ISSN 2449-8955 European Journal of Biological Research *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.4488096

Efficacy of *Detarium microcarpum* extracts and synergistic effect of combine extract and ivermectin against *Caenorhabditis elegans*

Haladu Ali Gagman [1,2], Nik Ahmad Irwan Izzaudin Nik Him [1], Bashir Mohammed Abubakar [2]*, Hamdan Ahmad [1]

[1] School of Biological Science, Universiti Sains Malaysia, 11800 Penang, Malaysia

[2] Department of Biological Sciences, Faculty of Science, Bauchi State University Gadau, 751 Itas Gadau, Nigeria

* Corresponding author: E-mail: bashiramohammed@basug.edu.ng; elbash1150@gmail.com

Received: 17 November 2020; **Revised submission:** 21 January 2021; **Accepted:** 01 February 2021

http://www.jbrodka.com/index.php/ejbr

ABSTRACT: This work tested the efficacy of crude methanol and aqueous extracts from the stem bark of *Detarium microcarpum* and the effect of combined extract and ivermectin *in vitro* against the motility of *C. elegans* Bristol N2 and *C. elegans* DA1316 L4 larvae. Series of concentrations (0.2, 0.6, 0.8, 1.0, and 2.0 mg/ml) of aqueous and methanolic extracts of *D. microcarpum* was used for the test. Counting of the motile larvae was carried out after 24 hours and 48 hours. A further test was carried out using a combination of plant extract and ivermectin. Both the aqueous and the methanolic extracts exhibited good anthelmintic activity in the inhibition of larval motility of *C. elegans* Bristol N2 as well of *C. elegans* DA1316 with a significant difference at $P < 0.05$ when compared to the negative control. However, a significant difference occurred between treatment with aqueous and methanolic extract at $P < 0.05$. The performance of the extracts was concentration and time-dependent. A combination of plant extract and ivermectin prove more potent than the pure extract against both strains of *C. elegans.* These extracts may be used to control parasitic nematodes including ivermectin resistant type. Treatment using combined plant anthelmintic and synthetic drugs should be encouraged as the combination was more promising. Further studies should be carried on the identification of active compounds in the extracts and studying the mode of action of the drugs on the nematodes and *in vivo* tests of the extract.

Keywords: Efficacy; Motility; Inhibition; Concentration; Extracts; Anthelmintic.

1. INTRODUCTION

The use of herbal medicine sorely or alongside synthetic drugs by the socioeconomically disadvantaged nations is on the increase. Increasing cases of drug resistance by the intestinal nematodes are responsible for significant economic losses within the veterinary industry and sometimes affects the Public health sector because control of the parasites using synthetic drugs often leads to environmental pollution [1, 2]. There is a need for scientific validation of the herbal remedies use by ethno-veterinary farmers. There is also the need to develop new effective and cheaper drugs from plants as a natural source [3]. These can be

achieved through research and drug development, which involved *in vitro* screening of plant extracts and other natural substances as the first line of action to identify the plant and plant products to be use for drug formulations [4].

The major challenge in drug development is the screening of substances for anthelmintics. For instance, it is expensive to screen in the natural host because special facilities are required which often make it expensive not possible for extensive screening. However, *in vitro* studies have been used as one of the best approach to reduce the problem to the minimum [4]. However, one of the obstacles in the application of *in vitro* process for drug screening is obtaining a suitable nematode of interest for the assay that can evaluate the efficacy of the substances against the parasitic nematodes [5]. The *in vitro* screening for anthelmintics against parasitic nematodes involves mostly developmental stages of nematodes such as egg hatch assay, larval development, and larval motility assay, but is difficult to obtained live adult parasitic nematodes worms to be used for the screening [6]. Where the adult parasitic nematodes are to use, such as the case of *Haemonchus contortus*, killing and dissecting the host become the only method of obtaining the adult worms for the assay, which makes it expensive and hectic [7].

The most fortunate development in the search for anthelmintics drugs today is the exhibition of similar anatomical and physiological effects by many anthelmintics on non-parasitic nematode *Caenorhabditis elegans* compared to that of parasitic nematodes such as *Haemonchus contortus, Ostertagia ostertagi* [8, 9]. *Caenorhabditis elegans* is a naturally occurring free-living nematode found in the temperate region of the world. *C. elegans* has been used as a model in biomedical and veterinary research for drug discovery [1, 8]. This is because of its unique characteristics as the only free-living none parasitic nematode with a full life cycle from egg to adult stage coupled with other characteristics such as ease of maintenance, low cost *in vitro* studies, fast rate of multiplication, and transparent body for easy study.

Detarium microcarpum is a leguminous tree of African origin [10]. The tree belongs to the family Fabaceae [11]. Both ethanolic stem bark's extract and the methanolic fruit coat extract has demonstrated antimicrobial action against a wide range of infectious microorganisms such as *Klebsiella pneumonieae*, *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* [12, 13]. This work was aimed at testing the efficacy of crude methanol and aqueous extracts from the stem bark of *D. microcarpum, in vitro* on *C. elegans* Bristol N2, which is susceptible to ivermectin and *C. elegans* DA1316 (ivermectin resistant strain).

2. MATERIALS AND METHODS

2.1. Collection of plants part and preparation of extracts

The stem bark of *D. microcarpum* was collected among the trees of the Savannah vegetation of Azare in Katagum Local Government Area of Bauchi State, Nigeria. The plant with the voucher specimen No. 3105 was authenticated at the Department of Biological Science, Bauchi State University Gadau, Bauchi State, Nigeria. The sample was crushed into pieces using pistil and mortar, shade dried at room temperature for three weeks before it was pulverized into powdered form. The extraction was carried out at the Universiti Sains Malaysia.

