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RESEARCH

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In vitro effect of three tropical plants on adult *Haemonchus placei*, an haematophagous nematode from cattle

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Abstract

Background: *Vernonia amygdalina* (leaf), *Garcinia kola* (seed), and *Leucaena leucocephala* (seed) are three well-known tropical plants used in African ethnomedicine to reduce parasitic worm burdens and are potential sources of alternative solution for controlling parasitic helminths infection in grazing livestock. This study investigated extracts from these plants for anthelmintic activity against adult *Haemonchus placei*, an haematophagous nematode from cattle abomasa. Powdered plant materials were macerated in acetone and the crude acetone extracts evaluated for anthelmintic activity using *H. placei* adult worm motility assay. Afterwards, fresh sample of *V. amygdalina* was macerated successively in chloroform and acetone and the extracts evaluated for anthelmintic activity. The chloroform extract was subjected to phytochemical and FT-IR analyses and fractionated by vacuum liquid chromatography. Anthelmintic data were fitted to a nonlinear regression equation (Log [extract or fraction] vs. lethality; variable slope) to produce best-fit sigmoidal curves and LC_{50} values computed with associated uncertainty.

Results: Of the three tropical plants, only *V. amygdalina* was active against adult *H. placei* with best-fit LC_{50} of 6.51 mg/mL (95% CI: 5.32–7.75). Evaluation of the two extracts obtained by successive maceration showed that chloroform extract (LC_{50}, 2.46 mg/mL, 95% CI: 1.87–3.28) was 11 times as potent as acetone extract (LC_{50}, 27.01 mg/mL, 95% CI: 21.32–48.57) ($a < 0.0001$). Chromatographic fractionation of the chloroform extract yielded four fractions (FA-FD) with FB (LC_{50}, 2.38 mg/mL, 95% CI: 1.76–3.28) 2.19 times as potent as FC (LC_{50}, 5.21 mg/mL, 95% CI: 4.40–5.79) against *H. placei*, while FA and FD were inactive. Phytochemical evaluation of the chloroform extract revealed the presence of saponins, steroids, terpenoids, cardiac glycosides, and the absence of tannins, flavonoids, alkaloids, and anthraquinones. FT-IR structural analysis of chloroform extract indicated the presence of key functional groups which are chemical fragments/ structural motifs known to be present in the two major classes of bioactive compounds (sesquiterpene lactones and steroid glucosides) reportedly to be found in *V. amygdalina*.

Conclusions: The findings showed that chloroform extract of *V. amygdalina* leaf possessed relatively good anthelmintic activity against adult *H. placei*. This could be indicative of its potential usefulness as an anthelmintic phytomedicine to control gastrointestinal nematodes infection in cattle.

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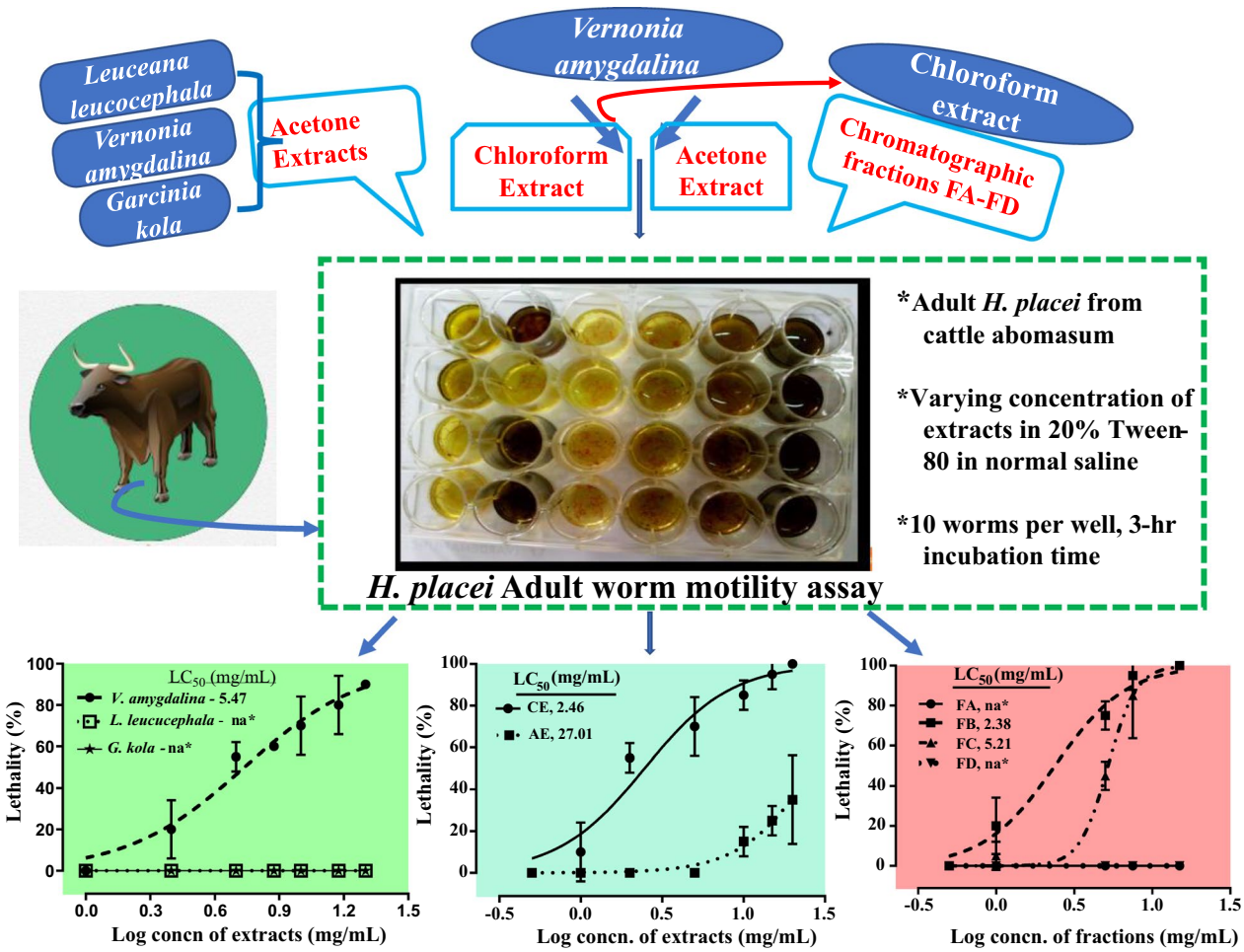
Key highlights

