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Anthelmintic Efficacy of *Brysocarpus coccineus* Leaf and Fractions on *Haemonchus contortus*

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ABSTRACT

The anthelmintic efficacy of Brysocarpus coccineus leaves were carried out to establish the inhibitory anthelmintic activities of the crude extract and fractions against the gastrointestinal nematode Haemonchus contortus. At the concentration of 12.5 mg/ml, the crude extract of B. coccineus showed a significant different (p<0.05) on the ovicidal and larvicidal inhibition of the parasite at 99.3% and 96.8% respectively, when compared with the 100% inhibitory effect of albendazole. The ovicidal fractions of B. coccineus had no significant different (P>0.05) from the activity of albendazole, while significant different (P<0.05) was observed with all the larvicidal fractions of B. coccineus when compared with albendazole against H. contortus. However, at the concentration ranges 0.78- 12.5 mg/ml, the crude extracts of B. coccineus and the fractions showed an anthelmintic activity against H. contortus. The results of this study revealed that the anthelmintic efficacy of the crude extract and fractions of B. coccineus significantly (p < 0.05) inhibited the hatching of H. contortus eggs and larvae in a concentration-dependent manner. Therefore, this study requires further in vivo study to confirm the toxicity activities of this plant for anthelmintic purposes.

Keywords: *Brysocarpus coccineus, Anthelmintic, Ovicidal, Larvicidal, Haemonchus contortus, Crude, fractions.*

INTRODUCTION

Parasitic diseases such as helminthosis have been implicated as one of the health problems constraining productivity of ruminant livestock [1]. Parasitic diseases are inherent ecological problems and their endemicity or suppression is dependent upon man's ability to control his environment; a variety of parasitic helminths are known to infest animals, birds or man [2].

Haemonchus contortus is the major cause of morbidity and mortality in livestock and responsible for a considerable economic loss in productivity to farmers; the control of this nematode has relied largely on the use of a limited number of anthelmintics [3].

The most common diseases affecting the productivity of sheep and goats are pneumonia and helminthosis, with *Haemonchus contortus* as the most pathogenic and highly prevalent gastrointestinal nematodes encountered in small ruminant livestock production in the tropical and subtropical regions [4].

In tropical Africa, intestinal parasites are regarded as an important health problem because many parasitic infections especially those of the helminths are usually asymptomatic or produce only mild symptoms and are often neglected until serious complications or chronic clinical features begin to appear. Infections by helminthic parasites are among the most common and economically important diseases of grazing livestock [5; 6].

Chemotherapy of parasitic diseases would involve interference with the life processes of the parasite and as such it is imperative to explore their life cycle and metabolic activities and host-parasite relationships [2; 7]. The development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintics [8].

In respect of the development of anthelmintic resistance in the parasites of higher economic significance, chemotherapy is still used widely for the purpose of controlling the helminths [9]. To explore the potentials of indigenous knowledge, both basic and applied research is important. As an applied research, evaluation of medicinal herbs has been carried out by several studies against various parasites and/or infectious agents. Some of these led to the discovery of effective cure for hepatitis B virus infection in humans [10; 11; 12]. A number of medicinal plants have been used to treat parasitic infections in man and animals [13]. *Dregea volubilis* (family Asclepediaceae) is widely used as anthelmintic in folkoric medicine in India [14]. *Vernonia lasiopus*, *Entanda leptostachya* and *Prosopis juliflora* as anthelmintics in Kenya [15; 16; 17].

Research have shown that plant natural products have pharmacological activities and are also useful as ethno-veterinary remedies and the advancement of natural products reveals the efficacy of isolated compounds in the treatment of gastrointestinal infections [18]. *Brysocarpus coccineus* is a scandant shrub or liane of savannah thickets and secondary jungle with delicate pink-tinged and sweet-scaled flowers [21]. The plant is commonly found across west and tropical Africa.

B. coccineus is popularly known in Ghana by the Twi and Gar people as "awenda" or "awende." In Northern Nigeria, it is referred to by the Hausas as "Tsamiyar kasa or kimbar maharba. The Yoruba tribe from the western Nigeria call it

Orikoteni; Amuje [22; 23].

Previous studies have shown that the plant extract possesses analgesic [24], antimicrobial [25. 26], anti-inflammatory [25], antipyretic [24], anxiolytic and sedative effects [27]. The plant is also used by traditional African healers for the management of mental illnesses and disorders of the central nervous system [28]. In Zimbabwe, ethno-veterinary medicine is gaining widespread recognition at the expense of conventional drugs especially because of its greater accessibility, lower costs and apparent effectiveness [19]. The cost of modern medicines is increasing with trends in technology and also the cost of modern health care has equally increased in recent times due to high cost of basic commodities [20]. This has prompted the need to investigate and validate the folkoric claims of the anthelmintic activity *Byrsocarpus coccineus* leaves against the eggs and the larval stages of *Haemonchus contortus*.