2.2. Phytochemical extraction

Methanol extraction was carried out as described by Paritala et al. [14]. 50 g of the plant's powdered sample was macerated in 300 ml (1:6 v/v) of 80% methanol for three days at room temperature. The infusion

was filtered through a Whatman No. 1 filter paper. The filtrate was dried in an oven at 45ºC. The dry extract was preserved in a labeled sterile specimen vial at 4ºC until further use. The same procedure was applied for aqueous extractions, where distilled water was used instead of 80% methanol.

2.3. Phytochemical screening

The preliminary phytochemical screening was carried out by mixing extracts solutions with various reagents such as Dragendorff's reagents (alkaloids), Salkowski's test using chloroform and concentrated sulfuric acid (terpenoids), extract solution mixed with ferric chloride ($FeCl_3$) solution (phenols) extract solution mixed with 10% potassium hydroxide (tannins). Froth formation on shaking with water (saponins), Acetic anhydride $(CH_3CO)_2O$) mixed with concentrated sulfuric acid (H_2HSO_4) (steroids) as described by Tadesse [15] and Cocan [16].

2.4. Total phenolic content (TPC) and total tannin content (TTC)

The method of Orak [17] was adapted using Folin-Ciocalteau reagent in the determination of total phenolic and total tannins content of methanol and aqueous extracts of *D. microcarpum*. Calibration curve Gallic acid serial concentration of (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 mg/ml) in 50 % (v/v) methanol was prepared and used as a standard. Absorbance values against the varying concentrations of the gallic acid standard were used to produce a calibration curve from which the regression equation (y = ax + b) of the curve was obtained using Microsoft Excel software 2016. The regression equation was used to calculate the phenolic content of the extracts, and the results were expressed as Gallic Acid Equivalence in mg (GAE/mg).

Folin-Denis spectrometric method described by Oliveira et al. [18] was used to determine total tannic content. Still, the procedure was the same as that of the determination of total phenolic content. However, the calibration curve was obtained using various concentrations of the Tannic acid solution (0, 0.5, 1, 1.5, 2, 2.5 mg/ml) instead of gallic acid. Folin-Denis reagent was used instead of, and the tannin content of the extract was expressed as Tannic Acid Equivalent (TAE/ mg/ml).

2.5. Maintenance of *C. elegans* and synchronization

The strains of *C. elegans* used for this work, *C. elegans* Bristol N2 and *C. elegans* DA1316 resistant were obtained from *C. elegance* genetic center (CGC), USA. Synchronized populations of the required strains *C. elegans* was used for this experiment as suggested by Baugh [19]. Synchronization of the *C. elegans* was carried out by adding 5 ml of fresh alkaline bleaching solution (a mixture of 1N NaOH and hypochlorite in the ratio of 1:2) to about 1 ml of a pelleted mixture of eggs and gravid adults in a 15 ml centrifuge tube. The content was shaken vigorously with occasional vortexing for about 5 minutes until most of the bacteria and the adult worms were dissolved. About 8 ml of M9 solution (3g KH_2PO, 6 g Na_2HPO_2, 6 g NaCl and 1 M $MgSO_4$ in 1 liter of distilled water) was added to the content to stop the bleaching process. The content was centrifuged at 1500 rpm, aspirated, and the pellet was re-suspended in M9 solution, shaken, centrifuged, and aspirated. The pelleted eggs were suspended in 1 ml of M9 buffer and transferred to the unseeded nematodes growth medium NGM (mixtures of 3 g NaCl 17 g agar and 2.5 g of Bacto peptone in 1000 ml of distilled water autoclaved and 1 ml of 1 M $CaCl_2$, 1 ml of 5 mg/ml cholesterol, 1 ml of 1 M $MgSO_4$, 25 ml of 1 M KPO_4 buffer, all autoclaved except cholesterol). The plate was kept at 20ºC in a shaker incubator overnight and the L1 larvae were observed the next day [19]. The L1 larvae were washed by centrifugation and transferred to a new NGM plate seeded with *E. coli* OP50 and incubated overnight at 20ºC until the L4 larvae emerged [20, 21].

2.6. *In vitro* bioassay of crude methanol and aqueous extracts on the motility inhibition of L4 larvae of *C. elegans* Bristol N2 and *C. elegans* DA1316

Evaluation of the efficacy of the extracts was based on the standard of the World Association for the advancement of veterinary Parasitology (WAAVP). The standard considers the ovicidal or larvicidal efficacy of the anthelmintic agent to be effective when it is up to 90% but moderately effective when it is lower than 90% but up to 80%. A total of 200 mg of each crude methanol and aqueous extracts of *D. microcarpum* was first dissolved in 10 ml of 1% Tween 80 solution and subsequently diluted with 90 ml of M9 buffer to give a stock solution of 2 mg/ml. Serial concentrations of 0.2, 0.6, 0.8, 1.0, and 2.0 mg/ml were prepared from the stock solution according to the method of Kumarasingha et al. [4]. A solution of 0.02 µg/ml of ivermectin was prepared by dissolving 1 mg solid sample of ivermectin in 1 ml of 1% DMSO and subsequently diluted with the M9 solution to 0.02 µg/ml.

The test was conducted on 24 macro-wells plates. Suspension of 50 µl containing approximately 100 L4 larvae of the strain of *C. elegans* to be tested was added to 24 wells. 1 ml of each of the tested extract concentrations (0.2, 0.6, 0.8, 1.0, and 2.0 mg/ml) was added to the larvae in the wells in triplicate. Larvae in the wells treated with 0.02 µg/ml of ivermectin served as the positive control. Larvae in wells treated with only M9 solution served as the negative control. The setup was incubated at 20ºC. Observation and counting of motile larvae were done after every 24 and 48 hours post-exposure to the extracts using an inverted microscope. The larvae were considered immotile in the absence of movement of any of the following; tail, head, or pharyngeal movement within at least five seconds of careful examination. Each experiment was repeated three times, and the average results were recorded. Percentage worm motility (%WM) was calculated based on the formula used by Tariq et al. [22] as follows:

WM % = [(number of worms in negative control well – number of mobil worms in treatment well) / number of worms in negative control well] x 100

