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RESEARCH PAPER

EVALUATION OF INSECTICIDAL AND ANTI-HOOKWORM ACTIVITIES OF CRUDE EXTRACTS AND ISOLATES FROM *DICHAPETALUM MADAGASCARIENSE* STEM BARK

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ABSTRACT

The constituents of the roots of Dichapetalum madagascariense have been investigated for their anti-tumour and anti-proliferative activities on some cancer cell lines and anti-parasitic activities against some causative pathogens of selected neglected tropical diseases. The most active constituents were the dichapetalins. To investigate the constituents of the stem bark of the plant for potential insecticidal and anti-hookworm activities, chromatographic separation of the stem bark extracts gave four commonly occurring triterpenoids; friedelan-3β-ol (1), friedelan-3-one (2), the relatively rare triterpenoid zeylanol (3), β-sitosterol (4) and stigmasterol (5), which was obtained as a mixture with (4). This is the first report of the presence of zeylanol* (3) *in D. madagascariense. Activity against the maize weevil, Sitophilus zeamais gave LD_{50} values (µg/mL) of 0.48 (compound 1), 0.56 (2), 0.52 (mixture of 1 and 2), and 0.98 (mixture of 4 and 5). Crude extract-treated maize grains at doses ≥ 2 g per 100 g grain provided the most effective protection to maize against hidden eggs and immature stages of S. zeamais compared to complete protection at the highest dosage (10 g/100 g grain). The IC_{50} of tested compounds against human hookworm Necator americanus showed lower potencies (0.64-1.33 µg/µL) than the standard, albendazole (0.0024 µg/µL). This study has established the presence of zeylanol* (3) *in D. madagascariense and shown that the insecticidal activity of the tested compounds and extracts against S. zeamais could provide some protection against maize grains but at levels that are uneconomical for incorporation into sustainable pest management programmes. Anti-hookworm activities obtained may serve as leads for further optimization and development in the anthelmintic drug discovery efforts.

Keywords: *Dichapetalum madagascariense*, *friedelan-3-one, zeylanol*, *Sitophilus zeamais*, *Necator americanus*

INTRODUCTION

Dichapetalum madagascariense Poir (Dichapetalaceae) Keay occurs in tropical Africa including Ghana (Burkill, 1985). The plant has found folkloric use in the treatment of viral hepatitis, jaundice, sores, bacterial infections and urethritis (Burkill, 1985; Lewis and Elvin-Lewis, 1977). The major chemical constituent of the plant has been identified as triterpenoids of oleananes, an example of which is friedelan-3-one (2), and that of dammarane types (dichapetalins A to H, and M) (Achenbach *et al.*, 1995; Addae-Mensah *et al*., 1996; Osei-Safo *et al.*, 2008, Chama *et al*., 2015). Friedelan-3-one (2) has been tested to be active as antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic and antihypertensive (Antonisamy *et al*., 2011; Ghosh *et al*., 2011). Dichapetalin A has shown both *in vitro* and *in vivo* anticancer activities (Addae-Mensah *et al.,* 1996), anti-hookworm (Chama *et al*., 2015), inhibition of fungal growth as well as antifeedant activity (*Spodoptera exigua*) (Jing *et al*., 2014).

The human hookworm, *Necator americanus*, Stiles. 1902 (Nematoda: Ancylostomatidae) has been reported to have significant adverse impact on health and education (Anderson and May, 1991). With reports of the low efficacies of albendazole and mebendazole which are currently the primary drugs for the treatment of hookworm infection (Keiser and Utzinger, 2008), potential new leads from plants are necessary. Also, the maize weevil *Sitophilus zeamais* Mots., 1855 (Coleoptera, Curculionidae) is among the primary pests for maize, wheat, rice and sorghum. The Food and Agriculture Organization (FAO) has indicated that infestation and damage to cereals by *S. zeamais* may account for 10% or more global loss in production (Gallo *et al.,* 2002). It is for this reason that as part of our current investigation of the constituents and biological activities of the Dichapetalaceae, we evaluated the insecticidal activities of the crude extracts and isolated compounds with respect to *S. zeamais*, and the anti-parasitic activity on the *N. americanus.*