MATERIAL AND METHODS

Study area and location

The first phase of the study was carried out at the Helminthology laboratory of the Department of Parasitology and Entomology, Faculty of Veterinary Medicine and in Pharmacognosy and drug development unit, Faculty of Pharmaceutical Sciences, both in Ahmadu Bello University, Zaria, Nigeria. Zaria is located in the North Central part of Nigeria on the Kubbani river (a tributary of river Kaduna). It has coordinates of 11°04´N 7°42´E and a total land square meter of 563km² (217sq m) [29]. The inhabitants of Zaria are livestock farmers, and are also known for leather work, dyeing and cap making [30]. Zaria has a tropical wet and dry climate with warm weather all year-round; a wet season lasting from April to September and a drier season from October to March.

Plant collection and identification

The plant and part of *Byrsocarpus coccineus* used for this study were collected in April, 2024 from Orba in Udenu Local Government Area of Enugu State, Nigeria. The plant material was identified by Mr. Alfred Ozioko a botanist with Bio-resources Development and Conservation Program (BDCP), Nsukka. The plant materials were air-dried, pulverized using mortar and pestle, then stored for extraction purpose.

Extraction of plant materials

The pulverized plants materials were weighed and extracted according to method described by [31]. Three hundred grams of each plant materials were weighed, wrapped in the tumble sack, placed in the soxhlet apparatus and extracted with one litre of 80% methanol. The crude plant extract was collected in a flask attached to the soxhlet apparatus; the methanol extract obtained was concentrated to dryness over water bath at 40-50 ˚C. The brown, semi-solid, oily extract obtained was weighed and stored in a labeled bottle at room temperature (27˚C).

Solvent partitioning of the crude extract

The crude extracts of the selected plants were subjected to column fractionation and partitioning with the solvents, petroleum ether, ethyl acetate and n-butanol in order of increasing polarity, using 150 ml of each solvent. The whole process was repeated three times for each solvent and before it was concentrated to dryness as described by Simon *et al*. (2008) and Olayemi *et al*. (2020).

Data analysis

Data generated were subjected to statistical analysis on SPSS version 20. The number of helminth eggs hatched and larval development inhibited at different concentrations of the extract were analyzed using one-way analysis of variance (ANOVA) and LSD post hoc test. Mean differences at p < 0.0.5 were considered significant.

RESULTS

The preparation of the crude extract of *B. coccineus* leaves gave a yield of 15% (Table 1).

Qualitative phytochemical analysis of the extract of *Byrsocarpus coccineus* leaves revealed the absence of anthraquinone, while other metabolites including saponins, alkaloids, carbonhydrates, steroids, triterpenes, flavonoids and phenols were present (Table 2). Quantitative phytochemical analysis of some metabolites in *B. coccineus* revealed that phenols had the highest percentage abundance of 21.7 %, whereas alkaloids had the lowest value of 5.00 % (Table 3). The mean (±s.e.) percentage inhibition of egg hatching and larval development assay of crude extract of *B. coccineous* against *H. contortus* at various concentrations are shown in Tables 4 and 5 respectively, while the Mean (±s.e.) Percentage inhibition of egg hatch and the larval development by the fractions are shown in Tables 6 and 7 respectively. However, the crude extract and fractions of the selected plants displayed effective anthelmintic activities against *H. contortus*. The anthelmintic activity of the crude and fractions of *B. coccineus* leaves in this study was observed to be concentrations dependent.

Table 1: Percentage yields of crude extract of *B. coccineus* leaves

Plant sample	Plant part	Quantity of plant sample (g)	Percentage yield
B. coccineus	Leaves	300	15

Table 2: Qualitative phytochemical analysis of *Byrsocarpus coccineus* leaves

Compound	*Byrsocarpus coccineus*
Saponins	+
Alkaloids	+
Carbonhydrates	+
Steroids	+
Triterpenes	+
Cardiac glycosides	+
Tannins	+
Flavonoids	+
Anthraquinones	-
Phenols	+

Key: + = Presence; - = Absence

Table 3: Quantitative phytochemical analysis of *Brysocarpus coccineous* metabolites.

Plant material	Flavonoids (%)	Alkaloids (%)	Tannins (%)	Phenols (%)	Saponins (%)
B. coccineus	9.3	5.00	10.0	21.7	9.83

Inhibition of *H. contortus* egg hatch by the crude extract of *B. coccineous*

At the lowest concentration (0.78 mg/ml), the crude extract of *B. coccineous* produced 94.17 ± 1.04% relative to Albendazole (96.8 ± 1.04%) which was significantly ($p<0.05$) lower. And at the highest test concentration of 12.5 mg/ml, the crude extract of *B. coccineous* also caused a significantly ($p<0.05$) reduced egg hatch inhibition (99.33 ± 0.28%) compared to Albendazole (100 **± 0.0**0%) (Tables 4).