2.7. Synergistic effect of combined plant extract and ivermectin motility of *C. elegans*

The bioassay for the synergistic effect of combined plant extract and ivermectin was carried out by mixing 0.02 µg/ml of ivermectin with the lowest concentration (0.2 mg/ml) of methanol extract of *D. microcarpum.* The methanol extract of *D. microcarpum* was chosen because it proved to be more effective than the aqueous extract. The effect was confirmed using the following methods:

2.7.1. Treatment with ivermectin after exposure to plant extracts (indirect combination)

The synchronized population of *C. elegans* Bristol N2 and *C. elegans* DA1316 were first incubated in 0.2 mg/ml methanol extract of *D. microcarpum* for 24 hours. A total of 100 larvae of the required strain of *C. elegans* incubated in 0.2 mg/ml of *D. microcarpum* for 24 hours were added to macro-wells in three replications followed by the addition 1 ml of 0.02 µg/ml of ivermectin. A total of 100 untreated larvae were added to wells in 3 replications, and 0.2 mg/ml of methanol extract of *D. microcarpum* was added to the larvae in each of the wells. Another set of 100 untreated larvae of the required strain was added to another sets of well in 3 replications followed by the addition of 0.02 µg/ml of ivermectin, and these served as positive controls. The last set of 100 untreated larvae added to each well in 3 replications were treated with M9 buffer. These served as negative control. The set up was incubated for 48 hours at 20ºC. The motile larvae in all the various treatments and the negative control wells were counted, and the percentage motility was computed.

2.7.2. Direct combination of plant extract and ivermectin

A total of 100 larvae of the required strain of the *C. elegans* were added to each of the 12 wells. One ml of a mixture of 1:1 of 0.2 mg/ml of *D. microcarpum* and 0.02 µg/ml ivermectin was added to the larvae in 3 of the wells. 1ml of 0.2 ml of *D. microcarpum* was added to larvae in the wells in 3 replications. Three (3) of the well containing the larvae were treated with 1 ml of 0.02 µg/ml of ivermectin and served as the positive control. 1 ml of M9 buffer was added to the fourth set of the larvae in 3 replications and served as the negative control. The setup was incubated for 48 hours, after which the motile larvae in each well were counted, and the percentage motility was computed.

2.8. Statistical analysis

Microsoft® Excel 2016 software was used to compute the percentage mean/standard error (SE) of the motility inhibitory efficacy of the various extracts ivermectin and other treatments against the larvae. IBM SPSS® statistic version 24 was used for the Statistical analysis. The comparison of the mean percentage motility inhibitory efficacy among different concentrations of the extracts and between the extract concentrations and the control was done using one–way ANOVA. The post hoc statistical significance used was the least square difference (LSD), and the difference between the means was considered significant at $P < 0.05$. Inhibitory concentration (IC50) was computed using probit analysis.

3. RESULTS

3.1. Results of phytochemical screening

The phytochemical screening of the extracts revealed more varieties of secondary metabolites in the methanol extract than in the aqueous extract of *D. microcarpum.* The secondary metabolites confirmed in the methanol extract include alkaloids, saponins, tannins, terpenoids, flavonoids, and phenols. Alkaloids, saponins, tannin sand phenols were reveled in aqueous extract.

3.2. Results total phenolic and total tannins contents

The highest phenolic and tannins content was recorded in the methanol extract than in the aqueous extract ($P < 0.01$). The methanol extract of *D. microcarpum* has the total phenolic content of 484.91 GAE/mg/ml and total tannins content of 6.23 TAE/mg/ml. On the other hand, the total phenolic content computed in the aqueous extract was 376.74 GAE/mg/ml, whereas 4.79 TAE/mg/ml was recorded as the total tannins content in the aqueous extract.

3.3. Results of *in vitro* bioassay of crude methanol and aqueous extracts against the motility of L4 larvae of *C. elegans* Bristol N2 and *C. elegans* DA1316

Both the methanol and the aqueous extracts of *D. microcarpum* demonstrated good inhibitory activity against the motility of the larvae of *C. elegans* DA1316 as well as *C. elegans* Bristol N2. The inhibitory activity of both the aqueous and methanol extracts increased with increase in the concentrations of the extracts and time (Figure 1-4). This is indicated by the gradual decrease in the percentage motility as concentration and time increased. The lowest performance of the extracts was recorded at 24 hrs at the concentration of 0.2 mg/ml as up 78.6% and 70.6% of *C. elegans* Bristol N2 in aqueous and methanol extracts, respectively were motile (Figure 1). Similarly, up to 80.8% and 70.2% motility was recorded for aqueous and methanol extract, respectively, against *C. elegans* DA1316 at 0.2 mg/ml in 24 hours (Figure 2). Generally the performance of

both the aqueous and methanol extract against the both strains of *C. elegans* within 24 hours was ineffective as less than 80 % of *C. elegans* were inhibited (Figure 1-2).

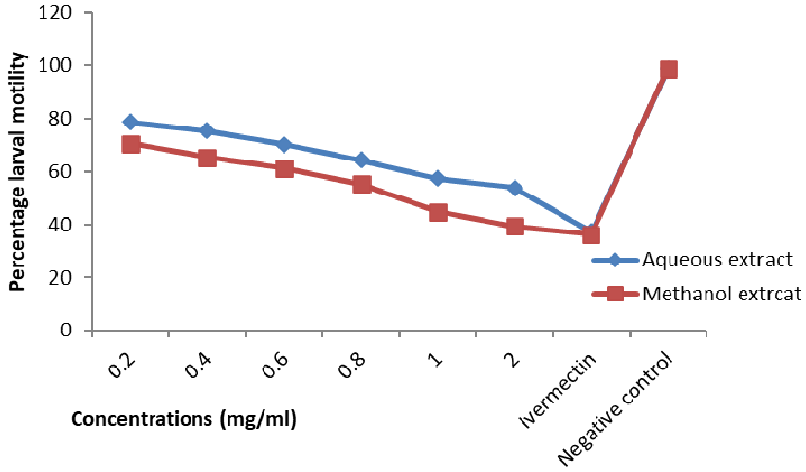

Figure 1. The efficacy of aqueous and methanol extract of *D. microcarpum* against *C. elegans* Bristol N2 after 24 hours.

