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Research Article

Anthelmintic Properties of Methanol Extract of *Bridelia micrantha* (Hochst) Baill Leaves

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Abstract: Helminths in recent times are said to be highly resistant to anthelmintic therapeutics. The level of attention paid to this area is quite low, therefore causing a serious threat to humans and livestock. Helminth's infection is rampant in developing countries contributing significantly to economic losses and food security in general. This study aims to determine the Anthelmintic properties of methanol extract of *Bridelia micrantha* (Hochst) Baill leaves. **Methods**: The anthelmintic activity of helminths was determined by exposure to various concentrations of reference anthelminthic (albendazole, praziquantel and mebendazole) drugs and plant extract. Measurements were taken based on times for death and paralysis. **Results**: The extract exhibited a concentration-dependent anthelmintic activity against *Lumbricus terrestris* with significant ($p < 0.0001$) paralysis and death times when the extract concentrations were 4, 8, 16 and 32 mg/mL respectively. In the presence of 0.125 mg/mL of the extract the reference anthelmintic (albendazole), showed a potentiated activity against the test organism. In the presence of 0.25 mg/mL of the extract the reference anthelmintic (mebendazole), also showed a potentiated activity against the test organism. In the presence of 0.125 and 0.25 mg/ml of the extract, the reference anthelmintic (praziquantel) showed similar results. **Conclusion**: The extract had anthelmintic activity against *L. terrestris* and modified the resistance of the organism to albendazole, mebendazole and praziquantel.

***Keywords*:** *Bridelia micrantha*, anthelminthic activity, potentiation

1. Introduction

Helminthiasis is one of the most common degenerative diseases, afflicting a large portion of the population. In developing nations, they pose a significant threat to public health, causing stomach discomfort, queasiness, vomiting,

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loss of appetite, cerebral pain, and lose of bowels, as well as eosinophilia and pneumonia [1]. As a result, in different tissues, larvae are active for varying amounts of time. When the larvae of the common human parasites infiltrate the tissues of a hyperimmune person, they cause similar but less severe and protracted reactions. Most helminth infections are persistent and weakening in nature [2]; they are likely to be more dreadful and inflict more financial and social misery among people and animals than any one group of parasites. Many people all around the world are infected with parasitic worms, which are harmful to their health [3].

Anthelmintic resistance has thus far received far less attention than antibacterial or other anti-infective agents, even though it has now become a global problem requiring similar attention to bacterial resistance, particularly among humans [4]. Given the high level of resistance among human helminth infections including soil-transmitted helminths, STH, (Ascaris lumbricoides, Hookworms - *Necator americanus* and *Ancylostoma duodenale* - and *Trichuris trichiura*) [5]. This resistance comes as a result of selective pressure that rose from periodic mass administration of anthelmintic medicines to children and other at-risk groups. This has resulted in resistance modulatory activities of natural products on traditional medicines, which have gained scientific interest in recent years [6].

Bridelia is a plant genus of the family Phyllanthaceae, which was first described as a genus in 1806. Geographically *B. micrantha* is widespread in Africa, especially in tropical Africa and Ghana as well. *B. micrantha* is extensively distributed all over the mainland in tropical Africa with some exceptions. These exceptions include countries with extremely low annual rainfall as the plants, which love to grow in rainy areas [7]. The *B. micrantha* species has now been familiarized with places like Reunion Island as a therapeutic plant and has been finally established on the Island. The genus *Bridelia* has more than 75 species that are highly prevalent in the tropics and about 15 species which are common in Africa and two species, which are also usually found in the Ocean islands in India. It's also one of the world's largest diversity of plants and animals species of organism [8]. Figure 1 below depicts the shoot of *B. micrantha*.

