Malaysian Applied Biology (2023) 52(4): 57-64
https://doi.org/10.55230/mabjournal.v52i4.d119

Research

Evaluation of The In-Vitro Anthelmintic Activity of *Leucas zeylanica* Extracts on Earthworms

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ABSTRACT

Leucas zeylanica known as Ketumbit has a wide range of medical applications. This fact includes the traditional practice of *L. zeylanica* for the treatment of worm infections successfully, however to date, the anthelmintic effect has still not been scientifically proven. Therefore, the present study aimed to evaluate the anthelmintic activity of *L. zeylanica*. The study began with the extraction of *L. zeylanica* extracts using methanol, ethanol, and aqueous. Subsequently, experiments were conducted to evaluate the possible in vitro anthelmintic activity of various extracts of *L. zeylanica* against earthworms. Various concentrations (25, 50 & 100 mg/mL) of extracts were tested and results were expressed in terms of the time of paralysis and time of death of worms. All extracts of the plant exhibited considerable anthelmintic activities in a dose-dependent manner. Of them all, methanolic extract at 100 mg/mL showed the most efficacious anthelmintic activity that was comparable to the reference drug (albendazole, 25 mg/mL). Therefore, we conclude that methanolic extract at the concentration of 100 mg/mL could be considered a candidate for worm treatment apart from the standard medication. This work may provide a framework for further study of *L. zeylanica* as an alternative treatment for worm infection.

Key words: Anthelminthic activity, earthworms, *in vitro*, *Leucas zeylanica*, medicinal plant

Article History
Accepted: 11 July 2023
First version online: 31 October 2023

Cite This Article:
Manisekaran, H., Selahuddeen, M.L., Rahmat, Z., Abdullah, F., A Samad, A.F. 2023. Evaluation of the in-vitro antihelmintic activity of *Leucas zeylanica* extracts on earthworms. Malaysian Applied Biology, 52(4): 57-64. https://doi.org/10.55230/mabjournal.v52i4.d119

INTRODUCTION

Helminthiasis commonly known as worm infection is a concerning public health crisis in either developed or underdeveloped countries, particularly among children (Lim & Chua, 2016). Almost 2 billion children globally are infected with diseases triggered by human intestinal parasitic worms. The World Health Organization (WHO) reported that by 2025, parasitic helminth illnesses will impact roughly 57% of the population in poor countries (Suresh *et al*., 2016). Even though intestinal worm infections are not considered the major parasites affecting children, their profoundly devastating numbers in pediatric cases globally require special attention (Murugamani *et al*., 2012).

Currently, the main anthelminthic drugs approved by WHO against intestinal nematodes are mebendazole, albendazole, pyrantel pamoate, and levamisole (Lloyd *et al*., 2014). However, these present treatment regimens are being hampered by limitations where they have minimal effects on adult worms which tend to live in the host for several years (Radha *et al*., 2018). Additionally, prior research suggested that anthelminthic medications can cause side effects such as nausea, vomiting, diarrhea, headaches, and stomach pain (Suresh *et al*., 2016). Besides, it was stated that mebendazole and albendazole documented side effects mainly involving abdominal problems despite being considered the most effective drugs for pinworm infection (Wendt *et al*., 2019). In addition, helminths have mutated and developed resistance against anthelmintic drugs (Suresh *et al*., 2016). The reason for, the increasing resistance to anthelmintic drugs, side

effects as well and their ineffectiveness there is an urgent need to look for alternative strategies against intestinal parasitic worms.

Leucas zeylanica or "Ceylon slitwort" locally known as "Ketumbit" (Malay) is a plant native to Tropical Asia and Malaya, abundantly distributed in the bushes of Malaysia, especially Kelantan (Abdullah *et al*., 2019). This plant is often cultivated in home gardens for use in local medicine and as a pot herb where this popular, multi-purpose medical plant has a variety of medicinal uses for different ailments including treating colds, expelling intestinal worms, ameliorating cough, and promoting digestion. In Malaysia, leaves are used to cure wounds and taken as a sedative while traditional and Ayurvedic physicians in Sri Lanka employ *L. zeylanica* in the treatment of worm infections successfully. However, the effect it has as an anthelmintic has still not been scientifically proven (Ediriweera, 2014).

