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Research Article

THERAPEUTIC POTENTIAL AND *IN VITRO* ANTHELMINTIC ACTIVITY OF RIDGE GOURD FRUIT

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ABSTRACT

Objective: The objective of the study was to evaluate the therapeutic potential and *in vitro* anthelmintic activity of ridge gourd fruit (*Luffa acutangula*) against Indian earthworms.

Methods: For anthelmintic activity against Indian earthworms (*Pheretima posthuma*, *Ascaridia galli*, and *Raillietina spiralis*), various different extracts concentration of *L. acutangula* fruit have been taken. Five concentrations as 10, 20, 30, 40, and 50 mg/ml of various extracts were tested and results were expressed in terms of time for paralysis and time for the death of worms. Albendazole (20 mg/ml) was used as reference standard and water (0.5%) as a control group.

Results: Preliminary phytochemical screening of the different extracts of ridge gourd fruit was shown to produce anthelmintic activities. In the present study, it was observed that all the extracts of ridge gourd fruit have exhibited a positive response to a certain degree of anthelmintic activity. Ethyl acetate extract exhibited more potent activity at the lower concentration of 10 mg/mL against *A. galli* (Roundworm). The anthelmintic activity of *L. acutangula* fruit extract has, therefore, been demonstrated.

Keywords: Helminth infections, Medicinal plant, *Luffa Acutangula,* Earthworm.

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INTRODUCTION

Helminthiasis is the most common infection caused by worms that is a contaminant to human body parts [1-3], and its one of the most prevalent infections; children are often infected with several species and a degenerative disease that affects a large percentage of the population in the worldwide. The available today anthelmintic treatment, including several drugs as albendazole, mebendazole, thiabendazole, niridazole, diethylcarbamazine, ivermectin, and praziquantel, is widely used to control of helminthiasis. It may cause and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia in developing nations [4,5]. Furthermore, the development of resistance to conventional anthelmintics in helminths [6,7] is a major problem in the treatment of helminths infections [8,9]. It is now important to seek alternative strategies against all of the gastrointestinal nematodes, which have already led to the plan to screen medicinal plants for their anthelmintic activity. About 2000 species of medicinal plants in India have high potential values; unfortunately, very few of them were studied chemically and pharmacologically for their medicinal benefit. *Luffa acutangula* (family: Cucurbitaceae) referred to as ridge gourd, angled or angled luffa (Fig. 1), is one of Asia's potential herbs. It is a common vegetable grown in India, China, Southeast Asia, Egypt, Japan, and other African countries, which has a various pharmacological activities includes antidiabetic activity [10,11], antioxidant activity, hepatoprotective activity [12], central nervous system [13], anticancer [14,15], and fungistatic property [16]. Its chemical constituents were found to be carbohydrates, carotene, fat, protein, phytin, amino acids, alanine, arginine, cysteine, glutamic acid, lysine, hydroxyproline, leucine, lectin, serine, tryptophan, and pipecolic acid [17,18]. Ancient literature concludes that the plant is used extensively as an antifungal and abortive agent [19]. LA as a whole is the source of the secondary metabolites, i.e., amino acids, carbohydrates, proteins, flavonoids, anthraquinones, terpenoids, tannins, and saponins [20]. Seeds of this plant show the presence of saturated and unsaturated fatty acids, linoleic, palmitic, stearic, oleic, and traces of lignoceric acid while fruits contain cucurbitacin B, E, and oleanolic acid [21]. Furthermore, various biological activities of this plant were reported above including its use in weight loss, jaundice, blood purification. As our ongoing work for a search of new drug [22-25], we have focusing in booth synthetic and nature products. Keeping in mind such astounding properties exhibited by this plant, the present study was intended to investigate the anthelmintic activity of ethyl acetate extract of ridge gourd fruit in the Indian earthworm.

METHODS

Drugs and chemicals

Solvents were obtained from SD Fine Chem Ltd. and Avra Synthesis Pvt Ltd., Mysore, India. Albendazole standard was purchased from Sigma Chemical Co., USA. Moreover, all other chemicals used in this study were analytical grade with high purity.

Administration of albendazole

Albendazole (20 mg/ml) was prepared using 0.5% w/v sodium carboxymethyl cellulose as a suspending agent as administered as per the method of extract.

Collection and identification of the plant

The fresh ridge gourd fruits were collected during the month of February (2018), from the area of Mandya district, Mysore, Karnataka, India. The fresh fruits of the plants were first washed with water to remove adhering dirt and then peeled off the skin manually, and then sun-dried for (10) days as shown in Fig. 2. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container for further use.