Figure 1. Shoot of *Bridelia micrantha* plant

The plant can be found in numerous habitats including savanna lands, various types of forests, riverine forests, mangrove swamps, etc. *B. micrantha* species is a forerunner species that can survive in numerous environments, including soils, forest types, and water regimes, and even tolerate moderate frost [9]. *Bridelia micrantha* is known to be one of the perennial and monoecious trees that grow to about twenty-seven meters tall. With a bowl of 100 cm in thickness which is twisted but short and also has a rounded crown. The bark is usually dark somewhat silver-grey with lenticels, which are usually smooth to rough, reticulated cracked and desquamating in nature. Spines are a feature of the branches of *Bridelia micrantha* both young and older branches have spines even though sometimes older branches have blunt or dull spines [10]. The leaves of *B. micrantha* are simple, complete and unique, and are usually replaceable, dazzling, slightly hairy, and elliptical to oviform. The flowers mostly arise in clusters at the leaf area or simply leaf axils, their colour is usually yellow, with cuneate sepals and are usually unisexual. Ripen fruits of *Bridelia micrantha* habitually have brownish seeds and are fleshy, bulbous and black in colouration [11].

Curing diseases and improving health status is generally one of the most important uses of plants in Africa. And *Bridelia* species, of no doubt, is among these plants. *Bridelia* species have been utilized in traditional medicine, mostly

as an antidote, a cathartic or purgative to treat various ailments such as eye infections, constipation, common cold, and gastritis and are also used as a mouthwash [12]. The treatment of wounds, aphrodisiacs and purgatives are mostly by the bark, whiles some African countries boil the bark, making it drinkable, to treat sore throat and cough [13]. The cure for headache, stomachache, diarrhoea, sore joints, fever, tapeworms, and sore eyes are also by the utilization of the bark in some African countries such as South Africa. The decoction of Leaf and root are applied as Anti - parasitic in other African countries to treat Trypanosomiasis [14].

The leaves of *Bridelia micrantha* are a good meal for cattle and a favourite for the *Anaphe* genus, especially the wild silkworm of the *Anaphe* spp. Also, edible caterpillars and silk are usually harvested from wild strands of *Bridelia micrantha*. The sweet fruits obtained from *Bridelia micrantha* are edible in numerous cultures, therefore, in other places like East Africa. And the Massai group people usually use it as a flavour of milk [15].

B. micrantha stems have been used as a taste improvement agent in West Africa. With its good shade, it has been grown in most agroforestry systems as a shade-providing plant and sometimes as a mulch, hedge, etc. One of the large sugarcane farms located in South Africa uses *Bridelia micrantha* to help restore and stabilize some deep canals caused by irrigation. It is also used as an ornamental plant and is indorsed for areas where there is a waterlogging condition [16].

Numerous studies have been conducted in the area of phytochemicals, which are naturally occurring in the roots, stem, and leaves of *Bridelia micrantha* [11, 17, 18]. These studies have shown that the plant contains essential oil, saponins, terpenoids, cyanogenic glycoside, ester, phenolic compounds, alkaloids, tannins, flavonoids, anthraquinones, sterols, oxalate, carbohydrates, minerals, anthocyanidin have all been determined to be present in *Bridelia micrantha* [19]. As a plant that is used as food in some cultures, *Bridelia micrantha* has been found to contain an extensive range of typical nutrients that includes carbohydrates, proteins, and various alcohol especially hexahydroxy alcohol. In terms of chemical constituents, the plant has been found to contain cobalt, magnesium, potassium, calcium lead, phosphorus, manganese, chromium, iron, sodium, copper and several others [19].

Bridelia micrantha anthelmintic properties have been tested on a variety of helminths including Schistosoma species that are currently causing devastating infections in Africa and around the world [19]. Research has been undertaken in African countries such as Cameroon, Kenya, Ghana, Nigeria, South Africa, Uganda, and Tanzania to evaluate the anthelmintic relevance of *Bridelia micrantha* in various cultures. However, research indicates that not all pathogenic worms have been thoroughly investigated, calling for additional practical studies on Bridelia micrantha anthelmintic and resistance-modifying activities [20, 21]. Hence, this study gives baseline information on several strategies for “fighting” worm diseases in Africa, particularly in Sub-Saharan African countries.

2. Methods

2.1 *Collection and identification of samples*

Fresh matured *Bridelia micrantha* leaves were collected between September 24 and 26, 2019 at Kwahu Asakraka in Ghana’s Eastern Region’s Kwahu South District (latitude: N 6° 37' 45.9048" and longitude: W 0° 41' 11.30253"), with Mpraeso as the capital (latitude: N 6° 37' 45.9048" and longitude: W 0° 41' 11.30253"). The plant samples were authenticated by Mr. Clifford Osafo Asare, a botanist at KNUST’s Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences (FPPS). In the Department’s herbarium, a specimen sample with the voucher number KNUST/HM1/2020/L008 has been deposited.