To counteract the limitation of the chemical drugs present on the market, a new approach is expected to be employed where natural medicinal plants are used as a treatment for this parasitic infection. On that account, the research was conducted to study the anthelminthic activity of *L. zeylanica* at different extraction solvents and concentrations in eluting worms based on the traditional Ayurvedic practice of this plant (Ediriweera, 2014). The success in proving its efficacy as an alternative treatment would be a stepping stone towards advancement in the development of a novel herbal medicinal plant-based product in the medical industry for helminthiasis.

MATERIALS AND METHODS

Plant material

The mature, healthy *L. zeylanica* plant was harvested from Pulau Gajah, 16100 Pengkalan Chelpa, Kelantan (6˚09‟40.9" N). The plant's identification was authenticated at the herbarium of UKM with voucher number UKMB40376. The roots were chopped off and only the ariel parts of the plants were thoroughly washed and removed from all earthy and foreign matter with running tap water. The plants were then air-dried on the bench at room temperature (30 °C) for 7 days until fully dried. The ariel part of the plants was dried until crispy texture (Abdullah *et al*., 2019).

Plant extraction

The maceration extraction method was used for plant extraction. Completely air-dried plant samples for 7 days were reduced into a more homogenized particle using an electrical blender. Grounded plant samples (100 g) were then soaked in 500 mL (80% v/v) of three different types of solvents, methanol, ethanol, and aqueous separately in 1 mL airtight Schott bottles for 72 h at room temperature 25 °C on an incubator shaker for continuous agitation. After 3 days, the soaked mixtures were filtered using a filter funnel with Whatman filter paper. The filtrates were further filtered using a 10 mL Terumo® syringe with a nylon millipore syringe filter of 0.22 µm for even purified filtrates. The filtrates are stored at 4 °C till further use (Das *et al*., 2019).

Collection of the test organism

Adult earthworms were collected from the moist soil areas around Universiti Teknologi Malaysia Skudai, Johor. The average size of worms was 5 - 8 cm in length and 0.2-0.3 cm in width weighing 0.8 - 2.0 g were used in this study. The maintenance of earthworms collected is under normal vermicomposting soil medium from where it was collected with an adequate supply of nourishment and water. The earthworms were collected freshly each time one day before the anthelmintic assay was done. Earthworms have traditionally been used as models for preliminary evaluation of anthelmintic activity owing to their anatomical and physiological resemblance with intestinal worms, parasites of human beings, and easy availability (Agarwal *et al*., 2011).

Preparation of drug and extracts solution

Albendazole is the standard anthelminthic drug chosen to be used as the reference and positive control in this evaluation of the anthelminthic test. The drug albendazole was obtained from ZENTEL® GlaxoSmithKline Pharmaceuticals Sdn Bhd. Albendazole solution was prepared at a concentration of 25 mg/mL in 10 mL. All extracts were prepared at a volume of 10 mL in concentrations ranging from 25 mg/mL, 50 mg/mL, and 100 mg/mL. Methanol, ethanol, and aqueous extracts of 1000 mg, 500 mg, and 250 mg that have been concentrated were weighed. Both methanolic and ethanolic extracts were dissolved in 5% dimethyl sulphoxide (DMSO) and then the volume was adjusted to 10 mL with normal saline (v/v) to prepare extract concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL. Meanwhile, aqueous extract solutions of *L. zeylanica* were prepared at different concentrations using water as a solvent (Gupta *et al*., 2012).

Evaluation of anthelmintic activity

The anthelmintic activity of the aerial part of plant extracts of *Leucas zeylanica* was evaluated

using earthworms. Earthworms were prepared in eleven groups (three earthworms per group). Each earthworm was separately released into 10 mL of the desired formulation of control, reference drug, and extracts in clean Petri dishes. Group 1 earthworms were released in 10 mL of distilled water and 5% of DMSO in a clean petri dish respectively which acts as a negative control. Group 2 earthworms were released in 10 mL normal saline containing the standard drug albendazole (25 mg/mL), the reference drug in this study. Group 3, 4, and 5 earthworms were released in every 10 mL methanolic extracts solution while Group 6, 7, and 8 earthworms were in 10 mL ethanolic extracts solution, both at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL respectively. Similarly, Group 9, 10, and 11 earthworms were released in 10 mL of normal saline containing 25 mg/mL, 50 mg/mL, and 100 mg/mL of aqueous extracts respectively. Before the initiation of the experiment, the earthworms were washed with normal saline to remove all the fecal matter and waste surrounding their body. Earthworms were observed; the time taken for paralysis and the time taken for death were monitored and documented in minutes. Paralysis was said to occur based on the behavior of earthworms with no revival body state in the normal saline medium. Death was concluded after ascertaining that the earthworm neither moved when shaken vigorously nor when dipped in warm water (50 °C) with faded body color (Agarwal *et al*., 2011).