Plant extraction and processing

The powdered plant material (20 g) was used for extraction by Soxhlet apparatus have packed in filter paper and loaded into the thimble,

which is placed inside the Soxhlet extractor. Following this, the solvents as 250 ml of hexane, dichloromethane, ethyl acetate, methanol, and water were added to the round bottom flask in the sequential order, starting from the non-polar, and ending with the polar solvent. The thimble then attached to a Soxhlet extractor and condenser Fig. 3 on an isomantle. The solvent is heated using the isomantle at a boiling point of hexane (69.1°C), dichloromethane (39.6°C), ethyl acetate (77.1°C), methanol (64.7°C), and water (100°C). After each extraction, the plant material was dried and used again for the next extraction. Extraction was considered to be complete when the plant materials become exhausted of their constituents that were confirmed from cycles of colorless liquid siphoning in the Soxhlet apparatus. All extracts of the plant were filtered individually through a fresh cotton bed. The filtrates obtained were dried at the temperature of 40±2°C to have a gummy concentrate of the crude extracts. After extraction, the solvent was removed from samples using a rotary evaporator apparatus. Each extract was kept in a suitable container with proper labeling and stored in a cold and dry place.

Fig. 1: Ridge gourd fruit

Fig. 2: Collection and packaging of ridge gourd fruit

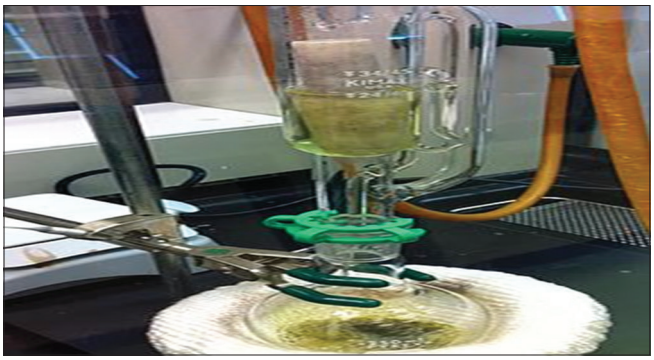

Fig. 3: Soxhlet extractor of plant

Experimental worms

All of the experiments were carried out in Indian adult earthworms (*Pheretima posthuma, Ascaridia galli,* and *Raillietina spiralis*) by reason of its anatomical resemblance with the intestinal roundworm parasites of human beings. They have been collected from moist soil and washed probably with water to remove any fecal matters.

Experimental section

Hexane, dichloromethane, ethyl acetate, methanol, and water extracts from the skin powder of ridge gourd fruit were investigated for anthelmintic activity against *P. posthuma*, *A. galli,* and *R. spiralis.* Various concentrations of 1, 20, 30, 40, and 50 mg/ml each for extract were tested by bioassay, which involved time determination of paralysis and death time of the worms. Albendazole was used as the standard reference and saline water as a control. Adult earthworms (*P. posthuma),* Roundworm (*A. galli), and* Tapeworms (*R. spiralis)* were used to evaluate the anthelmintic activity *in vitro*. Earthworms were collected from moist soil and washed properly with normal saline to remove; all fecal matters were used for the anthelmintic studies. The earthworms of 3–5 cm in length and 0.1–0.2 cm in width were used for all the protocol experiments. Roundworms and tapeworms were collected from the intestines of freshly slaughtered fowls. Infested fowl intestines were obtained from the local slaughterhouse and cleaned with normal saline solution to eliminate all fecal matters. These intestines were then dissected and worms were collected and kept in normal saline solution. The average size of the roundworm was 5–7 cm and the average size of the tapeworm was in the rate of 6–8 cm. Earthworm and helminths were identified in the Maharani Science College for women, Mysore. Albendazole was used as standard anthelmintic during the experimental protocol.

