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# Anthelmintic Activity of *Nauclea diderrichii* Leaf Extracts and Fractions Against Adult *Haemonchus placei*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

## Abstract

**Background:** *Nauclea diderrichii* is used in West and Central Africa countries for its insecticidal and antiparasitic properties. There is, however, no documentation on its activity against parasitic nematodes.
**Objectives:** This study investigated *N. diderrichii* leaf extracts and fractions for anthelmintic activity against adult *Haemonchus placei*, an abomasal parasitic worm that infect cattle.
**Materials and Method:** Pulverized dried leaves of *N. diderrichii* was defatted using *n*-hexane, followed by successive extraction using chloroform and acetone. The extracts were evaluated for anthelmintic activity by incubating ten (10) adult *H. placei* in a range of concentrations (1−20 mg/mL) for 3h and then counting the number of confirmed dead worms. The most active extract was subjected to chromatographic fractionation, and the thin layer chromatographic (TLC) profile of the fractions was documented. The dried fractions were then evaluated for anthelmintic activity and anthelmintic data were fitted to a non-linear regression equation (Log [extract or fraction] vs. response; variable slope) to produce best-fit sigmoidal curves and $LC_{50}$ values computed.
**Results:** Anthelmintic assay shows that acetone extract is worm-active with a best-fit $LC_{50}$ of 16.24 mg/mL, while the chloroform extract was inactive. Fractionation of the acetone extract yielded three fractions (FA, FB and FC) and TLC profile revealed the various secondary metabolites in the fractions. Of the fractions, only fraction B was active against *H. placei* with $LC_{50}$ of 12.24 mg/mL.
**Conclusion:** Acetone extract of *N. diderrichii* leaf possessed anthelmintic activity against adult *H. placei*, and could be useful as anthelmintic phytomedicine to deworm cattle.

**Keywords:** Anthelmintic, *Haemonchus placei*, *Nauclea diderrichii*, Chromatography

## INTRODUCTION

The discovery and introduction of modern anthelminthic drugs starting with thiabendazole in the early 1960s have meaningfully helped to curtail the negative impacts of parasitic gastrointestinal worms on livestock production (Sargison, 2012; Vercruysse *et al.*, 2018). However, their widespread use has become a contributory factor to emergence of resistance which is now the *status quo* (Kaplan and Vidyashankar, 2012; Geurden *et al.*, 2015). These compounds were generally not used in a rational, sustainable manner, being often overused, applied incorrectly or adulterated (Van Wyk *et al.*, 1997). To this end, alternative control measures are strongly being advocated (Molento, 2009; Hoste and Torres-acosta, 2011).

Medicinal plants, with their rich and structurally diverse secondary metabolites, represent a potential means of controlling parasitic nematode infection and discovering new anthelmintic agents (Anthony *et al.*, 2005; Pan *et al.*, 2013). A number of these plants are

being used in ethnomedicine to control worm burdens (Githiori *et al.*, 2005). Already, this has spurred a renewed interest in the exploration of medicinal plants for their activity against many parasitic helminths (Athanasiadou *et al.*, 2007; Ademola, 2016). This approach becomes even more imperative because of public health and safety concerns regarding veterinary drug residues in livestock products (Sutherland *et al.*, 2013).

*Nauclea diderrichii* (De Wild.) Merr. (Rubiaceae) is a widely used medicinal plant from West and Central Africa (Lamidi *et al.*, 1995). Its natural habitat is sub-tropical or tropical moist lowland forests. The plant has a broad spherical crown with thick foliage and is known to be widely used in traditional medicine for its insecticidal and antiparasitic properties (Di Giorgio *et al.*, 2006). The bark is reported to be used for the treatment of abdominal pains and as a vermifuge (aqueous decoction) in Congo (Lamidi *et al.*, 1996; Mbuta *et al.*, 2012). Literature search, however, revealed no scientific documentation on the anthelmintic activity of any part of this plant against parasitic nematodes. Since it is a well-established fact that harvesting of leaves on sustainable basis for medicinal purpose is less damaging to the plant (Gaoue and Ticktin, 2007), this study investigated two different solvent extracts and fractions of *N. diderrichii* leaf against adult *Haemonchus placei*, an haematophagous, abomasal nematode of cattle (Giudici *et al.*, 1999).

