

**ANTHELMINTIC ACTIVITY OF BLACK MANGROVE OF
*AEGICERAS CORNICULATUM*****N. J. Raju* and L. R. Patro**

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Pharmacy, Narapally,
Hyderabad.**ABSTRACT**

This study evaluated the scientific basis for the traditional use of *Aegiceras corniculatum* (Myrsinaceae) stem extract as an anthelmintic agent. The study design were investigation of the traditional anthelmintic medicinal plant *Aegiceras corniculatum* using *in vitro* anthelmintic properties of four extracts by using earthworms. The earthworm (*Pheretima posthuma*) resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings. The four crude stem extracts of *Aegiceras corniculatum* were Petroleum Ether extract (50 &100 mg/ml), Chloroform extract (50

&100 mg/ml), Methanol extract (50 &100 mg/ml) and Aqueous extract (50 &100 mg/ml). The paralysis time of Petroleum Ether extract (96 ± 6.33 & 76 ± 2.31), Chloroform extract (116 ± 4.36 & 92 ± 7.50), Ethanol extract (62 ± 3.20 & 34 ± 3.52) and aqueous extract (200 ± 2.22 & 180 ± 3.19) were compared to the standard drug Albendazole suspension 100mg/5ml (5 ± 1.00). The results shown that the four extracts produced dose dependent and significant anthelmintic activities. From these findings the four extracts of *Aegiceras corniculatum* are rich source of naturally occurring anthelmintic activity. Further work is recommended to evaluate the *in vivo* anthelmintic activity and toxicity of the extracts.

KEYWORDS: *Aegiceras corniculatum*, earthworms, anthelmintic activity.**INTRODUCTION**

Helminth infections resulting to diseases such as ascariasis, hookworm infection and schistosomiasis constitute the bulk of the 13 diseases classified as neglected tropical diseases (NTDs) by the WHO.^[1] These incapacitating diseases have continued to inflict severe disability and often deaths. It is more pronounced among the impoverished population living in marginalized areas of the world.^[2] In most developing countries, intestinal helminth

infections are a major health concern because factors that pre-dispose humans to these infections abound in these areas. Children, especially those at a preschool age have been identified as the most vulnerable group with very high rates of infection. Due to the asymptomatic nature of these diseases, the helminths remain undetected and children born in an endemic region may harbour the worms for the most part of their lives.^[3] The manifestation of most parasitic diseases is due to the host responses to the presence of the parasite.^[4] Also worth considering is the fact that the immune response triggered by helminth infection may drain the body's ability to fight other diseases, making affected individuals more prone to co-infection.^[5]

Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminthes^[6-7] against conventional anthelmintics is a foremost problem in treatment of helminthes diseases. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity. Mangrove forests are a special type of vegetation found in the coastal regions of the tropical and subtropical parts of the world. Global area that comprises mangrove forest is about 181000 square km. Mangrove forests are still quite unfamiliar to a vast population due to their limited distribution. However, the people inhabiting areas near mangrove forests heavily depend on these forests to meet their needs including their healthcare. During the early stage of human civilization, mangrove forests drew very little or no attention. This is to some extent because of the difficulty to access these areas. As the population continued to grow, people had to find new and unexplored sources including mangrove forests.^[8] Like other terrestrial plants, many mangrove plants have ethnopharmacological relevance and have also been exploited by the local people in the search for remedies for various ailments. However, only a few of the mangrove plants have so far been included in any books listing medicinal plants. This may be due to the difficulty in collecting and identifying these plant species and lack of adequate information available about their uses.^[9]

The preliminary studies of *A. corniculatum* bark showed the presence of alkaloids, glycosides, steroids, flavonoids, saponins and tannins.^[10] The therapeutic applications of the black mangrove like *Aegiceras corniculatum* (Linn.) (Myrsinaceae) distributed in coastal and estuarine areas of India are well studied. Also, the ethnopharmacological consequence

pointed out that the mangrove plants are traditionally used for the treatment of rheumatism, painful arthritis, inflammation, asthma antioxidant, free radical scavenging, anti-inflammatory, antinociceptive, diabetes and hepatoprotective actions.^[11] However, there are no proper scientific reports available regarding the effects of *Aegiceras corniculatum* on Diabetes mellitus. Mangroves and associated plants provide a wide domain for therapeutic application in recent years, most yet to be explored. The leaves of *A. corniculatum* are reported that it have rich in flavonoids with proven anti-inflammatory and antioxidant property.^[12-13] *Aegiceras* has been used as traditional medicine for years with array of biological activity such as antioxidant, antibacterial, antifungal, antiulcer, anticancer, antiplasmodial and antitumour properties.^[14-15] The current investigation used to assess anthelmintic activity of black mangrove of *Aegiceras corniculatum* using *in vitro* anthelmintic properties of four extracts by using earthworms.

MATERIALS AND METHODS

Plant Material Collection

Aegiceras corniculatum stem were collected from the coringa mangrove forest, Andhra Pradesh and authenticated by the taxonomist, Dept. of Botany, Andhra University in the month of November 2016 and the specimen voucher (AC01/2016) were preserved in the herbarium.