Anthelmintic assay

The anthelmintic assay was performed as per the reported method [26]. The *in vitro* assay was accomplished using adult earthworm (*P. posthuma*) because it also has highly functional and biological similarity with

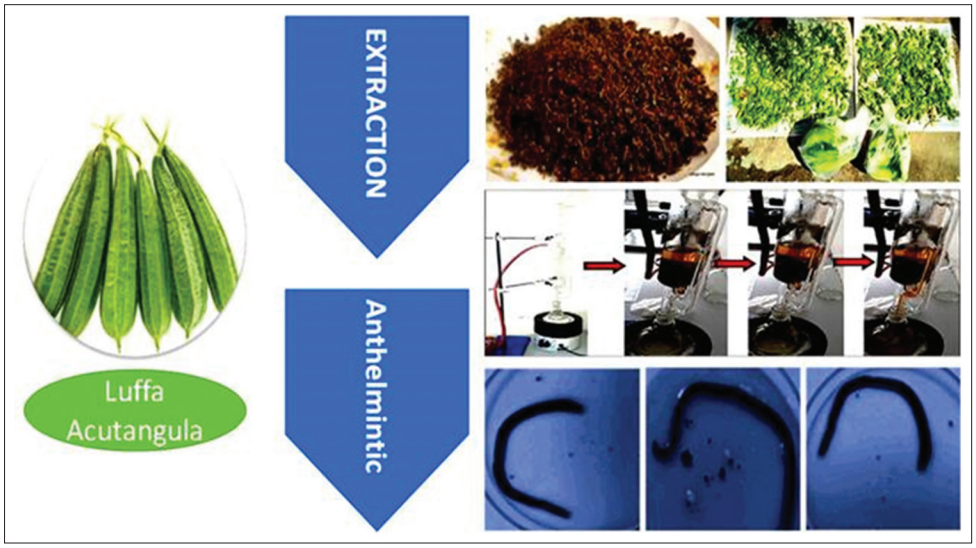

Fig. 4: Study design flow chart

human intestinal roundworm parasites for the confirmatory evaluation of anthelmintic activity [27,28]. The use of *A. galli* and *Raillietina* species as an appropriate model for screening of anthelmintic drug was encouraged by the earlier report [29]. The test samples of the plant extract were prepared at concentrations of 10, 20, 30, 40, and 50 mg/ml in the distilled water and three worms, i.e., *P. posthuma, A. galli,* and *R. spiralis* of approximately equal with size were placed in each of 9 cm Petri dish containing (25 ml) of above test solution of plant extracts. Albendazole (20 mg/ml) was used as the standard reference and double distilled water as control [30]. This procedure has been decided to adopt for all of the three different types of worms. Before starting the experiments, all the test solutions and standard drug solution were prepared freshly. Observations were clearly made for the time taken for paralysis when no movement of any kind could still be observed except for when the worms were strenuously shaken. The time of the worms' death was recorded after it was found that the worms did not move when shaken strenuously or when dipped in warm water (50°C). All the results were shown in Table 1 and expressed as a mean±SEM of six worms in each group.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of different extracts of ridge gourd fruit was shown to produce anthelmintic activities. In the present study, it was observed that all the extracts of ridge gourd fruit have exhibited a positive response to a certain degree of anthelmintic activity.

We have extracted the ridge gourd fruit from the different solvent as moving non-polar solvent to polar solvent as the separate non-polar and polar compounds by different extract of ridge gourd fruit and also to know the biological activity in them. As during the experiment, we have to get more compounds in the polar extract than the non-polar extract (hexane, dichloromethane, ethyl acetate, methanol, and water). The extract was minted on thin-layer chromatography by choosing different mobile phase as hexane:ethyl acetate (6:3).

Ethyl acetate extract exhibited more potent activity at a lower concentration of 10 mg/ml against *A. galli* (Roundworm). Evaluation of anthelmintic activity was compared with reference albendazole as shown in Table 1. The ethyl acetate extract of ridge gourd demonstrated paralysis as the well as the death of worms in less time as compared to other extracts, especially at the lower concentration. While water extract showed significant activity, hexane extract comprised the midget activity among all of the extracts.

The predominant action of albendazole on the worm is inhibitory action on microtubular function. The plant extracts not only showed paralysis but also the death of the organism with a decrease in concentrations. In conclusion, the traditional use of ridge gourd as an anthelmintic has been confirmed as the skin extracts displayed activity against the worms used in this study. Further studies need to establish the mechanism(s) of action is required.