2.1.1 *Preparation of methanol extract of plant material*

The leaves of *B. micrantha* were cleaned properly under running faucet water to eliminate extraneous items and left to drain before being air-dried at 28 °C for 5 days. An ultra-fine grinding mill was used to ground the dried leaves into a fine powder (ufg mill, DFL 18 Pulverizer, China). *Bridelia micrantha* powdered leaves (500 g) were cold macerated for 72 hours at 4 °C in 2.5 litres of 70% methanol with frequent intervals of shaking. The extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated at 40 °C using a rotary evaporator (BUCHI Rota vapor R - 114) before being dried entirely in the oven at 40 °C. *Bridelia micrantha* Extract (BME) dried methanol extract was weighed, maintained in Falcon Tubes, labelled, and stored in a desiccator for usage.

2.1.2 *Collection of adult Lumbricus terrestris*

The earthworms *Lumbricus terrestris*, measuring 9 cm to 13 cm in length [22] have not gotten the expected level of attention, because despite their customary abundance in our gardens, our initial fascination with them fades before they reach adulthood [23]. The worms were collected from the soil behind the Department of Theoretical and Applied Biology lecture hall at Kwame Nkrumah University of Science and Technology. The worms were identified and authenticated at the Theoretical and Applied Biology Department.

2.2 *Determination of in vitro anthelmintic activity of methanol extract of leaves of Bridelia micrantha*

The *in vitro* anthelmintic bio-assay was performed according to the method described by Bharathi et al. [3]. Ten grams (10 g) of the methanol whole plant extract of *B. micrantha* Extract (BME) were mixed in 1 mL DMSO and diluted with Ringer's lactate solution (Amanta Healthcare Ltd, Gujarat, India) to 500 mL to produce 20 mg/mL extract solution. Using Ringer's lactate solution, it was serially diluted to generate 32, 16, 8, 4, 2, 1, 0.5, 0.25, and 0.125 mg/mL solutions. As a positive control, albendazole, mebendazole, and praziquantel (10 mg/mL) were made in the same way. Ringer's lactate was used as a negative control. The *L. terrestris* worms were cleaned of all debris using 0.9% w/v NaCl solution (Amanta Healthcare Ltd, Gujarat, India) and placed in separate Petri dishes (3 worms per petri dish) into which the various extract solutions and reference standard solution (50 mL in each petri dish) had been added. The period is taken for the various solutions to cause paralysis and the death of the individual worms. *Lumbricus terrestris* was found to have a maximal paralysis and death time of 400 minutes at a concentration of 0.5 mg/mL. When no movement was noticed, except when the worm wriggled when shaken, the time for paralysis was recorded. After determining that the worm does not move when shook forcefully or immersed in the ringer's dextrose solution, the time for the death of the worm was recorded. Death was deemed to have occurred when the worm lost motility followed by a fading away of its body colour which was confirmed by shaking the worm in warm water at 50 °C with no response [24].

2.2.1 *Determination of the influence of methanol extract of B. micrantha on the anthelmintic activity of albendazole, mebendazole and praziquantel*

The anthelmintic assay was performed with sub-inhibitory concentrations of methanolic extracts at MICs of 0.25 and 0.125 mg/mL in combination with each of the standard drugs (Albendazole, Mebendazole, and Praziquantel) at concentrations of 10, 5, 2.5, 1.25, and 0.625 mg/mL. 50 mL of these solutions were dispensed into separate Petri dishes and three worms were added to each petri dish. The time taken for the worms to paralyse was recorded when no movement was detected except when the worms were shaken vigorously. After determining that worms do not move when shaken vigorously or immersed in the ringer's dextrose solution, the death time of the worms was recorded (Sigma - Aldrich, London, UK).

2.3 *Statistical analysis*

Data obtained was analysed using Graph Pad Prism Version 6.0 for Windows (Graph Pad Software Inc, San Diego, CA, USA) statistical package programme. Two-way ANOVA followed by Bonferroni's post hoc test was employed in the analysis of data.