MATERIALS AND METHODS

General experimental procedure

TLC was performed on aluminium foil slides pre-coated with silica gel (thickness 0.2 mm, type Kiesegel $60F_{254}$, Merck, Rogers, AR); detection: I_2 vapour and anisaldehyde spray reagent. Column chromatography was carried out on silica gel 60 (Fluka Analytical, Bellefonte, PA). Melting points (uncorrected) were determined on a Stuart Scientific Melting Point Apparatus (Sigma Aldrich, St. Louis, MO). IR spectra were recorded in KBr discs on a Shimadzu IR-408 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). ^{1}H NMR was run at 600 MHz and ^{13}C NMR at 150 MHz in $CDCl_3$ with TMS as the internal standard on a Brücker Avance 600 spectrometer (Brücker Inc., Fremont, CA). MS were obtained by electron impact at 70eV using Finnigan mass spectrometer. The optical rotation was determined in $CHCl_3$ on a Bellingham Stanley Polarimeter (ADP220, PE02037) (Bellingham and Stanley Ltd, Kent, UK). Hand micro-applicator was obtained from Burkard Manufacturing Co, Ltd. UK.

Chemicals and reagents

All solvents were of analytical grade from Sigma-Aldrich. Solvent systems were prepared as v/v. Anisaldehyde reagent was prepared by adding 1 mL of concentrated sulphuric acid to 50 mL glacial acetic acid. A volume of 0.5 mL anisaldehyde was added to the mixture.

Materials

The stem bark of *D. madagascariense* was collected at the Legon Botanical Garden and identified by the late Mr. J. Y. Amponsah of the National Herbarium at the Department of Plant and Environmental Biology, University of Ghana where a voucher specimen (DM02) has been deposited. The plant material was chopped into pieces, air-dried for four weeks and then pulverised. Adult *S. zeamais* were obtained from the Entomology Laboratory of the Crop Science Department, University of Ghana. Disease-free maize (*Zea mais)* was obtained from Madina market, a suburb of Accra. For the anthelmintic activity which involved human subjects, study consent was obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research of the University of Ghana (CPN 006/12-13). Field isolates of human hookworm (*N. americanus*) were obtained following the

collection of faecal samples from 231 consenting volunteers of rural communities in two villages on the Kintampo to Tamale highway, namely, Bawa Akuraa (N 08.23888, W 001.63234) and Jato Akuraa (N 08.25099, W 001.61732) in the Brong Ahafo Region of Ghana. Stool samples from 146 females (63.2 %) and 85 males (36.8 %) aged 4 to 95 years were obtained upon oral adult and parental consent for the study. All infected individuals were subsequently treated to make them totally free of the infection, and duly advised on how to avoid or prevent possible re-infection.

Extraction and isolation of compounds

The pulverised plant material (5.0 kg) was exhaustively Soxhlet extracted with 10 L of 60-80 °C petroleum ether (PE) for 72 h. Evaporation of the solvent under reduced pressure afforded 15.80 g of crude extract. The rest of the defatted plant material was re-extracted with 7.5 L of chloroform for another 72 h to afford 13.0 g of extract upon evaporation of the solvent. The chloroform-extracted residue was air -dried and then extracted with 7.5 L of acetone to obtain 11.0 g of crude extract. Another 5.0 kg fresh pulverized plant material was exhaustively Soxhlet-extracted with 8 L of ethanol for 72 h. This gave 31.0 g extract after evaporation. The plant material after the ethanol extraction was allowed to air-dry and then exhaustively re-extracted with methanol for 72 h to give 21.7 g of extract after evaporation.

Solids that precipitated from the petroleum ether extract during solvent concentration were re-crystallized from a mixture of petroleum ether and chloroform to afford friedelan-3β-ol (1). Its TLC profile and all spectroscopic properties (IR, NMR and MS) were consistent with reported literature values and authentic samples (Kundu *et al*., 2000). Five grams of the acetone extract was chromatographed over silica gel 60 (Fluka) with petroleum ether and ethyl acetate gradient to give fractions A to F. Fraction D precipitated solids which were re-crystallized from methanol to give friedelan-3-one (2) and zeylanol (3). Spectroscopic data of both compounds were consistent with those reported in literature (Escobedo-Martinez *et al.,* 2012, Fang *et al*., 2006 respectively), Table 1. β-sitosterol (4) and a mixture of β-sitosterol and stigmasterol (5) of undetermined percentage composition were obtained from the separation of the combined acetone-chloroform extract on 14.0 g silica gel with petroleum ether and ethyl acetate solvent mixtures. The two were characterised by chromatographic retardation factor (R_f) value comparison in various solvents, as well as their spectroscopic characteristics (Pateh *et al*., 2009; Chaturvedula and Prakash, 2012).

