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Research Article

Bridelia ferruginea Leaf Fractions Ameliorates Helminth Infections

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Abstract

Bridelia ferruginea leaf generally used in indigenous folk medicine for diverse purpose was evaluated scientifically to elucidate its anthelmintic and antioxidant activity of various fractions *in vitro*. Antioxidant properties of the fractions were evaluated using total phenol (mg/GAE g), total flavonoids (mg/QUE g), and ferric reducing ability (mg/g). The total antioxidant activities results indicated that, n-hexane fraction has significant higher antioxidant properties compared to other fractions (n-butanol, ethyl acetate and residual aqueous). The *in vitro* anthelmintic activities of the plant fractions were carried out on Pheretima Posthuma at varying concentrations of 25 mg/ml - 100 mg/ml in three replicates. The plants fractions caused a dose-dependent motility inhibition with highest effect from n-hexane fraction. The results confirm that *B. ferruginea* leaves are potential sources for novel anthelmintics and their varied degrees of antioxidant activity has the potential to be developed into dietary supplements and synergically modified with synthetic antioxidants.

Keywords: Helminthes; *Bridelia ferruginea*; Pheretima posthuma; Anthelmintic; Antioxidants

Introduction

Helminth infections remain a global burden in terms of its widespread and its effect on human and animal health [1]. More than a quarter of human population is infected, leading to serious morbidity and mortality [2]. Helminth infections continue to be the most substantial cause of economic losses in livestock industry [3]. Although the greater numbers of infections due to worms are extensively restricted to tropical regions, they impinge upon travelers, who have visited those areas and some of them can flourish in temperate regions [4]. The exploration and commercialization of synthetic drugs, though numerous, have not ameliorated the persistent dilemma due to rapid development of resistance in helminth parasites to all kinds of commercial drugs as well as accessibility problem for farmers, particularly native ones [3]. Therefore, farmers are compelled to rely heavily on ethno-medicines for controlling helminthiasis for their livestock.

Considering the inevitable problems, there has been renewed interest in the evaluation of traditional helminthes remedies as an alternative to synthetic drugs and use of the well-established medicines. Diverse kinds of plants and plant parts are employed in traditional medicines for the treatment of helminth infections. Thus, an earnest search for prospective anthelmintic phyto-medicines has been considerably accelerated. Over the last few years, medicinal plants have captured the attention of plant scientists, nutritionists, and growers. Findings from traditionalist on some plants showed that *B. ferruginea* is one of the most promising plants which could help to ameliorate these infections that pose great risk to human and livestock.

B. ferruginea belongs to the family Euphorbiaceae which is commonly found in the Savannah regions especially in the moister regions extending from Guinea to Zaire and Angola [5]. It is usually a gnarled shrub which sometimes reaches the size of a tree in satisfactory condition. Its common names are Kizni (Hausa), Marehi (Fulani), Iralodan (Yoruba), and Ola (Igbo). The tree is 6 m to 15 m high, up to 1.5 m in girth and bole crooked branching low down with dark grey bark, rough and often marked scaly [6].

In Ethno medicine, decoction of the leaves has been used to treat diabetes [7]. The bark extract was reported to have potential for water treatment [8]. In Togo, the roots of the plant are used as chewing sticks and the root bark is used for intestinal and bladder disorder remedies as well as skin diseases [9]. Its antimicrobial and anti-inflammatory properties have been well explored and documented [10,11]. Previous phytochemical attention on *B. ferruginae* has led to identification of flavonoids, triterpenoids, glucosides, bioflavonoids, phenols and tannins from various morphological parts of the plant [6].

In spite of numerous pharmacological and phytochemical reports on *B. ferruginae*, there is a dearth of literature report on the anthelmintic potency of the plant. Hence, it is of great significance and necessity that research focuses on discovering potency of *B. ferruginae* against helminthic infections. This research therefore sought to determine the anthelmintic and antioxidant activities of n-hexane, ethyl acetate, butanolic and residual aqueous fractions of leaves *B. ferruginae*.

