuma aromatica Salisb tuber on earthworms was carried in on month period of time. The earth worms were collected and adapted for 2 weeks and then authenticated by the zoologist as Lumbricus rubellus. As like as the Curcuma aromatica Salisb tubers were collected and authenticated by the Department of Gunapadam, The extract was prepared in 4 different concentrations. Earthworms with the average length of 5-8 cm in and 0.2-0.3 cm in width were divided into control(distilled water), test groups in four different concentration (100%, 75%, 50%, 25%) of solutions of aqueous extract of rhizomes of Curcuma aromatica Salisb and standard group(albandazole solution) containing 3 earthworms in each group. Before starting the experiment the earthworms were washed in normal saline to evacuate the fecal matter.Data was collected based on the observation. The results obtained were statistically analyzed by SPSS, IBM, Version 23. Using one way-ANOVA method and paired sample t-test analysis method.

Plant Material

Fresh rhizomes of Curcuma aromatica Salisb. were collected from the Batticaloa district, Sri Lanka.The plant was authenticated by the Department of Gunapadam and preserved as herbarium.

Preparation of extract

Rhizomes of Curcuma aromatica Salisb were chopped into small pieces and washed thoroughly in tap water followed by distilled water to remove adhered material and soaked in paper towels. Cleaned pieces of rhizomes were crushed manually by motor and pestle. Obtained material was filtered through whatman no.1 filter paper and the filtrate was collected and used as the stock solution(100%).The prepared extract was diluted with the help of distilled water in varying concentrations as follows, 100% concentration, 75% concentration, 50% concentration and 25%.(14)

Chemicals and drugs:

Standard drug: five tablets of albendazole(400mg) and suspending agent Carboxy methyl cellulose (100g) were purchased from an authorized pharmacy.

Others: Distilled water was purchased from a reliable seller.

Experimental Animal

100 adult earthworms were collected from the moist lands of Batticaloa on January,2021 due to their similarity in anatomical and physiological resemblance with the intestinal roundworm parasite of human beings and they were authenticated as Lumbricus rubellus by a zoologist at Eastern University, Sri Lanka. Intestinal round worms and earthworms share more similarities as follows both Insects with low body structure, vertebrates with bilateral symmetry include both types, the digestive system of both is fully functional. Even though, they cannot breathe as they have no respiratory system. Instead, they breathe through the skin. (15)

Adaptation of animal

Earthworms were kept in an aerated container with moist soil of their origin. The availability of the food was ensured by adding some plants in to the container. Adaptation was continued for about one week. They were kept away from direct sunlight and below the temperature of 25 degrees Celsius. The good health of the earthworms during one week adaptation period was ensured by looking for markers of great wellbeing like turgidity, normal body shape without localized constrictions, absence of wounds and epidermal injuries, and versatility. Healthy earthworms were respond to touch, attempt to elude from light, and begin swimming in water.(16)

Preparation of animals

Before starting the experiment the earthworms were washed in normal saline to evacuate the fecal matter. The earthworms of 5-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol.(17)

Grouping of animals

Animals were divided into seven groups, each group consisting 3 earthworms.

Group I: Control

Group II: Standard 20 mg/mL Albendazole solution was prepared by using 0.5 w/v of Carboxy Methyl Cellulose as a suspending agent.

Group III, IV, V and VI: Four different concentrations of solutions were prepared by diluting the prepared aqueous extract of rhizomes of Curcuma aromatica Salisb with the help of distilled water in varying concentrations as follows, 100% concentration, 75% concentration, 50% concentration and 25%.(14)

Treatment for each groups: The earthworms of group I were kept untreated in a petri dish containing 20 mL of distilled water and considered as control.

The earthworms of group II were served as a standard and received albandazole solution prepared by dissolving albandazole in 0.5 w/v of Carboxy Methyl Cellulose suspending agent in the concentration of 20mg/mL.

The earthworms of group III, IV, V and VI were considered as test and treated with four different concentration accordingly (100%, 75%, 50% and 25%) of rhizomes of Curcuma aromatica Salisb aqueous extract. Treatment procedure: All the test solutions and standard drug solutions were prepared freshly before starting the experiments. Then 20 mL of each solutions were poured in to separate labeled petri dishes and then three worms were placed into each.

Assessment: Data was collected based on the observation. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the worms will be shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). This method was triplicated for accuracy.(18) In vitro anthelmintic activity was performed and paralysis time and death time were obtained and recorded. The results obtained were statistically analyzed by SPSS, IBM, Version 23. Using one way-ANOVA method and paired sample t-test analysis method.

