Hindawi
Journal of Parasitology Research
Volume 2023, Article ID 9545565, 6 pages
https://doi.org/10.1155/2023/9545565

Research Article

Nematocidal Activity of Ethanol and Aqueous Extracts of *Persea americana* Seeds against *Heligmosomoides polygyrus* using the Worm Microtracker Method

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Received 10 April 2023; Revised 6 June 2023; Accepted 21 August 2023; Published 14 September 2023

Academic Editor: María Eugenia López-Arellano

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Background. Infections with gastrointestinal helminths constitute a serious obstacle to the good health of the local population in most African Countries. The aim of this study was to evaluate the anthelminthic activity of *Persea americana* ethanol and aqueous extracts against *Heligmosomoides polygyrus* using the worm microtracker. *Method.* Aqueous and ethanolic extracts of *P. americana* were prepared. Different concentrations of the extracts were tested against the egg and larvae stages of *H. polygyrus* using an automated high-throughput method. Briefly, embryonated eggs and larvae of this parasite were obtained after the incubation of fresh eggs at 25°C for 24, 48, and 96 hours for embryonated eggs, L_1 and L_2 larvae, respectively. One hundred microliters of the plant extracts at various concentrations were put in contact in a 96-well microplate with a suspension of 100 embryonated eggs in a total volume of 200 μL and incubated in a worm microtracker where the motility of the worms was recorded every 30 minutes for the ovicidal activity. The final tested extract concentration was 5, 2.5, 1.25, 0.625, and 0.3125 mg/mL, whereas ringer solution (0.95%) and 1.5% Dimethyl sulfoxide (DMSO) were used as negative controls and levamisole as positive control. The same method was used for larvicidal activities. The anthelmintic activity was determined using the average movement of the worms in the tested product compared with the negative control (1.5% DMSO and ringer solution). *Results.* The egg hatching rates of *H. polygyrus* had IC_{50} of 0.49 mg/mL (95% confidence interval: 71.70–92.03) and 0.22 mg/mL (95% confidence interval: 74.28–86.18) for the ethanol and aqueous extract, respectively. These IC_{50} indicate that the aqueous extract is more active for the inhibition of hatching at a 95% confidence interval. The aqueous and ethanol extracts presented mean inhibitory hatching rates of $78.33 \pm 1.67\%$ and $75.67 \pm 1.15\%$ at 5 mg/mL, respectively, with no significant differences. The highest percentage of inhibition of L_1 larva was observed at 5 mg/mL with $89 \pm 2.3\%$and $85 \pm 2.7\%$ for the ethanol and aqueous extracts, respectively. The lowest percentage of inhibition was observed at 0.3125 mg/mL, with $54.67 \pm 3.38\%$ and $49 \pm 2.64\%$ for the ethanol and aqueous extract, respectively. No significant differences were observed between the two extracts at 5 mg/mL with an inhibitory percentage of $90.67 \pm 3.05\%$ (ethanol) and $89.33 \pm 2.08\%$ (aqueous). *Conclusion.* Extracts of *P. americana* seeds possess nematocidal activity, however, further *in silico* and *in vivo* investigations are necessary to confirm their anthelminthic activity.

1. Introduction

Helminthiases constitute a serious obstacle to the good health of the local population in most African Countries [1]. Furthermore, they are considered neglected tropical diseases partly because little attention is directed toward them, whereas more attention is directed toward other diseases, such as COVID-19, malaria, turberculosis, and Human immunodeficiency virus (HIV) [2]. These infections are responsible for great mortality and morbidity in humans and affect mostly school-age children by inhibiting their intellectual and growth rate rendering them vulnerable to other infections [3]. In African countries, helminthiasis is one of the neglected tropical diseases that need to be eradicated [4]. The study of Adoubryn et al. [5] presented a very high prevalence of parasite infection of 55.2% in Biankouma, Ivory Coast. In Cameroon, according to the National Program of Schistosomiasis and Gastrointestinal Helminth Control reported in 2006 that more than 10 million people were infected with various parasitic worms [6]. Cedric et al. [6, 7] demonstrated in the North West region of Cameroon a high prevalence of 23.5% among children in Bambili. The same observations were recorded by Igore et al. [8] in the Tonga Sub division, West Region of Cameroon. All this high prevalence indicates that Cameroon has a serious problem with helminthiases and is neglected in Cameroon despite all efforts being put in place by the government to eradicate this disease.

