IN-VITRO VALIDATION OF THE USE OF JACKFRUIT (*ARTOCARPUS HETEROPHYLLUS* LAM) AS AN ALTERNATIVE FOR ANTHELMINTIC DRUGS FOR THE CONTROL OF PARASITIC NEMATODES IN RUMINANTS

MING JIUN, J. L. AND NIK HIM, N. A. I. I.*

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Penang
**Corresponding author:* nikirwan@usm.my

ABSTRACT. The aim of this study is to determine the phytochemical constituent of *Artocarpus heterophyllus* Lam (Jackfruit) and its effectiveness as an alternative for the anthelmintic treatment of parasitic nematodes in ruminants. *Artocarpus heterophyllus* Lam leaves were used to test on L3 nematode larva harvested from fecal culture. There are four species of parasitic nematodes identified, namely: *Haemonchus contortus*, *Trichostrongylus* sp., *Cooperia* sp., and *Oesophagostomum* sp. *Haemonchus contortus* was found to be the most dominant, followed by *Trichostrongylus* sp., *Cooperia* sp., and *Oesophagostomum* sp. The phytochemical test of *Artocarpus heterophyllus* Lam leaves was revealed to possess alkaloids, flavonoids, tannins, tripertenes and steroids. These components were shown to be effective at causing paralysis and deaths of parasitic nematode in the larval motility assay, where 100 % of the nematodes tested were killed below 6 hours at 5.0 mg/ml concentration; and inhibit migration in the larval migration assay, where migration of the larva was completely inhibited in 2 hours using 2.0 mg/ml concentration. The results suggest that *Artocarpus heterophyllus* Lam leaves can be used as an alternative for the anthelmintic treatment in goats and sheep.

Keywords: Artocarpus heterophyllus, phytochemical, anthelmintic, ethnoveterinary, nematodes

INTRODUCTION

Most rural people rely on conventional medicines to alleviate a range of ailments in many developing countries with restricted access to orthodox health care facilities (McGaw & Eloff, 2010). In the same way, many pastoralists use common remedies to treat their sick animals. With anthelmintic drug resistance being a serious and real threat nowadays, it is with no doubt that there is great importance to test and search for alternative ways to treat the ever-looming threat of gastrointestinal parasites in ruminants. In fact, in Malaysia, there are numerous reports on the emergence of anthelmintic drug resistance. The first case was reported by Dorny et al. (1994) on the benzimidazole resistance in goat farms followed by a report by Pandey et al. (1994) and Chandrawathani *et al.,* (1999) regarding the nematode-resistant to levamisole, closantel and ivermectin.

Furthermore, a study by Khadijah *et al.,* (2006) showed the nematode population of the small ruminant farms in Peninsular Malaysia which indicated the presence of resistance problem in goats to oxfendazole, moxidectin, levamisole and closantel. The study suggested that anthelmintic resistance of nematode populations at government farms may be due to the regular treatment of the anthelmintic over period of time. Based on their record, deworming frequency ranged from twice per year to 12 times per year. In addition, the benzimidazoles drug was frequently used throughout the period. Study by Nor-Azlina *et al.,* (2011) showed that management practices did contribute to the emergence of the resistance in nematode

population to anthelmintic treatment. The morning grazing practice had significantly increased the worm burden and the high degree of resistance towards ivermectin and benzimidazoles was present in all the farms.

As the situation stands, there is a need to reduce the reliance on anthelmintic drugs in treating the gastrointestinal infection in ruminants. There were studies on the use of certain plants as a potential candidate for the treatment of gastrointestinal infections in goats and sheep that have been documented and reported (Chandrawathani *et al.,* 2002; Al-Rofaii *et al.,* 2012a, 2012b; Siti Futri *et al.,* 2018). When fresh leaves of the neem tree (*Azadirachta indica*) were fed to a group of trichostrongyle-infected sheep, faecal egg counts, and larval recoveries were reduced (Chandarawathani *et al.,* 2002). In addition, *in vitro* study of methanol extracts of neem (*Azadirachta indica*) and cassava (*Manihot esculenta*) showed significant activity against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* (Al-Rofaii *et al.,* 2012a, 2012b). *in vitro* assay by Siti Futri *et al*. (2018) showed that the plant extract of the *Punica granatum* inhibited and killed the L3 larvae *in vitro* at different concentrations. In Malaysia, some of the farmers resort to inexpensive alternative ways for controlling gastrointestinal infection in their animals by feeding the animals with leaves of jackfruit (*Artocarpus heterophyllus* Lam). However, the anthelmintic efficacy of this plant has not been extensively tested against parasitic nematodes in goats and sheep. Because of that, this study was carried out to investigate the potential use of *Artocarpus heterophyllus* Lam against parasitic nematodes *in vitro* in different concentrations by using larval motility and larval migration inhibition assays.