Statistical analysis

The data of evaluation on the anthelmintic activity of plants were expressed as mean ± S.E.M of the three earthworms in each group. The statistical analysis was carried out by one-way analysis (ANOVA) followed by Tukey's *t*-test at the significance level (where, *p<0.05, **p<0.01) stands for significant and more significant respectively. The analyses will be done using IBM SPSS version 22 to determine the mean and standard error of paralysis and death time of the earthworms (Agarwal *et al*., 2011).

RESULTS AND DISCUSSION

Leucas zeylanica is a well-known medicinal plant and is widely used in folks' medicine and the Ayurvedic system of medicine. In the present study, three types of solvents, methanol, ethanol, and water were used sequentially for crude extraction of *L. zeylanica* aerial part of plants. The concentrated extracts were then used to efficiently evaluate the anthelmintic properties of this plant at varying concentrations (25 mg/mL, 50 mg/mL & 100 mg/mL) to justify the ethnomedicinal claims of *L. zeylanica*. Earthworms were used for the initial evaluation of anthelmintic because of their anatomical and physiological resemblance with other intestinal helminths and ease of availability (Omale & Ojonugwa, 2014). Earthworms possess the ability to move by ciliary movement. Earthworms have a slimy mucilaginous layer composed of complex polysaccharides as their outermost layer which enables their free movement. When damage occurs to the mucopolysaccharide membrane, irritation leads to restricted movement and paralysis which eventually leads to the death of the worm (Kumar *et al*., 2010). Based on the literature study, this was the first report on the anthelmintic property of this plant. Therefore, *L. zeylanica* was expected to have application as an anthelmintic drug in man and mammals like various other medicinal plants that have been reported.

Table 1 shows the anthelmintic effect on earthworms when tested with *L. zeylanica* extracts, standard drug (albendazole), and control at different concentrations. The effects on the earthworms were observed once earthworms were placed in test samples, after the paralysis and death of earthworms. Table 1 shows that the earthworms of all extracts and reference drugs experienced fading in body color which indicated death (Agarwal *et al*., 2011). Besides, slimy discharge was noticed surrounding the earthworms after paralysis and death, possibly indicating the damage to the earthworm's outermost layer, which controls free movement. The rupture caused by the membrane might cause restriction of movement that leads to paralysis and death of the worm (Kumar *et al*., 2010).

Table 2 depicts the results of in-vitro anthelmintic activity of *L. zeylanica* extracts on earthworms in terms of time taken for paralysis and death after treatment with the test extracts and reference drug (albendazole). Methanol, ethanol, and aqueous extracts of the aerial part of *L. zeylanica* samples showed variable time taken at different concentrations, and the mean values were calculated for each parameter. Besides, the significant difference between groups of extract means for the time taken for paralysis and death were labeled in Table 2.

Based on Table 2, it is evident that all extracts of *L. zeylanica* showed anthelmintic properties causing death to earthworms at all concentrations however the time taken for paralysis and death vary significantly among extracts. The general trend shows that all three extracts have a concentration-related anthelmintic activity. Meanwhile, earthworms belonging to the control group showed paralysis and death only after a week which occurred under natural circumstances. The results obtained for the test extracts were compared with a standard drug. In this study, albendazole was used as a reference drug at a concentration of 25 mg/mL. which recorded paralysis and death time of 17.33 ± 0.88 (min) and 23.00 ± 1.53 (min) respectively. The predominant effect of albendazole is to cause paralysis of worms so that they are expelled in the feces of man and animals. The action of anthelmintic drugs like

albendazole is by inhibiting microtubule polymerization after binding to the colchicine-sensitive site of β-tubulin. This results in a decrease in microtubules in the intestinal cells, absorptive function, and uptake of glucose by the adult and larval forms of the helminths thereby depleting glycogen storage. This reduces excitability which leads to muscle relaxation and flaccid paralysis that results in the expulsion of the worm by peristalsis (Das *et al*., 2017).

Table 1. Anthelmintic effect of various extracts and concentrations of *L. zeylanica*

Types of extracts	The concentration of extracts (mg/ml)	Initial	After Paralysis	After Death
Distilled water + 5% DMSO (control) Group 1	N/A	 The worms survived for 24 hours	N/A	N/A
Albendazole (reference drug) Group 2	25			
Methanolic extract	25 Group 3			
	50 Group 4			
	100 Group 5			

Table 1. Continued...