3. Results

3.1 *The anthelmintic activity of methanol extract of B. micrantha*

The extract although exhibited good anthelmintic activity, it, therefore, recorded a very high paralysis and death time at a concentration of 0.125, 0.25, 0.5 and 1 mg/mL at 400 mins exposure time. Figure 2 ($P = 0.0001$), Figure 3 ($P = 0.0265$), Figure 4 ($P = 0.0159$), and Figure 5 ($P < 0.0001$) all show that the extract's paralysis period was much shorter than that of the control medicines. Death time declined with increasing concentrations in all of these figures. The highest concentration dose of 32 mg/mL in Figure 2 reported the shortest paralysis and death time after 8 hours. Praziquantel

had the shortest paralysis and death times among the control medications, as seen in Figure 5.

3.2 *Determination of resistance modulation of methanol extract of B. micrantha on the anthelmintic activity of albendazole, mebendazole and praziquantel*

A modulatory activity for the extract and control drugs recorded a synergistic activity whereby as the concentration of the drug decreased, the time required for paralysis and death increased. The shortest death and paralysis time was observed for 0.125 mg/mL of extract and praziquantel (Figure 6) with $P < 0.0001$ while the highest death time and paralysis time was identified for 0.125 mg/mL of extract and Albendazole (Figure 7) with $P = 0.0105$. Significant differences of $P = 0.0012$, and $P < 0.0001$ were also found for 0.25 mg/mL of BME and mebendazole (Figure 8) and 0.25 mg/mL of BME and praziquantel respectively (Figure 9), which demonstrate that at those concentrations, the extract and the various standard drugs exhibited anthelmintic activity. The modulation of 0.25 mg/mL BME and albendazole (Figure 10) with $P = 0.2988$ and 0.125 mg/mL BME and mebendazole (Figure 11) showed no significant changes with $P = 0.0857$.

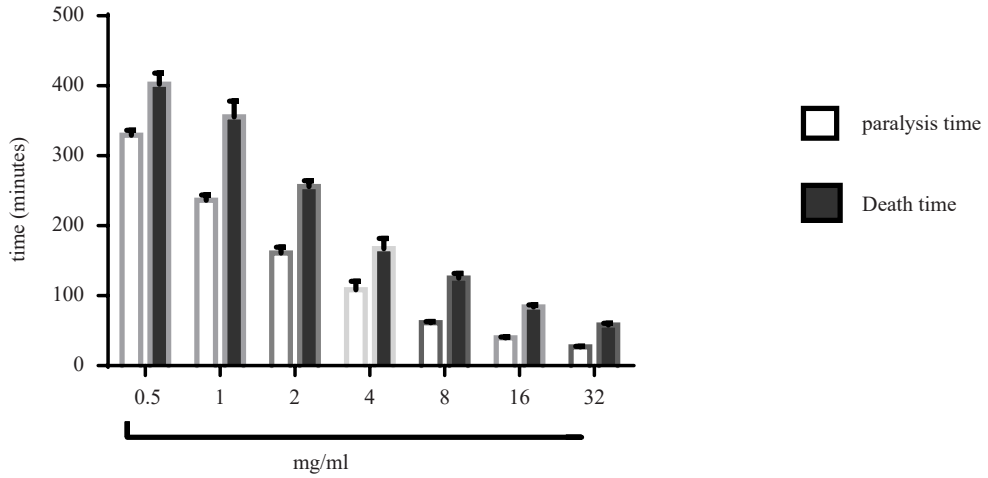

Figure 2. Paralysis and death time of leaves of *Bridelia micrantha* extract. Data represent mean ± Standard Error Mean (SEM), n = 2. $P < 0.0001$ as against each concentration (0.5 mg/mL - 32 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