- Extracts of three different plant materials (one leaf, two seeds) were tested against adult *Haemonchus placei* in vitro;
- Chloroform extract of *Vernonia amygdalina* was 11 times as potent as acetone extract;
- Fractionation of the chloroform extract yielded a bioactive fraction responsible for about 90% of the total lethal effect of the chloroform extract.
- Bioprocessing of *V. amygdalina* leaf could produce phytomedicines for organic livestock farming.

Keywords: *Vernonia amygdalina*, *Haemonchus placei*, Anthelmintic activity, Metabolites

Graphical abstract



1 Background

Food products from farm animals are important contribution towards the realization of global food security [1, 2]. While many factors threaten this food supply source, gastrointestinal nematode infection (GIN) of farm animals represents a fundamental constraint [2, 3]. Gastrointestinal nematode infection occurs as a result of grazing of animals on pasture contaminated with infective larvae of nematodes. Infection generally results in weight loss, decreased reproductive performance,

poor food conversion, and anaemia [4, 5]. The infection is dominant in areas where inadequate livestock extension services, poor environmental hygiene, and prevailing weather conditions, altogether favour the development and transmission of infective larvae. Even though grazing animals usually suffer from mixed infection of nematodes, adult stages of the abomasal nematode *Haemonchus* species are devastatingly pathogenic as they feed on blood, resulting in anaemia and related complications [2, 6, 7].

With the advent of chemotherapy, the control of GIN has been mainly by the use of modern anthelmintic drugs. The hefty reliance and widespread application of these drugs, however, has inevitably led to the emergence of anthelmintic resistance [3, 8, 9]. Furthermore, this deplorable state is aggravated by the presence of drug residues in animal products, prompting heightened health and environmental concerns [10]. Thus, there is a resurgence of interest in the potential usefulness of plant extractives for effective control of gastrointestinal nematodes.

Vernonia amygdalina Del. is a tropical, edible vegetable whose leaves are used ethnomedicinally as remedies for gastrointestinal disorders, fever, general tonic, treatment of wound, venereal infections, as an anthelmintic and as a laxative [11]. This plant has been observed to be eaten by wild chimpanzees, possibly in a self-medicative behaviour against parasite-related illnesses [12]. *Garcinia kola* Heckel is a West African plant used as remedies for diarrhoea, worm infection, gonorrhea, stomach ache, jaundice, high fever, as chewing stick and to alleviate colic, chest colds and cough [13]. *Leucaena leucocephala* (Lam) De Wit leaf is commonly used as fodder for ruminants because of its high nutritive value. Its fresh seeds are used in Nigeria to deworm animals [14]. This paper reports the effect of extracts of these three tropical plants (*V. amygdalina*, *G. kola* and *L. leucocephala*) against adult *H. placei*, as none of these plants has ever been tested against this nematode which infect cattle primarily [6].

2 Methods

2.1 Plant materials

Vernonia amygdalina (leaves) and *L. leucocephala* (seeds) were obtained within the premises of University of Ibadan, Ibadan, Nigeria, while *G. kola* (seeds) were bought from Bodija Market, Ibadan, Nigeria. They were identified at Forestry Research Institute of Nigeria (FRIN), Ibadan. *V. amygdalina* leaves were air-dried under shade for four weeks, while *G. kola* seeds (chopped into smaller pieces) and *L. leucocephala* seeds were dried under shade for eight weeks. Afterwards, they were milled into coarse powder.