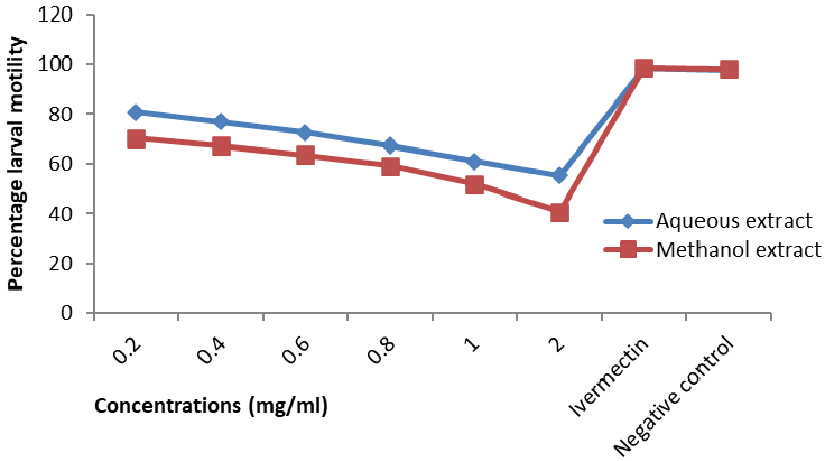

Figure 2. The efficacy of aqueous and methanol extracts of *D. microcarpum* against *C. elegans* D1316 after 24 hours.

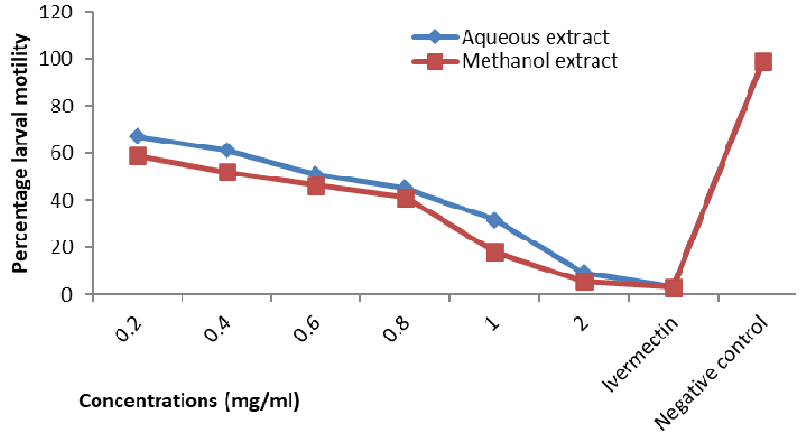

Figure 3. The efficacy of aqueous and methanol extracts of *D. microcarpum* against *C. elegans* Bristol N2 after 48 hours.

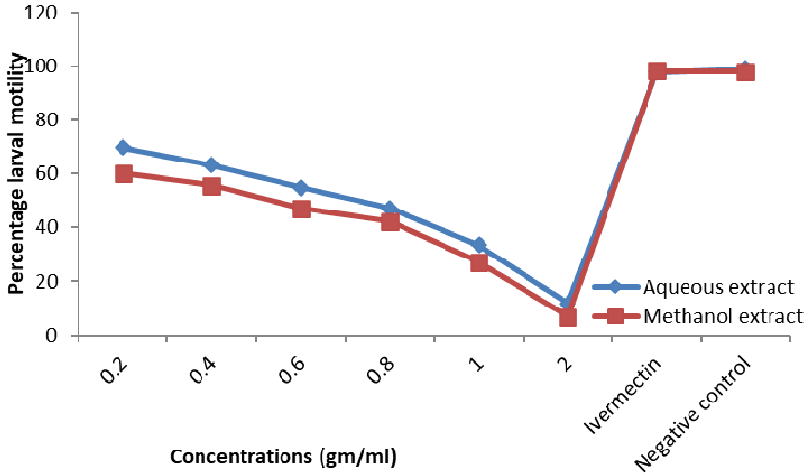

Figure 4. The efficacy of aqueous and methanol extracts of *D. microcarpum* against *C. elegans* D1316 after 48 hours.

The highest performance of the extracts against the motility of both strains of *C. elegans* was recorded at the highest concentration of 2.0 mg/ml after 48 hours, where the difference between the treatment and control became highly significant at $P < 0.001$ (Figure 3-4). This is evidenced as only 9.20% and 5.60% of *C. elegans* Bristol N2 motility was recorded in aqueous and methanol extract (Figure 3). On the other hand 11.80% and 6.80% motility of *C. elegans* DA1316 was recorded in aqueous and methanol extracts at 2.0 mg/ml after 48 hours (Figure 4).

A significant difference was established in the motility of the larvae between aqueous and methanol extracts ($P < 0.05$) at 2.0 mg/ml after 48 hours. There was no significant difference in the performance of the extract against the motility of *C. elegans* Bristol N2 and *C. elegans* DA1316 ($P > 0.05$). The difference between the extracts performance and ivermectin against *C. elegans* DA1316 was highly significant (0.001) as ivermectin was ineffective against *C. elegans* DA1316. This is also evidenced as there was no significant difference between the performances of ivermectin as compared to negative control against *C. elegans* DA1316. However, ivermectin was more effective against the motility of *C. elegans* Bristol N2 than the plant extracts at $P < 0.0\ 5$.