MATERIALS AND METHODS

Plant Collection

The *Artocarpus heterophyllus* leaves were collected in Universiti Sains Malaysia. The collected leaves were washed with running tap water and left to dry in an oven at 40 °C for 2 days. The dried leaves were then ground into fine powder using the blender. The powder was transferred into a tight air beaker and kept at room temperature prior to use for the extraction.

Plant extraction

Approximately 50 g of the ground powder of the leaves was weighed and dissolved in 250 ml of distilled water in a conical flask. The mixture was left for 24 hours at 50 °C. After 24 hours, the dissolved plant materials were filtered then centrifuged for 15 minutes at 2500 rpm. The aqueous filtrate was then kept in universal bottles to be freeze-dried. After freeze-drying, the aqueous filtrate formed into powder form (Siti Futri *et al.,* 2018).

Phytochemical constituent tests

For the phytochemical constituent, five different tests were conducted to determine the presence of the secondary metabolites in the aqueous extract of the jackfruit leaves.

(i) *Alkaloids*

Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of potassium in 100 ml distilled water. Then 2 drops of Wagner's reagent were added to 1 ml of aqueous extract of jackfruit. If alkaloids are present, a reddish-brown precipitate will be formed (Raaman, 2006).

(ii) *Flavonoids*

An amount of 1 ml 10 % ammonia was added to 1 ml of aqueous extract. If flavonoids are present, yellow colouration is formed.

(iii) *Saponins*

A total amount of 2 ml aqueous extract and 5 ml of distilled water were mixed in a test tube. The test tube was shaken vigorously and observed. If there is persistent frothing for 15 minutes, then saponins are present (Kaushik *et al.,* 2012).

(iv) *Tannins*

1 ml aqueous extract and 2 drops of 1 % ferric chloride solution were mixed in a test tube. If tannins are present, there will be blue-black precipitate formation (Evans *et al.,* 2009).

(v) *Tripertenes and steroids*

Salkowski test was used for this, where 1 ml of aqueous extract and 1 ml of chloroform were dissolved in a test tube. This was followed by the addition of 1 ml concentrated sulphuric acid with the content. If tripertenes present, reddish-brown colouration will form (Adesegun *et al.,* 2008).

Faecal Collection

The faecal samples were collected from three places, namely: the private goat farm in Teluk Kumbar, Balik Pulau, and Bukit Mertajam. Approximately 10 g of faecal samples were collected immediately after observing defecation. All fresh samples were kept in the icebox to minimise the hatching process and directly transported to the lab the same day, where they were subjected to a preliminary investigation for the presence of eggs and larvae culture preparation.

Fecal Floatation and Sample Screening

All collected samples were subjected to preliminary study to determine the presence of the eggs for the culture by using the simple floatation techniques (Zajac & Conboy, 2012). 2 g of faeces samples were weighed and transferred into a cup. A saturated sodium chloride solution was added in the cup and the mixture was stirred. The mixture was strained through tea strainer and the filtered solution was kept. The filtered solution then was poured into test tube and the floatation solution was added in the tube until the reverse meniscus level. The coverslip was placed on the fluid drop at the top and the test tube was left to stand for 10 minutes on the bench. After 10 minutes, the coverslip was removed and placed on a slide. Any presence of the egg was recorded. These procedures were applied to all samples prior to use in the larvae culture.

Larvae culture

The collected faecal samples were subjected to the culture by referring to Harada and Mori (1995). Faeces samples were mixed with a little water before being spread evenly on the filter paper. The filter paper was rolled and inserted into the test tube where water was poured in until it touched the bottom of the filter paper. Cotton wool was used to cover the mouth of the test tube to prevent contamination. The test tubes were left for 7–10 days at room temperature. Then the filter paper was removed, leaving the water filled with larvae. The water filled with larvae were identified for the larvae species by morphological characteristic using the slide microscopically. 50 larvae were identified based on the morphological identification keys from Dickman and Andrew (1933) and Gordon (1933).