2.2 Source of adult nematodes

Actively moving adult *H. placei* nematodes were collected from the abomasal content of freshly slaughtered cattle at the Bodija Abattoir, Bodija Market, Ibadan, Nigeria, and maintained in normal saline solution. The identity of the worms was confirmed at the Parasitology Research Unit, by Prof I. O. Ademola, a parasitologist and head of the Department of Veterinary Parasitology and Entomology, University of Ibadan, Ibadan, Nigeria.

2.3 Chemicals and reagents

Acetone, *n*-hexane, chloroform, ethyl acetate, methanol, Tween 80, sodium chloride, vanillin, sulphuric acid (solvents and reagents were of analytical grades, Sigma-Aldrich, UK), silica gel 60 G (5–40 µm, Merck, Germany) and pre-coated TLC silica gel 60 (F_{254}, aluminium sheets 10×20 cm, Merck, Germany).

2.4 Solvent extractions (crude and successive)

For the crude extraction, powdered materials (200 g each) of the three plants were extracted twice by maceration using acetone (1 L for 24 h in each instance). Afterwards, a fresh powdered material of *V. amygdalina* (300 g), after initial defatting using *n*-hexane (*n*-hex, 1L) for 24 h, was subjected to successive maceration in chloroform, and acetone (1L each, 2 times for 24 h), respectively. The various solvent extracts, filtered with the aid of filter paper into clean glass bottles, were concentrated into smaller volumes using rotary evaporator (40 °C), and subsequently evaporated to dryness under reduced pressure at 40 °C for 48 h. They were further dried in vacuum desiccator until constant mass.

2.5 Vacuum liquid chromatographic fractionation of *V. amygdalina* chloroform extract

Vernonia amygdalina chloroform extract (1 g, obtained by successive extraction) was dissolved using chloroform and then adsorbed on silica gel 60 G (5 g). The adsorbed sample, dried under reduced pressure at 40 °C, was packed onto sintered glass earlier prepacked with silica gel 60 G (25 g) with the aid of a vacuum pump and eluted using 200 mL each of varying solvent mixtures [(*n*-hexane → *n*-hexane/ethyl acetate (1:1) → ethyl acetate → ethyl acetate/methanol (1:1)]. The eluates were monitored by thin-layer chromatography analysis using ethyl acetate/methanol (90/10) as mobile phase, while the chromatogram was viewed under ultra violet light (356 nm), day light, and after spraying with vanillin-sulphuric acid reagent (0.1 g vanillin, 28 mL methanol, 1 mL sulphuric acid). The fractions, concentrated into smaller volumes under reduced pressure, were later dried *in vacuo* at 40 °C.

2.6 Structural profile and phytochemical analysis of *V. amygdalina* chloroform extract

In other to obtain structural/ organic functional groups information on the *V. amygdalina* chloroform extract, it was subjected to Fourier transform infrared (FT-IR) spectroscopic characterization. About 1 mg of the extract was smeared on a KBr salt disc, placed in a disc holder and inserted into the sample beam of the FT-IR instrument, Perkin-Elmer® spectrum 2. The phytochemical analysis of the extract was done following standard procedures [15, 16].

2.7 Anthelmintic evaluation

Varying test concentrations of the crude acetone extracts (1–20 mg/mL; *V. amygdalina*, *L. leucocephala*, and *G. kola*), chloroform and acetone extracts (0.5–20 mg/mL) of *V. amygdalina*, and chromatographic fractions (0.5–15 mg/mL) of the chloroform extract were prepared in 20% Tween-80 in normal saline. Each concentration (0.5 mL, in duplicates) was dispensed into wells of 24-well standard plates (each well: diameter-14.00 mm, depth-16.10 mm, volume-2.39 mL), followed by placing ten adult *H. placei* nematodes into each well. The nematodes were exposed to these test concentrations for 3 h at ambient temperature (26–30 °C). Afterwards, the nematodes were removed into Petri dishes containing distilled water, cleansed of the extracts and then exposed to warm (40 °C) normal saline for 10 min and observed for any revival of motility. At the end of the experiments, the nematodes were categorized as dead if there were no revival of mobility plus a complete lack of response to poking with a pick. The number of dead worms was recorded. Levamisole (as hydrochloride salt, from Reals Pharmaceutical Ltd, Nigeria) and 20% Tween-80 in normal saline were used as positive and negative controls, respectively. All experiments were replicated twice [17].

2.8 Statistical analysis

Statistical analysis was conducted using the GraphPad Prism Software 7 (GraphPad Software Inc., California, USA). Anthelmintic data were fitted to a nonlinear regression equation (Log [extract or fraction] vs. lethality; variable slope) to produce best-fit sigmoidal curves from which median lethal concentration (LC_{50}) values were computed with associated uncertainty.