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Materials and Methods

Collection of plant and preparation of plant fractions

Fresh green leaves of *B. ferruginea* were collected from a local farm in the suburb of Ado Ekiti, Ekiti State, Nigeria. Identification and authentication of the plant was carried out at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria by Mr. Omotayo F.O and a voucher specimen number (UHAE.2017/065) was deposited at the herbarium of the Department for future references.

Sample preparation: The plant material were shredded with a knife and air-dried at room temperature for 45 days to get rid of its water content. The air-dried leaves were weighed using electronic weighing balance, pulverized using a laboratory mechanical grinder and the fine powders obtained stored until further use, 840 g of the powdered sample was extracted with solvent combination (*via* maceration) of 70% ethanol for 48 h. Seven liters of 70% ethanol was used. The mixture was decanted and filtered using sterile Whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer to obtain ethanolic residue. The crude extract was later subjected to further fractionation processes**.**

Experimental protocol

Determination of total phenol content: The phenolic contents were determined using Follin-Ciocalteu reagent and expressed as Garlic Acid Equivalents (GAE) [12]. The extracts were diluted with methanol, by taking 3 ml of methanol and 1 ml of crude extract solution. To this sample solution, 1 ml of 5-fold diluted Folin-Ciocalteu's reagent was added. The contents were mixed well, kept for 5 min at room temperature followed by the addition of 1 ml of 10% aqueous sodium carbonate. After incubation at room temperature for one and half hour the absorbance of the developed blue color was read at 760 nm (Shimadzu UV-1650 PC Shimadzu Corporation, Kyoto, Japan) against reagent blank. Garlic acid (100 mg/mL to 1000 mg/mL) was used to construct the calibration curve. Results were calculated as garlic acid equivalent (mg/g) of samples. The determination was done in triplicates and concentrations of phenolic compounds were calculated from obtained standard garlic acid graph.

Determination of total flavonoids content: Total Flavonoids Content (TFC) was determined spectrophotometrically using the method of Zhishen et al. [13] based on the formation of flavonoid-aluminium complex. An aliquot (0.5 ml) of the extract solution were mixed with 2 ml double distilled water, followed by 0.15 ml of 5% $NaNO_3$ solution. After 6 min, 2 ml of $AlCl_3$ (10%) was added, followed by addition of 0.5 ml of NaOH (1M) to the mixture. The mixture was diluted by adding 2.5 ml of double distilled water immediately, and then mixed thoroughly. Absorbance of the mixture, pink in color, was determined at 510 nm against reagent blank without extract. The absorbance of each blank consisting of some mixture in which $AlCl_3$ solution was substituted with double distilled water which was subtracted from the test absorbance. Rutin (0.04 µg/ml - 2.5 µg/ml) was used as standard and TFCs from extracts were expressed as µg-rutin equivalent TR/g dry weight of fruit sample. The concentrations of the flavonoids were calculated from obtained standard rutin graph.

Determination of ferric reducing power: The reducing power of the sample was determined according to the method described by [14], 1 ml of the extracts was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min. After incubation period, 2.5 ml of 10% trichloroacetic acid (TCA) was added and the reaction mixture was centrifuged at 2000 rpm for 10 min. The upper 2.5 ml layer was mixed with 2.5 ml of deionized water and 0.5 ml of ferric chloride and thoroughly mixed. The absorbance was measured spectrophotometrically at 700 nm. A higher absorbance indicates a higher reducing power.