## METHODOLOGY

### *Plant material*

Fresh leaves of *N. diderrichii* were collected from the trees growing within the University of Ibadan, Ibadan, Nigeria. The plant was authenticated and herbarium specimen (FHI-110022) deposited at Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan.

*Extraction*

The leaves were air-dried in the shade at room temperature (26-30 ºC) over two weeks and thereafter blended into coarse powder using laboratory blender (Elgento-125, China). The powdered plant material (1.06 kg) was first defatted using *n*-hexane (2 L) for 24 h. Afterwards, the dried marc was macerated twice using two different solvents, chloroform and acetone, in succession (2 L x 2, for 24 h). The supernatants were filtered through Whatman No 1 filter papers into clean glass containers. The extracts were concentrated under vacuum at 40°C into smaller volumes and then evaporated to dryness on a water bath at 50°C. The quantities extracted and the percentage yields were determined.

### *Source of Adult worms*

Adult worms were collected from the abomasum of freshly slaughtered cattle at the Bodija Abattoir, Bodija Market, Ibadan, Oyo state, Nigeria. The worms were identified as *Haemonchus placei* at the Parasitology Research Unit, Department of Veterinary Parasitology and Entomology, University of Ibadan.

### *Anthelmintic evaluation of extracts*

Stock solutions (20mg/mL) of each extract were prepared in 20% Tween 80 in normal saline. Test concentrations (1−20 mg/mL, 0.5mL), prepared from the stocks, were placed into wells of a 24-well flat bottom microtiter plate. Ten (10) adult worms per well, in duplicates, were incubated in the test concentrations at ambient temperature (26-30 ºC) for 3 h. Worms were thereafter moved into Petri dishes containing warm (40°C) normal saline and observed for any revival of motility. After 30 minutes in warm normal saline, worms were categorized as dead following no revival of motility, and no noticeable movement on being pin-pricked. 20% Tween 80 in normal saline were used as control (Ogedengbe *et al.*, 2019). Anthelmintic activity data were fitted to a non-linear regression equation (Log [compound] vs. response; variable slope) to produce best-fit sigmoidal curves from which median Lethal Concentration ($LC_{50}$) values were computed with associated uncertainty using GraphPad Prism 7 (GraphPad Software Inc., California, USA).

### *Vacuum Liquid Chromatographic fractionation of acetone extract*

The outcome of the initial anthelmintic evaluation of the two solvent extracts informed the choice of acetone extract for chromatographic fractionation. One gram (1 g), dissolved in acetone, was adsorbed on $SiO_2$ (5 g). The dried, adsorbed extract was then packed onto sintered glass earlier prepacked with $SiO_2$ (30 g) with the aid of a vacuum pump and eluted using varying solvent mixtures [(*n*-Hex: EtOAc (5:5) → EtOAc → MeOH), 300 mL each] to yield three fractions (FA, FB, and FC). The eluates were monitored by thin layer chromatography using EtOAc/ *n*-Hex (80:20, 10ml) as mobile phase, while the developed chromatogram was visualized under UV-light at wavelength of 365 nm. The fractions were concentrated under vacuum and dried *in-vacuo* at 40°C.

### *Anthelmintic evaluation of chromatographic fractions*

Stock solutions (20 mg/mL) of the fractions (A-C) were prepared in 20% Tween 80 in normal saline.

From these stocks, varying concentrations (1 – 20 mg/mL, 0.5mL), in duplicates, were prepared and placed into the wells of a 24-well flat bottom microtiter plate. Thereafter, ten adult worms per well were placed in the test concentrations and incubated for 3 h at room temperature. 20% Tween 80 in normal saline was used as control. The number of dead worms were determined and the anthelmintic data processed as stated.