3 Results

3.1 Solvent extractions

The percentage yields of all the extracts—crude acetone extracts of the three screened plant species, and the

Table 1 Details of medicinal plants and the percent yield of both crude and successive extraction

S/no.	Plant species [extraction solvent*]	Family name	Common name	Part used	FHI no.**	Percent yield (*w*/*w*)	
						Crude extraction	Successive extraction
(I) Crude extraction							
1	*Leucaena leucocephala* (Lam) De Wit [b]	Fabaceae	Lead tree	Seed	112,022	4.60	–
2	*Garcinia kola* Heckel [b]	Guttiferae	Bitter kola	Seed	110,593	6.20	–
3	*Vernonia amygdalina* Del. [b]	Asteraceae	Bitter leaf	Leaf	110,023	6.40	–
(II) Successive extraction							
1	*V. amygdalina* Del. [a]	〃	〃	〃	〃	–	3.43
2	*V. amygdalina* Del. [b]	〃	〃	〃	〃	–	5.64

*Extraction solvent—[a], chloroform; [b], acetone

**Forest Herbarium Ibadan number

Table 2 Yield of FA-FD and lethal effect per fraction that kills 50% of *Haemonchus placei*

Fractions of CE	Eluting mobile phase	Yield (%)	Mass (mg)	LC_{50} (mg/mL)	Lethal effect per fraction that kills 50% of worms (mL)*	% of Total
FA	*n*-Hex	13.5	135	na	–	–
FB	*n*-Hex/EtOAc (1:1)	52.7	527	2.38	221.43	89.18
FC	EtOAc	14.0	140	5.21	26.87	10.82
FD	EtOAc/MeOH (1:1)	19.0	190	na	–	–
Total			992		248.30	

*Lethal effect calculated by: Mass × $1/LC_{50}$

na not active; *CE* chloroform extract; *n-Hex n*-hexane; *EtOAc* ethyl acetate; *MeOH* methanol

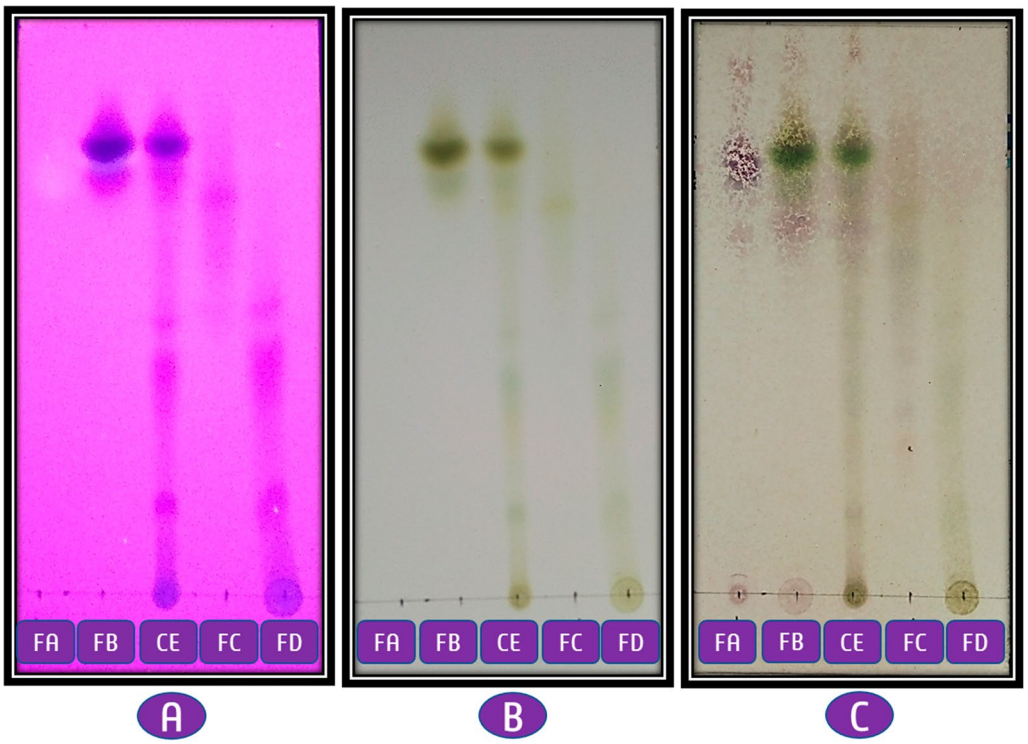


Fig. 1 TLC profiles of fractions (FA-FD) obtained from chloroform extract (CE) of *Vernonia amygdalina* leaf. **A** Profile at 365 nm under UV light; **B** Profile in daylight; **C** Profile after spraying with vanillin-H_2SO_4 reagent and heating plate in the oven at 105 °C for 5 min; mobile phase: EtOAc/MeOH (90/10)