Motility assay

Motility of larvae was examined as described by Stepek et al. (2005) and Siti Futri et al. (2018). Only the healthy and active larvae were chosen for the assay. A total of five third stage (L3) larva were placed in 1 ml distilled water in a 24-well test plate. 1 ml of *Artocarpus heterophyllus* leaves extract at 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, and 5.0 mg/ml were added into each well and left to incubate at 27 °C. The motility of the nematode was recorded every 6, 12, 18, 24, 30, 36, 42, and 48 hours and the data were tabulated to show the results.

Larval migration assay

Molan *et al.* (2000) methods were used to test larval migration. Five third stage (L3) larvae were placed in 1 ml distilled water in a 24-well test plate. One ml of *Artocarpus heterophyllus* leaves extract at 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, 2.0 mg/ml, and 5.0 mg/ml was added into each well and left to incubate at 27 °C for 2 hours. After that, the incubated larvae were transferred to a muslin cloth and left to incubate for another 2 hours at 27 °C. The number of larvae that migrated through the muslin cloth was recorded.

RESULTS

The aim of this study is to investigate the effectiveness of *Artocarpus heterophyllus* leaves extract and to determine the potential use of *Artocarpus heterophyllus* leaves extract against parasitic nematodes of goats. In this study, the *Artocarpus heterophyllus* leaves extract was prepared using an aqueous as a solvent. Results in Table 1 below show the presence of alkaloids, flavonoids, tannins, saponins, tripertenes and steroid in the *Artocarpus heterophyllus* leaves extract.

In the preliminary study, helminth eggs were observed in the faecal samples and based on the culture, four species of parasitic nematodes were identified (*Trichostrogylus sp., Haemonchus contortus, Oesophagostomun sp., and Cooperia* sp.

Table 1. Phytochemical constituents of *Artocarpus heterophyllus* leaves aqueous extract.

Phytochemical Constituent	Observation	Test Result
Alkaloids	Reddish-brown precipitate	+
Flavonoids	Yellow coloration	+
Saponins	Persistent frothing	+
Tannins	Blue-black precipitate formed	+
Tripertenes and steroids	Yellow layer formed	+

+ Indicated the presence of phytochemical constituents.

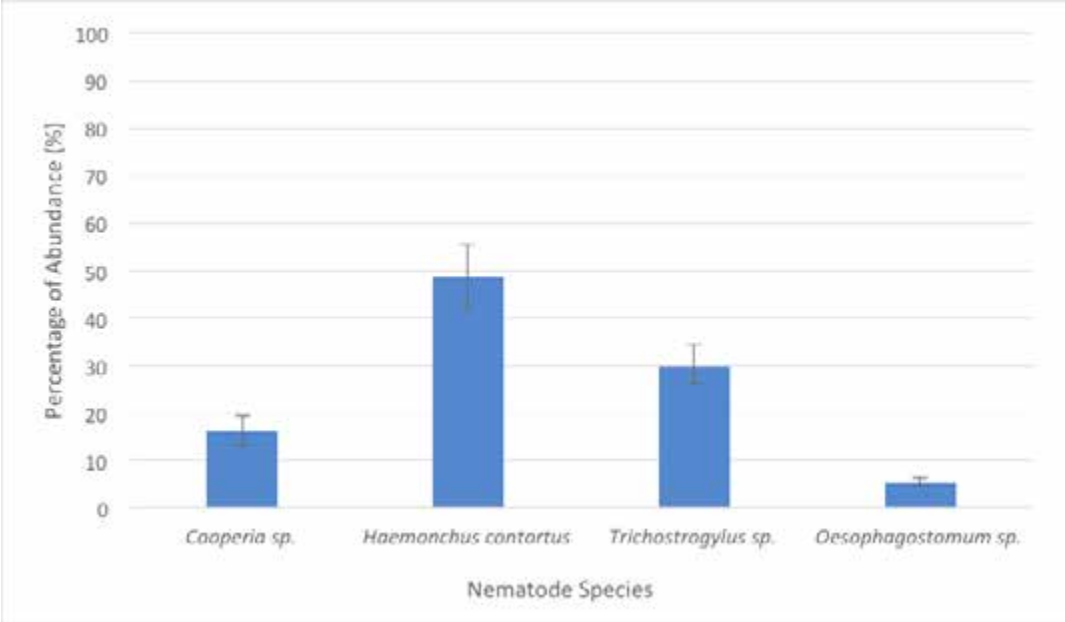

Figure 1. The abundance of parasitic nematodes from the faecal culture.

