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Research Article

In-vitro Anthelmintic activity of *Limnophila repens* and *Argyreia cymosa* against *Pheretima posthuma*

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Abstract

Objective: To carry out a thorough study to establish the therapeutic of the methanolic and aqueous extract of whole plants of *Limnophila repens* (*L. repens*), *Argyreia cymosa* (*A. cymosa*) against *Pheretima posthuma* which is utilized as the experimental model for helminthic.

Methods: The whole plants of *Limnophila repens, Argyreia cymosa* and Indian adult earthworms (*Pheretima posthuma*) were collected and identified by the approved taxonomist. Earthworms had been grouped and treated with extracts at the concentration of 10, 20, 40 and 80 mg/mL; albendazole of 40 mg/mL as standard and normal saline as a control. The paralysis and death time was regarded as the indicator of anthelmintic activity.

Results: Crude methanolic and aqueous extracts with concentrations of 10, 20, 40 and 80 mg/ml of L. repens and *A. cymosa* showed concentration dependent activity, but significant activity was observed at 80 mg/mL in both of the plants. The methanolic extract showed better activity than aqueous extracts at all concentrations. The methanolic extract of *L. repens* [P (min) = 1.01, D (min) = 9.52] and *A. cymosa* [P (min) = 3.45, D (min) = 13.25] correspondingly, the activity was identified being comparative along with the standard drug Albendazole [P (min) = 2.58, D (min) = 5.23].

Conclusion: From the investigation, the conclusion can be drawn that methanolic extracts showed better activity than aqueous extracts of *L. repens* and *A. cymosa* to treat intestinal worm infections. Since this is a preliminary evaluation; isolation of chemical constituents which are responsible for the activity could be done in the future.

Keywords: *Limnophila repens; Argyreia cymosa; Anthelmintic; Pheretima posthuma; Albendazole*

Introduction

Anthelmintic resistance is a global issue, and new plant-derived substances will be analyzed because of their potential utilization towards gastrointestinal nematodes. The majority of illnesses brought on by helminths are of chronic in nature; they most likely lead to morbidity and even fiscal and interpersonal deprivation among humans beings and animals than any kind of solitary group of parasites. Helminthiasis is an issue influencing a huge populace around the globe. In helminthiasis, a part of the body is overwhelmed with worms like pinworm, round worm or tape worm. Mostly, the worms reside in the gastrointestinal tract, but may also burrow into the liver as well as other organs.

Parasitic diseases might result in serious morbidities, such as lymphatic filariasis, onchocerciasis and schistosomiasis. Many developing nations are poverty prone, malnutrition and don not comply with minimal sanitary hygiene conditions that are the main reason for worm infections. Helminthic infections result in deficiency conditions such as malnutrition, anemia, and worsening the immune system. The current synthetic anti-helminthic agents produce several side effects, and they are not cost effective. The broad spectrum anti-helminthic drug like albendazole is noted to produce nausea, vomiting, dizziness and gastrointestinal irritation in some patients.

As a result, there is certainly a rise in dependence on using natural medicines as an anti-helminthic agent. Herbal medicines will be reasonably economical and also have lesser unwanted effects in comparison to synthetic drugs. The anthelmintic activity had been evaluated on adult Indian earthworm, *Pheretima posthuma* (*P. posthuma*) because of its anatomical and physiological likeness with all the intestinal round worm parasites of human beings and easy availability [1].