Ethanolic extract	25 Group 6			
	50 Group 7			
	100 Group 8			
Aqueous extract	25 Group 9			
	50 Group 10			

Table 2. In vitro anthelmintic activity of different solvent extracts of *L. zeylanica* on earthworms

Types of extracts	The concentration of extracts (mg/mL)	Time is taken for paralysis (min)	Time is taken for death (min)
Control(Distilled water + 5% DMSO)	-	-	-
Albendazole	25	17.33 ± 0.88	23.00 ± 1.53
Methanolic extract	25	45.67 ± 3.48	54.00 ± 2.08
	50	31.00 ± 1.15	43.00 ± 1.53
	100	15.67 ± 1.76	23.00 ± 1.53
Ethanolic extract	25	49.67 ± 0.88	64.00 ± 2.08**
	50	41.33 ± 0.88	61.33 ± 0.88**
	100	23.33 ± 1.76	42.00 ± 1.15**
Aqueous extract	25	54.67 ± 2.40*	85.00 ± 2.89**
	50	48.00 ± 1.53*	63.67 ± 2.03**
	100	33.33 ± 2.40*	45.00 ± 2.89**

#Values are presented as Mean ± S.E.M (n=3) analyzed by one-way ANOVA followed by the Tukey test. *p<0.05 and **p<0.01 significant compared to standard (albendazole)

Based on Table 2, it is evident that all extracts of *L. zeylanica* showed anthelmintic properties causing death to earthworms at all concentrations however the time taken for paralysis and death vary significantly among extracts. The general trend shows that all three extracts have a concentration-related anthelmintic activity. Meanwhile, earthworms belonging to the control group showed paralysis and death only after a week which occurred under natural circumstances. The results obtained for the test extracts were compared with a standard drug. In this study, albendazole was used as a reference drug at a concentration of 25 mg/mL. which recorded paralysis and death time of 17.33 ± 0.88 (min) and 23.00 ± 1.53 (min) respectively. The predominant effect of albendazole is to cause paralysis of worms so that they are expelled in the feces of man and animals. The action of anthelmintic drugs like albendazole is by inhibiting microtubule polymerization after binding to the colchicine-sensitive site of β-tubulin. This results in a decrease in microtubules in the intestinal cells, absorptive function, and uptake of glucose by the adult and larval forms of the helminths thereby depleting glycogen storage. This reduces excitability which leads to muscle relaxation and flaccid paralysis that results in the expulsion of the worm by peristalsis (Das *et al*., 2017).

All types of *L. zeylanica* extracts showed better anthelmintic activity from the highest concentration of 100 mg/mL to the lowest concentration of 25 mg/mL proving a dose-dependent activity. The anthelmintic activity of methanolic, ethanolic, and aqueous extracts was observed to range from loss of movement to loss of response to external stimuli, which gradually led to death at 25 mg/mL to 100 mg/mL. The results indicated that a higher concentration of all plant extracts produced a paralytic effect much earlier meanwhile the time taken for death was shorter than the lower concentration of test plants for all worms. Methanolic and ethanolic extracts were dissolved in 5% DMSO. When earthworms were treated with 5% DMSO in normal saline, the earthworms did not report immediate paralysis or death. Therefore, indicating 5% DMSO was not toxic to the earthworms anthelmintic action was caused by extracts alone (Gupta *et al*., 2012).

Among the different solvent extracts of *L. zeylanica*, methanol extracts exhibited the most effective anthelmintic activity at all concentrations ranging from 25 mg/mL to 100 mg/mL. Previously, three other species of Leucas (*L. lavandulifolia*, *L. cephalotes*, and *L. aspera*) also concluded that the methanolic extract of the plant has better anthelmintic activity in a dose-dependent manner (Gupta *et al*., 2012). The effect of extracts on the paralysis or helminthiasis and death of the worm, according to the results in Table 2 may be indicated as methanol > ethanol > aqueous extracts. Of them all, methanolic extract at 100 mg/ml concentration reported the earliest paralysis time of 15.67 ± 1.76 min and the shortest time taken for death which was observed to be at 23.00 ± 1.53 min. Despite methanolic extract at 100 mg/mL having the best overall anthelmintic effect recorded that was comparable to the time taken for paralysis and death by albendazole at 25 mg/mL, it was still not as potent as the reference drug. This was because methanol extract only took a comparatively shorter time of action when a higher dose was applied, whereas albendazole was effective as an anthelmintic at a lower dose (25 mg/mL). Besides methanolic extracts also showed a shorter time taken for both worm paralysis and death at concentrations of 25 mg/mL and 50 mg/mL compared to ethanolic and aqueous. Methanolic extract at 25 mg/mL and 50 mg/mL recorded earthworm paralysis times of 45.67 ± 3.48 and 31.00 ± 1.15 minutes respectively while death times of 54.00 ± 2.08 and 43.00 ± 1.53 min, respectively.

