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Original Article

In vitro Anthelmintic activity of *Aralia racemosa*. L (root) and *Argyreia Pilosa* Wight & Arn. (Whole plant) against *Pheretima Posthuma*

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Abstract

To carry out a thorough study to establish the therapeutic of methanolic and aqueous extract of root of Aralia racemosa and whole plant of Argyreia pilosa against Pheretima posthuma, which is utilized as an experimental model for helminthic. The *Aralia racemosa* (Root), *Argyreia pilosa* (whole plant), and Indian adult earthworms (*Pheretima posthuma*) were collected and identified by an approved taxonomist. Earthworms had been grouped and treated with extracts at a concentration of 10, 20, 40, and 80mg/mL; albendazole of 40mg/mL as standard and normal saline as a control. The paralysis and death time was regarded as an indicator of anthelmintic activity. Crude methanolic and aqueous extracts with concentrations of 10, 20, 40, and 80mg/ml of *A. racemosa* and *A. pilosa* 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 *A. racemosa* [P (min) = 2.01, D (min) = 10.52] and *A. pilosa* [P (min) = 2.45] respectively, the activity was found to be equivalent as compared to the standard drug Albendazole [P (min) = 2.28, D (min) = 5.44]. From the investigation, a conclusion can be drawn that methanolic extracts showed better activity than aqueous extracts of *A. racemosa* and *A. pilosa* to treat intestinal worm infections. Since this is a preliminary evaluation, isolation of chemical constituents that are responsible for the activity could be done in the future.

Keywords: *Aralia racemosa* L., *Argyreia pilosa* Wight & Arn., Anthelmintic, *Pheretima posthuma*, Saponins, and Albendazole.

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1. Introduction

Anthelmintic resistance is a really 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 a 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 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. Usually, the worms live in the gastrointestinal tract, however could also burrow into the liver along with other organs.

The parasitic worms are classified into three groups: cestodes or tapeworms, nematodes or roundworms, and trematodes or flukes. Parasitic diseases might result in serious morbidity, such as lymphatic filariasis, onchocerciasis and schistosomiasis. Many developing nations are poverty prone, malnutritioned and don not comply with minimal sanitary hygiene conditions that is 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 antihelmintic drug like albendazole is noted to produce nausea, vomiting, dizziness and gastrointestinal irritation in some patients. Consequently, there is an increased in need for utilizing natural drugs as antihelmintic agent. Herbal drugs are reasonably inexpensive and have fewer side effects compared to synthetic ones. The anthelmintic activity had been assessed on adult Indian earthworm, *Pheretima posthuma* (*P. posthuma*) because of its anatomical and physiological resemblance with the intestinal round worm parasites of human beings and easy availability [1].

Araliaceae is an extensive family consists of 254 species. *Aralia racemosa*. L is a perennial herb in this family and is distributed in America, Africa, Australia, New Zealand and Pacific Islands. The genus Aralia of the family Araliaceae is contained up of 71 species of plants scattered over Asia, North America and South America i.e., *A. armata, A. bipinnata, A. chinensis, A. continentalis, A. cordata, A. dasyphylla, A. echinocaulis, A.elata, A. fargensii, A. nudicaulis.* One genus of *Aralia* found in India i.e., *Aralia racemosa* L. It is commonly known as American Spikenard. The plants of this family have a significant contribution in the treatment of respiratory inflammation, diabetes, cancer, and parasitic infections [2]. The genus *Aralia* is rich in triterpenoidal saponins chemically. Phytochemical investigation on *Aralia racemosa* L. revealed the presence of triterpenoidal saponins i.e., Oleanolic Acid, Sterols i.e., β-sitosterol and Diterpenoids i.e., ent-Kaurenoic acid, Continentalic acid [2, 3]. In conventional system of medicine the various parts of *A. racemosa.* L can be used in the remedy of Rheumatism, Whooping cough, skin diseases, pleurisy, diaphoretic, diuretic, pulmonary diseases, asthma, rheumatism, diarrhea, stimulant, expectorant, syphilis, Inflammation and Hay fever [4, 5].