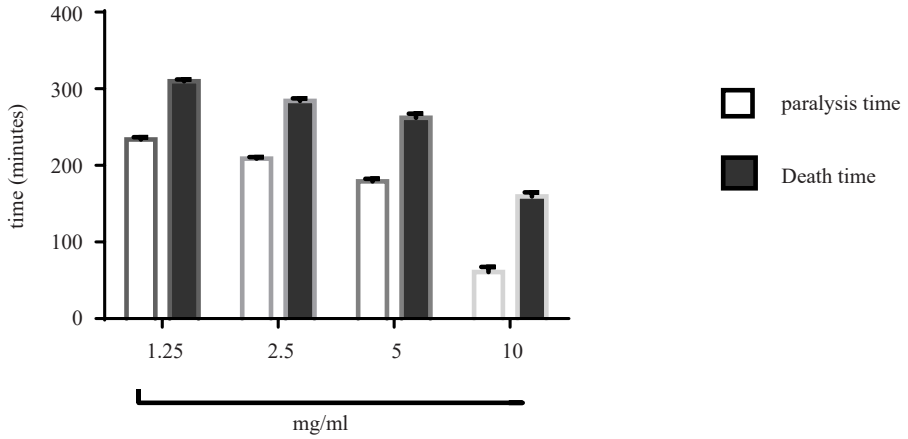

Figure 3. Paralysis and death time of albendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. $P = 0.0265$ as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

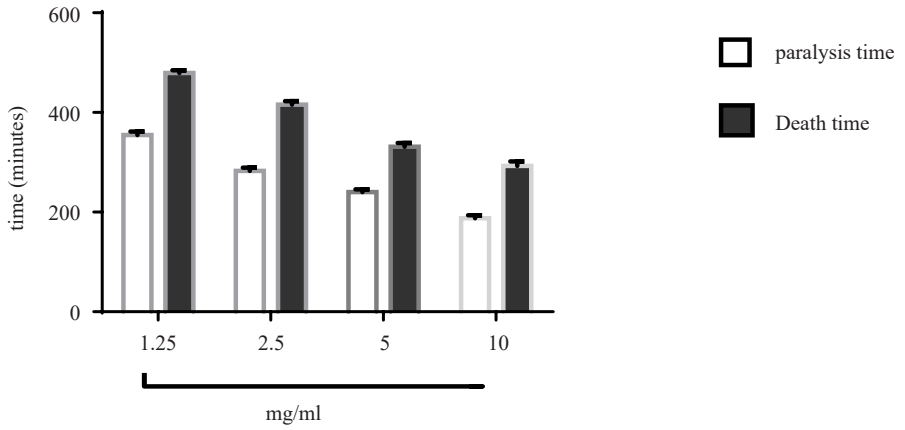

Figure 4. Paralysis and death time of mebendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. P= 0.0159 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

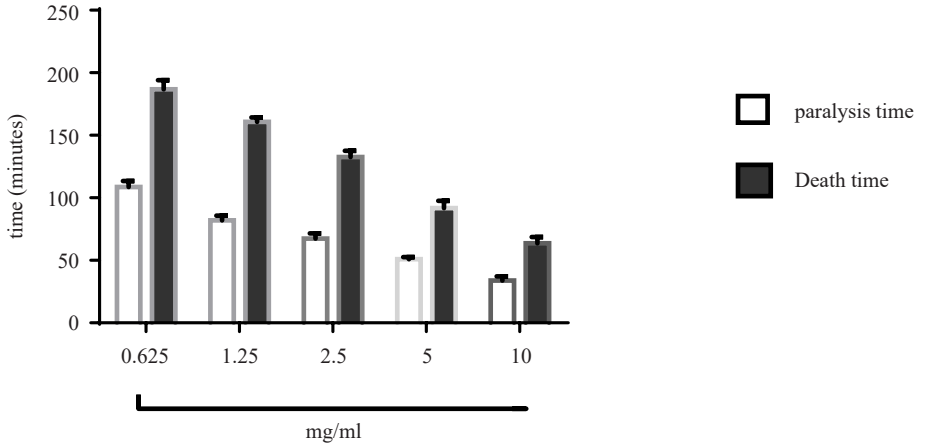

Figure 5. Paralysis and death time of praziquantel. Data represent mean ± Standard Error Mean (SEM), n = 2. P < 0.0001 as against each concentration (0.625 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