Considering IC_{50} (concentration at which the extract inhibited 50% of the larvae), the Lower IC_{50} value indicates the higher performance of the extract, whereas higher IC_{50} value indicates the lower performance of the extract. Based on the IC_{50}, the methanol extract was more efficient than the aqueous extract ($P < 0.05$). This is proven by the exhibition of lower IC_{50} value of 1.181 mg/ml by methanol extract compared to the higher IC_{50} value of 1.84 mg/ml for aqueous extracts at 24 hours against *C. elegans* Bristol N2. Similarly, a lower IC_{50} value of 0.443 mg/ml was recorded by the methanol extract compared to the higher IC_{50} value of 0.652 recorded by aqueous extract after 48 hours against *C. elegans* Bristol N2 (Table 1).

Table 1. IC_{50} of aqueous and methanol extract of *D. microcarpum* against *C. elegans* Bristol N2 and *C. elegans* DA1316.

Extracts type	***C. elegans* Bristol N2**		***C. elegans* DA1316**	
	IC_{50} mg/ml		IC_{50} mg/ml	
	24 hours	48 hours	24 hours	48 hours
Aqueous	1.842	0.652	2.112	0.717
Methanol	1.181	0.443	1.396	0.516

A similar trend was observed where the IC_{50} value of 2.112 mg/ml and 1.396 mg/ml was recorded by aqueous and methanol extract, respectively, against *C. elegans* DA1316 after 24 hours. On the other hand, the methanol extract with the lower IC_{50} value of 0.516 mg/ml was more efficient than the aqueous extract with a higher IC_{50} value of 0.717 mg/ml after 48 hrs against *C. elegans* DA1316 (Table 1).

3.4. Results of the synergistic effect of combined plant extract and ivermectin

The combined extract and ivermectin yielded a good result. The first assay (indirect combination) showed a moderately effective activity against both strains of *C. elegans.* This is because only 16.6% (83.4% inhibition) of *C. elegans* Bristol N2 were motile and 18.3% (81.7% inhibition) of *C. elegans* DA1316 were motile at 2.0 mg/ml after 48 hours (Table 2). In the extract of *D. microcarpum,* up to 65.5%, *C. elegans* Bristol N2 were motile, whereas 67.3% of *C. elegans* DA1316 were motile at 2.0 mg/ml after 48 hours (Table 2). In ivermectin, only 3.5% of *C. elegans* Bristol N2 were observed to be motile after 48 hours, whereas *C. elegans* DA1316, as usual, was insensitive to ivermectin as the larval motility remained as high as 99.4% after 48 hours.

A significant difference was established between the sensitivity of *C. elegans* DA1316 larvae treated with extract and ivermectin ($P < 0.05$). Also, the difference between *C. elegans* DA1316 treated with ivermectin after exposure to plant extract and those treated with ivermectin was significant at $P < 0.05$. However, there was no significant difference in sensitivity between *C. elegans* Bristol N2 and *C. elegans* DA1316 treated with ivermectin after exposure to plant extract ($P > 0.05$) (Table 2).

Table 2. Effect of treatment with ivermectin after exposure of the larvae to *D. microcarpum* extract.

Treatment	*C. elegans* Bristol N2	*C. elegans* DA1316
	% motility	% motility
Extract	65.5 ± 0.90	67.3 ± 0.92
Ivermectin after 24 hours	16.6 ± 0.85	18.3 ± 0.55
Ivermectin	3.57 ± 0.91	99.4 ± 0.61
Negative control	98.4 ± 0.38	98.2 ± 0.96

Data are presented as a percentage means ± standard error for three independent experiments. L4 larvae of *C. elegans* Bristol N2 and DA1316 were incubated for 48 hours in extract, indirectly combined extract, ivermectin, and M9 buffer. Counting of the immotile worms was carried out after 48 hours.

For direct combination, the mixture of ivermectin and plant extract yielded the most effective result as only 0.97% (99.03% inhibition) of *C. elegans* Bristol N2 while on the other hand, 1.2% (9.8% inhibition) of *C. elegans* DA1316 were motile (Table 3). The performance of a mixture of *D. microcarpum* extract and ivermectin was higher than ivermectin against *C. elegans* Bristol N2. The sensitivity of *C. elegans* Bristol N2 to treatment with a mixture of ivermectin and extract was the same when compared to that of *C. elegans* DA1316 ($p>0.05$). In the extract, up to 59.2% of *C. elegans,* Bristol N2 motility was recorded, and 61.1% was recorded against *C. elegans* DA1316. As usual, ivermectin was significantly effective against *C. elegans* Bristol N2 as only 4.2% of them were motile after exposure to ivermectin for 48 hours. The ineffectiveness of ivermectin against *C. elegans* DA1316 remained clear as there was no significant difference between the larvicidal efficacy of ivermectin and the negative control against *C. elegans* DA1316 (Table 3).

Table 3. Synergistic effect of direct combined of plant extract and ivermectin against the motility of *C. elegans.*

Treatment	*C. elegans* Bristol N2	*C. elegans* DA1316
	% motility	% motility
Extract	64.2 ± 0.86	66.1 ± 0.9
Ivermectin and extract	0.97 ± 0.41	1.20 ± 0.58
Ivermectin	4.20 ± 1.05	97.6 ± 0.83
Negative control	98.6 ± 0.52	98.2 ± 0.55

Data are presented as a percentage means ± standard error for three independent experiments. L4 larvae of *C. elegans* Bristol N2 and DA1316 were incubated for 48 hours in the extract, the mixture of ivermectin and extract, ivermectin, and M9 buffer. Counting of the immotile worms was carried out after and 48 hours.

4. DISCUSSION

This work was aimed at tested the efficacy of crude methanol and aqueous extracts from the stem bark of *D. microcarpum* and the effect of combined extract and ivermectin *in vitro* against the motility of *C. elegans* Bristol N2 and *C. elegans* DA1316 L4 larvae. The different diluents used in the preparation of the extracts' solution did not interfere with the natural potential of the extracts against larval motility. This is evidence because the percentage of larval motility remained high in the negative control experiment throughout the assay, coupled with the variation in the percentage larval motility in accordance with the various concentrations of the plant extracts.