Plant Extract preparation

Aegiceras corniculatum stem was dried under shade then made into coarse powder and was weighed for 250gm and it was soaked in 1litres of Petroleum ether by maceration. The mixture were kept for 3 days in tightly sealed vessels at room temperature and stirred several times daily with a glass rod. This mixture was filtered through What man No-1 filter paper. The extract was subjected to Rotavapour evaporation to remove the solvent. The same procedures were used for the chloroform, methanol and aqueous extraction. The extracts obtained were kept in dessicator containing calcium carbonate.

These extracts were stored at -4°C until use and dissolved in normal saline on the day of the experiments for the purpose of evaluating pharmacological activity.

Parasites

Adult earthworms (*Pheretima posthuma*) of 8-10cm in length were used to evaluate *in vitro* anthelmintic activity.^[16]

Experimental Treatments

The earthworms were divided into six groups containing six earthworms in each group. Since the earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, Adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity.

All the earthworms were washed in normal saline solution before they were released into 10ml of respective formulation as follows: Vehicle (5% DMF in 10ml normal saline) used as control, Albendazole oral suspension (100 mg/5ml) used as standard. The selected amount of stem extracts of *Aegiceras corniculatum* were weighed and taken into the test tubes containing 10ml of normal saline. The extracts were Petroleum Ether extract (50 &100 mg/ml), Chloroform extract (50 &100 mg/ml), Methanol extract (50 &100 mg/ml) and Aqueous extract (50 &100 mg/ml). All the extracts and the standard drug solution were freshly prepared before starting the experiments. Different extracts and the standard drug solution were poured in different petridishes. Observations were made for the time taken to paralyze (Paralysis is when the worm did not revive even in normal saline) and Death (Death is when the worms lost their motility followed with their body colors fading away) the results of each petridish were recorded.^[17-20]

Statistics

All the results was expressed as mean \pm SEM and followed by student 't' test (n = 6).

RESULTS AND DISCUSSION

The crude extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The crude extracts of ethanol showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 25, 50, 100 and 200mg/ml. The ethanol extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of aqueous extracts.

Table-1: An effect of *A. corniculatum* Pet.ether and Chloroform extracts on earthworms

Extracts (mg/ml)	Pet. Ether extract		Chloroform extract	
	Paralysis time (min)	Death time (min)	Paralysis time (min)	Death time (min)
50	96 ± 6.33	125 ± 7.03	116 ± 4.36	138 ± 2.62
100	76 ± 2.31	98 ± 1.21	92 ± 7.50	126 ± 7.89
Albendazole				
Suspension	5 ± 1.00	23 ± 6.34		
100mg/5ml				

Each value represents mean ±SEM (N=6).

Table- 2: An effect of *A. corniculatum* Aqueous and Ethanol extracts on earthworms

Extracts (mg/ml)	Aqueous extract		Ethanol extract	
	Paralysis time (min)	Death time (min)	Paralysis time (min)	Death time (min)
50	200 ± 2.22	240 ± 2.12	62 ± 3.20	84 ± 8.29
100	180 ± 3.19	210 ± 3.62	34 ± 3.52	56 ± 4.36
Albendazole				
Suspension	5 ± 1.00	23 ± 6.34		
100mg/5ml				

Each value represents mean ±SEM (N=6).

P<0.001 significantly different compared with reference compound, Albendazole, student's *t*-test.

The four crude stem extracts of *Aegiceras corniculatum* were Petroleum Ether extract (50 & 100 mg/ml), Chloroform extract (50 & 100 mg/ml), Methanol extract (50 & 100 mg/ml) and Aqueous extract (50 & 100 mg/ml). The paralysis time of Petroleum Ether extract (96 ± 6.33 & 76 ± 2.31), Chloroform extract (116 ± 4.36 & 92 ± 7.50), Ethanol extract (62 ± 3.20 & 34 ±

3.52) and aqueous extract (200 ± 2.22 & 180 ± 3.19) were compared to the standard drug Albendazole suspension 100mg/5ml (5 ± 1.00). Results were tabulated in Table 1 & 2.

Mangroves and associated plants provide a wide domain for therapeutic application in recent years, most yet to be explored. The leaves of *A. corniculatum* are reportedly rich in flavonoids with proven anti-inflammatory and antioxidant property.^[12]

CONCLUSION

The results of the present study clearly indicated that the crude methanol extract of *A. corniculatum* produced significant anthelmintic activity against Indian earthworms *Pheretima posthuma*. Nevertheless, activities of extracts of the plant investigated on the earthworms were lower than that of the reference compound, Albendazole suspension. Thus this plant having a potential novel and cheap source of potent anthelmintic agent.

To conclude, in the future studies, there is need for thorough phytochemical, clinical and possible studies on molecular mechanism of action. At the same time efforts should be made to standardize the plant extract and formulate the best alternative herbal preparations to replace or complement the synthetic drugs which are in current use.

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CONFLICT OF INTERESTS

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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