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In vitro determination of the anti-helminthic properties of *Anthocleista djalonensis*

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Abstract

Medicinal plants have been identified and used throughout human history for its different activities. This study was carried out to screen the aqueous, butanol, ethyl acetate, methanol and n-hexane fractions of the leaves of *Anthocleista djalonensis* for anti-helminthic activity across various concentrations (10–50 mg/ml) of the crude extract and 10 mg/ml of the different fractions. Albendazole (10 mg/ml) was used as reference standard drug while 20% Tween 80 was used as control. Determination of paralysis time and death times of the worms were recorded. In vitro treatment of the parasite with the highest dose of crude extract (50 mg/ml) resulted in paralysis and death at 20 min and 110 min respectively. Butanol showed greatest anti-helminthic activity even higher than that of the reference drug with paralysis and death times of 13 min and 51 min respectively. The order of sensitivity of the fractions to the worms was butanol > n-hexane > ethyl acetate > aqueous. The overall finding of this study demonstrates that the leaves possess anthelminthic compounds and should be further evaluated as a remedy to various disease conditions associated with worm infestation.

Keywords: Medicinal plants; Anti- Helminthic; Helminthiasis; *Anthocleista djalonensis*

1. Introduction

Helminthiasis/worm infection is a type of macro parasitic disease of humans and other animals which involves a part of the body being affected by parasitic worms also known as helminths. The most important helminthiases are the soil-transmitted helminthiasis and schistosomiasis and these diseases are among the neglected tropical diseases. The main species that infect people are the roundworms (*Ascaris lumbricoides*), the whipworm (*Trichuris trichuria*) and hookworms (*Necator americ*anus and *Ancylostoma duodenale*) [1]. They often invade the gastrointestinal tract of their hosts, but they may burrow into other organs, where they can also produce physiological damage.

According to WHO estimates, about 870 million children live in areas of high prevalence of helminth infections [2]. Africa, South Asia and South America are the most affected regions of the world [3]. Helminthiasis has been associated with poor birth outcome, poor cognitive development, poor school and work performance, poor socio- economic development and poverty. Secondary effects include chronic illness, malnutrition and anaemia. Soil transmitted helminthic infections rarely cause mortality. Acute symptoms include diarrhea, abdominal pain and low haemoglobin levels however, the long-term effects are dangerous as those with infections show reduced cognitive abilities, intellectual capacity and lower work productivity [4].

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The prevalence and control of soil-transmitted helminth infections is inextricably linked with water quality, sanitation, hygiene practices and socio-economic status in the affected areas [5]. Despite the fact that infection can be cured with either albendazole or mebendazole, eradication is difficult given soil-transmitted infection faeco-oral and penetration-via-skin transmission pattern as the chances of reinfection are very high in population living in affected areas [6]. Also, the broad-spectrum anti-helminths are expensive, unaffordable, inaccessible or inadequately available to less privileged people in developing countries [7]. This issue has been compounded by the development of anthelmintic resistance of the parasites [8]. Resistance has affected all major broad spectrum anthelmintic types: Morantel, trichlophon, levamisole, benzimidazoles and the other nicotinic agonists, in addition to the avermectins and milbemycins [9]. hence the need to develop new anti-helminthic compounds from plant materials.

Many studies on natural products have shown interesting biological and pharmacological activities as they have been used as chemotherapeutic agents for centuries to treat variety of diseases or they serve as starting materials in the development of modern medicines [10]. Natural products from plants have traditionally played important roles in the treatment of human diseases. Currently, about 80% of the world population residing in third world countries still relies almost entirely on plant product for their primary health care. The remaining 20% of individuals living in the first world countries use more than 25% of the pharmaceuticals which have been directly derived from plant products [11].

Anthocleista djalonensis is one of the medicinal plants under study for its anthelmintic effect. The plant is commonly called cabbage tree. This is because the stem is branched only at the top with huge leaves clustered at the end of the shoot [12]. The *Anthocleista species* have been reported in literature to be widely used in traditional medicine for the treatment of various diseases, amongst the therapeutic species which have been evaluated is *A. djalonensis* [13]. The in vitro and in vivo pharmacological studies on the crude extract, fractions and few isolated compounds of *Anthocleista species* shown anti-diabetic, anti-plasmodial, anti-microbial, anthelmintic, hypotensive, spasmogenic, anti-obesity, anti-ulcerogenic, analgesic, anti-inflammatory, anti-oxidant, fertility, diuretic and laxative activities which supports most of their uses in traditional medicine [13]. High level of alkaloids in the leaves of *A. djalonensis* in addition to flavonoids, saponins and tannins in all the parts, could be the reason for the traditional usefulness of the plant as a medicine [14].