Physical characteristics

Zeylanol (3), 10 mg as white powder; mp: 236-240 °C (Lit. 276-278 °C); $[\alpha]_D^{20}$ -0.8° (Conc. = 0.44, $CHCl_3$) (Lit. $[\alpha]_D^{20}$-0.95° (Gunatilaka *et al*., 1983), anisaldehyde: purple; IR (KBr) ν_{max} cm^{-1}; 3500 (OH), 1695 (C=O), 1375 ($C(CH_3)_2$), 1240 (C-O); ^1H NMR (600 MHz, $CDCl_3$); δ_H 3.68 (1H, H6), 2.40 (1H, H4/H10), 2.25 (1H, H2), 2.00 (1H, H18), 1.96 (1H, H1), 1.22 (3H, H17), 1.16 (3H, H14), 1.05(3H, H13), 1.00 (3H, H20), 0.95(3H, H9),0.85(3H, H4), 0.75 (3H, H5), 1.19-1.53(20H) ppm; EIMS m/z (rel. int.) (%); (M^+): 442 (10), 427 (3), 274 (10), 123 (46), 95 (38), 85 (56), 69 (100), TLC: 100 % $CHCl_3$, R_f 0.69; PE/EtOAc (3:1), R_f 0.56 and PE/$CHCl_3$ (1:1), R_f 0.18.

Bioactivity studies

Insect culture

Adults of *S. zeamais* were cultured in the insectary of the Department of Biochemistry, Cell and Molecular Biology, University of Ghana. The parent stocks of *S. zeamais* were reared on maize sterilized by heat disinfestation for 4 hours at 60 °C and then stored in the freezer. Sixty to seventy randomly selected unsexed adults of *S. zeamais* were introduced into 400 g of sterilized maize in rearing jars and maintained at 27 ± 1 °C and 60-70 % relative humidity in the insectary. The maize grains were sieved to remove the parent adult insects after 14 days. Progeny emergence began after 21 days and adults that emerged were used for the various assays.

Table 1: Comparison of ^{13}C NMR of zeylanol (3) with that of literature

Carbon-No.	^{13}C NMR (ppm) of zeylanol, 150 MH_Z, $CDCl_3$	Multiplicity	^{13}C NMR (ppm), 75 MH_Z, pyridine-d5, Lit. (Fang *et al*, 2006)
	21.9	CH_2	29.8
	41.3	CH_2	41.5
	213.0	C	212.0
	58.3	CH	58.5
	47.7	C	47.9
	79.5	CH	78.7
	29.3	CH_2	22.0
	49.8	CH_2	49.7
	37.0	C	37.3
	58.6	CH_2	58.4
	35.3	CH_2	35.5
	30.4	CH_2	30.5
	38.1	C	39.7
	39.7	C	38.0
	32.4	CH_2	32.3
	35.9	CH_2	36.0
	30.0	C	29.9
	42.8	CH	42.8
	35.5	CH_2	35.3
	28.2	C	28.1
	32.7	CH_2	32.8
	39.2	CH_2	39.2
	10.4	CH_3	11.1
	9.1	CH_3	9.7
	17.5	CH_3	17.5
	20.2	CH_3	18.6
	18.6	CH_3	20.1
	32.1	CH_3	32.0
	31.8	CH_3	31.7
	35.0	CH_3	34.8

Insecticidal activity of crude extracts and isolated compounds on *S. zeamais*

General toxicity assay

The method reported earlier by Bisseleau *et al* (2008) for contact toxicity was used. A hand micro-applicator was used to topically apply 1.0 µL acetone solutions of the test samples to the thorax of mixed sexes of randomly selected four-day old adult *S. zeamais*. Geometrically varied doses (applied as acetone solutions) from 0.005, 0.05, 0.5 and 5.0 µg/µL for the crude extracts and 0.001, 0.01, 0.1 and 1.0 µg/µL for the isolated compounds and petroleum ether extract were used per insect. Ten insects of four replicates were used for each treatment kept in petri dish (8.5 cm diameter) and the number of dead insects for each treated group was recorded after 48 h. Treatment series included four groups of *S. zeamais* treated with acetone alone to serve as controls. The mean mortality and percentage mortality were calculated.