Table 4: Mean (± s.e.) Percentage inhibition of egg hatch by crude extract on *H. contortus*.

Treatment groups	Concentrations				
	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
B. coccineus	94.17 ± 1.04^{a}	95.83 ± 0.76^{a}	97.5 ± 0.50^{a}	97.83 ± 0.28^{a}	99.33 ± 0.28^{a}
Albendazole	96.8 ± 1.04^{b}	98 ± 0.50^{b}	99 ± 0.00^{b}	99.8 ± 0.289^{b}	100 **± 0.00** ^{b}
Distill water	6.2 ± 2.33	-	-	-	-

Means with different superscript letter (a, b) differ significantly ($p<0.05$) from each other and from distilled water-treated group

Inhibition of *H. contortus* larval development by crude extract

At 0.78 mg/ml, *B. coccineus* crude extract caused larval development inhibition of 54.2 ± 2.03% compared to Albendazole (73.47 ± 2.25%); while at 12.5 mg/ml, crude extract also caused larval development inhibition of 96.8 ± 0.0% relative to Albendazole (100 ± 0.0%). The larval development inhibition produced by the reference anthelmintic (albendazole) both at the lowest and highest concentrations were significantly ($p<0.05$) higher (Table 5).

Table 5: Mean (± s.e.) Percentage inhibition of larval development by crude extract on *H. contortus*

Treatment groups	Concentrations				
	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 mg/ml	12.5 mg/ml
B. coccineus	54.2 ± 2.03^{a}	53.2± 2.15^{a}	60.6 ± 2.99^{a}	85.3 ± 2.50^{a}	96.8 ± 0.00^{a}
Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.2^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
Distill water	13 ± 1.15				

Means with different superscripts (a, b) differ significantly ($p<0.05$) from each other and from distilled water-treated group

Inhibition of *H. contortus* egg hatch by the fractions

N-butanol and Ethyl acetate fractions exhibited comparable percentage inhibition of *H. contortus* egg hatch (92.3 ± 1.04 and 92.3 ± 1.76 respectively) at the lowest test concentration (0.78/ml) and both fractions also produced a complete (100%) inhibition of the parasite's egg hatch at 12.5 mg/ml. This contrasts significantly ($p<0.05$) with lower percentage inhibition of egg hatch produced with petroleum ether fraction (90.07 ± 4.43%) or the aqueous fraction (86.3 ± 0.76%) at the lowest test concentration (0.78/ml) and petroleum ether fraction (98.7 ± 0.46%) or aqueous fraction (97.6 ± 1.70%) at 12.5 mg/ml, respectively (Table 6).

Table 6: Mean (± s.e.) Percentage inhibition of *H. contortus* egg hatch with *B. coccineus* fractions

Fractions	**Treatment**	**Concentrations (mg/ml)**				
		0.78	1.56	3.125	6.25	12.5
N-Butanol	*B. coccineus*	92.3±1.04^{a}	94.17 ± 1.26^{a}	96.5 ± 1.32^{a}	98.3 ± 1.26^{b}	100.0 ± 0.87^{b}
Ethyl acetate	*B. coccineus*	92.3 ± 1.76^{a}	94.3 ± 1.26^{a}	96.5 ± 0.50^{a}	98.3 ± 0.289^{b}	100 ± 0.00^{b}
Petrol. Ether	*B. coccineus*	90.07 ± 4.43^{d}	91.83 ± 3.30^{d}	93.67 ± 3.2^{d}	94.3 ± 2.08^{d}	98.7 ± 0.46^{c}
Aqueous	*B. coccineus*	86.3 ± 0.76^{e}	88.7 ± 0.87^{e}	90.2 ± 0.87^{e}	93.6 ± 0.69^{e}	97.6 ± 1.70^{d}
	Distilled water	6.2 ± 2.33				

Means with different superscript letter (a, b, c, d and e) differ significantly ($p<0.05$) from each other and from distilled water-treated group.

Inhibition of *H. contortus* larval development by fractions

N Butanol, Ethyl acetate, Petroleum ether and Aqueous fractions inhibited larval development by 90 ± 3%, 90 ± 0%, 85 ± 3%, 93.17 ± 3% respectively relative to Albendazole (100 ± 0%) at 12.5 mg/ml (Table 7).