**RESULTS**

The percentage yields were 3.3 and 5.2 (% w/w) for chloroform and acetone, respectively. The result of the anthelmintic evaluation solvent extracts is shown in Figure 2. Of the extracts of *N. diderrichii* evaluated, acetone extract gave a best-fit $LC_{50}$ of 16.24 mg/mL, while chloroform extract was inactive against adult *H. placei* at 20 mg/mL, the highest concentration tested. Three fractions were obtained from the chromatographic fractionation of the acetone extract with the following yields: *n*-Hex/EtOAc (FA), 44.6; EtOAc (FB), 8.0; and MeOH (FC), 45.0 (% w/w). Thin layer chromatographic profile of the fractions, shown in Fig. 1, revealed the various secondary metabolites in the fractions. Anthelmintic evaluation of the fractions revealed that only FB was active against adult *H. placei* (Figure 2) with best-fit $LC_{50}$ of 12.24 mg/mL. Fractions FA and FC were inactive.

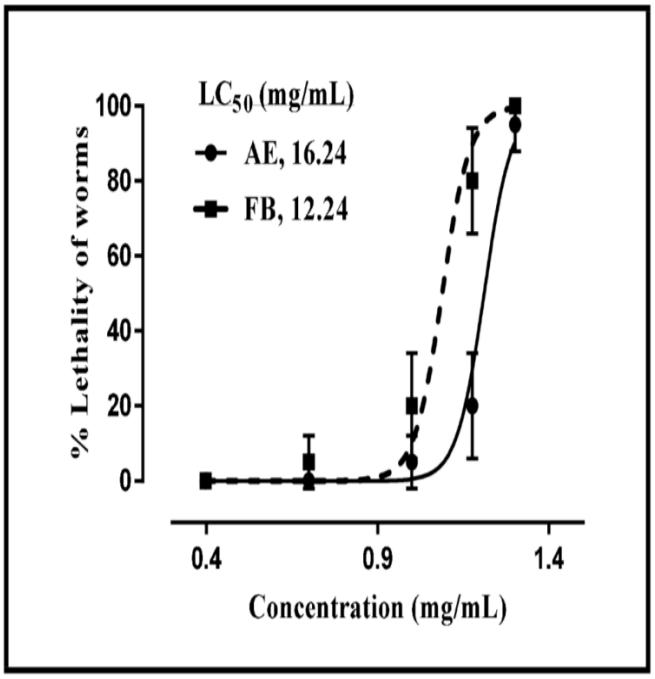


**Figure 1**. Anthelmintic activity profiles of AE (Acetone Extract) and FB (Fraction B, obtained from AE) of *Nauclea diderrichii* against adult *Haemonchus placei* exposed to varying concentrations (1–20 mg/mL) for 3 h at room temperature (26 ºC - 30 ºC). The stock solutions (20 mg/mL) of the AE and FB were made in 20 % Tween-80 in normal saline

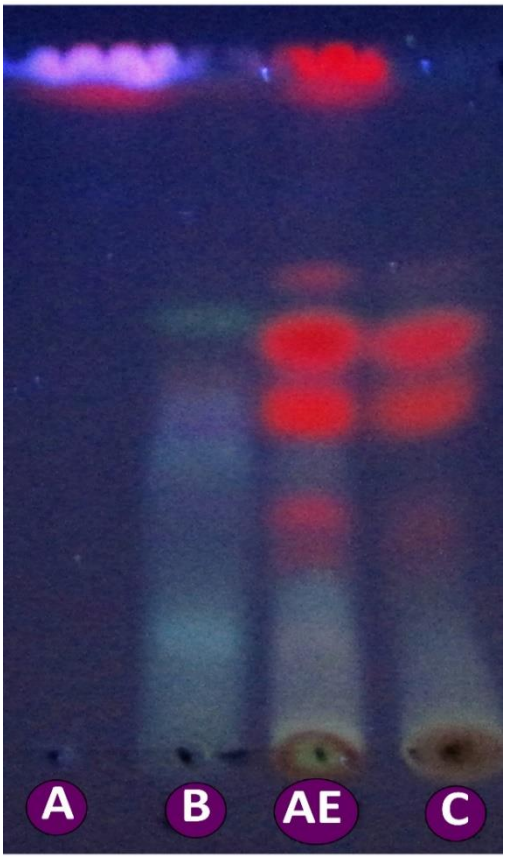


**Figure 2:** TLC profile of the chromatographic fractions A to C along with the acetone extract (AE) showing the various secondary metabolites present in the them as viewed under UV at 365 nm