Ovicidal and larvicidal activity of the crude extracts

The toxicity of the extracts to eggs (ovicidal action) and immature stages (larvicidal action) were investigated. Five hundred grams (500 g) of equilibrated maize placed in a 1-liter glass jar were infested with 250 adults of *S. zeamais* to allow for egg laying. The parent adults were removed after seven days. Twenty-four h after adult removal, 25 g of the infested maize were treated with 1 g/mL each of the petroleum ether, acetone and methanol extracts dissolved in their respective solvents. Controls were also set up by treatment of the infested maize (25 g) with only the respective solvent (petroleum ether, acetone and methanol). Thereafter, these treatments were repeated 1 and 2 weeks after adult removal to determine the effect of the extracts on the early and late instar larvae of *S. zeamais*. Each treatment was replicated four times. Counts were taken of adults emerging after 2 weeks following the last treatments.

Progeny production and damage assessment

The effects of crude extracts on F_1 progeny produced by adult *S. zeamais* were investigated on maize treated with extract concentrations of 0.016, 0.08, 0.4, 2, and 10 g per 100 g of maize grains by the method reported earlier (Bisseleua et al., 2008). One hundred grams (100 g) of pre -equilibrated maize grains were treated with the different doses of the extracts in 2 mL of the respective solvents (petroleum ether, acetone and methanol). The solvents were allowed to completely evaporate within 3 h after application and 20 adult *S. zeamais* were introduced into the grains. The containers were covered with white muslin cloth held in place with rubber bands. Control treatment consisted of grains treated only with the solvents (petroleum ether, acetone and methanol). After 21 days oviposition period, the parent adults were removed and insects subsequently emerging were counted to estimate F1 progeny production. Counting began after 43 days and stopped after 50 days. Thereafter, the damage caused to the grains by *S. zeamais* was assessed.

Damage assessment

Percent weight loss and seed damage were calculated by the 1985 United Nations Food and Agricultural Organization (FAO) formulae as follows:

$$\% \text{ Weight loss} = \frac{(UNd - DNu)\,x100}{U(Nd+Nu)}$$

$$\% \text{ seed damaged} = \frac{Nd\,x100}{(Nd + Nu)}$$

Where

U = weight of undamaged grains

D = weight of damaged grains

Nd = number of damaged grains

Nu = number of undamaged grains

Anti-hookworm activity of crude extracts and isolated compounds

PCR method for hookworm identification

Identification of *Necator americanus*; Stiles 1902 (Nematoda: Ancylostomatidae) was by the PCR method described in our earlier report (Chama *et al*., 2015).

Egg hatch inhibition test

Detection of the presence of hookworm eggs was carried out with the Kato–Katz faecal smear technique (Katz, Charves and Pellagrino, 1972) and faecal samples with more than 1200 eggs were pooled for egg extraction. Purification of eggs was by the density float method with the mean number of eggs per millilitre calculated. The *in vitro* anthelmintic activity test was conducted in 96-well flat bottom Microtest™ Tissue culture plates (Becton Dickinson Labware Europe, BD Biosciences, Bedford, MA). Egg hatch inhibition (EHI) assay was set up by plating the purified eggs suspension (approximately 50 eggs per well) in a 96-well plate with concentration ranges of test samples selected after some initial trials. Concentration-dependent activities were obtained from serial dilutions of the lowest concentration that gave 100 % Egg Hatch Inhibition (EHI) for each treatment group. Stock solutions (5 mg/mL) of each of the crude extracts (petroleum ether, acetone and methanol) and isolates (compounds 1, mixture of 1 and 2, and mixture of 4 and 5) were prepared in dimethyl sulfoxide (DMSO). Six dilutions (dilution factor of 0.5) of the stock solution of each test sample were pre-

pared in DMSO and added to the wells. Each of the tests was performed in duplicate. Albendazole and water were used as positive and negative controls respectively. A stock solution of 5 mg/mL of albendazole (400 mg) in DMSO was also prepared for use as the positive control. The plates were incubated at a temperature of about 27 °C for 24 h after which a drop of 2 % Lugol's iodine was added to stop hatching of eggs.

The number (#) of hatched and unhatched eggs was obtained by physical counting under light microscopy and the percent egg hatch inhibition values were calculated as:

% Egg Hatch Inhibition, EHI =

$$\frac{\#\text{ of unhatched eggs}}{\#\text{ of unhatched} + \#\text{ larvae}} \times 100$$

Median Inhibitory concentration (IC_{50}) of test samples were then determined.