The following methanolic extract was ethanolic extract which recorded better paralysis and death time for earthworms at every concentration compared to that of aqueous extracts. Ethanolic extracts of *L. zeylanica* plant of concentration (25, 50 & 100 mg/mL) showed paralysis at 49.67 ± 0.88,

41.33 ± 0.88 and 23.33 ± 1.76 (min) and death at 64.00 ± 2.08, 61.33 ± 0.88 and 42.00 ± 1.15 min. Meanwhile aqueous extract of concentration (25, 50 & 100 mg/mL) paralysis at 54.67 ± 2.40, 48.00 ± 1.53, and 33.33 ± 2.40 min and death at 85.00 ± 2.89, 63.67 ± 2.03 and 45.00 ± 2.89 min, respectively. Based on the time taken for paralysis and death of worms, the aqueous extract was considered to have the least effective anthelmintic activity.

Hypothetically, the anthelmintic property of plants was related to their secondary metabolites. Many plant constituents are known to be responsible for anthelmintic, these include alkaloids, flavonoids, terpenoids, and others (Kalpana & Rajeswari, 2016). Tannins, the polyphenolic compounds in plants are typically shown to have desired anthelmintic properties in many reports (Islam *et al*., 2017). The anthelmintic mechanism of action of tannins is by interfering with the energy production in the helminthic parasites either by connecting with free proteins in the gastrointestinal tract of the host animal or by binding to the glycoprotein on the cuticle of the parasite which leads to death (Islam *et al*., 2017). In addition, tannins or their derivatives have a direct effect on the viability of the pre-parasitic stages of helminths. Meanwhile, saponins mainly act by parallel integration of mucus membranes leading to parasite death (Omale & Ojonugwa, 2014). The difference in the degree of paralysis and death of worms was also due to the different extract's levels of phytochemicals like tannins present in compounds. Therefore, phytochemical identification of active compounds in plant extracts is a necessary step to properly identify and isolate the compounds. of *L. zeylanica* as an alternative treatment for worm infection.

CONCLUSION

Leucas zeylanica is a multi-purpose perennial medicinal plant herb of the family Lamiaceae. In-vitro analysis using earthworm as an animal model for an anthelmintic activity study depicted that the methanolic, ethanolic, and aqueous extract of the aerial part of *L. zeylanica* exhibited anthelmintic potential in a dose-dependent manner. Nevertheless, a clear review of the current investigation demonstrated that, among the extracts examined, methanol extracts displayed high potential anthelmintic activity among the extracts with longer timing for both worm paralysis and death time at all concentrations. Furthermore, the methanolic extract of *L. zeylanica* showed the most significant anthelmintic activity at 100 mg/mL with the shortest results compared with the reference drug, (albendazole) at 25 mg/mL. Besides, the results obtained from the study also supplicate its traditional uses in treating worm infestation, thus validating that *L. zeylanica* has anthelmintic efficacy. With this validation, the future works of our research group are focused on utilizing crude methanolic extracts of *L. zeylanica* as a new source of active compounds for the development of natural product-based therapy for the management of helminthic infection.

ACKNOWLEDGEMENTS

We would like to extend our gratitude to the staff at Plant Biotechnology Lab for their assistance during this study being carried out. Besides, we also would like to thank the respective editors and reviewers for their critical comments and valuable suggestions. This work on the evaluation of anthelmintic properties in *L. zeylanica* was funded by Tier 2 UTM Grant (Q.J130000.2654.16J57) and UTM Encouragement Research Grant (Q.J130000.2654.17J96) awarded to Faizuan Abdullah and Abdul Fatah A. Samad, respectively.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdullah, F., Nasir, S.N.A.M., Han, D.K., Appalasamy, S., Nor, M.M. & Rak, A.E. 2019. Potential of *Leucas zeylanica* extract to eliminate *E. coli* and *S. aureus* in *Corbicula fluminea* (Etak) tissue. Malaysian Journal of Fundamental and Applied Sciences, 15(4): 597–599. https://doi.org/10.11113/mjfas.v15n4.1248