Table 3 Absorption peak frequency data from FTIR spectrum of the chloroform leaf extract of *Vernonia amygdalina*

S/no.	Absorption frequency (cm^{-1})*	Chemical bond (s)**	Ref [45]	Putative class of compounds
1	3442.93	O–H, broad; non-phenolic, SV	3300–3700	Alcohols in steroids, sugars (glucosides)
2	3009.50	sp^2 C–H, Vinylic; epoxy ring, SV		Alkene, epoxy
3	2918.81	sp^3 C–H, SV	2700–3000	CH_2, Methylene grp
4	2849.78	sp^3 C–H, SV	2700–3000	CH_2, Methylene grp
5	1731.30	C = O; unsaturated ester, SV	1715–1730	Carbonyl
6	1712.43	C = O; conjugated ester, SV		Carbonyl
7	1649.50	C = C; unconjugated; weak	1640–1680	Alkene
8	1619.20	C = C; conjugated	1620–1670	Alkene
9	1492.00	C–H, deformation, BV		
10	1453.75	C–H, deformation; BV		CH_2
11	1161.80	C–O; ester, lactone, SV		
12	1046.70	C–O; alcohol, SV	1000–1050	1050–1150

SV strong vibration; *BV* bending vibration

chloroform and acetone extracts of *V. amygdalina* with (after initial defatting with *n*-hexane) are presented in Table 1.

3.2 Vacuum liquid chromatographic fractionation of *V. amygdalina* chloroform extract

After the anthelmintic evaluation of the two extracts (CE and AE) of *V. amygdalina* obtained by successive extraction, CE was selected and subjected to vacuum liquid chromatographic fractionation. This yielded four

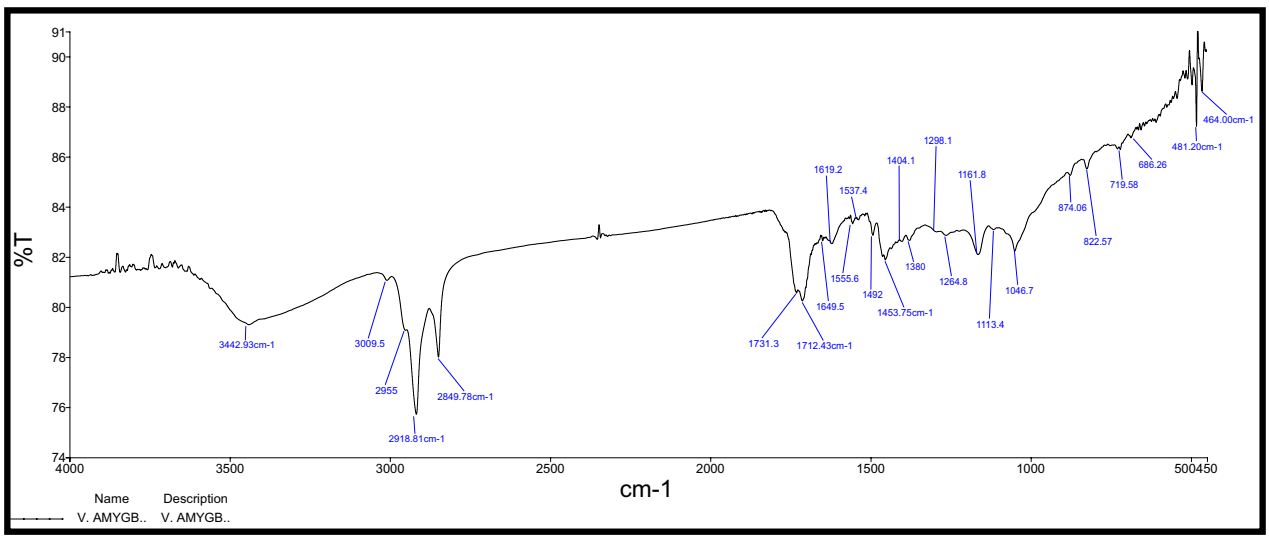


Fig. 2 FT-IR spectrum of *V. amygdalina* leaf chloroform extract showing the various absorption peak frequencies

Table 4 Potency parameters of the three plants extracts and successive extracts and fractions of *Vernonia amygdalina*

Assay parameters	VA	GK	LL	CE	AE	FA	FB	FC	FD
LC_{50} (mg/mL)	6.51	NA	NA	2.46	27.01	NA	2.38	5.21	NA
Standard error	0.04	–	–	0.06	0.07	–	0.06	0.02	–
95% CI	5.32–7.75	–	–	1.87–3.28	21.32–48.57	–	1.76–3.28	4.40–5.79	–
R^2	0.83	–	–	0.93	0.81	–	0.97	0.97	–

VA Vernonia amygdalina, *GK Garcinia kola*, *LL Leucaena leucocephala*, *CE* Chloroform extract of *Vernonia amygdalina*; *AE* Acetone extract *Vernonia amygdalina*, Chromatographic fractions of CE (FA, FB, FC, FD)

fractions, FA-FD (Table 2), with FB having the highest yield (52.7%). Chromatographic fractionation of CE resulted in good fractional separation of this bioactive extract as shown by the thin-layer chromatographic fingerprint of the fractions that revealed the various secondary metabolites in them (Fig. 1). Based on the solvent strength of the eluting solvents, the fingerprint revealed gradient elution as attested to by the relative positions of the metabolites on the plate (Fig. 1).