The genus *Limnophila* is frequently used in the traditional medicine against cardiovascular diseases, stomach disorders, elephantiasis, diarrhoea, dyspepsia, fever, dysentery, indigestion, Dysmenorrhoea and abdominal pain [2-4]. Phytochemical analysis of genus *Limnophila* revealed the presence of the number of phytoconstituents such as flavonoids, tannins, alkaloids, terpenoids, steroids, and glycosides [5]. This diversity in compounds could justify the traditional use of *L. repens*. The genus *Limnophila* is relatively abundant and widely used in folk medicine as an antioxidant [6,7], antimicrobial [8], anticancer [9], antimycobacterial [10], as on to date no biological studies have been conducted on this plant. Consequently, this exploration was performed to evaluate the anti-inflammatory potential and antinociceptive properties of *L. repens.*

Argyreia cymosa is an decorative, as well as a therapeutic herb. Every part of the herb will be traditionally used being a folk tradition remedies for the management of several problems. The root is usually employed to get rid of a different health problems like sexually transmitted disorders i.e. gonorrhea and syphilis, blood disorders [11]. Young wines are mixed together with rhizome of ginger are spread all around the body to relieve from fever. The decoction of its root used to treat diarrhea and cathartic [12]. A vast range of phytochemical constituents has been separated from the genus *Argyreia* i.e. glycosides, alkaloids, amino acids, proteins, flavonoids, triterpene and steroids [13]. The genus *Argyreia* has been reported for treatment of various disorders including ulcer, hepatotoxicity, inflammation, hyperglycemic, diarrheal, microbial, viral, bacterial infections, pain, wound healing, helminthiasis, nervous disorders [13-15]. However the drug has its own benefits, it's medicinal and phytochemistry is extremely poorly investigated.

Traditionally, the two plants had been employed for anthelmintic activity, however, till date, no scientific data has been reported. Therefore, the current study has been carried out with the methanolic and aqueous extract of *L. repens* and *A. cymosa* to investigate its anthelmintic activity against *P. posthuma* using albendazole as a reference standard.

Materials and Methods

Procurement and authentication of crude drug

The plants *L. repens* and *A. cymosa* were collected from Tirupati, Chittoor district of Andhra Pradesh was authenticated at Botany Division, Sri Venkateswara University. The voucher specimen (No. 1568 and 1043) were deposited at herbarium and raw drug depository respectively. The plant components had been dried out under shade for Two weeks, coarsely powdered and then kept in air restricted canisters guarded from humidity and sunlight for further study.

Worms collection

Indian adult earthworms i.e. *Pheretima posthuma* had been utilized to assess anthelmintic activity. The earthworms were being collected from the water logged area of soil, Machilipatnam, rinsed with normal saline to get rid of all fecal matter. The earthworms of 5 - 8 cm in length and 0.2 - 0.3 cm in width were utilized for all experimental protocol.

Preparation of methanolic and aqueous extracts

About 250g of the powdered crude drug of *L. repens* and *A. cymosa* were extracted by cold maceration with 1000 mL of methanol for 18h after pretreatment with petroleum ether. The aqueous extract was prepared by cold maceration utilizing the solvent like water. All of the extracts obtained had been concentrated to dryness in the vacuum at 40°C and kept at 4°C inside the refrigerator till further used. The extracts had been subjected to phytochemical and pharmacological evaluation [16].

Phytochemical screening

The different extracts of *L. repens* and *A. cymosa* were afflicted by qualitative chemical evaluation by utilizing standard methods [17-24].

Indian adult earthworm as model for the experiment

All the studies had been performed in Indian adult earthworms (*P. posthuma*) gathered from damp soil and washed along with normal saline to eliminate all waste matter which was utilized for anthelmintic activity because to its anatomical and physiological similarities with the intestinal roundworm parasite *Ascaris lumbricoids* of human beings. Due to its easy availability, earthworms have been utilized extensively for the preliminary evaluation of the anthelmintic activity. Adult earthworms of about 3.5 - 5.0 cm in length and 0.2 - 0.5 cm in width were utilized for the studies [16].