Various pharmacological activities of Aralia racemosa have been reported such as antioxidant, ant diabetic [6, 7] and anti tubercular [8].

Argyreia pilosa Wight & Arn. is an ornamental, in addition to a medicinal plant. All parts of this plant are widely used as a folklore

medicine for the treatment of various ailments by the Indian traditional healer. Its root is utilized to cure a various illness like sexually transmitted diseases viz., gonorrhea and syphilis, blood diseases. Traditionally, the paste of the leaves is applied to the neck region for cough, quinsy and applied externally in case of itch, eczema and other skin troubles, antidiabetic, antiphlogistic, rheumatism and reduce burning sensation [9]. 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 [10]. A vast range of phytochemical constituents has been separated from the genus *Argyreia* i.e., glycosides, alkaloids, amino acids, proteins, flavonoids, triterpene and steroids [11]. The genus *Argyreia* has been reported various biological activities including nootropic, aphrodisiac, antioxidant, antiulcer, immunomodulatory, hepatoprotective, anti-inflammatory, antihyperglycemic, antidiarrheal, antimicrobial, antiviral, nematicidal, anticonvulsant, analgesic, anti-inflammatory , wound healing, anthelmintic and central nervous depressant activities [11-13]. Even though the drug has many uses, it's pharmacological and phytochemistry is very poorly explored.

Traditionally, both plants were utilized for anthelmintic activity but till date no scientific evidence has been reported. Therefore, the current study has been carried out with the methanolic and aqueous extract of root of *Aralia racemosa* L. and whole plant of *Argyreia pilosa* Wight and Arn. with a view to investigate its anthelmintic activity against *P. posthuma* using albendazole as a reference standard.

2. Material and Methods

2.1. Procurement and Authentication of Crude Drug

The plants *A. racemosa* and *A. pilosa* were collected from Tirupathi during the month of September, 2016. The plants were identified and authenticated by Dr K. Madhava chetty; plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh and voucher specimen of the plant (No 1489 and 1922) were deposited at the herbarium for future references. The plant materials were dried under shade for 15 days, coarsely powdered and stored in air tight containers protected from humidity and sunlight for further study.

2.2. 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.

2.3. Preparation of Methanolic and Aqueous Extracts

About 250g of powdered crude drug of *A. racemosa* and *A. pilosa* were extracted by cold maceration with 1000 mL of methanol for 18 h after pretreatment with petroleum ether. Aqueous extract was also prepared by cold

maceration by using solvent as water. All the extracts acquired were concentrated to dryness in vacuum at 40°C and stored at 4°C within the refrigerator until further used. The extracts were subjected to phytochemical and pharmacological assessment [14].

2.4. Phytochemical Screening

The various extracts of *A. racemosa* and *A. pilosa* were subjected to qualitative chemical analysis using standard procedures.

The phytochemical screening of carbohydrates was detected by Molisch's test; proteins were detected by using two tests namely Biuret test and Millon's test and amino acids by Ninhyrdin's test; Steroids was detected by Salkowski, Liebermann- Bur chard's and Liebermann's test; alkaloids were identified with freshly prepared Dragendroff's Mayer's, Hager's and Wagner's reagents and observed for the presence of turbidity or precipitation. The flavonoids were detected using four tests, namely Shinoda, sulfuric acid, aluminum chloride, lead acetate, and sodium hydroxides. Tannins were detected with four tests namely gelatin, lead acetate, potassium dichromate and ferric chloride. The froth, emulsion, and lead acetate tests were applied for the detection of saponins. The steroids were detected by (acetic anhydride with sulfuric acid) and (acetic chloride with sulfuric acid) tests. Sample extracted with chloroform was treated with sulfuric acid to test for the presence of terpenoids. Ammonia solution and ferric chloride solutions were used for the presence of anthraquionones [15-22].