**DISCUSSION**

*Nauclea diderrichii*, an important medicinal plant, is reported to be rich in bioactive secondary metabolites (Haudecoeur *et al.*, 2018). Specifically, the leaf has been reported to contain the following class of secondary metabolites compounds – alkaloids, saponins, phenolics, tannins, flavonoids, phytosterols and terpenoids (methanol extract); phenolics, phytosterols and terpenoids (ethyl acetate extract); alkaloids, saponins and flavonoids (aqueous extract) (Mbuta *et al*., 2012; Agnaniet *et al.*, 2016; Isa *et al.*, 2017). These structurally diverse phytochemicals have disparate solubilities in different solvents due to varying solute-solvent interactions partly influenced by their individual chemical structure (Aulton, 2002). Thus, successive maceration with solvents of varying polarity translates to selective extraction yielding semi-purified extracts containing structurally diverse active principles. Most of the non-polar constituents of the plant material devoid of hydroxyl, amino and other hydrophilic groupings are expected to be extracted into chloroform, a non-polar solvent; while most of intermediate polar and some polar constituents are expected to be extracted into acetone, an intermediate polar solvent with high solvent strength (Watson, 1999).

The anthelmintic evaluation of these two extracts led to an inactive chloroform extract and a worm-active acetone extract. The anthelmintic evaluation of the three chromatographic fractions helps to further narrow down on FB as the worm-active fraction. This fraction was eluted by 100% EtOAc, and based on the report of Isa et al. (2017), phenolics, phytosterols and terpenoids might be suggested as the bioactive compounds in this fraction. In addition, judging by the $LC_{50}$ of acetone extract and FB, FB was only slightly more potent than the acetone extract. However, both were far less potent when compared to the activity of levamisole, a known synthetic anthelmintic, against adult *H. placei* as reported by Ogedengbe et al. (2019). While one might judge it needful to put into consideration the way the plant is used ethnomedicinally – as barks decoction (Mbuta *et al.*, 2012), many studies have shown that water extracts usually have relatively poor bioactivity (Eloff, 1998; Ncube *et al*., 2011; Eloff *et al.*, 2017). This is further corroborated by work of Lamidi *et al*. (1996) which reported that aqueous-methanol extract (2:8, v/v) of *N. diderrichii* back (containing saponins and alkaloids)

exhibited potent antimalaria activity relative to stem and leaf decoction (100% aqueous) which showed weak antimalarial activity (Mustofa *et al.*, 2000). Moreover, an earlier study revealed that aqueous extracts of the back and leaf of *N. diderrichii* contain essentially similar secondary metabolites (Agnaniet *et al*., 2016).

Apart *N. diderrichii*, two other *Nauclea* spp which are known to be used traditionally in some African nations as anthelminthic agents are *N. latifolia* and *N. pobeguinii* (Haudecoeur *et al.*, 2018). In particular, *N. latifolia* has been reported to elicit anthelmintic activity against different livestock parasitic nematodes (Asuzu and Njoku, 1996; Onyeyili *et al.*, 2001; Ademola *et al.*, 2007; Josué *et al.*, 2012).

## CONCLUSION

This represents the first anthelmintic report on *N. diderrichii* leaf. The study shows that *N. diderrichii* leaf acetone extract possess anthelmintic activity against adult *H. placei* and could be useful as an anthelmintic phytomedicine to reduce parasitic worm burden in cattle.

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