3.3 Structural and phytochemical analysis of *V. amygdalina* chloroform extract

Infrared spectroscopy detects the molecular vibrations (stretching and bending) of bonds within functional groups present in organic molecules. The FT-IR spectrum of *V. amygdalina* chloroform extract (Fig. 2) with its complexity of vibrational nodes represents a sum of the spectra of the individual constituent molecules present in the extract [18, 19]. The absorption peak frequency data are presented in Table 3.

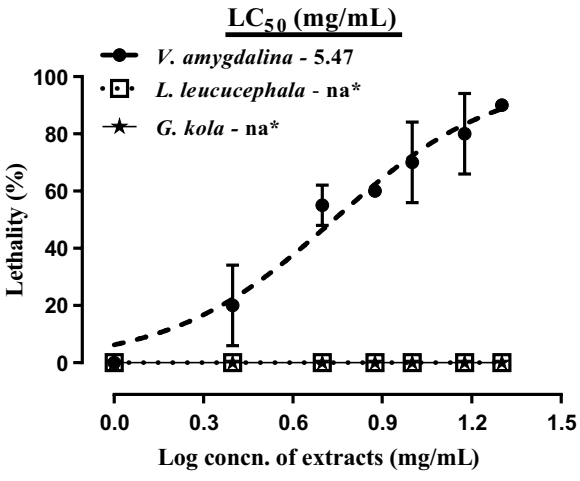


Fig. 3 Anthelmintic activities of the plants' acetone extracts (1–20 mg/mL) against adult *Haemonchus placei*. Each value represents two replicates of two independent experiments after 3-h exposure at ambient temperature (26–30 °C). *na indicates 'not active'

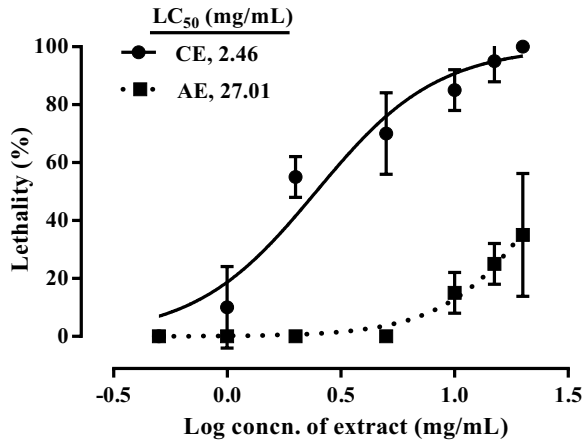


Fig. 4 Anthelmintic activities of *Vernonia amygdalina* leaf extracts (CE—chloroform extract; AE—acetone extract) against adult *Haemonchus placei*. Each value represents two replicates of two independent experiments after 3-h exposure to varying concentrations (0.5–20 mg/mL) at ambient temperature (26–30 °C)

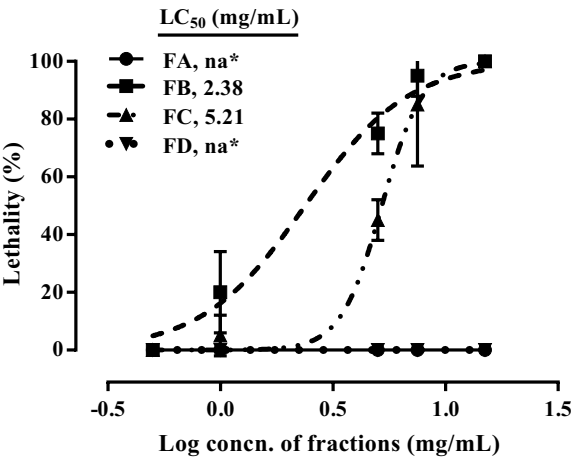


Fig. 5 Anthelmintic activities of FA-FD, obtained by vacuum liquid chromatographic fractionation of *Vernonia amygdalina* chloroform extract. Each value represents two replicates of two independent experiments after 3-h exposure to varying concentrations (0.5–15 mg/mL) at ambient temperature (26–30 °C). *na indicates 'not active'