2.5. 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 were 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 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 [14].

2.6. Assessment of Anthelmintic Activity

The anthelmintic activity of methanol and aqueous extracts of *A. racemosa* and *A. pilosa* were evaluated as per the method reported by Barnali Gogoi [1]. Eighty Indian adult earthworms were collected, and divided into twenty groups containing four worms in each group (Figures 1 and 2). A volume of 10 mL of each different concentration of standard drug albendazole and test drugs. Four earthworms were released in each of the nine clean Petri dishes. Earthworms were observed; the time taken for paralysis and death was monitored and documented in 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 color [14].

Figure 1. Anthelminthic activity of (a) Albendazole (40mg/ml), (b) Control (Distilled Water)

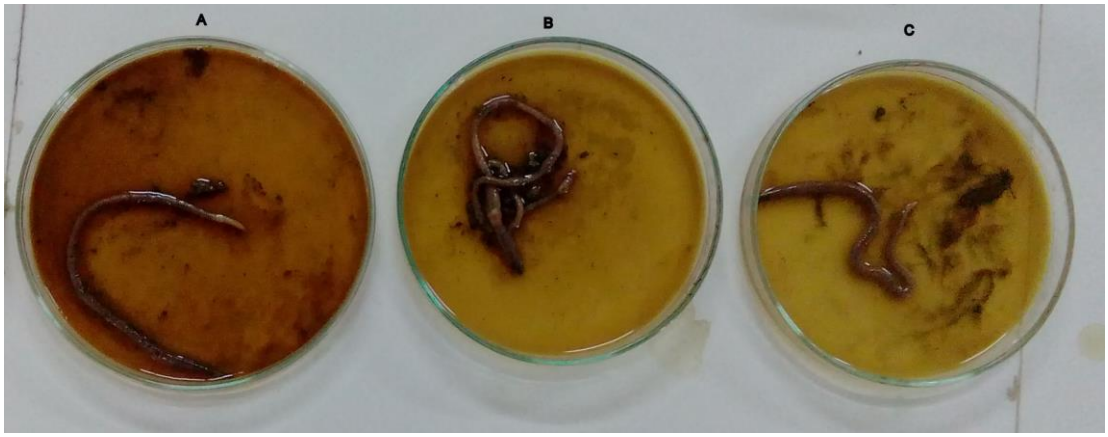

Figure 2. Anthelmintic activity of methanolic root extract of Aralia racemosa at different concentrations (a) 80mg/ml (b) 40mg/ml (c) 20mg/ml

2.7. Statistical Analysis

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

3. Results and Discussion

Preliminary phytochemical evaluation of methanolic and aqueous extracts of *A. racemosa* revealed the presence of flavonoids, tannins, phenols, saponins, steroids and glycosides [23]; *A. pilosa* revealed the presence of flavonoids, alkaloids, tannins, phenols, acid compounds, glycosides, amino acids and proteins like Phytoconstituents in the methanolic extracts and aqueous extract respectively(Table 1, 2) [14, 24].

Table 1. Phytochemical analysis of various extracts of *Argyreia pilosa* Wight & Arn. Leaves.

Phytoconstituents	Method	Aqueous Extract	Methanolic Extract
Flavonoids	Shinoda Test	+	+
	Zn. Hydrocholride 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	+	+

"+" Present; "-" Absent

Table 2. Phytochemical analysis of various extracts of *Aralia racemosa* L. root.