To control these parasitic infections, patients used synthetic anthelmintic drugs. However, unfortunately, the parasite has developed resistance to the available synthetic drugs [9]. These substances are also less available and sometimes have side effects. Some side effects have been reported and are equally toxic to the natural environment [10]. The search for newer sources of drugs to overcome this issue of resistance is now a global challenge preoccupying pharmaceutical companies and other research institutions [11]. This issue of resistance, poverty, low efficacy, and scarcity of synthetic drugs has drawn back the population, especially those living in tropical countries due to their dependence on natural plants to overcome helminthiasis [12].

Medicinal plants are often used by traditional healers in the western region of Cameroon to overcome this problem of resistance, high cost, and availability [13]. The misuse of synthetic drugs is now a serious challenge in the domain of drug discovery. It becomes imperative to screen local medicinal plant beliefs to possess anthelminthic activities by the local population to deeply study them and scientifically validate their usage in traditional medicine.

Avocado (*Persea americana*) peels, seeds, fruits, and leaves are commonly used in America and Africa for the treatment of hypertension, stomach pain, bronchitis, diarrhea, and diabetes [14]. Avocado seeds equally possess insecticidal, fungicidal, and antimicrobial activities [15]. Study shows that *P. americana* contains carbohydrates, proteins, vitamins, and phytochemicals like saponins, phenolics, alkaloids, tannins, and flavonoids, which contributes to its high nutritional and pharmaceutical habits [16]. Helminthiasis is treated in the western region of Cameroon with the help of *P. americana*.

Most anthelminthic *in vitro* tests are done on Petri dishes, lack repeatability, and are time-consuming [17].

The WMicroTracker (Phylumtech, Argentina), is an apparatus that measures the average motility of worms when in contact with a test product [18]. *Heligmosomoides polygyrus* mimics the infection of human parasites and is used as a model in anthelminthic drug discovery. Ngouateu et al. [19], Wabo et al. [10], and Komtangi et al. [20] used *Heligmosomoides bakeri* when evaluating the anthelminthic activity of some medicinal plants used in Cameroon. This study aimed to evaluate the anthelminthic activity of *P. americana* ethanol and aqueous extracts using the worm microtracker against *H. polygyrus* to deeply study them and scientifically validate their usage in traditional medicine.

2. Materials and Methods

2.1. Parasite. Swiss albinos mice were infected with L3 larva. After a prepatent period of 10 days, the infected mice started producing fresh eggs. The different stages of development of *H. polygyrus* were cultured on Petri dishes under ideal conditions, such as wet filter paper, following the method described by White [21]. *H. polygyrus* is a nematode used by pharmaceutical industries for the evaluation of anthelminthic compounds [22].

2.2. Collection and Identification of Plant Species. P. americana seeds, commonly known as avocado seeds, were bought from avocado sellers in Bamenda, and identified at the National Herbarium with the identification number 18604/sfr/Cam.

2.3. Preparation of Extracts. The method described by Wabo et al. [23] was used for the preparation of different extracts. Briefly, the seeds were grated using a kitchen grater and dry to obtain a powder. The plant powder (100 g) was introduced into 1 L of ethanol at 95% for three days and 1 L of boiled water (100°C) for 3 hours until the obtention of the filtrate. The filtrate was then dried at 50°C (drying at more than 50°C will denature the active principles in the extracts) to obtain the extract.

2.4. Production of Embryonated Eggs. The collection of eggs was done using the flotation technique described by Cédric et al. [13]. After the collection of fresh eggs, it was incubated at 25°C for 24 hours for the obtention of embryonated eggs and used for the evaluation of hatching.

2.5. Collection of H. Polygyrus L_1 and L_2 Larvae. Embryonated eggs were incubated 25°C in ringer solution for 24 and 72 hours for the obtention of L_1 and L_2 larvae, respectively.