Assessment of Anthelmintic activity

The anthelmintic activity of methanol and aqueous extracts of *L. repens* and *A. cymosa* were evaluated as per the method reported by Barnali Gogoi [1]. 80 Indian mature earthworms had been gathered, and split up into 20 groups comprising four worms in every group (Figures 1 and 2). A amount of 10 mL of each different concentration of standard medicine albendazole and test drugs. 4 earthworms had been released in each of the nine clean Petri dishes. Earthworms had been noticed; the time taken for paralysis and fatality was supervised and recorded in the minute. Paralysis time was examined based on the behavior of the earthworm without any revival of body condition in normal saline medium and no movements when shaken vigorously. Death was concluded depending on total loss of motility without any movements even if dipped in warm water at 50 - 60°C temperature and pale body colour [16].

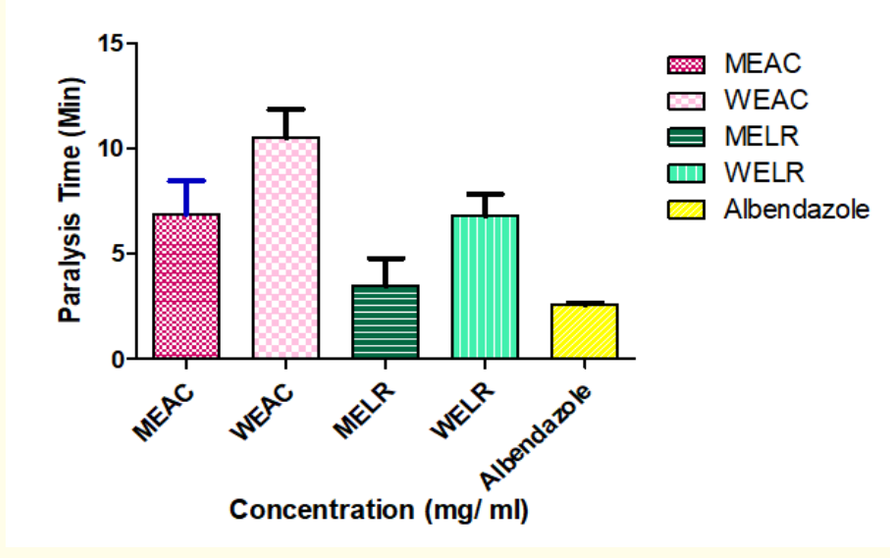

Figure 1: *Paralysis time for aqueous and methanolic extracts of Argyreia cymosa and Limnophila repens with respect to albendazole (40 mg/ml).*

Statistical analysis

The results are listed as mean ± SEM of four worms in each group. Comparisons have been made between standard and test treated group using Dunnett test. The difference in values at $P < 0.05$ was considered statistically significant.

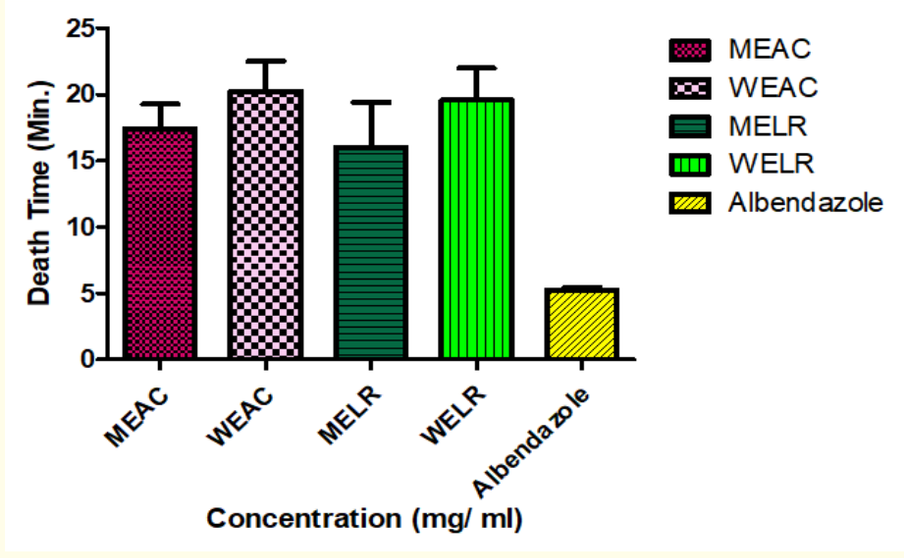

Figure 2: *Death time for aqueous and methanolic extracts of Argyreia cymosa and Limnophila repens with respect to albendazole (40 mg/ml).*