Phytoconstituents	Method	Aqueous Extract	Methanolic Extract
Flavonoids	Shinoda Test	+	+
	Zn. Hydrocholride 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	-	-

"+" Present; "-" Absent

Methanol and aqueous extracts had been utilized to assess anthelmintic activity, indicates dose dependant activity. The Mean ± S.D. values (statistical analysis) had been calculated for each extracts. The outcomes of anthelmintic activity on earthworm *Pheretima posthuma* was presented in Table 3, shows that, the various concentration utilized for both aqueous and alcoholic extracts has shown paralysis and death of earthworms and it also compared 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 colors [14]. The methanolic extract of *A. racemosa* [P (min) = 2.01, D (min) = 10.52] and *A. pilosa* [P (min) = 2.45] respectively, the activity was found to be equivalent as compared to the standard drug Albendazole [P(min) = 2.28,D(min) = 5.44] and aqueous extract of *A. racemosa*, *A. pilosa* was found to be [P(min) = 5.26,D(min) = 15.23], [P(min) = 6.56,D(min) = 14.23] (Figure 3,4).

Table 3. Anthelmintic activity of aqueous and methanolic extract of *Argyreia pilosa* Wight & Arn. and *Aralia racemosa* L. against *Pheretima*.

	Paralysis Time (min)				Death Time (min)				Albendazole		
Conc. (mg/ml)	APME	APAE	ARME	ARAE	APME	APAE	ARME	ARAE	Conc. (mg/ml)	Paralysis Time (min)	Death Time (min)
10	9.55± 0.32	12.42 ±0.47	8.16± 0.63	10.21 ±0.26	21.05 ±0.24	25.02 ±0.77	25.12 ±0.47	26.51 ±0.55	40	2.28±0 .33	5.44±0. 23
20	7.22± 0.66	11.05 ±0.47	4.44± 0.36	8.30± 0.32	17.37 ±0.66	20.15 ±0.63	20.01 ±0.44	22.05 ±0.22			
40	4.46± 0.77	8.25± 0.77	2.32± 0.55	7.52± 0.47	15.02 ±0.55	17.53 ±0.24	12.42 ±0.55	18.53 ±0.22			
80	2.45± 0.72	6.56± 0.44	2.01± 0.66	5.26± 0.77	12.25 ±0.47	14.23 ±0.66	10.52 ±0.32	15.23 ±0.44			

Values are expressed as mean ± SEM (n=4). APME=Crude Methanolic extract of *Argyreia pilosa* Wight & Arn; APAE= Crude Aqueous extract of *Argyreia pilosa* Wight & Arn; ARME= Crude Methanolic extract of *Aralia racemosa* L.; ARAE= Crude Aqueous extract of *Aralia racemosa* L.

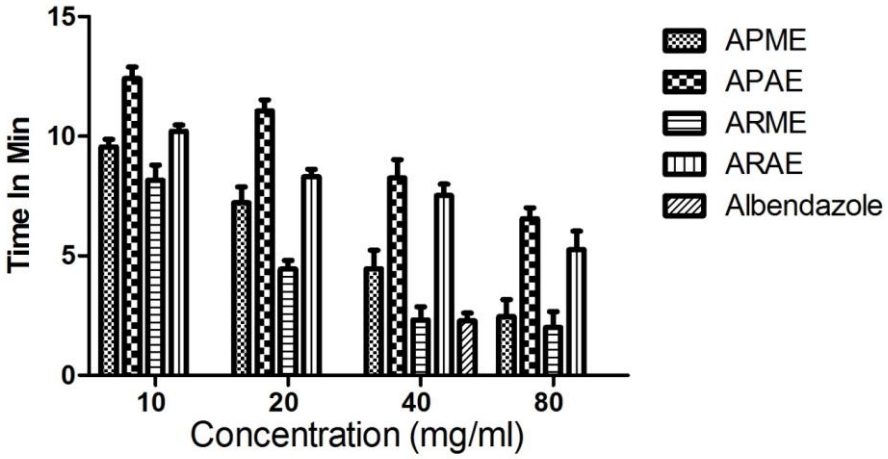

Figure 3: Paralysis Time for Aqueous and Methanolic Extracts of *Aralia racemosa* L. and *Argyreia pilosa* Wight & Arn. concerning Albendazole (40mg/ml).