2.6. Egg Hatching Test. The egg hatching test was evaluated using the worm microtracker method [13]. The final tested extract concentration ranged from 5 to 0.3125 mg/mL. Levamisole (5 μg/mL) and 0.5% Dimethyl sulfoxide (DMSO) were used, respectively, as positive and negative controls in a 98-well microplate titer. The plates were incubated at 25°C for 24 hours in a worm microtracker where the movement of the new larvae were recorded every 30 minutes. The

inhibition percentage was calculated as follows:

$$\%\text{Inhibition} = \frac{\text{Mobility activity of Control} - \text{msobility activity of the test sample}}{\text{Mobility activity of Control}} \times 100. \quad (1)$$

2.7. Larvicidal Activity. The larvicidal activity was determined using the worm microtracker method described by Cédric et al. [13]. The L1 and L2 larvae were put in contact with extracts with the same concentrations as the ovicidal activity (5–0.3125 mg/mL) and incubated for 24 hours in a final volume of 200 μL. The percentage of inhibition of larvae motility was determined [24].

2.8. Qualitative Phytochemical Screening. The method described by Harbone [25] was used to test for the presence of anthraquinones, anthocyanins, saponins, triterpenoids, sterols, and alkaloids.

2.9. Total Phenolic and Flavonoid Contents. The total phenolic and flavonoid contents were determined according to the method described by Sidiki et al. [26].

2.10. Ethical Considerations. The animals were well treated according to the guideline EEC Directive 86/609/EEC of the 24th November, 1986.

2.11. Statistical Analysis. The data was analyzed using the GraphPad Prism software version 8.0. The 50% inhibitory concentrations (IC 50) at a 95% confidence interval were determined using a standard curve obtained from the GraphPad.

3. Results

Table 1 shows the effect of *P. americana* extracts on the mean inhibitory hatching percentage and larvae motility. From the analysis of Table 1, the mean inhibitory hatching percentage of ringer solution and 1.5% DMSO were 0.00% (negative control) and 100% for levamisole (positive control). No significant difference was observed for the mean inhibitory hatching rate of the aqueous extract (78.33 ± 1.67%) and the ethanol extract (75.67 ± 1.15%) at 5 mg/mL.

The concentration of 5 mg/mL presented the highest percentage of inhibition for the L1 larva with 89 ± 2.3% (ethanol) and 85 ± 2.88% (aqueous). The inhibition was concentration-dependent. There was no significant difference in terms of their inhibition at the highest level of inhibition. Levamisole, which was the drug of choice (positive control), had the highest percentage (100.0%) of L_1 larva. This value indicated that levamisole has an inhibitory effect on the L_1 larva of *H. polygyrus*. For the L2 larva, the highest percentage of inhibition was observed at 5 mg/mL with 89.33 ± 1.2% (aqueous) and 90.67 ± 1.7% (ethanol). The lowest inhibition was observed at 0.3125 mg/mL, with 54.67 ± 5.8% and 49 ± 4.5% for the ethanol and aqueous extracts, respectively. From the different IC_{50} 0.49 mg/mL for the ethanol extract at 95% confidence interval (71.70–92.03) and 0.22 mg/mL for the aqueous extract at 95% confidence interval (74.28–86.18) it indicates that the aqueous extract is more active on egg hatching inhibition. A similar observation is noticed in the L1 and L2 larvae where the aqueous extract is more active than the ethanol extract. Table 2 shows the phytochemical screening of the aqueous and ethanol extracts of Persea americana. It follows from the analysis of this table that the aqueous extract contains all the screen phytochemical constituent except sterols. Similarly, the ethanolic extract contains the same compounds except saponins and sterols.

3.1. Total Phenolic and Flavonoid Contents. More flavonoid content extract (453.9 ± 43.46 mg/g) was observed in the ethanol extract compared with the aqueous extract (174.54 ± 38.10 mg/g). The ethanol extract equally had more phenolic content (381.10 ± 9.23 mg/g) with respect to the aqueous extract (321.9 ± 11.14 mg/g).

4. Discussion

No significant difference was observed for the mean inhibitory hatching rate of the aqueous extract (78.33 ± 1.67%) and the ethanol extract (75.67 ± 1.15%) at 5 mg/mL. This mean hatching inhibition rate seen in the wells containing the extracts confirms the fact that *P. americana* seed extracts possess egg hatching inhibitory power. Wabo et al. [27] obtained similar results with *Chenopodium ambrosiodes* on *H. bakeri* eggs and Rosa et al. [28] when evaluating *Persea willdenovii* extracts on gastrointestinal nematodes. The egg hatching rates of *H. polygyrus* had IC_{50} of 0.49 mg/mL and 0.22 mg/mL for the ethanol and aqueous extracts, respectively. These IC_{50} indicate that the aqueous extract is more active for the inhibition of hatching at a 95% confidence interval. According to Adamu et al. [29] crude extracts with IC50 ≥ 6 mg/mL are considered to have weak anthelmintic activity. Therefore, it can be concluded from these criteria of classification that the aqueous (0.49 mg/mL) and ethanol (0.22 mg/mL) extracts showed high ovicidal activity against *H. polygyrus.*

Similar *in vitro* activities were obtained by Rosa et al. [28] where all extracts of *P. willdenovii* inhibited the egg hatching of gastrointestinal nematodes ($p < 0.05$), in a concentration-dependent manner.