Results

Preliminary phytochemical evaluation of methanolic and aqueous extracts of *L. repens* revealed the presence of alkaloids, tannins, phenols, steroids, flavonoids, glycosides, carbohydrates and proteins; *A. cymosa* revealed the presence of flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins like Phytoconstituents in the methanolic extracts and aqueous extract respectively (Table 1 and 2) [16,25]. Methanol and aqueous extracts had been utilized to assess anthelmintic activity, indicates dose dependent activity. The Mean ± S.D. values (statistical analysis) had been calculated for each extract. The final results of anthelmintic activity on earthworm *Pheretima posthuma* was presented in table 3, implies that, the different concentration employed for both aqueous and alcoholic extracts indicates paralysis and death of earthworms and in addition it examined in the same concentration with albendazole as standard drug. Indian adult earthworms have been utilized for the evaluation of anthelmintic activity as experimental animals. Albendazole (40 mg/ml) was used as standard, and normal saline water was used as vehicle respectively. Observations were made for the time taken to paralysis and death. Paralysis was said to take place when the worm failed to revive even in normal saline solution and death was concluded when the worms lost their motility even if vigorously shaken or dipped body colours [16]. The methanolic extract of L. repens [P (min) = 1.01, D (min) = 9.52] and A. cymosa [P (min) = 3.45, D (min) = 13.25] respectively, the activity was found to be equivalent as compared to the standard drug Albendazole [P (min) = 2.58, D (min) = 5.23] and water extract of L. repens, A. cymosa was found to be [P (min) = 4.56, D (min) = 14.23], [P (min) = 7.56, D (min) = 15.23] (Figure 1 and 2).

Discussion

Earthworms have anatomical similarities with the intestinal worms like tapeworms, roundworms, pinworms etc. Additionally, they show physiological similarity in mechanism of action. The current research revealed that both the plants showed potent anthelmintic activity. The methanolic extract had revealed promising result as anthelmintic activity, and aqueous extracts also have proven activity up to the minimal level. Findings were made for time taken to paralysis and fatality of individual worms in the standard drug that is Albendazole. It binds to free β-tubulin, curbing its polymerization and therefore interfering with microtubule-dependent glucose uptake by the worm. It has a selective inhibitory activity on helminths microtubular function, being 250 - 400 times more potent in helminths compared to mammalian tissue [16].

Phytoconstituents	Method	Aqueous Extract	Methanolic Extract
Flavonoids	Shinoda Test	+	+
	Zn. Hydrochloride test	+	+
	Lead acetate Test	+	+
Volatile oil	Stain test	-	-
Alkaloids	Wagner Test	+	+
	Hager's Test	+	+
Tannins & Phenols	$Fecl_3$ Test	+	+
	Potassium dichromate test	+	+
Saponins	Foaming Test	-	-
Steroids	Salkowski test	+	+
Carbohydrates	Molish test	-	-
Acid compounds	Litmus test	+	+
Glycoside	Keller-Killani Test	+	+
Amino acids	Ninhydrin test	+	+
Proteins	Biuret	+	+

Table 1: *Phytochemical analysis of various extracts of Argyreia cymosa.*

Phytoconstituents	Method	Aqueous Extract	Methanolic Extract
Flavonoids	Shinoda Test	+	+
	Zn. Hydrochloride test	+	+
	Lead acetate Test	+	+
Volatile oil	Stain test	-	-
Alkaloids	Wagner Test	+	+
	Hager's Test	+	+
Tannins & Phenols	$Fecl_3$ Test	+	+
	Potassium dichromate test	+	+
Saponins	Foaming Test	-	-
Steroids	Salkowski test	+	+
Carbohydrates	Molisch test	+	+
Acid compounds	Litmus test	-	-
Glycoside	Keller-Killani Test	+	+
Amino acids	Ninhydrin test	+	+
Proteins	Biuret	+	+