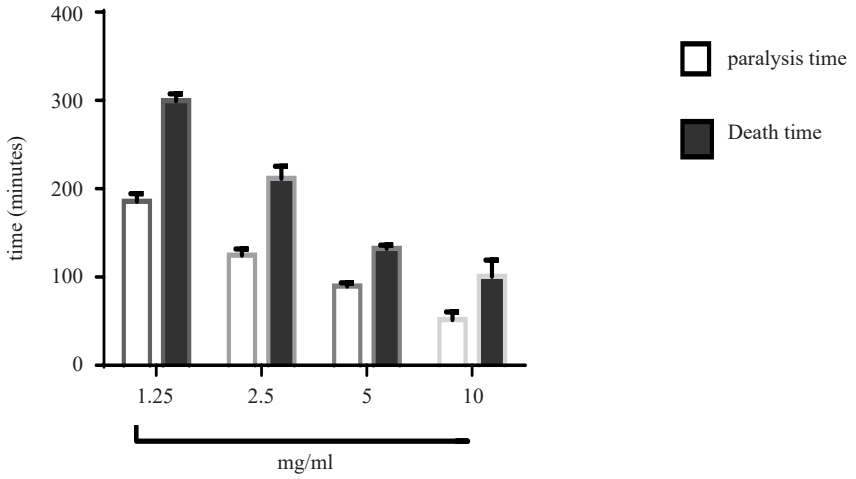

Figure 6. Paralysis and death time of modulation for 0.125 mg/ml of *Bridelia micrantha* extract and praziquantel. Data represent mean ± Standard Error Mean (SEM), n = 2. P < 0.0001 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

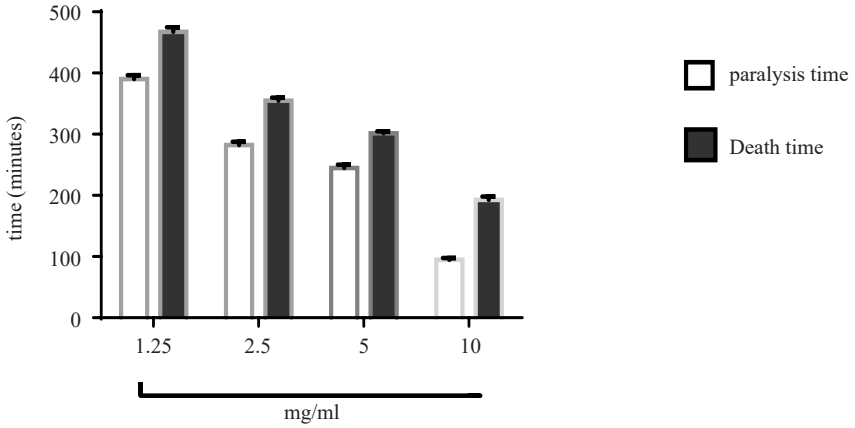

Figure 7. Paralysis and death time of modulation for 0.125 mg/ml of *Bridelia micrantha* extract and albendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. P = 0.0105 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

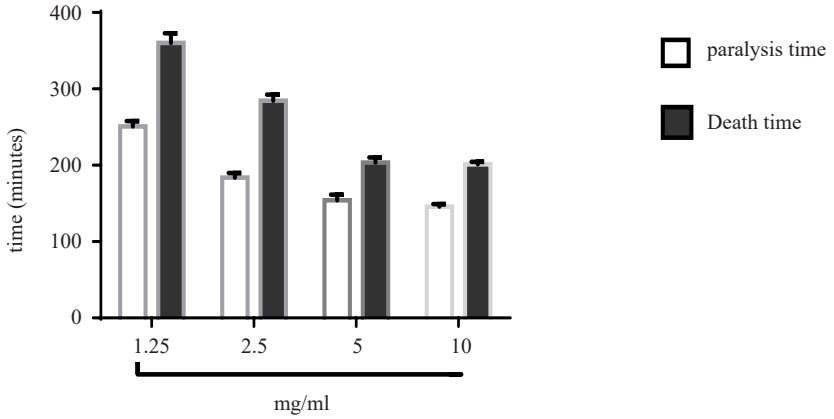

Figure 8. Paralysis and death time of modulation for 0.25 mg/ml of *Bridelia micrantha* extract and mebendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. P = 0.0012 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