Previous studies conducted by Henry et al. [16] have shown that *P. americana* seed extracts contain lipid substances, alkaloids, phenols, and hydro-soluble compounds. From the analysis of the different IC_{50}, it is clear that the aqueous extract is more active for the inhibition of hatching at 95% confidence interval. This high ovicidal activity of the aqueous extract may be due to saponins present in their phytochemical composition which is absent in the ethanol extract.

Phytochemical screening revealed the presence of compounds, such as saponine, which is well known for its hatching inhibitory activity [30]. The embryonic development of structures used for hatching, such as the protractible stylet, which is used for hatching may have been inhibited by the plant extract. Furthermore, the extracts may have influenced the osmotic pressure and the shape of the egg by inhibiting the exchange of molecules across the eggshell. However,

Table 1: Percentage (%) inhibition of hatching, larvae motility, and half maximal inhibitory concentration (IC_{50}).

Anthelminthic test	Extract	Concentrations (mg/mL)					IC_{50}	95% confidence interval	Controls		
		0.312	0.625	1.25	2.5	5			Levamisole	1.5% DMSO	Ringer 0.9% NaCl
% Inhibition of hatching	Aqueous	49.00 ± 1^{e}	62.33 ± 1.45^{d}	67.33 ± 1.45^{a}	70.33 ± 0.88^{a}	78.33 ± 1.67^{b}	0.22	74.28–86.18	100.00 ± 0^{f}	0.00 ± 0^{g}	0.00 ± 0^{g}
	Ethanol	45.67 ± 2.33^{e}	58.33 ± 1.67^{d}	62.33 ± 1.46^{c}	67.33 ± 1.45^{b}	75.67 ± 1.15^{a}	0.49	71.70–92.03	100.00 ± 0^{f}	0.00 ± 0^{g}	0.00 ± 0^{g}
% Inhibition of L1 larva motility	Aqueous	49 ± 2.64^{c}	55.33 ± 2.9^{bi}	67 ± 1.52^{ghi}	75.67 ± 2.84^{fh}	85 ± 2.88^{a}	1.443	86.49–127.4	100 ± 0^{d}	0.00 ± 0^{e}	0.00 ± 0^{e}
	Ethanol	54.67 ± 3.38^{h}	59.67 ± 2.60^{g}	71.33 ± 1.86^{ci}	78.67 ± 2.02^{bi}	89 ± 2.3^{a}	1.75	90.71–143.7	100 ± 0^{d}	0.00 ± 0^{e}	0.00 ± 0^{e}
% Inhibition of L2 larva motility	Aqueous	55.67 ± 2.96^{dj}	62.33 ± 3.28^{cj}	74.67 ± 0.6^{bi}	82.67 ± 3.05^{ai}	89.33 ± 1.2^{abf}	0.97	88.78–120.9	100 ± 0^{f}	0.00 ± 0^{e}	0.00 ± 0^{e}
	Ethanol	62 ± 4.04^{dj}	65.00 ± 2.88^{chj}	77.00 ± 1.73^{bgh}	84.67 ± 0.6^{ag}	90.67 ± 1.7^{abf}	1.34	91.21–129.5	100 ± 0^{f}	0.00 ± 0^{e}	0.00 ± 0^{e}

a,b,c,d,e,f,g,h,i,j: values carrying the same superscript letter on the same row at different concentrations are not significantly different at $p \geq 0.05$.

Table 2: Qualitative analysis of *P. americana* extracts.

Phytochemicals	Extracts	
	Aqueous	Ethanol
Polyphenol	+	+
Flavonoids	+	+
Anthraquinone	+	+
Triterpenoids	+	+
Saponins	+	−
Alkaloids	+	+
Sterols	−	−

−: absent; +: present.

since the layers of the eggshells are hydrophilic, the aqueous extract can penetrate the eggshell better than ethanol extract. According to Caroline et al. [31], phenolic compounds do not influence egg hatching, which may be due to the eggshell, which is impermeable to phenolic compounds. The observation that the aqueous extract was more active was equally observed by Komtangi et al. [20].