Statistical analyses

Probit analysis based on the methods of Finney (1971) in MINITAB software was used to estimate LD_{50} and IC_{50} for the toxicity assays at 95 % confidence interval and corrections for natural control mortality were made using Abbott's formula (1925) (Abbot, 1987).

RESULTS

Identification of compounds

Spectroscopic analyses involving IR and NMR coupled with mass spectrometry, comparative thin layer chromatography and melting point with authentic samples and literature data identified structures 1, 2, 3, and 4 as the known compounds; friedelan-3β-ol (Kundu *et al*., 2000), friedelan-3-one (Escobedo-Martinez *et al.,* 2012), zeylanol in Table 1 (Fang *et al*., 2006), and β-sitosterol (Fig.1) respectively. Stigmasterol (5) (Fig. 1), was also obtained as a mixture with β-sitosterol (Pateh *et al*., 2009).

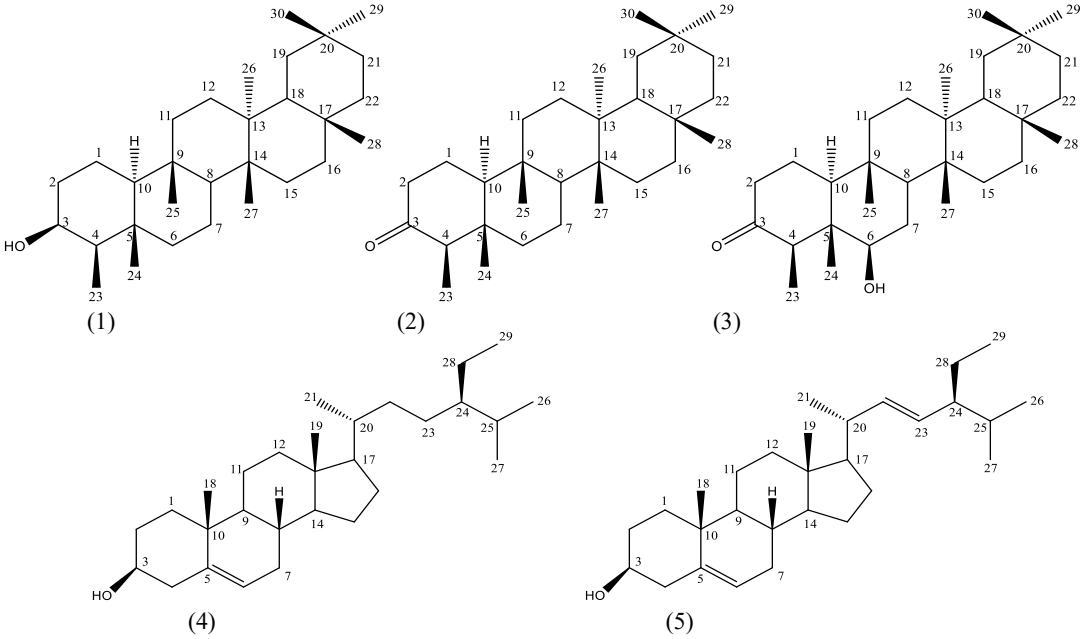

Fig.1: Structures of friedelan-3β-ol (1), friedelan-3-one (2), zeylanol (3), β-sitosterol (4) and stigmasterol (5)

Insecticidal activity

Toxicity of test samples on adult S. zeamais

Among the extracts, the petroleum ether extract demonstrated the highest toxicity (LD_{50} = 0.86 µg/mL) against adult *S. zeamais* while the methanol extract showed the lowest toxicity (LD_{50} = 1.25 µg/mL), Table 2. Remarkably, it was observed that the less polar crude extracts (petroleum ether and acetone) generally exhibited higher toxicity than the more polar methanol crude extract in the order; methanol<acetone<petroleum ether. This observation is consistent with the fact that low LD_{50} values were also obtained for compounds obtained from the less polar petroleum ether extracts. Friedelan-3-one (2), friedelan-3β-ol (1), and a mixture of the two compounds, recorded LD_{50} values of 0.48, 0.56 and 0.52 µg/mL respectively.

Ovicidal and larvicidal effects of crude extracts on S. zeamais

The effect of the extracts: methanol, acetone and petroleum ether crude extracts on the development of hidden eggs and immature stages of *S. zeamais* inside grains was generally similar. Maize grains treated with the crude extracts significantly inhibited the eggs and immature stages of *S. zeamais* ($p < 0.05$) as indicated in (Fig. 2). Treatments applied 14 days after oviposition recorded higher numbers of emerged adult *S. zeamais* than in treatments applied earlier than 14 days. Also, none of the extracts completely inhibited the development of *S. zeamais*; nevertheless, these extracts reduced the progeny emergence during the trial.