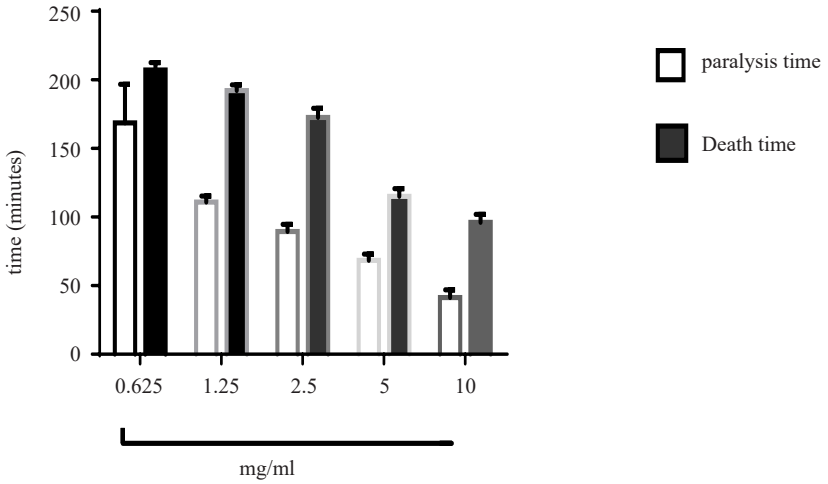

Figure 9. Paralysis and death time of modulation for 0.25 mg/ml of *Bridelia micrantha* extract and praziquantel. Data represent mean ± Standard Error Mean (SEM), n = 2. P < 0.0001 as against each concentration (0.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

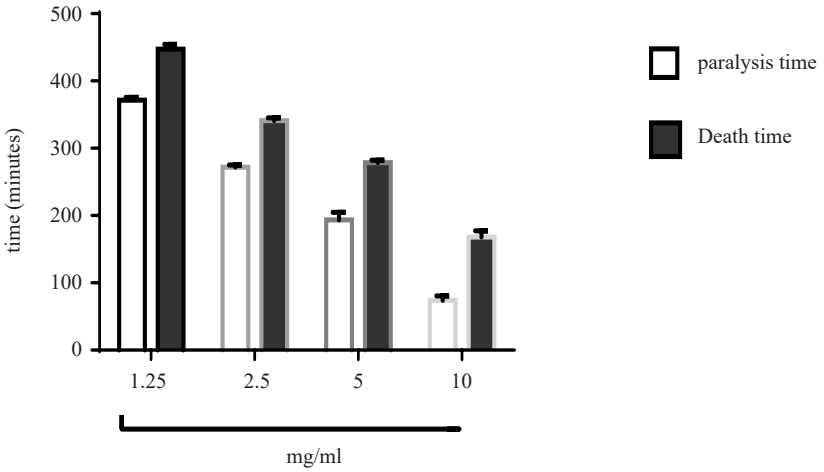

Figure 10. Paralysis and death time of modulation for 0.25 mg/ml of *Bridelia micrantha* extract and albendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. P = 0.2988 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