The IC_{50} for aqueous extract was 1.443 mg/mL (95% confidence interval: 86.49–127.4) and 0.97 mg/mL (95% confidence interval: 88.78–120.9), respectively, for the L1 and L2 larvae, and 1.75 mg/mL (95% confidence interval: 90.71–143.7) and 1.34 mg/mL (95% confidence interval: 91.21–129.5) for the ethanol extract, respectively, for the L1 and L2 larvae, respectively. This result shows that L_2 larva are more sensitive than L1 larva. These findings were similar to earlier reports by Ngouateu et al. [19]. According to these authors, the L1 stage is still having some food or energy reserved since they are just from hatching, and as time goes on, the food reserved and energy gets finished, making them to be more sensitive to the tested compounds. The diffusion and absorption of the extract across the cuticle of *H. polygyrus* may be responsible for the observed larvicidal activity. The negative controls were free of dead larvae proving the fact that the high mortality (100%) rate in the wells containing the extracts was due to the active phytochemicals present in the extract validating its larvicidal activity. High mean inhibitory mobility concentration was observed at 5 mg/mL in a concentration-dependent manner.

Similar observations were made by Payne et al. [32]. According to Payne et al. [32], the active compounds found in the extracts may paralyze the larvae by blocking the postsynaptic receptors. Furthermore, active compounds may diffuse across the cuticle of the larvae, hence increasing the cell permeability which leads to the death of the larvae [33]. The results obtained at this stage were similar to that obtained by Wabo et al. [27] with *C. ambrosiodes* on the different larvae stages of *H. bakeri* and contrary to that obtained by Rosa et al. [28] where a low *in vitro* activity was observed for all the extracts and fractions of *P. willdenovii* leaves on the L_3 larva of gastrointestinal nematodes.

5. Conclusion

This study validates the use of *P. americana* seeds by traditional healers in the fight against helminthiasis. *In silico* and *in vivo* investigations are necessary to confirm their anthelminthic activity. However, validation of a method or technique implies multiple experiments with a deeper statistical analysis.

Data Availability

All data generated and analyzed are included in this research article.

Conflicts of Interest

The author(s) declare(s) that they have no conflicts of interest.

Authors' Contributions

The experiments were developed by Yamssi Cédric, Noumedem Anangmo Christelle Nadia, and Vincent Khan Payne. The experiments were carried out by Sandra Nfufu, Mounvera Abdel Azizi, and Tientcheu Noutong Jemimah Sandra. Data were analyzed by Yamssi Cédric and Noumedem Anangmo Christelle Nadia. The manuscript was written by Yamssi Cédric, Noumedem Anangmo Christelle Nadia, and Vincent Khan Payne. The final manuscript was read and approved by all authors.

Acknowledgments

The authors would like to thank Phylumtech in Argentina for donating the mini WMicroTracker devices utilized in this investigation. We would also like to thank Prof. Walter Luyten of Belgium, for initiating us in this domain and providing the required equipment and chemicals.

References

[1] A. Ahmed, H. M. Al-Mekhlafi, A. H. Al-Adhroey, I. Ithoi, A. M. Abdulsalam, and J. Surin, "The nutritional impacts of soil-transmitted helminths infections among Orang Asli schoolchildren in rural Malaysia," *Parasites and Vectors*, vol. 5, no. 1, pp. 1–9, 2012.

[2] D. K. de Souza, A. Picado, S. Biéler, S. Nogaro, and J. M. Ndung'u, "Diagnosis of neglected tropical diseases during and after the COVID-19 pandemic," *PLoS Neglected Tropical Diseases*, vol. 14, no. 8, article e0008587, 2020.

[3] L. Rodríguez, E. Cervantes, and R. Ortiz, "Malnutrition and gastrointestinal and respiratory infections in children: a public health problem," *International Journal of Environmental Research and Public Health*, vol. 8, no. 4, pp. 1174–1205, 2011.

[4] G. Martelli, C. Di Girolamo, L. Zammarchi et al., "Seroprevalence of five neglected parasitic diseases among immigrants accessing five infectious and tropical diseases units in Italy: a cross-sectional study," *Clinical Microbiology and Infection*, vol. 23, no. 5, pp. 335.e1–335.e5, 2017.