Effect of plant extracts on F1 progeny production

As shown in Fig. 3, the extracts showed a dose-dependent activity particularly for petroleum ether with respect to F1 progeny production by the treated grains. While lower concentrations of the plant extracts were less effective in reducing the number of F1 progeny produced by the *S. zeamais,* acetone extract completely inhibited F1 progeny production at 2 g/100 g grain. Methanol and petroleum ether also provided complete F1 inhibition at 10 g/100 g grain.

Damage assessment

The effects of extracts on weight loss and seed damage on grains showed that damage caused by the insects to the seeds was more in the various controls than maize treated with the different extracts (Table 3). More grains were damaged at lower concentrations (0.016 and 0.08 g/100 g) of the extracts while no damage was recorded for the highest dose of 10 g/100 g grain.

Anti-hookworm activity of crude extracts and isolated compounds

The results of the crude extracts and isolated compounds evaluated in the human hookworm

Table 2: Lethal dosage (LD_{50} (µg/mL) ± SEM) of test samples of *D. madagascariense* on *S. zeamais*

Samples	LD_{50} (µg/mL) ± SEM
Petrol ether extract	0.86 ± 0.18
Acetone extract	0.94 ± 0.30
Methanol extract	1.25 ± 0.34
Mixture of β-sitosterol & stigmasterol(5)	0.98 ± 0.19
Friedelan-3-one(2)	0.48 ± 0.08
Mixture of Friedelan-3-one & friedelan-3β-ol	0.52 ± 0.09
Friedelan-3β-ol (1)	0.56 ± 0.09

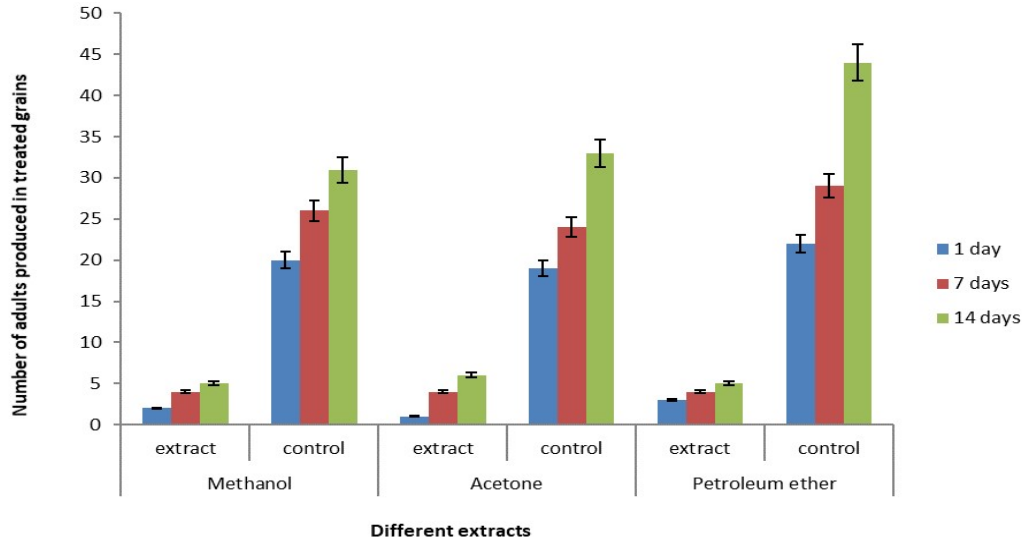

Fig. 2: Mean number of adult *S. zeamais* produced in *D. madagascariense* extract-treated maize after oviposition period