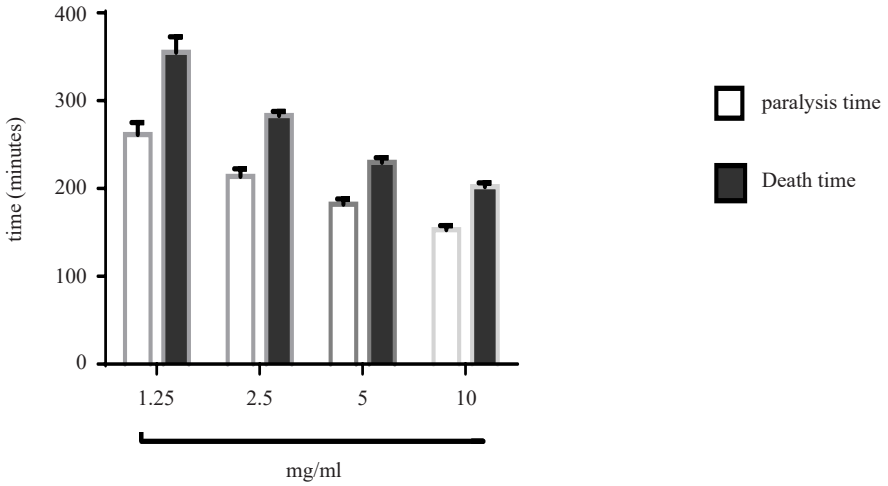

Figure 11. Paralysis and death time of modulation for 0.125 mg/ml of *Bridelia micrantha* extract and mebendazole. Data represent mean ± Standard Error Mean (SEM), n = 2. P = 0.0857 as against each concentration (1.25 mg/mL - 10 mg/mL), the data was analysed using Two - way ANOVA followed by Bonferroni's post hoc test

4. Discussion

The duration of paralysis and death times of the adult earthworm (*Lumbricus terrestris*) at 4, 8, 16, and 32 mg/mL of BME were compared to albendazole, mebendazole, and praziquantel, indicating that the extract had a good anthelmintic activity which represented low paralysis and death time. Low concentrations of (0.5 and 1 mg/mL) had low activity which represented high paralysis and death time. It also revealed that there was concentration-dependent activity. This research backs up a study by Waterman et al. [25] that found that the anthelmintic effects of aqueous and back extracts of BME against a Levamisole-resistant strain of the worm *Caenorhabditis elegans* were concentration dependant. Albendazole, mebendazole and praziquantel are used for the treatment of helminth infections [26]. Albendazole and mebendazole belong to a class of benzimidazoles and their effect of its results in the death of helminths. At all concentrations tested the reference drugs (albendazole, mebendazole, and praziquantel) had a paralytic effect on the test worms. Praziquantel consistently outperformed mebendazole and albendazole in terms of activity showing smaller paralysis and death times than mebendazole and albendazole. This is in line with a report published by

Hailegebriel et al. [27] wherein His meta-analysis revealed that praziquantel has an 89.8% cure rate for treating human schistosomiasis in Ethiopia after a single dosage. The existence of secondary metabolites that rendered the medications more available at their site of action, thereby potentiating anthelmintic drugs, might be attributable to the elevated activities of albendazole and mebendazole in the presence of sub-anthelmintic concentrations of BME (0.125 and 0.25 mg/mL) [28].

Praziquantel alone produced characteristic paralytic activity and in the presence of BME, its activity was enhanced against the *L. terrestris* compared to albendazole and mebendazole indicating a resistance modulation activity of the extract. From the mechanism of action of praziquantel, it may be due to the extract making the acetylcholine gated channels more open to praziquantel enhancing the effects of the latter, allowing more depolarization and entry of Ca^{+2} or enhancing the sensitivity of the contractile proteins to Ca^{2+}. It may also contain some cholinergic agonists that help amplify the effects of Praziquantel [29]. The findings indicate that feeding *B. micrantha* to animals can boost the activity of anthelmintic drugs. The bioactive compounds that are responsible for the anthelmintic activity of *B. micrantha* extract must be isolated and characterized. It is also necessary to conduct a toxicological test and an in vivo assay to understand better the potential of BME in treating subclinical and clinical infections of helminthiasis.

5. Conclusion

Bridelia micrantha extract had anthelmintic activity against *L. terrestris* and exhibited resistance modulation activity on *L. terrestris* in the presence of the reference anthelmintic agents. The phytochemicals present in the BME could be responsible for its anthelmintic activity. These findings support the folkloric use of BME as an anthelmintic agent.

Authors' Contributions

Philip Asumang and Jibira Yakubu designed and conducted all laboratory experiments, analyzed and interpreted experimental results. William Gariba Akanwariwiak, Francis Adu, Yaw Duah Boakye and Philomena Entsie participated in the conceptualization and designing of the research protocol, supervision and analysis of experimental results. Philip Asumang, Yaw Duah Boakye and Francis Adu wrote the first and final draft of the manuscript, Emmanuel Oppong, Gadafi Iddrisu Balali, Vera Afua Dela Gobe Job Donkor and Amihere Cobbinah Emmanuel also did proofread the manuscript. All authors read and approved the final manuscript.

Data availability statement

All data used for the study are available upon a reasonable request.

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Conflict of interest

The authors declare they have no competing interests.

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