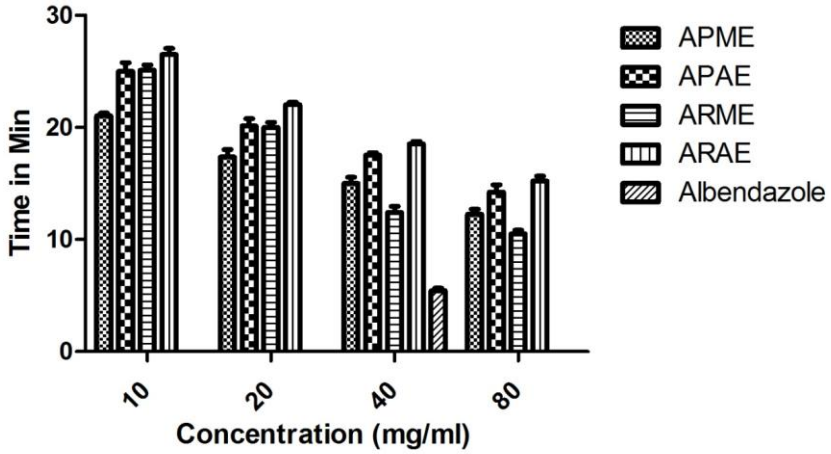

Figure 4. Death Time for Aqueous and Methanolic Extracts of *Aralia racemosa* L. and *Argyreia pilosa* Wight & Arn. with respect to Albendazole (40mg/ml).

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 root of *Aralia racemosa* L. and whole plant of *Argyreia pilosa* showed potent anthelmintic activity. Methanolic extract had revealed promising result as anthelmintic activity and aqueous extracts has also shown activity up to lesser extent. Observations were made for the time taken to paralysis and death of individual worms in standard drug that is Albendazole. It binds to free β-tubulin, inhibiting its polymerization and hence 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 [14].

The plant extract comprises of many secondary metabolites which are responsible for anthelmintic activity. Preliminary phytochemical screening of *A. racemosa* revealed the presence of flavonoids, tannins, phenols, saponins, steroids and glycosides; *A. pilosa* revealed the presence of flavonoids, alkaloids, tannins, phenols, acid compounds, glycosides, amino acids and proteins. The Phytoconstituents showing anthelmintic effect includes alkaloids, saponins, polyphenols, steroids tannins etc. [24]. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds. Certain synthetic phenolic anthelmintics (*eg*. niclosamide, oxyclozanide and bithionol) are proven to hinder energy generation in helminthic parasites through uncoupling oxidative phosphorylation [14, 25]. It is possible that tannins contained in the extract of *A. racemosa* and *A. pilosa* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastro intestinal tract of host animal and cause death. Also steroids are known to have an effect on membrane permeability and pore formation of parasites thereby leads to mortality of parasites. Steroidal saponin also produces disruption of monogenea teguments, microvilli which will act as absorptive surface within the earth worm. Alkaloids in parasite diminishes nitrate generation thus reduces ribosomal and mitochondrial protein synthesis as well as disrupts the synthesis and activities of genetic material i.e., DNA and RNA, inhibits glucose supply to result in paralysis of worms through acting on central nervous system [26]. Therefore, it may be plausible that the relative anthelmintic activity of *A. racemosa* and *A. pilosa* could be attributed to presence of phytochemicals like tannins, alkaloids, steroids and saponins. The investigational confirmation attained in this bioassay might give a rationale for the conventional utilization of *A. racemosa* and *A. pilosa* plants as anthelmintic potential. But further research is essential to isolate, characterize and assess the actual bioactive components and their mechanism of actions.

4. Conclusion

It is concluded based on the results of the present study that the plants *A. racemosa* and *A. pilosa* are potent anthelmintic. Phytochemical screening states the presence of tannins and flavonoids in both the extracts that may produces 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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