Before this experiment, such insufficient reports were to evaluate the biological activity in depth for every part of the ridge gourd plant. In the previous studies, less investigation on ridge gourd fruit was published to estimate biological activity against some of the pathogenic infections. Fruit powder has the capacity to cure this health issue of anthelmintic, and it took less time to cause paralysis and death of the earthworms compared to the relative of pharmacy standard drug. The present study provides efficient evidence for indicated that the crude ethyl acetate extract of ridge gourd fruit did produce anthelmintic activity against Indian earthworms (*P. posthuma*), Roundworm (*A. galli*), and

Table 1: Anthelmintic activity of ridge gourd fruit

Groups	Conc. (mg/ml)	*P. posthuma* (Earthworm)		*A. galli* (Roundworm)		*R. spiralis* (Tapeworm)	
		Time taken for paralysis (P) in min (Mean and SEM)	Time taken for death (D) in min (Mean and SEM)	Time taken for paralysis (P) in min (Mean and SEM)	Time taken for death (D) in min (Mean and SEM)	Time taken for paralysis (P) in min (Mean and SEM)	Time taken for death (D) in min (Mean and SEM)
Hexane extract	10	50±0.94	60±0.66	47±0.47	65±0.67	50±1.58	68±1.59
	20	46±0.87	58±0.65	41±0.97	59±0.72	41±0.77	58±0.59
	30	39±0.74	49±0.65	34±0.74	43±0.53	38±1.46	41±1.54
	40	29±0.65	50±0.55	28±0.86	47±1.33	32±0.67	53±0.65
	50	25±0.78	37±0.66	25±0.78	50±1.46	30±0.48	55±1.88
Dichloromethane extract	10	44±0.80	58±0.59	39±0.93	60±0.58	47±1.86	57±1.52
	20	40±0.34	48±0.43	31±0.67	42±0.42	37±0.56	42±0.51
	30	27±0.68	35±0.72	27±0.61	38±0.46	25±1.78	33±1.33
	40	22±0.56	36±0.43	24±0.74	39±1.29	28±0.55	40±0.62
	50	18±0.36	29±0.47	19±0.56	46±1.45	28±0.84	43±1.53
Ethyl acetate extract	10	29±0.39	49±0.21	26±0.34	50±0.41	31±1.49	55±1.49
	20	24±0.11	44±0.27	21±0.74	50±0.30	23±0.39	44±0.51
	30	17±0.43	29±0.61	14±0.17	38±0.49	19±1.61	39±1.09
	40	16±0.3	29±0.10	13±0.83	37±1.19	18±0.49	39±0.49
	50	16±0.43	27±0.24	10±0.36	35±1.11	17±0.72	38±1.19
Methanol extract	10	32±0.42	52±0.31	30±0.54	58±0.55	33±1.52	61±1.52
	20	27±0.12	46±0.38	25±0.88	52±0.39	25±0.41	47±0.62
	30	21±0.55	32±0.78	18±0.21	40±0.53	21±1.72	42±1.16
	40	20±0.51	33±0.25	15±0.91	41±1.25	20±0.53	41±0.52
	50	19±0.45	31±0.35	13±0.48	36±1.25	20±0.83	41±1.25
Water extract	10	38±0.98	57±0.55	36±0.95	52±0.45	36±1.82	55±1.44
	20	30±0.22	50±0.48	29±0.96	45±0.32	29±0.51	49±0.55
	30	20±0.58	38±0.88	21±0.41	40±0.45	20±1.71	39±1.45
	40	19±0.54	31±0.34	17±0.54	33±1.19	18±0.35	37±0.32
	50	16±0.35	25±0.25	15±0.56	42±1.35	24±0.93	39±1.22
Control (Water only)	-	-	-	-	-	-	-
Albendazole standard	10	21±1.09	58±0.72	11±1.41	36±1.09	23±0.46	50±1.09

All values represent Mean±SD; n=7 in each group. Comparisons made between standard and treated groups, $P<0.05$ was considered significant

Tapeworms (*R. spiralis*). The plant possesses significant anthelmintic activity at 100 mg/ml concentration measured by time is taken for paralyze/death of the earthworms. The current investigation leads to a conclusion that the fruit of ridge gourd has potent anthelmintic activity when compared with the conventionally used drug. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. Further studies using *in vivo* models and to isolate active constituents from extract are required to carry out and established the effectiveness and pharmacological rationale for the use of ridge gourd fruit as an anthelmintic drug.

CONCLUSION

The traditional use of the fruit of ridge gourd as anthelmintic has been confirmed using the different extracts and showed significant anthelmintic activity. Further, it would be interesting to isolate the responsible phytoconstituents, which are responsible for the anthelmintic activity and the mechanism of action, which is being attempted in the laboratory. It indicates that the ethyl acetate extract is more potent as compared to other extracts as it took less time to cause paralysis and death of the earthworms as compared to the standard reference drug Fig. 4.

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AUTHOR'S CONTRIBUTIONS

The author declares that this work was done by the authors named in this article.

CONFLICTS OF INTEREST

No conflicts of interest are associated with this work.

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