2. Material and methods

2.1. Reagents and Equipment

2.1.1. Solvents and reagents

Methanol, ethyl acetate, n-hexane and butanol produced by Guangdong Guanghua Sci-Tech Co. Ltd. China, distilled water, tween 80.

2.1.2. Equipment

Rotary evaporator, water bath, separating funnel, weighing balance, measuring cylinder, beakers, conical flasks, cotton wool, milling machine, filter paper, maceration bottle.

2.2. Test animals

Adult earthworms were used for this study. They were obtained from the waterlogged areas of Agulu Lake, Anambra state. All worms were authenticated at the Department of Zoology, Nnamdi Azikiwe University, Awka. The average length of the earthworms was 6-8cm and the earthworms were washed with water to remove adhering dirt.

2.3. Sample collection and preparation

The leaves of *Anthocleista djalonensis* plant were obtained from Umuchu, Anambra state. It was identified and authenticated by a plant technologist in the Department of Pharmacognosy and Traditional medicine, Nnamdi Azikiwe University, Awka. Samples of the plants were deposited at the herbarium of Department of Pharmacognosy and Traditional Medicine as voucher specimen Psidium guajava: PCG474/A/069. The leaves were properly washed with clean water to remove traces of dust and dirt on them and they were spread under shade to dry. After drying, the leaves were grinded using a mechanical grinding mill. The powdered sample obtained was stored in air tight container under room temperature prior to extraction.

2.4. Extraction of Active Constituents

The leaf sample was extracted using the cold maceration method. The powdered leaf sample was divided into three parts, each weighing 1kg and transferred to different maceration bottles and 1.5 liters of methanol was added to each.

The mixture was left to stand for 72hours. The extract was obtained by sieving using muslin cloth. It was further filtered three times with Whatman filter paper. The filtrate was further concentrated using water bath at 50 ^{0}C. The weight of the concentrated crude extract was obtained and it was stored in a closed container for further use.

2.5. Fractionation of crude extract

The crude extract was fractionated into aqueous, n-hexane, ethyl acetate and butanol fractions by liquid-liquid fractionation method with the aid of a separating funnel. The concentrated crude extract was reconstituted with 50ml of methanol and 100ml of distilled water. Then 500 ml of n-hexane was added and shaken vigorously releasing the pressure at intervals. The mixture was then allowed to stand for two hours for proper separation and then n-hexane fraction was collected into a clean beaker. Another 500ml of n-hexane was added to the extract in the funnel and the fraction was collected again. Then 500 ml of ethyl acetate was added to the residue in the separating funnel and shaken vigorously and then allowed to stand for 2 hours. The ethyl acetate fraction was then collected into a clean beaker. Another 500ml of ethyl acetate was added and the fraction was collected. The above procedure was repeated for butanol to obtain the butanol and aqueous fractions.

2.6. Anti-helminthic Assay

The anthelmintic assay was carried out using the method of Ajaiyeoba et al [15]. The assay was done using adult earthworms due do its anatomical and physiological resemblance to the intestinal roundworm parasites of human beings.

2.6.1. Preparation of sample

Albendazole 10mg/ml was prepared using 20% tween 80. Crude sample was prepared at concentrations of 10mg/ml, 20mg/ml and 50mg/ml. butanol, aqueous, ethyl acetate and n-hexane fractions were prepared at 10mg/ml each using 20% tween 80. 20% tween 80 was used as negative control.

2.6.2. Experimental design

Sterilized Petri-dishes were labeled into 7 groups.

- Group 1: control (20% tween 80)
- Group 2: 10mg/ml crude extract
- Group 3: 20mg/ml crude extract
- Group 4: 50mg/ml crude extract
- Group 5: 10mg/ml aqueous fraction
- Group 6: 10mg/ml butanol fraction
- Group 7: Standard (10mg/ml albendazole)

The samples were prepared using 20% tween 80 and 25ml of each sample was transferred to the labelled Petri-dishes. Four earthworms were placed in each of the Petri-dishes. They were closely observed and time of paralysis was recorded as when the worms stopped moving except when touched by a metallic object. Death time was recorded when no movement was observed in the earthworms when shaken vigorously and when dipped in 50^{o}C warm water. Albendazole as used as the standard control.