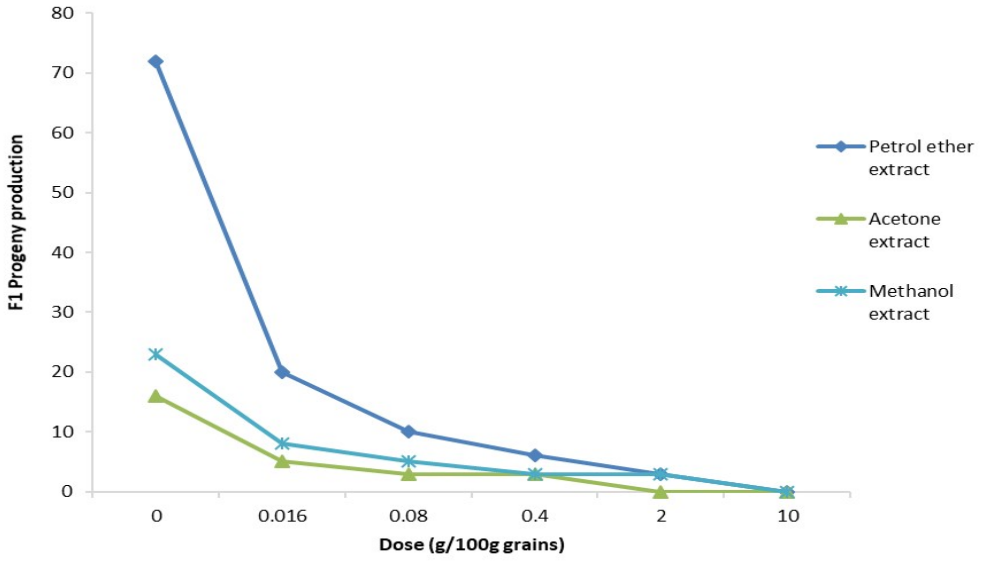

Fig. 3: Effect of plant extracts on F1 progeny production

Table 3: Percentage weight loss caused *by S. zeamais* in *D. madagascariense* extract-treated

Dose extract (g/100 g grain)	% weight loss of grain in different extracts		
	Petroleum ether extract	*Acetone extract*	*Methanol extract*
10	0	0	0
2	0.14	0.01	0.01
0.4	0.33	0.07	0.02
0.08	2.43	0.09	0.06
0.016	2.44	0.16	0.14
0 (control)	4.60	2.42	2.38

Table 4: IC_{50} values (±SEM) for samples and albendazole against the egg hatch inhibition of *N. americanus*

Samples	IC_{50} (µg/µL) ± SEM
Petroleum ether extract	1.33 ± 0.079
Acetone extract	1.13 ± 0.074
Friedelan-3β-ol (1)	0.64 ± 0.037
Friedelan-3-one (2)	0.95 ± 0.048
β-sitosterol (4)	0.75 ± 0.061
Mixture of Friedelan-3-one and friedelan-3β-ol	1.72 ± 0.13
Mixture of β-sitosterol and stigmasterol (5)	9.41 ± 2.06
Albendazole	0.0024 ± 0.0005

egg hatch inhibition assay are shown in Table 4.

In general, pure compounds (IC_{50} 0.64-0.95 µg/µL) were found to show more inhibitory activity towards the hookworm, *N. americanus* compared with the crude extracts (1.13-1.33 µg/µL) and mixtures (1.72-9.41 µg/µL) of the pure compounds**.** The most potent among the pure compounds was friedelan-3β-ol (1) and the least was friedelan-3-one (2), while the acetone extract was the most active extract. The mixture of β-sitosterol (4) and stigmasterol (5) was the least potent (9.41 µg/µL) among all the tested samples. None of these tested substances however had any significant activity compared with the selected drug of choice, albendazole, used as the positive control.

DISCUSSION

Zeylanol (3), a relatively rare triterpenoid, is reported for the first time from *D. madagascariense* in addition to the relatively more common triterpenoids friedelan-3β-ol (1), friedelan-3-one (2) and β-sitosterol (4). This is chemotaxonomically significant since the compound has also been isolated from *D. gelonioides* (Fang *et al.*, 2006) and other families such as Celastraceae and Balanopaceae (Gunatilaka *et al*., 1983; Setzer *et al*., 2000). Toxicity of the petroleum ether and the acetone extracts as well as the compounds isolated from them to adult *S. zeamais* was higher in potency than the more polar methanol extract. Generally, topically applied insecticides need to penetrate the non-polar insect cuticle to cause any toxicological effect that may be lethal. The different extracts applied to maize grains at doses ≥ 2 g/100 g of grain also provided much better protection to maize against hidden eggs and immature stages of *S. zeamais* attack compared to the lower doses. *S. zeamais* killed in treated grains mostly appeared paralyzed before death, suggesting that toxicity was not only due to ingestion of treated grains but also through contact toxicity (Obeng *et al*, 1997). The significant reduction in feeding damage, number of F1 progeny produced, and inhibition of the development of eggs and immature stages of *S. zeamais* by the extracts indicate the higher protectant potential of these extracts against insect damage to stored grains, suggesting the presence of growth regulatory and ovicidal metabolites in *D. madagascariense* (Ogunwolu and Idowu, 1994). Hence, the plant extracts can inhibit egg -hatching and control the growth of *S. zeamais* to some extent.