Agarwal, S., Jacob, S., Chettri, N., Bisoyi, S., Badarinath, D.K., Vedamurthy, A.B., Krishna, V. & Hoskeri, H.J. 2011. Evaluation of in vitro anthelmintic activity of *Leucas aspera* extracts. Pharmacognosy Journal, 3(24): 77–80. https://doi.org/10.5530/pj.2011.24.15

Das, S., Ray, A., Nasim, N., Nayak, S. & Mohanty, S. 2019. Effect of different extraction techniques on total phenolic and flavonoid contents, the antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. 3 Biotech, 9(1): 37. https://doi.org/10.1007/s13205-018-1565-8

Das, S., Rumana, A., Huque, S., Anwar, R., Das, P., Tanni, K.A. & Shahriar, M. 2017. *In vitro* anthelmintic

activity of leaf extracts of four different types of calamus species. Pharmacy & Pharmacology International Journal, 5(2): 72–76. https://doi.org/10.15406/ppij.2017.05.00118

Ediriweera, S. 2014. Effect of decoction of *Leucas zeylanica* in worm infestation with special reference to *Enterobius vermicularis*. Journal of Ayurveda and Holistic Medicine, 2: 11–17.

Gupta, D.K., Agrawal, K.K., Anju, V. & Kishan, S. 2012. Investigation of in-vitro anthelmintic activity of *L. lavandulifolia*, *L. cephalotes* and *L. aspera*. Journal of Pharmacy Research, 5(1): 212– 213.

Islam, A., Hussain, M., Sen, N., Abedin, F., Millat, M.S., Islam, M.S., Das, A., Kar, A. & Hossain, M.M. 2017. Investigation of in vitro thrombolytic and anti-helminthic activity and in vivo anxiolytic and antidepressant potentiality with phytochemical nature of methanolic extract of *Leucas lavandulifolia*. Sustainable Chemistry, and Pharmacy, 6: 61–66. https://doi.org/10.1016/j.scp.2017.09.001

Kalpana, V.N. & Rajeswari, V.D. 2016. Phytochemical and Pharmacological investigation of an indigenous medicinal plant *Leucas aspera*. International Journal of PharmTech Research, 9(8): 2455–9563.

Kumar, B.S.A., Lakshman, K., Jayaveera, K.N., Velmurugan, C., Manoj, B. & Sridhar, S.M. 2010. Anthelmintic activity of methanol extract of *Amaranthus caudatus* Linn. Journal of Food Safety, 12: 127–129.

Lim, L.A. & Chua, T.H. 2016. Worm infection among children in Malaysia. Borneo Journal of Medical Sciences, 10(1): 59–74. https://doi.org/10.51200/bjms.v10i0.568

Lloyd, A.E., Honey, B.L., John, B.M. & Condren, M. 2014. Treatment options and considerations for intestinal helminthic infections. The Journal of Pharmacy Technology, 30(4): 130–139. https://doi.org/10.1177/8755122514533667

Murugamani, V., Raju, L., Anand Raj V.B., Sarma Kataki, M. & Sankar G.G. 2012. The new method was developed for evaluation of anthelmintic activity by housefly worms and compared with conventional earthworm method. International Scholarly Research Notices, 2012: 709860. https://doi.org/10.5402/2012/709860

Omale, J. & Ojonugwa, T.G.U. 2014. In vitro antihelmintic activity of *Saba florida* (benth) extracts against Nigerian adult earthworm (*Terrestris lumbricoides*). Planta Medica, 2014: 80. https://doi.org/10. 1055/s-0034-1394734

Radha, P., Nur Hafizah, R., Nagavalli, D., Pruthvi, N., Prabhu, M. & Joysa Ruby, J. 2018. *In vitro* anthelmintic study of *Bauhinia acuminata* Linn. leaf extracts against the housefly worms, Indo American Journal of Pharmaceutical Sciences, 5(6): 5082–5089. https://doi.org/10.5281/zenodo.1287887

Suresh, K., Priyanka, K., Ramanjaneyulu, K. & Himabindhu, J. 2016. Phytochemical screening and in vitro anthelmintic activity of *Alysicarpus monilifer*. International Journal of Pharmacognosy and Phytochemical Research, 8(10): 1630–1633.

Wendt, S., Trawinski, H., Schubert, S., Rodloff, A.C., Mössner, J. & Lübbert, C. 2019. The diagnosis and treatment of pinworm infection. Deutsches Arzteblatt International, 116(13): 213–219. https://doi.org/10.3238/arztebl.2019.0213