3.4 Anthelmintic evaluation of extracts and fractions

The results of anthelmintic evaluation of the extracts and fractions against *H. placei* are presented in Table 4. Of the three plants, only *V. amygdalina* was active with best-fit LC_{50} of 6.51 mg/mL (Fig. 3). The other two (*L. leucocephala* and *G. kola*) were inactive, even at the highest concentration (20 mg/mL). The best-fit LC_{50} values of the two extracts of *V. amygdalina* obtained by successive extraction were found to be significantly different (alpha < 0.0001): 2.46 mg/mL and 27.01 mg/mL for CE and AE, respectively (Fig. 4). For the four chromatographic fractions obtained after fractionation of CE, only FB and FC were active against *H. placei* (best-fit LC_{50} of 2.38 mg/mL and 5.21 mg/mL, respectively (Fig. 5). FA and FD were, however, not active. Levamisole, a reference anthelmintic, expectedly exhibited high potency with LC_{50} of 11.74 ng/mL. There was 100% survival of the worms in the 20% Tween 80 in normal saline used as the negative control.

4 Discussion

The three plants were selected for this study based on their ethnomedicinal uses and available documentation in the literature. The need to extract out much of their secondary metabolites content irrespective of their chemical classes informed the choice of acetone as solvent for the crude extraction. Acetone seems to have higher capacity to extract more bioactive secondary metabolites for screening purposes relative to other solvents [20, 21]. Acetone's organic and polar character (methyl hydrocarbon skeletons interspersed with polarized carbonyl functional group) enables the extraction of both polar and non-polar secondary metabolites by solvation through dipole–dipole interactions with solute molecules [22, 23]. Based on the outcome of the anthelmintic evaluation of the three selected plants, fresh dried sample of *V. amygdalina* leaves was successively macerated using solvents of different polarities. This process afforded selective separation of the various secondary metabolites in *V. amygdalina* into two broad categories: less polar/intermediate-polar (Chloroform Extract, CE); and intermediate-polar/polar (Acetone Extract, AE). The two extraction solvents, chloroform and acetone, possess good selectivity property based on different dielectric constant (4.81 and 21.01, respectively), and relatively high solvent strength (4.1 and 5.1, respectively) [24, 25]. Accordingly, macerating first with chloroform facilitated the selective extraction of most non-polar compounds and some intermediate-polar compounds; macerating afterwards with acetone facilitated selective extraction of most intermediate-polar and polar compounds. Increasing the number of extraction solvents to three by macerating further with any of ethanol, methanol or water was considered, but dropped because these are ionic, amphiprotic solvents [18] with inherent capacity to extract out the more polar primary and secondary metabolites like sugars, amino acids, tannins and glycosides. The potential outcome will be increased extraction mass, and this often does not translate to higher bioactivity [20, 21, 26].

Analysis of the fundamental vibration frequencies that the spectrum revealed could yield structural information regarding the constituent's mixture of phytochemicals in this bioactive extract. A number of

fundamental frequency bands were identifiable in the spectrum and these include: 3442.93 cm^{-1}, represents OH stretching vibration; 3009.50 cm^{-1}, represents C–H stretching vibration coming from vinylic group or epoxy ring; 2918.81 and 2849.78 cm^{-1}, represent C–H stretching vibrations from methylene group; 1731.30 and 1712.43 cm^{-1}, are carbonyl stretching vibrations from ester group and lactone ring, respectively; 1649.50 and 1619.20 cm^{-1} can be assigned to C=C from unconjugated and conjugated alkene group, respectively; 1492.00, 1453.75 are frequency bands from C–H deformations; 1264.80 cm^{-1}, representative of C–O stretching vibration from ester group and lactone ring; 1046.70 can be assigned to C–O stretching vibration from an alcohol, ester or lactone ring. All these functional groups are chemical fragments or structural motifs present in the two major classes of bioactive compounds (sesquiterpene lactones and steroid glucosides) reported to have been isolated from *V. amygdalina* [27, 28]. This was further affirmed by the result of the phytochemical analysis of the extract which revealed the presence of saponins, steroids, terpenoids and cardiac glycosides, as well as absence of tannins, flavonoids, alkaloids and anthraquinones.