Animal studies

Anthelmintic activity: Mature life Nigeria earthworm Pheretim. Posthuma (Annelid) was used to determine the effect of plant extracts by the method described by Ajaiyeoba et al. [15]. For this purpose, Nigeria earthworm P. posthuma (Annelid) was collected from moist garden soil of AfeBabalola University, Ado-Ekiti, Ekiti State, Nigeria. The average size of worms was 6 cm to 8 cm. The worm was washed in cold distilled water to remove dirt and finally suspended in Phosphate Buffered Saline (PBS). Worm was authenticated at the Parasitological Research Unit, Biological science Department, AfeBabalola University, Ado-Ekiti, Ekiti State, Nigeria. Five worms were exposed in three replicates to each of the following treatments in separate Petri dishes/test tubes at room temperature (25°C - 30°C): n-hexane fraction, ethyl acetate fraction, n-butanol fraction and residual aqueous fraction each at 100 mg/ml, 50 mg/ml and 25 mg/ml; levamisole at 0.55 mg/ml and PBS. The inhibition of motility/paralysis and/or mortality of worms kept in different treatments were used as criterion for the anthelmintic activity.

Results

Figure 1A presents the *in vitro* anthelmintic activity of n-butanolic fraction on Pheretima postuma. The response to treatment was concentration dependent (25 mg/ml, 50 mg/ml, 100 mg/ml). Group 2 (25 mg/ml) is significantly different from group 4 (100 mg/ml) at $p<0.05$ while group 1 (standard) is not significantly different from group 3 (50 mg/ml) ($p<0.05$).

Figure 1B presents the *in vitro* anthelmintic activity of n-hexane fraction on Pheretima postuma. The response to treatment was concentration dependent (25 mg/ml, 50 mg/ml, 100 mg/ml) with significant differences in all the groups (standard, 25 mg/ml, 50 mg/ml and100 mg/ml) at $p<0.05$.

Figure 1C presents the *in vitro* anthelmintic activity of ethyl acetate fraction on Pheretima postuma. The response to treatment was concentration dependent (25 mg/ml, 50 mg/ml, 100 mg/ml). There is no significant difference between group 1 (standard) and 4 (100 mg/ml) but significantly different when compared to group 2 (25 mg/ml) and 3 (50 mg/ml) at $p<0.05$.

Figure 1D presents the *in vitro* anthelmintic activity of residual aqueous fraction on Pheretima postuma. The response to treatment was concentration dependent (25 mg/ml, 50 mg/ml, 100 mg/ml). There is no significant difference between group 1 (standard) and 4 (100 mg/ml) at $p<0.05$ while group 2 (25 mg/ml) is significantly different from group 3 (50 mg/ml) at $p<0.05$.

Discussion

Helminthes infections, like other parasitic infections remain one of the most widespread infections in humans, owing to its effect on large population of the world [16]. There is therefore a need to pay attention to the existing helminthic infections as the majority of infections due to helminthes are injurious to health. Helminthes has its link to the development of anemia, pneumonia, undernourishment, eosinophilia and some other secondary complications [4]. In this study, the anthelmintic activities of four fractions (n-hexane, ethyl acetate,

n-butanol and residual aqueous) of the leaf extract of *B. ferruginea* were investigated and result shows potency against the investigated worm (Pheretima posthuma). At concentration above 50 mg/ml, the fractions demonstrated higher anthelmintic potency against all the investigated worms compared to the reference anthelmintic drug (Figure 1A-D). The anthelmintic potential of *B. ferruginae* leaves varied with solvent used in extraction of active ingredients with n-hexane fractions being the most potent (Figure 1A-D). This could probably be related to the different chemical ingredients extracted in the different solvents and their biological effects on parasites. The variation in potency may also be attributed to the sources of parasite and previous exposure to the plants. Similar variation in potency and efficacy was observed by Costa et al. [17] and Gakuya [18], when they used different solvents for extraction of active ingredient and observed varying bioactivity results. Similarly, Tuwangye and Olila [19] used methanol to extract *vernonia amygdalina* for anthelmintic bioassay and achieved 50% death. The study showed that efficacy of fractions increased with increasing concentration of fractions. Increasing motility inhibition with increasing concentration could be due to the saturation of target receptors. Similar observation were made by Lullman et al. [20] who said that the receptors get saturated with increasing dose of active ingredient that increases with incubation period. It is likely that at higher concentration, all binding receptors on the worms were occupied; thus leading to hyper polarization of membranes thereby limiting excitation and impulse transmission which leads to flaccid paralysis of worm muscles [21].