3. Results and discussion

3.1. Extraction yield of plant material

57.3 g (2.9% w/w) of crude methanol extract was recovered from 2000 g of dried powdered plant sample. Weight of fractions and their yields calculated from 57.3 g of crude methanol extract are: ethyl acetate fraction (9.1 g, 15.9 % w/w), butanol fraction (6.3 g, 11.0 % w/w), aqueous fraction (14.1 g, 24.6% w/w) and n-hexane fraction (5.0 g, 8.7 % w/w).

3.2. Result of *in vitro* anthelminthic activity

Table 1 below shows the average paralysis and death time of the earthworms when administered with different test agents. The results reveal that the butanol possessed the greatest potency causing paralysis at 13 min and death at 51 min. the aqueous fraction showed lowest potency with paralysis time of 23 minutes and death time of 78 min. The

butanol fraction performed better than the standard agent used, albendazole. It is obvious that within two hours all the earthworms administered with the extracts and fractions died showing a 100% efficacy.

Anthocleista djalonensis has been reported to possess many secondary metabolites including; saponins, flavonoids, tannins, reducing sugars, steroids, alkaloids and phlobatanins [16]. Some of the phytochemicals present in the plant have been associated with anthelmintic activity. Tannins have been reported to have anthelmintic effects against nematode parasites by interfering with coupled oxidative phosphorylation thus blocking ATP synthesis in parasites [17,18]. Other studies have shown alkaloids to have anthelmintic properties [19,20]. Alkaloids have the ability to intercalate with DNA synthesis of parasites [21].

Table 1 Effects of the different standards, extracts and fractions of *Anthocleista djalonensis* on motility/survival of *Pheretima posthuman.*

Extract	Concentration (mg/ml)	Paralysis time in min (Mean and SEM)	Death time in min (Mean and SEM)
Control (20% tween 80)	–	34.00 ± 13.09 *94.00 ± 6.54*	133.50 ± 17.31 *233.50 ± 8.66*
Crude extract	10	23.75 ± 1.50	100.00 ± 8.76
Crude extract	20	30.00 ± 10.86	113.75 ± 5.97
Crude extract	50	20.50 ± 4.93	110.25 ± 6.99
Aqueous fraction	10	23.75 ± 9.07	78.00 ± 0.00
Butanol fraction	10	13.50 ± 2.08	51.25 ± 0 .96
N-hexane fraction	10	19.03 ± 2.12	61.00 ± 0.00
Ethyl acetate fraction	10	21.28 ± 0.91	56.95 ± 0.00
Standard (albendazole)	10	16.25 ± 5.80	97.00 ± 15.21

All values are represented as mean ± SEM (n=4).

Previous work done on the HPLC analysis indicated that the butanol fraction of the plant contains kaempferol which has been reported to possess anti-helminthic activity [22]. and apigenin, a type of glycoside which has also been reported to possess anthelminthic activity [23, 24]. Butanol fraction with the greatest activity indicated that the bioactive constituents were selectively separated into the butanol fraction. Butanol is a moderately polar compound and thus has both hydrophilic and lipophilic components.

Reports have indicated that trans-cuticular diffusion is a common means of entry for non-nutrient and non-electrolyte substances in helminths [25]. It has been observed that this route is predominant for the uptake of major broad spectrum anthelmintics as opposed to oral ingestion. Lipophilic compounds have greater ability to cross the external surface of helminths than hydrophilic compounds [26]. This may be the major reason for the grater potency of the butanol fraction which contains some lipophilic constituents compared to the aqueous extract which contains majorly hydrophilic constituents.

The crude extract showed slight anthelmintic activity with longer paralysis and death time than albendazole. This could be due to the presence of many compounds which could mask the bioactive components of the plant drug.

The findings from this study will be valuable to the field of plant anthelmintics to provide sustainable, effective and safer alternatives to conventional anthelmintics.

4. Conclusion

The results obtained from the study revealed that butanol, aqueous fractions and crude extract all possess anthelmintic activity with varying potencies. The butanol fraction showed the best activity with lower paralysis and death times than the standard drug albendazole. This indicates that the active constituents responsible for anthelmintic activity of *Anthocleista djalonensis* are present in the butanol fractions of the plant. The activity of the butanol fraction may be due to its partial lipophilic nature which encourages Tran's cuticular diffusion into the parasite's membrane. The

anthelmintic activity of the plant could be due to its high content of tannins, saponins, alkaloids and other phytochemicals.

This study supports the claim of the anthelmintic efficacy of *Anthocleista djalonensis* and justifies the use of this plant as an anthelmintic agent in traditional medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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