The potency of the plant extracts as grain protectant and insect-toxins might also be due to the oily nature of the petroleum ether and the acetone extracts which may block the insect spiracles, coat the seed testae, or act as ovicides by plugging the egg micropyle thus hindering oxygen supply to the embryo (Schoonhoven, 1978; Pereira, 1983). The complete protection of grains by the different extracts applied at the highest dosage of 10 g per 100 g of grain, may suggest the presence of anti-feedant properties by the plant extracts. Whereas some amount of feeding by the insect after treatment with the extracts was reflected in weight losses, the weight losses recorded were minimal. It was also observed that though the insects were able to bore into the seeds at lower doses, these grains were not necessarily eaten up by the *S. zeamais* as would be expected. At lower concentrations, the extracts did not adversely inhibit progeny production of *S. zeamais*. However, under practical storage conditions, relatively higher doses may be required as compared to lower doses applied in experimental conditions which may then increase potency. Insecticidal activity has been carried out on friedelan-3-one (2) and stigmasterol (5) against *Musca domestica* L. and *Aedes albopictus* (Skuse). Friedelan-3-one (2) was toxic to both species whereas stigmasterol (5) was not. The LC_{50} value of friedelan-3-one (2) against adult *M. domestica* 48 hours after treatment was 129.27 µg/g (Huang *et al,* 2009); while the LD_{50} of the compound against *S. zeamais* in the current study was 0.48 µg/mL, an indication of the significant toxicity of the compound to *S. zeamais* as compared with *M. domestica*.

The observations of antifeedancy as demonstrated by the ability of the extracts to protect maize grains from attack by the weevils, the oviposition deterrence ability and reduced F1 progeny production are indicators of the insecticidal potential of these constituents and the crude extracts of *D. madagascariense* stem. Such cannot be suggested for the hookworm activity carried out for the extracts and isolates as their potencies compared to the standard albendazole was very low. The most potent pure compound for the hookworm activity was friedelan-3β-ol (1) (IC_{50} = 0.64 g/mL), which was 1.5 times more potent than friedelan-3-one (2) and 2.7 more potent when in a mixture with the ketone form. Similarly, β-sitosterol (4) was 12.5 times more potent than its mixture with stigmasterol (5). The control standard, albendazole, showed a remarkably high potency (IC_{50} = 0.0024) exceeding over 260 times that obtained for the most potent tested compound, friedelan-3β-ol (1). Although the biological activity of the tested compounds was far less than that of the standard drug for the specific test helminths, the compounds demonstrated some activity to serve as lead compounds for further optimization and development in the

anthelminthic drug discovery efforts.

Even though the topical application method is concentration independent, the technique is usually laborious (Moyses and Gfeller, 2001). Also, the reduction in the rate of cuticular penetration of extracts gives the metabolic system of the insect the opportunity to degrade the extract into innocuous materials (Georghiou and Saito, 1983). Thus, only a small concentration of the active compound gets to the insects to elicit activity. The Kato-Katz method as a semi quantitative technique is usually not useful for routine tests in primary health care. This is attributed to the low reproducibility of the technique which results in diagnostic errors (Kongs *et al.,* 2001). The technique also relies on a single Kato-Katz thick smear procedure, this leads to low sensitivity with respect to the detection of the parasite (Barenbold *et al*., 2017).

CONCLUSION

This study has established the presence of zeylanol (3) in the stem bark of *D. madagascariense* and also shown that the crude extracts and compounds from the stem bark of *D. madagascariense* are toxic to the different stages of *S. zeamais* in its growth cycle. Thus, the isolates and extracts from the stem of *D. madagascariense* can give some protection to grains from the maize weevil. However, the level of activity of extract at over 100 g/kg maize grain for complete protection of maize destruction against *S. zeamais* and production for F1 progeny makes it uneconomical for inclusion of the stem bark of *D. madagascariense* in a pest management programme. Also, the extracts and isolates demonstrated low activity compared to the standard, albendazole, hence cannot be considered as potential candidates for the development of anthelmintic drugs.

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