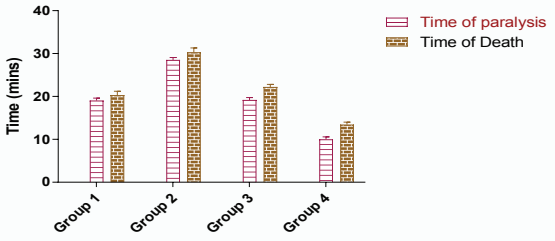

Figure 1A: *In vitro* anthelmintic potential of n-butanol fraction of the leaves of *Bridellia ferruginea* on *Pheretima posthuma* (Earthworm).
Values are expressed as mean ± standard error of mean (p<0.05)
Legend:
Group 1: Received a standard drug (levamisole) (0.55 mg/ml)
Group 2: Received 25 mg/ml n-butanolic fraction of the leaf extract
Group 3: Received 50 mg/ml n-butanolic fraction of the leaf extract
Group 4: Received 100 mg/ml n-butanolic fraction of the leaf extract

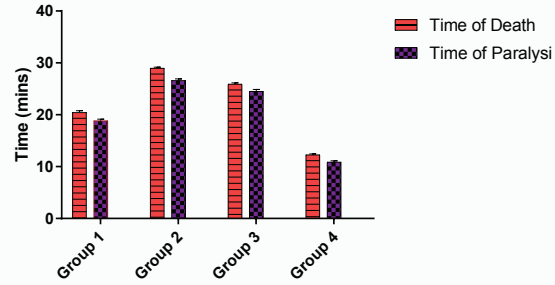

Figure 1B: *In vitro* anthelmintic potential of n-hexane fraction of the leaves of *Bridellia ferruginea* on *Pheretima posthuma* (Earthworm).
Values are expressed as mean ± standard error of mean (p<0.05)
Legend:
Group 1: Received a standard drug (levamisole) (0.55 mg/ml)
Group 2: Received 25 mg/ml n-hexane fraction of the leaf extract
Group 3: Received 50 mg/ml n- hexane fraction of the leaf extract
Group 4: Received 100 mg/ml n- hexane fraction of the leaf extract

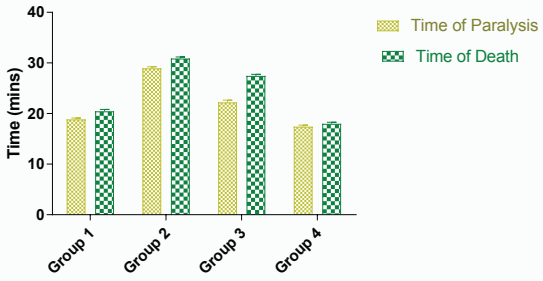

Figure 1C: *In vitro* anthelmintic potential of ethyl acetate fraction of the leaves of *Bridellia ferruginea* on *Pheretima posthuma* (Earthworm).
Values are expressed as mean ± standard error of mean (p<0.05)
Legend:
Group 1: Received a standard drug (levamisole) (0.55 mg/ml)
Group 2: Received 25 mg/ml ethyl acetate fraction of the leaf extract
Group 3: Received 50 mg/ml ethyl acetate fraction of the leaf extract
Group 4: Received 100 mg/ml ethyl acetate fraction of the leaf extract

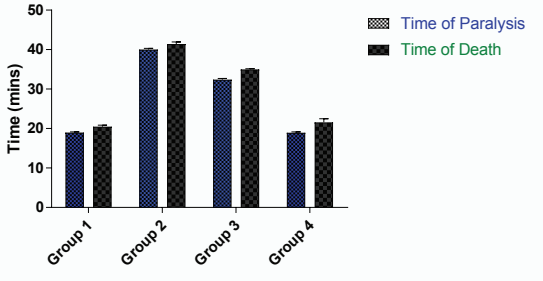

Figure 1D: *In vitro* anthelminticpotential of residual aqueous fraction of the leaves of *Bridellia ferruginea* on *Pheretima posthuma* (Earthworm).
Values are expressed as mean ± standard error of mean (p<0.05)
Legend:
Group 1: Received a standard drug (levamisole) (0.55 mg/ml)
Group 2: Received 25 mg/ml residual aqueous fraction of the leaf extract
Group 3: Received 50 mg/ml residual aqueous fraction of the leaf extract
Group 4: Received 100 mg/ml residual aqueous fraction of the leaf extract