RESULTS

The Present study revealed that all concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb possess anthelmintic activity As mentioned in figure 4.1, the minimum time for paralysis (15.5 min) and death (34.7 min) was observed in 100% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb, followed by 75% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb, time for paralysis (6.9 min) and death (57.6 min). Standard drug albandezole 20mg/mL showed paralysis time of 144.1min and death time of 224.0 min, which is greater than the 25% concentration of aqueous extract of rhizomes of Curcuma aromatica Salisb P time for paralysis (46.2 min) and death (105.0 min).

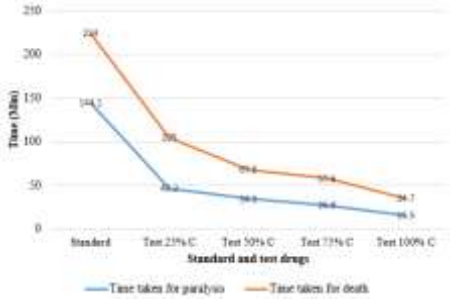

Figure 4.1 In vitro evaluation of anthelmintic activity of aqueous extract of rhizomes of Curcuma aromatica Salisb

The standard drug used was albendazole at the concentration of 20mg/mL showed a significant value of 0.049 which is P<0.05. Where as aqueous extract of Rhizomes of Curcuma aromatica Salisb was used as the test drug at the different concentration gradients of 25%, 50%, 75% and 100% against the Earth worms belongs to the species of Lumbricus rubellus which indicates the P values less than 0.05.

DISCUSSION

More over 1.5 billion individuals, or 24% of the global population, are afflicted with helminths, which are spread from nation to country.(19) In Sri Lanka, Ascaris lumbricoides, Trichuris trichiura, and Necator americanus diseases were recorded recently.(20) In this study the standard used was albendazole at the concentration of 20mg/mL showed a significant value of 0.049 which is P<0.05 which indicates that the standard drug used for the research is an efficient and effective drug which elicited anthelmintic activity. Where as aqueous extract of Rhizomes of Curcuma aromatica Salisb was used as the test drug at the different concentration gradients of 25%, 50%, 75% and 100% against the Earth worms belongs to the species of Lumbricus rubellus which indicates the P values as for 25% (p=0.033, p<0.05), for 50% (p=0.022, p<0.05), for 75% (p=0.014, p<0.05) and 100% (p=0.009, p<0.05).

When compared to the standard drug the test drug all percentage of concentrations indicates effective anthelmintic activity. Among the different concentration gradients with the increase in concentrations, the anthelmintic activity also increased. Therefore aqueous extract of rhizomes of Curcuma aromatica Salisb which is used as a test drug for this study clearly illustrates that it is a very effective and efficient drug to elicit anthelmintic activity and can be used in the treatment and prevention of worm infections. However, further study will be needed to get vast insights of anthelmintic activity. The paralysis and death of the worms happen due to the presence of phytochemicals such as Flavanoids, Tannins and alkaloids.

Alkaloids, such as steroidal alkaloids and oligoglycosides, have neurotoxic properties that affect acetylcholine-stimulated body wall muscle contraction, acting as acetyl-cholinesterase inhibitors and causing worm paralysis. They also act

as an antioxidant, reducing nitrate production, which can interfere with local homeostasis, which is required for the development of helminthes.

Flavonoid compounds, such as apigenin, can inhibit larval growth and arachidonic acid metabolism, which can lead to neuro-degeneration in the worm's body and death. Tannins may have an anthelmintic effect by reducing the migratory ability and survival of newly hatched larvae. They reduced worm burden while causing damage to worm cuticle and digestive tissues. Furthermore, tannins inhibit worm energy generation by uncoupling oxidation and phosphorylation, and they bind to glycoproteins on the worm's cuticles, causing death.(21) Another study shows that chemically tannins are polyphenolic compounds and appeared to create anthelmintic activities. It is Reported anthelmintic impact of tannins is that they can attach to free proteins within the gastrointestinal tract of host animal or glycoprotein on the cuticles of the parasite and may cause death.(22)

CONCLUSION

According to the results of the current study concluded that the aqueous extract of rhizomes of Curcuma aromatica Salisb has anthelmintic activity against (Lumbricus rubellus). . The aqueous extract of rhizomes of Curcuma aromatica Salisb has showed increasing anthelmintic activity with increasing concentrations. However, Further studies to be conducted to determine the component responsible for the anthelmintic activity.

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