Results of phytochemical screening of *D. microcarpum* aqueous extracts revealed the presence of alkaloids, saponins, tannins, among other phenolic compounds. On the other hand, secondary metabolites confirmed in methanol extracts include alkaloids, saponins, tannins, terpenoids, flavonoids, and tannins, among other phenolic compounds. This finding is related to that of Irondi et al. [23] and Zakari and Kubmarawa [24] where they found all the above mentioned secondary metabolites in the extracts from different parts of *D. microcarpum* such as stem, leaves, fruit, and seed coats. More varieties of secondary metabolites were confirmed in methanolic extract than aqueous extract. This finding is in line with the observation of Truong et al. [25] in their work titled, the evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia.*

Also, phenolic and tannins quantification revealed higher phenolic and tannins content in methanol extract than aqueous extract. Neffati et al. [26] in similar work recorded a higher quantity of tannins compounds in the methanol extracts than the aqueous extract of several plants investigated. The quantity of the secondary metabolite in the aqueous and methanol extracts varied. This could be attributed to the variation in the polarity between water and methanol used as the extraction solvent. Water as a polar solvent extracts only polar compounds. On the other hand, methanol exhibits both polar and non-polar characteristics this make it possible for water to extract both polar, and none polar compound hence placed it at the advantage of extracting more varieties and quantities of plant's secondary metabolites than water [27, 28]. Furthermore, the non-polar characteristic of methanol gives it the advantage of degrading the plant cell walls because they are also non-polar and this released more secondary metabolites [29].

Both methanol and aqueous extract of *D. microcarpum* exhibited good potency against *C. elegans* Bristol N2 and *C. elegans* DA1316. Presently, there is a scarce scientific experimental report on the efficacy

of *D. microcarpum* against *C. elegans.* However, *in vitro* antimicrobial activity of *D. microcarpum* has been documented by Ebi et al. [12]. Ethno-veterinary application of stem bark of *D. microcarpum* for treatment of gastrointestinal disorder as well as a dewormer in many African countries was reported by Douche et al.

Similarly, crude extracts from plants such as *Kaya senegalensis, Annona senegalensis*, and *Annogeisus leiocarpus* were reported to inhibit egg hatch, larval development, and survival of adult *C. elegans* [30]. Furthermore, Ndjonka et al. [31] reported that ellagic acid and gentistic acid from *Anogeissus leiocarpus* exhibited strong anthelmintic activity against *C. elegans* DA1316 among other synthetic drugs resistant strains of *C. elegans* tested. Crude aqueous extract of Psidium *guajava* was also reported to be effective against levamisole resistant strain of *C. elegans* (CB193) [32].

The findings in this research were further compared to the effects of other plant extracts on parasitic nematodes belonging to the same Clad V with *C. elegans.* For instance, the potency of the methanol and aqueous extracts of the *D. microcarpum* could be due to the presence of active compounds such as alkaloids, saponins, tannins, and other phenolic compounds which were confirmed to exhibit the following anthelmintics characteristics: Tannins inhibits the process of energy phosphorylation in the nematodes thereby leading to energy depletion and starvation of the nematode, and this will eventually leads to paralysis and death of the nematode. Tannins forms complex by binding to the free protein of the nematodes and could also attach to some structure in the nematode, such as the cuticle, digestive system and reproductive tract of the nematodes, thereby inhibiting their functions [33, 34]. Tannins have been confirmed to inhibit egg hatch and larval development in nematodes [33, 34]. Wang et al. [35] reported that saponins enhance the formation of pore and permeability of the nematode's cell membrane leading to vacuolization and disintegration of the nematode's integument.

The efficacy of the extracts was time and concentration-dependent, evidenced by the percentage decreased in the motility of the larvae as the concentration of the extracts increase and treatment time. This is similar to the findings of Kanojiya et al. [36], who observed that the percentage paralysis of ovine gastrointestinal nematodes increased as the concentration increased and incubation time. The methanol extract was more potent against the motility of both strains of *C. elegans* than aqueous extract ($P < 0.05$). This is similar to the report of Kanojiya et al. [36], who observed high effectiveness of crude methanol extracts of *Eucalyptus globulus* compared to aqueous extract against ovine gastrointestinal nematodes. The higher potency of methanol extract than that of the aqueous extract could be due to more varieties of secondary metabolites and their quantities in the methanol extract than that of the aqueous extract [37, 38]. Furthermore, the effectiveness of methanol extract against larval motility could be attributed to the action of the enzyme polyphenol oxidase, which degrades polyphenol in water extracts, thereby reducing the effectiveness of the extract. In contrast, such enzymes are inactive in methanol extracts [29].

The extracts of *Detarium microcarpum* exhibited good anthelmintic activity against the motility of *C. elegans* DA1316, whereas the organism proved resistant to 0.2 mg/ml of ivermectin. This might be attributed to different modes of action exhibited by the extract of *D. microcarpum* on the organism compares to that of ivermectin. This assumption is similar to the report of Kumarasingha et al. [4], who observed that extracts from the plant *Picria fel-terrae* induced a stress response in *C. elegans* wild-type and stress reporter (GFP-tagged reporter strain) different from that of Doramectin and Levamisole.

There is presently scarce record on the synergistic effect of combined plant extracts and synthetic drugs against the larval activity of *C. elegans.* This investigation revealed that *C. elegans* DA1316 treated with pure ivermectin (0.02 µg/ml) for 48 hours after exposure of the larvae to 0.2 mg/ml of methanol extract

of *D. microcarpum* for 24 hours yielded a moderately effective result. Treatment of *C. elegans* DA1316 with a direct combination (mixture) of the plant extract, and ivermectin (0.2 mg/ml of methanol extract of *D. microcarpum* and 0.0 2 µg/ml of ivermectin) yielded significantly effective results against the motility of the larvae than the indirect combination ($P < 0.05$). Furthermore, the direct combination of ivermectin and plant extract yielded the most effective result against *C. elegans* DA1316 than the extract.