Table 7: Mean (± s.e.) Percentage inhibition of larval development by fractions on *H. contortus*

Fractions	Treatment	0.78 mg/ml	1.56 mg/ml	3.125 mg/ml	6.25 m/ml	12.5 mg/ml
N- Butanol	*B. coccineus*	62.3 ± 2.00^{a}	67 ± 2.00^{a}	82 ± 2.00^{a}	87± 2.00^{a}	90 ± 3.00^{a}
Ethyl acetate	*B. coccineus*	52 ± 6.08^{c}	59.6 ± 3.79^{c}	70 ± 0.00^{c}	81.3 ± 1.15^{c}	90 ± 0.00^{c}
Petroleum Ether	*B. coccineus*	55.1 ± 3.00^{d}	60.5 ± 2.00^{d}	65 ± 3.00^{d}	75 ± 3.00^{d}	85 ± 3.00^{d}
Aqueous	*B. coccineus*	53 ± 3.00^{e}	60 ± 4.00^{e}	68.5 ± 2.00^{e}	80.0 ± 0.00^{e}	93.17 ± 3.06^{e}
	Albendazole	73.47 ± 2.25^{b}	77 ± 1.73^{b}	91.3 ± 3.20^{b}	100 ± 0.00^{b}	100 ± 0.00^{b}
	Distilled water	13 ± 1.15				

Means with different superscript letter (a, b, c, d and e) differ significantly (p<0.05) from the positive control group.

Discussion

In this study, 300 g of *Byrsocarpus coccineus* leaves was weighed, pulverized and used for the phytochemical analysis. The percentage methanol crude extract (MCE) yields of *B. coccineus* was 15.0% as observed in the present study. Apart from the species difference, other factor that may affect biochemical profile and yields of plant extracts includes plant parts used, age, harvest season and habitat [32]. The MCE and the fractions (petroleum ether and ethyl acetate) were partitioned with different solvents to separate the phytochemical components based on their polarity and solubility [31].

The crude extracts and fractions of *B. coccineus* produced anthelmintic activity which inhibited hatching of eggs and larval development of *H. contortus*. The crude extracts of *B. coccineus* produced 99.3% and 96.8% for egg hatch and larval development inhibition at 12.5 mg/ml respectively. Statistically, there was significant difference (P<0.05) on the anthelmintic activity of *B. coccineus* as compared to albendazole against *H. contortus* eggs and larvae inhibition. The results of this *in vitro* anthelmintic study also revealed that the crude extracts and fractions significantly ($p < 0.05$) inhibited the hatching of *H. contortus* eggs and killed the larvae of the helminth in a concentration-dependent manner. *B. coccineus* extracts and fractions showed significant difference ($p < 0.05$) was statistically observed on the inhibitory effect on hatching of *H. contortus* eggs when compared with the anthelmintic activity of albendazole [33], while *B. coccineus* (aqeuous and petroleum ether) also showed a significant difference ($p<0.05$) from ovicidal activity of albendazole as observed in this study.

The fractions of *B. coccineus* produced significant ($p<0.05$) larvicidal activity and this compared with the larvicidal activity of albendazole on *H. contortus* parasite. The present study revealed that the tested crude extract and fractions were able to inhibit 50% egg hatching and mortality of larvae at the concentration ranges of 0.78-12.5 mg/ml as observed when compared with albendazole. This study also showed that the ovicidal inhibitory effects was higher than that of the larvicidal activity of *H. contortus*. The higher ovicidal inhibitory effect produced by these crude extracts and fractions could be attributed to the penetration of the active different phytochemicals such as tannins, flavonoids and saponins into the parasite's egg shell [33; 34]. The possible explanation could also be attributed to the difference in the structure of the egg shell as compared to the cuticle of the larvae and their individual mode of membrane absorption of phytochemical compounds [35].

Previous study on medicinal plant extracts have also been reported to affect the biology of the parasitic eggs [36]. The larvicidal effect produced by these extracts was likely due to the penetration of the active chemical constituents of the extracts across the cuticle of the larvae into their circulatory system when the larvae had contact with the extracts as reported earlier by [37] The tannins, alkaloids and saponins contained in plants have been reported to possessed anthelmintic efficacy against helminths activities [38; 39]. The anthelmintic effects of these metabolites may be attributed to their capacities to bind free protein available in the tubes for larval nutrition and thus reduced nutrient availability. This could have resulted in larval starvation and causing larval death [40].

The investigation of phytochemical metabolites from *B. coccineus* plants is fundamentally important for the development of new anthelmintic drugs and may offer an alternative source for the control of gastrointestinal nematodes of small ruminants. Therefore, the findings from this study suggest that the crude and fractions extracts of *B. coccineus* leaves possess anthelmintic activities for egg hatch inhibition and larval development inhibition of *H. contortus*. However more detailed studies are required to identify and evaluate the active constituents and mechanism of action of these crude extract and fractions.

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