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ANTHELMINTIC ACTIVITY OF BLACK MANGROVE OF *AEGICERAS CORNICULATUM*

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ABSTRACT: This study evaluated the scientific basis for the traditional use of *Aegiceras corniculatum* (Myrsinaceae) stem extract as an anthelmintic agent. The study design was an investigation of the traditional anthelmintic medicinal plant *Aegiceras corniculatum* using *in-vitro* anthelmintic properties of four extracts of the plant were evaluated 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 showed that the four extracts produced dose-dependent and significant anthelmintic activities. From these findings, the four extracts of *Aegiceras corniculatum* are a rich source of naturally occurring anthelmintic activity. Further work is recommended to evaluate the *in-vivo* anthelmintic activity and toxicity of the extracts.

INTRODUCTION: Helminth infections resulting in 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 predispose humans to these infections abound in these areas.

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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 harbor the worms for most 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 helminths coupled with improved management has been the important worm control strategy throughout the world. However, development of resistance in helminths [6, 7] against conventional anthelmintics is a foremost problem in the treatment of helminth 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. The 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, anti-nociceptive, 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 has rich in flavonoids with proven anti-inflammatory and antioxidant property [12, 13]. *Aegiceras* has been used as traditional medicine for years with an array of biological activity such as antioxidant, anti-bacterial, antifungal, antiulcer, anticancer, anti-plasmodial and antitumor properties [14, 15].

MATERIALS AND METHODS:

Plant Material Collection: *A. corniculatum* stem were collected from the coringa mangrove forest, Andhra Pradesh and authenticated by the taxonomist, Department of Botany, Andhra University in July 2016 and the specimen voucher (AC01/2016) were preserved in the herbarium.

Plant Extract Preparation: *A. corniculatum* stem was dried under shade then made into coarse powder and was weighed for 250 gm, and it was soaked in 1 litres of petroleum ether by maceration. The mixture was 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 Whatman 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 desiccator containing calcium carbonate. These extracts were stored at - 4 °C until use and dissolved in normal saline on the day of the experiments to evaluate pharmacological activity.

Parasites: Adult earthworms (*Pheretima posthuma*) of 8-10 cm 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 10 ml of the respective formulation as follows:

Vehicle (5% DMF in 10 ml normal saline) used as the control, Albendazole oral suspension (100mg / 5ml) used as a standard. The selected amount of stem extracts of *Aegiceras corniculatum* were

weighed and taken into the test tubes containing 10 ml 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 Petri dishes. 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 Petri dish were recorded [17-20].

Statistics: All the results were expressed as mean ± 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 the 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 200 mg/ml. The ethanol extract showed a 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

	Pet. ether extract		Chloroform extract	
Extracts (mg/ml)	**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 Suspension100mg / 5ml	5 ± 1.00	23 ± 6.34		

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

TABLE 2: AN EFFECT OF *A. CORNICULATUM* AQUEOUS AND ETHANOL EXTRACTS ON EARTHWORMS

	Aqueous extract		Ethanol extract	
Extracts (mg/ml)	**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 Suspension100mg / 5ml	5 ± 1.00	23 ± 6.34		

P<0.001 significantly different compared with the 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** and **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 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 a need for thorough phytochemical, clinical and possible studies on the 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 INTEREST: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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