The mechanism of ivermectin resistance in *C. elegans* DA1316 is complex due to a multiplicity of mutagenic genes involved [39, 40]. Such genes are associated with ivermectin resistance in *C. elegans* DA1316 and are responsible for encoding glutamate-gated chloride channels. These are; avr-14-(GluClα3), avr-15-(GluClα2) and glc-1-(GluClα1). Simultaneous mutation of these genes might have produced extreme resistance to ivermectin in *C. elegans* DA1316.

Mutation of two or any one of the genes does not confer resistance to ivermectin in *C. elegans* DA1316. This is because each of the genes is involved in parallel pathways. For instance, it has been postulated that avr-15 is responsible for regulating pharyngeal muscle, whereas avr-14 and glc-1 act in neurons controlling pharyngeal pumping [39]. Therefore, the effectiveness of the ivermectin against *C. elegans* DA1316 on exposure to combined plant extract might have resulted from the compromised of acquired resistance of the *C. elegans* DA1316.

Other reasons could result from several modes of action of the plant extract, which acted in synergy with ivermectin to yield a better result than ivermectin acting alone. This is in line with the findings of Hemaiswarya et al. [41]. Furthermore, Hemaiswarya et al. [41] and Ademola et al. [42] reported that synergism between several varieties of secondary metabolites could lead to several mechanisms of action against helminth. Clove oil and its major compounds combined with the synthetic drug were reported to produce the effective results of more than half the efficacy of pure synthetic antibiotics against oral bacteria [43].

5. CONCLUSION

The outcome of this research revealed good anthelmintic activity by both the methanolic and aqueous extract against the motility of *C. elegans* Bristol N2 and *C. elegans* DA1316. Combination of extract and ivermectin yielded a better larvicidal efficacy than the extract against both strains of *C. elegans.* These extracts could be used as an alternative control for parasitic nematode as well as ivermectin resistant types. The extract could be used in combination with commercial drugs. Further studies should be embarked upon to determine the active compounds from these extracts. Also, the mode of action of these extracts on the nematodes remains an important challenge to tackle in the future studies.

Authors' Contributions: NAIINH: Conception, designed of the experiment and supervision of the experiment and the writing. HAG: Development of methodology, conducted the experiment and provided the data and writing. BMA: Analysed the data and intemperate the result HA: Writing and review of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: This research was funded by Universiti Sains Malaysia (Research University Grant (1001/PBIOLOGI/811275). Haladu Ali Gagman was supported by the Authority of Bauchi State University Gadau, Bauchi state, Nigeria.

Acknowledgments: The authors are grateful to Universiti Sains Malaysia for their laboratorial and instrumental supports, and all laboratory technicians for technical aids in this project.

REFERENCES

1. Yan-Ping L, Zhang Y, Zhang HM, Zhang H, Zhang L, Hong-Juan Y, et al. Anti-parasitic effects of water-soluble alkaloid fractions from ethanolic extracts of *Sophora moorcroftiana* seeds in *Caenorhabditis elegans*. Chinese J Nat Med. 2018; 16(9): 665-673.
2. Aderibigbe SA, Idowu SO. Anthelmintic activity of *Ocimum gratissimum* and *Cymbopogon citratus* leaf extracts against *Haemonchus* placei adult worm. J Pharm Biores. 2020; 17(1): 8-12.
3. Taylor J, Rabe T, Mcgaw L, Jäger A, Van S. Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 2001; 34(1): 23-37.
4. Kumarasingha R, Palombo EA, Bhave M, Yeo TC, Lim DSL, Tu CL, et al. Enhancing a search for traditional medicinal plants with anthelmintic action by using wild type and stress reporter *Caenorhabditis elegans* strains as screening tools. Int J Parasitol. 2014; 44(5): 291-298.
5. Katiki LM, Ferreira JF, Zajac AM, Masler C, Lindsay DS, Chagas AC, Amarante AF. *Caenorhabditis elegans* as a model to screen plant extracts and compounds as natural anthelmintics for veterinary use. Vet Parasitol. 2011; 182(2-4): 264-268.
6. Brunet S, Jackson F, Hoste H. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. Int J Parasitol. 2008; 38(7): 783-790.
7. Marie-Magdeleine C, Hoste H, Mahieu M, Varo H, Archimede H. In vitro effects of *Cucurbita moschata* seed extracts on *Haemonchus contortus.* Vet Parasitol. 2009; 161(1-2): 99-105.
8. Burns AR, Luciani GM, Musso G, Bagg R, Yeo M, Zhang Y, et al. *Caenorhabditis elegans* is a useful model for anthelmintic discovery. Nature Commun. 2015; 6(1): 1-11.
9. Holden-Dye L, Walker R. Anthelmintic drugs and nematocides: studies in *Caenorhabditis elegans*. WormBook: the online review of *C. elegans* biology. 2014; 1-29.
10. Padonou EA, Teka O, Bachmann Y, Schmidt M, Lykke AM, Sinsin B. Using species distribution models to select species resistant to climate change for ecological restoration of bowé in West Africa. Afr J Ecol. 2015; 53(1): 83-92.
11. Paton AJ, Brummitt N, Govaerts R, Harman K, Hinchcliffe S, Allkin B, Lughadha EN. Towards Target 1 of the Global Strategy for Plant Conservation: a working list of all known plant species - progress and prospects. Taxon. 2008; 57(2): 602-611.
12. Ebi G, Afieroho O. Phytochemical and antimicrobial studies on *Detarium microcarpum* Guill and Sperr (Caesalpinioceae) seeds coat. Afr J Biotechnol. 2011; 10(3): 457-462.
13. Semde Z, Koudou J, Zongo C, Somda MK, Figueredo G, Ganou L, Traore AS. Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Detarium microcarpum* Guill. and Perr. leaves from Burkina Faso. Int J Pharmaceut Sci Res. 2018; 9: 956-964.
14. Paritala V, Chiruvella KK., Thammineni C, Ghanta RG, Mohammed A. Phytochemicals and antimicrobial potentials of mahogany family. Rev Brasil Farmacogn. 2015; 25(1): 61-83.
15. Tadesse B, Terefe G, Kebede N, Shibeshi, W. In Vivo anti-trypanosomal activity of dichloromethane and methanol crude leaf extracts of *Dovyalis abyssinica* (Salicaceae) against *Trypanosoma congolense*. BMC Complem Altern Med. 2015; 15(1): 1-11.
16. Cocan I, Alexa E, Danciu C, Radulov I, Galuscan A, Obistioiu D, Dehelean C. Phytochemical screening and biological activity of Lamiaceae family plant extracts. Exp Therap Med. 2018; 15(2): 1863-1870.