The observed activity of *V. amygdalina* extract in this study provided additional evidence justifying the ethnomedicinal use of the leaf in treatment of worm infection. The result suggests that the plant exhibited a fairly good potency against *H. placei* relative to acetone leaf extracts of *Ocimum gratissimum* and *Cymbopogon citratus* [29]. For the extracts obtained by successive extraction after the outcome of initial anthelmintic evaluation of the crude extracts, though CE and AE were both active, the result suggests that CE is 11 times more potent than AE against adult *H. placei*. In addition, CE's potency was 2.65 times higher relative to the crude acetone extract. Thus, the successive extraction was value adding. It showed clearly that the activity of this plant against *H. placei* is largely contributed by the less polar/intermediate-polar components selectively extracted out by chloroform. For the two bioactive fractions, judging by their best-fit LC_{50} values only, FB was 2.19 times as potent as FC against *H. placei*. If, however, we factor in the mass obtained per fraction and calculate the lethal effect per fraction that kills 50% of worms, the result suggests that FB is responsible for almost 90% of the total activity of CE against *H. placei* [30]. In addition, this activity against adult *H. placei* is attributable to some of the phytochemicals present in FB and FC. Considering the mobile phases (*n*-hexane/ethyl acetate (1:1) and ethyl acetate (100%)) used for their elution, and the result of the structural analysis of CE, we submit that these phytochemicals would be of intermediate or medium polar compounds such as saponins (stigmastane-type and steroidal) and sesquiterpene lactones which have been reported in the literature to be present in the leaf [11, 31]. The structural motifs and moieties of these class of compounds with embedded polar fragments such as carbonyls, lactones, and hydroxyl groups made them to be easily dissolved or solvated by the ethyl acetate-based eluting solvents.

Overview of literature reports on the anthelmintic activity of *V. amygdalina* leaf revealed that aqueous/aqueous-organic solvents extracts were used, instead of varying solvents of different polarities as we have used in the current study. Nalule et al. [26] reported that aqueous ethanol extract (70%) with ED_{50} = 5.94 mg/mL, was twice as potent as the aqueous extract (ED_{50} = 13.70 mg/mL) against adult *Ascaris suum* at 48 h post-treatment [26]. Against earthworm (*Lumbricus terrestris*) at 50 mg/mL, [32] reported about a half time to death of 37.46 ± 13.55 min for aqueous ethanol extract (70%) relative to aqueous extract of 76.65 ± 12.73 min [32]. Also, against *H. contortus* eggs and larvae, aqueous acetone extract (70%) gave LC_{50} values of 957.00 and 508.20 µg/mL, respectively, at 48-h exposure [33]. Three other studies indicated varying activities against nematodes: aqueous ethanol extract (80%) gave 71.43% mortality at 24 h (500 mg/mL; dose too high) post-treatment against *Heligmosomoides bakeri* infective larvae [34]; aqueous ethanol extract (50%) gave 1.70% mortality at 72 h (1 mg/mL; longer duration of exposure) against *Caenorhabditis elegans*, a free-living nematode model [35]; while hot water extract exhibited poor inhibitory hatching effect against *H. contortus* egg [36]. The root extracts (methanol, water and acetone) were also reported to be active against adult *H. contortus* in vitro with mean mortality of 20–33.3% at 6.25 mg/mL [37]. In addition, in vivo studies in goats showed the leaf aqueous extracts as efficacious against helminths [38, 39]. Overall, while all these studies used aqueous-based extracts, ours used primary extracts of different polarities and showed that the chloroform extract was more potent.

Garcinia kola seed did not show any activity in this study even though it was reported to possess anthelmintic activity (albeit weak) in some earlier studies using ethanol seed extracts. The seed exhibited 18.75% inhibition at 100 mg/mL and irreversibly paralyzed 76.52% at 50 mg/mL of *H. bakeri* egg and larvae, respectively [40]. Similarly, at 1.7 mg/ml, it was considered inactive against *Haemonchus contortus* larvae with less than 60% mortality [41]. Similarly, *L. leucocephala* seed was reported to exhibit good anthelmintic activity with the chloroform soluble alkaloidal seed extract furnishing an equivalent effect to mebendazole at 5 mg/mL using *Ascaris suum* [42]. Also, aqueous seed extract (LC_{50} = 0.586 mg/mL)

and chromatographic fractions of the ethanol extract were active against *H. contortus* larvae [14, 43], while an investigation of seed protein extracts on *H. contortus* larvae gave a 50% hatching inhibition (0.48 mg/mL, cotyledon extract; 0.33 mg/mL, total seed extract) [44].

5 Conclusions

Results from this study suggest that chloroform leaf extract of *V. amygdalina* possesses anthelmintic activity against adult *H. placei* nematode. Bioprocessing of this extract could produce phytomedicines for organic livestock farming. Further studies to better characterize this extract are on-going in our laboratory.

Abbreviations

VA: *Vernonia amygdalina*; GK: *Garcinia kola*; LL: *Leucaena leucocephala*; CE: Chloroform extract; AE: Acetone extract; TLC: Thin-layer chromatography; FT-IR: Fourier transform infrared; FA: Fraction A; FB: Fraction B; FC: Fraction C; FD: Fraction D; FHI: Forest Herbarium Ibadan; LC: Lethal concentration; UV: Ultra violet; MeOH: Methanol; Hex: Hexane; EtOAc: Ethyl acetate; SV: Strong vibration; BV: Bending vibration.

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Author contributions

SAA and SOI designed the experiment; and all authors carried out the experiment. All authors analysed the results and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and material

Yes, in the main manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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