Table 2: *Phytochemical analysis of various extracts of Limnophila repens.*

Conc. (mg/ml)	Paralysis Time (min)				Death Time (min)				Albendazole		
	MEAC	WEAC	MELR	WELR	MEAC	WEAC	MELR	WELR	Conc. (mg/ml)	Paralysis Time (min)	Death Time (min)
10	10.55 ± 0.23	13.42 ± 0.74	7.16 ± 0.36	9.21 ± 0.62	22.05 ± 0.42	26.02 ± 0.33	24.12 ± 0.42	25.51 ± 0.22	40	2.58 ± 0.23	5.23 ± 0.52
20	8.22 ± 0.63	12.05 ± 0.74	3.44 ± 0.63	7.30 ± 0.23	18.37 ± 0.25	21.15 ± 0.36	19.01 ± 0.45	21.05 ± 0.52			
40	5.46 ± 0.72	9.15 ± 0.62	2.32 ± 0.44	6.52 ± 0.52	16.02 ± 0.58	18.53 ± 0.42	11.42 ± 0.54	17.53 ± 0.54			
80	3.45 ± 0.27	7.56 ± 0.42	1.01 ± 0.23	4.26 ± 0.22	13.25 ± 0.46	15.23 ± 0.62	9.52 ± 0.23	14.23 ± 0.42			

Table 3: *Anthelmintic activity of the aqueous and methanolic extract of Argyreia cymosa and Limnophila repens against Pheretima posthuma.*

Values are expressed as mean ± SEM (n = 4). MEAC: Methanolic Extract of Argyreia cymosa; WXAC: Water Extract of Argyreia cymosa; MELR: Methanolic Extract of Limnophila repens.; WELR: Water Extract of Limnophila repens.

The plant extract comprises of many secondary metabolites which are responsible for the anthelmintic activity. Preliminary phytochemical screening of *L. repens* revealed the presence of alkaloids, tannins, phenols, steroids, flavonoids, glycosides, carbohydrates and proteins; *A. cymosa* revealed the presence of flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins. The Phytoconstituents showing anthelmintic effect includes alkaloids, phenols, steroids tannins etc [25]. Tannins had been proven to produce anthelmintic activities chemically tannins are polyphenolic substances. Certain synthetic phenolic anthelmintics are shown to impede energy generation in helminthic parasites via uncoupling oxidative phosphorylation [16,26]. It is possible that tannins contained in the extract of *L. repens* and *A. cymosa* produced similar effects. An additional feasible anthelmintic effect of tannins is they may bind to free proteins in the GIT the host animal and trigger fatality. Also, steroid drugs will be proven to have an effect on membrane permeability, and pore formation of parasites thereby contributes to fatality of parasites. Steroidal saponin likewise generates interruption of monogenean teguments, microvilli that will work as the absorptive surface area within the earth worm [27]. Consequently, it might be credible that the comparative anthelmintic activity of *L. repens* and *A. cymosa* could be caused by the existence of phytochemicals like tannins, alkaloids and steroids. The investigational confirmation attained in this bioassay might give a rationale for the conventional utilization of *L. repens* and *A. cymosa* plants as anthelmintic potential. But further research is essential to isolate, characterize and assess the actual bioactive components and their mechanism of actions.

Conclusion

It is concluded based on the results of the present study that the plants *L. repens* and *A. cymosa* are potent anthelmintic. Phytochemical testing claims the existence of tannins, phenols, steroids and flavonoids in both the extracts which may causes anthelmintic activity. But, dosage and the form in which they may be utilized need standardization. Since this is a preliminary evaluation; isolation of chemical constituents which are responsible for the activity could be done in the future.

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