17. Orak HH. Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and their correlations. Scientia Horticult. 2007; 111(3): 235-241.
18. Oliveira LMB, Bevilagne CML, Cosha CZC. Anthelmintic activity of *Cocos nucifera L.* against sheep gastrointestinal nematodes. Vet Parasitol. 2009; 159(1): 55-59.
19. Baugh LR. To grow or not to grow: nutritional control of development during *Caenorhabditis elegans* L1 arrest. Genetics. 2013; 194(3): 539-555.
20. Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974; 77(1): 71-94.
21. Radman I, Greiss S, Chin JW. Efficient and rapid *C. elegans* transgenesis by bombardment and hygromycin B selection. PLoS One. 2013; 8(10): e76019.
22. Tariq K, Chishti M, Ahmad F, Shawl A. Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet Parasitol. 2009; 160(1-2): 83-88.
23. Irondi EA, Oboh G, Akindahunsi AA. Methanol extracts of *Brachystegia eurycoma* and *Detarium microcarpum* seeds flours inhibit some key enzymes linked to the pathology and complications of type 2 diabetes in vitro. Food Sci Human Wellness. 2015; 4(4):162-168.
24. Zakari A, Kubmarawa D. In vitro cytotoxicity studies and qualitative investigation of phytochemicals of stem bark extracts of *Detarium microcarpum* (Caesalpinioideae), *Echinaceae angustifolia* (Compositae) and *Isoberlinia doka* (Fabaceae). In Vitro. 2016; 1(1):22-26
25. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH. Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. J Food Quality. 2019; 9: ID 8178294.
26. Neffati N, Aloui Z, Karoui H, Guizani I, Boussaid M, Zaouali Y. Phytochemical composition and antioxidant activity of medicinal plants collected from the Tunisian flora. Nat Prod Res. 2017; 31(13): 1583-1588.
27. Dailey A, Vuong QV. Effect of extraction solvents on recovery of bioactive compounds and antioxidant properties from macadamia (*Macadamia tetraphylla*) skin waste. Cogent Food Agricult. 2015; 1(1): 1115646.
28. Simon BI, Lidianys ML, Claudia LL, Armida AG, Daniela F, Jose LR, David DH. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*. BMC Res Notes. 2015; 8(1): 396.
29. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharmac Sci. 2011; 1(1): 98-106.
30. Ndjonka D, Agyare C, Luersen K, Djafsia B, Achukwi D, Nukenine EN, et al. In vitro activity of Cameroonian and Ghanaian medicinal plants on parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes. J Helminthol. 2011; 85(3): 304-312.
31. Ndjonka D, Abladam E, Djafsia B, Ajonina-Ekoti I, Achukwi M, Liebau E. Anthelmintic activity of phenolic acids from the axlewood tree *Anogeissus leiocarpus* on the filarial nematode *Onchocerca ochengi* and drug-resistant strains of the free-living nematode *Caenorhabditis elegans*. J Helminthol. 2014; 88(4): 481-488.
32. Piña-Vázquez DM, Mayoral-Peña Z, Gómez-Sánchez M, Salazar-OlivoLA, Arellano-Carbajal F. Anthelmintic effect of *Psidium guajava* and *Tagetes erecta* on wild-type and Levamisole-resistant *Caenorhabditis elegans* strains. J Ethnopharmacol. 2017; 202: 92-96.
33. Debiage RR, Gonçalves FF, Pereira AR, da Silva RG, Yoshihara E, de Mello PET. Anthelmintic potential of *Psidium guajava* in sheep. Planta Med. 2016; 82(1): 997.
34. Williams AR, Fryganas C, Ramsay A, Mueller-Harvey I, Thamsborg SM. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum.* PloS One. 2014; 9(5): e97053.

35. Wang GX, Han J, Zhao LW, Jiang DX, Liu YT, Liu XL. Anthelmintic activity of steroidal saponins from *Paris polyphylla*. Phytomedicine. 2010; 17(14): 1102-1105.
36. Kanojiya D, Shanker D, Sudan V, Jaiswal AK, Parashar R. In vitro and in vivo efficacy of extracts of leaves of *Eucalyptus globulus* on ovine gastrointestinal nematodes. Parasitol Res. 2015; 114(1): 141-148.
37. Badar N, Iqbal Z, Khan MN, Akhtar MS. *In vitro* and *in vivo* anthelmintic activity of *Acacia nilotica* (L.) Willd. ex Delile bark and leaves. Pakistan Vet J. 2011; 31(3):185-191.
38. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. *Haemonchus contortus*: in vitro and in vivo anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix*. Exp Parasitol. 2007;116(4): 340-345.
39. Gilleard JS. Understanding anthelmintic resistance: the need for genomics and genetics. Int J Parasitol. 2006; 36(12): 1227-1239.
40. James CE, Hudson AL, Davey MW. Drug resistance mechanisms in helminths: is it survival of the fittest? Trends Parasitol. 2009; 25(7): 328-335.
41. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008; 15(8): 639-652.
42. Ademola IO, Eloff J. Anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. Trop Animal Health Prod. 2011; 43(2): 521-527.
43. Moon SE, Kim HY, Cha JD. Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria. Arch Oral Biol. 2011; 56(9): 907-916.