The anthelmintic properties of *B. ferruginae* leaf fractions could be attributed to the variety of secondary metabolites present. Previous studies have revealed the presence of several phytochemicals (flavonoids, alkaloids, tannins, and cardiac glycosides, anthraquinone, phlobatinnins and saponin) in various morphological parts of the plant [22]. Notwithstanding, Waterman [23] reported that plant metabolites are unstable molecules and their biological activity are dependent on their structure, physical and chemical properties. It is therefore possible that the parasite paralysis and/or death observed could be attributed to secondary metabolites [24] like tannins, alkaloids and saponins among others. These plant metabolites may have worked singly or in combination to cause the motility inhibition, paralysis or death of the worms that was achieved in all the studied plant fractions. Kaufman et al. [25] explained the synergistic interactions to underlie the effectiveness of phyto-medicines that lead to better activity of some individual constituents. Briskin [26], Wynn and Fougere [27] acknowledged that plant metabolites action may be additive, synergistic or antagonistic in manner acting at single or at multiple target sites. It is therefore likely that a number of compounds could have contributed to the anthelmintic activity observed in the studied plant fractions.

Antioxidants have a wide range of biochemical activities including

inhibition of the activities of Reactive Oxygen Species (ROS), direct or indirect scavenging of free radicals and alteration of intracellular redox potential [28]. Free radicals and other reactive oxygen species are generated continuously via normal physiological process, more so in pathological conditions. These free radicals are associated directly or indirectly with most of the pathologies known to date [29]. The use of natural antioxidants has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents [30,31]. In the present study, the fractions exhibited a strong antioxidant activity in the order of decreasing magnitude; n-hexane > ethyl acetate > residual aqueous > butanol (Table 1), hence conferring greatest potency on the n-hexane fraction and also showed its ability to quench the radicals. This agrees with similar studies by Kaibing et al. (2011) on the seed of Carica papaya.

Table 1: Total phenol, total flavonoid and ferric reducing ability of the leaf fractions of *Brideliaferruginea*.

Treatment	Total phenol (mg/GAE g)	Total flavonoid (mg/QUE g)	Ferric reducing ability (mg/g)
n-Hexane fraction	14.14 ± 0.30^{a}	7.82 ± 0.10^{a}	117.90 ± 1.13^{a}
n-Butanol fraction	8.42 ± 0.14^{c}	2.60 ± 0.11^{b}	45.67 ± 0.37^{d}
Ethyl acetate fraction	13.42 ± 0.41^{b}	5.76 ± 0.07^{c}	90.90 ± 0.13^{b}
Residual aqueous fraction	6.57 ± 0.04^{c}	3.78 ± 0.06^{b}	59.64 ± 0.01^{c}

Values are mean ± SEM (n=3).
Values that have the same superscript along the column are not significantly different ($p<0.05$).

Conclusion

The overall findings of the study showed that the investigated fractions (n-hexane, ethyl acetate, n-butanol and residual aqueous) of the leaf extract of *B. ferruginea* exhibit evidence of *in vitro* anthelmintic activity and antioxidant properties against Pheretima posthuma in a dose-dependent manner (25 mg/ml, 50 mg/ml and 100 mg/ml). This justifies their traditional ethno-veterinary use by pastoral communities around the world. However, potency of plant fractions was dependent on the solvent used to extract the active ingredients (n-hexane > ethyl acetate > residual aqueous > n-butanol). Further studies are needed to determine its activity against other developmental stages of parasites.

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