[5] K. Adoubryn, C. Kouadio-Yapo, J. Ouhon, N. Aka, F. Bintto, and A. Assoumou, "Parasitoses intestinales infantiles a Biankouma, région des 18 Montagnes (ouest de la Côte d'Ivoire): étude de l'efficacité et de la tolérance du praziquantel et de l'albendazole," *Médecine et Santé Tropicales*, vol. 22, no. 2, pp. 170–176, 2012.

[6] J. W. Pone, M. Mbida, P. N. E. Alango, and C. B. Bilong, "Prevalence and intensity of infections of three neglected tropical diseases in patients consulted at a Traditional Health Care Centre in Dschang West Cameroon," *Tropical Parasitology*, vol. 2, no. 1, p. 24, 2012.

[7] Y. Cedric, N. C. Nadia, C. Ebai, F. Ngoufo, G. Teke, and J. Dulafe, "Prevalence and risk factors of geohelminths in Ntamuchie, Mezam division, North West Region, Cameroon," *Open Public Health Journal*, vol. 2, no. 2, p. 1015, 2020.

[8] K. Igore, V. Payne, N. Nadia, and Y. Cedric, "Risk factors associated with prevalence and intensity of gastrointestinal parasitic infections within households in Tonga sub-division, West Region Cameroon," *Journal of Infectious Diseases and Epidemiology*, vol. 6, p. 123, 2020.

[9] R. K. Prichard and T. G. Geary, "Perspectives on the utility of moxidectin for the control of parasitic nematodes in the face of developing anthelmintic resistance," *International Journal for Parasitology: Drugs and Drug Resistance*, vol. 10, pp. 69–83, 2019.

[10] J. Wabo Poné, O. Fossi Tankoua, J. Yondo, M. C. Komtangi, M. Mbida, and C. Bilong Bilong, "The in vitro effects of aqueous and ethanolic extracts of the leaves of Ageratum conyzoides (Asteraceae) on three life cycle stages of the parasitic nematode *Heligmosomoides* bakeri (Nematoda: Heligmosomatidae)," *Veterinary Medicine International*, vol. 2011, p. 5, 2011.

[11] T. J. Sharpton, L. Combrink, H. K. Arnold, C. A. Gaulke, and M. Kent, "Harnessing the gut microbiome in the fight against anthelminthic drug resistance," *Current Opinion in Microbiology*, vol. 53, pp. 26–34, 2020.

[12] P. M. Cheuka, G. Mayoka, P. Mutai, and K. Chibale, "The role of natural products in drug discovery and development against neglected tropical diseases," *Molecules*, vol. 22, no. 1, p. 58, 2016.

[13] Y. Cédric, N. A. Christelle Nadia, S. N. Sylvain Raoul et al., "Antihelminthic activity of Lophira lanceolata on *Heligmosomoides* polygyrus using an automated high-throughput method," *Journal of Tropical Medicine*, vol. 2023, p. 7, 2023.

[14] K. Omeje, J. Ozioko, and H. Opmeje, "Pharmacological potentials, characterization and fatty acids profile of *Persea americana* mill. Avocardo seed oil using gas chromatography-mass spectroscopy," *Biochemistry and Analytical Biochemistry*, vol. 7, no. 4, 2018.

[15] D. Dabas, R. M. Shegog, G. R. Ziegler, and J. D. Lambert, "Avocado (*Persea americana*) seed as a source of bioactive phytochemicals," *Current Pharmaceutical Design*, vol. 19, no. 34, pp. 6133–6140, 2013.

[16] L. N. Henry, U. Y. Mtaita, and C. C. Kimaro, "Nutritional efficacy of avocado seeds," *Global Journal of Food Science and Technology*, vol. 3, no. 5, pp. 192–196, 2015.

[17] A. C. Taki, J. J. Byrne, T. Wang et al., "High-throughput phenotypic assay to screen for anthelmintic activity on Haemonchus contortus," *Pharmaceuticals*, vol. 14, no. 7, p. 616, 2021.

[18] R. L. Rollins, M. Qader, W. L. Gosnell, C. Wang, S. Cao, and R. H. Cowie, "A validated high-throughput method for assaying rat lungworm (Angiostrongylus cantonensis) motility when challenged with potentially anthelmintic natural products from Hawaiian fungi," *Parasitology*, vol. 149, no. 6, pp. 765–773, 2022.

[19] S. E. Ngouateu Teufack, G. N. M. Tayo, M. N. Alidou et al., "Anthelminthic properties of methylene chloride-methanol (1 : 1) extracts of two Cameroonians medicinal plants on *Heligmosomoides* bakeri (Nematoda: Heligmosomatidea)," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, pp. 1–7, 2017.

[20] M. Komtangi, J. Yondo, J. W. Poné, P. Tane, and M. Mbida, "In vitro anthelmintic activity of *Vepris louisii* Gilbert extracts on developmental stages of *Heligmosomoides* bakeri (Nematoda: Heligmosomoidae)," *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, vol. 6, no. 3, pp. 174–180, 2014.

[21] G. White, "A method for obtaining infective nematode larvae from cultures," *Science*, vol. 66, no. 1709, pp. 302–303, 1927.

[22] D. Alimi, A. Abidi, E. Sebai et al., "In Vivo nematicidal potential of camel milk on *Heligmosomoides* polygyrus gastrointestinal nematode of rodents," *Helminthologia*, vol. 55, no. 2, pp. 112–118, 2018.

[23] P. J. Wabo, N. J. Ngankam, B. C. Bilong, and M. Mpoame, "A comparative study of the ovicidal and larvicidal activities of aqueous and ethanolic extracts of pawpaw seeds *Carica papaya* (Caricaceae) on _*Heligmosomoides* bakeri_," *Asian Pacific Journal of Tropical Medicine*, vol. 4, no. 6, pp. 447–450, 2011.

[24] S. K. Panda, R. Das, A. H. Mai, W. M. De Borggraeve, and W. Luyten, "Nematicidal activity of *Holigarna caustica* (Dennst.) oken fruit is due to linoleic acid," *Biomolecules*, vol. 10, no. 7, p. 1043, 2020.

[25] J. Harbone, *Phytochemical Methods London*, p. 278, Chapman and Hall, Academic Press, London, 1973.

[26] N. N. A. Sidiki, N. A. C. Nadia, Y. Cedric et al., "Antimalarial and antioxidant activities of ethanolic stem bark extract of *Terminalia macroptera* in Swiss Albino mice infected with *Plasmodium berghei*," *Journal of Parasitology Research*, vol. 2023, p. 11, 2023.

[27] P. J. Wabo, J. Yondo, T. O. Fossi, C. K. Marie, B. C. Bilong, and M. Mpoame, "The in vitro effects of *Chenopodium ambrosioides* (Chenopodiaceae) extracts on the parasitic nematode *Heligmosomoides* bakeri (Nematoda, Heligmosomatidae)," *Journal of Pharmacognosy and Phytotherapy*, vol. 3, no. 4, pp. 56–62, 2011.

[28] S. S. S. Rosa, F. O. Santos, H. G. Lima et al., "In vitro anthelmintic and cytotoxic activities of extracts of *Persea willdenovii* Kosterm (Lauraceae)," *Journal of Helminthology*, vol. 92, no. 6, pp. 674–680, 2018.

[29] M. Adamu, V. Naidoo, and J. N. Eloff, "Efficacy and toxicity of thirteen plant leaf acetone extracts used in ethnoveterinary medicine in South Africa on egg hatching and larval development of *Haemonchus contortus*," *BMC Veterinary Research*, vol. 9, no. 1, pp. 1–9, 2013.

[30] M. Hernández-Villegas, R. Borges-Argáez, R. Rodriguez-Vivas, J. Torres-Acosta, M. Méndez-González, and M. Caceres-Farfan, "Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*," *Veterinary Parasitology*, vol. 179, no. 1–3, pp. 100–106, 2011.

[31] AnthelminticEffectPterogyne.pdf.

[32] V. Payne, N. K. Etung, J. P. Wabo et al., "In vitro comparative effect of extracts of the seeds of *Embelia rowlandii* (Myrsinaceae) on the eggs and L1 and L2 larval stages of the parasitic nematode *Heligmosomoides* bakeri (Nematoda; Heligmosomatidae)," *African Journal of Biotechnology*, vol. 12, no. 2, pp. 205–211, 2013.

[33] J. F. Garcia-Bustos, B. E. Sleebs, and R. B. Gasser, "An appraisal of natural products active against parasitic nematodes of animals," *Parasites and Vectors*, vol. 12, no. 1, pp. 1–22, 2019.