Haemonchus contortus was the most abundant species (49 ± 11.55 %), followed by *Trichostrongylus* sp. (30 ±6 .93 %). The less abundant species was *Oesophagustomun* sp. with 5 ± 1.15 %).

Two tests were carried out *in vitro* to investigate the effectiveness of the *Artocarpus heterophyllus* leaves extracts against parasitic nematodes. Results in this study indicated the effectiveness of the use of the leaves extract against parasitic nematodes of goats *in vitro.* At 0.5 mg/ml concentration, all tested L3 larvae were killed after 42 h incubation. At 1.0 mg/ml concentration, all L3 larvae were killed after 30 h incubation. At the highest concentration (5.0 mg/ml), all L3 larvae were killed after 12 h of incubation. Results show that as the concentration of the extract increased, the incubation time required to kill 100 % of the tested L3 larvae decreased. All L3 larvae in the control were able to survive within the 48 h incubation period.

In the larval inhibition migration assay, the same results were obtained. The L3 larvae were unable to migrate and were inhibited after being exposed to the leaves extract as how in Figure 2 and 3, even at the lowest concentration used for the assay. 60 % of the L3 larvae were inhibited at 0.5 mg/ml. At 2.0 and 5.0 mg/ml mg/ml concentrations, the incubation with the leaf extract completely inhibited the migration of the L3 larvae.

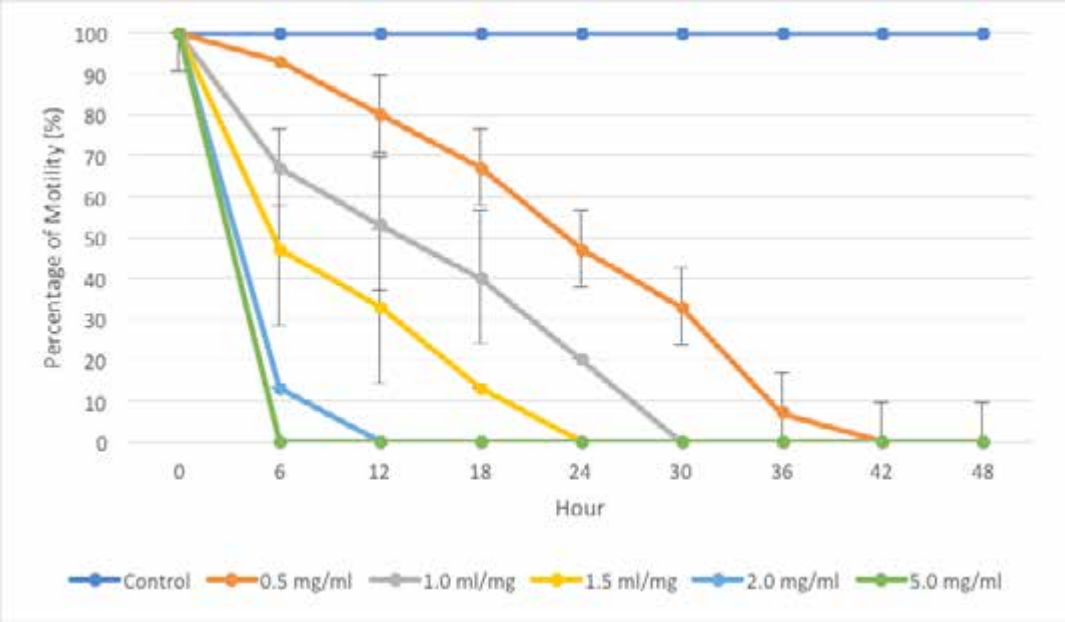

Figure 2. The effects of the aqueous extract of *Artocarpus heterophyllus Lam* (Jackfruit) leaves extract on larval motility. Graph represented the mean ± standard error based on three different experiments.

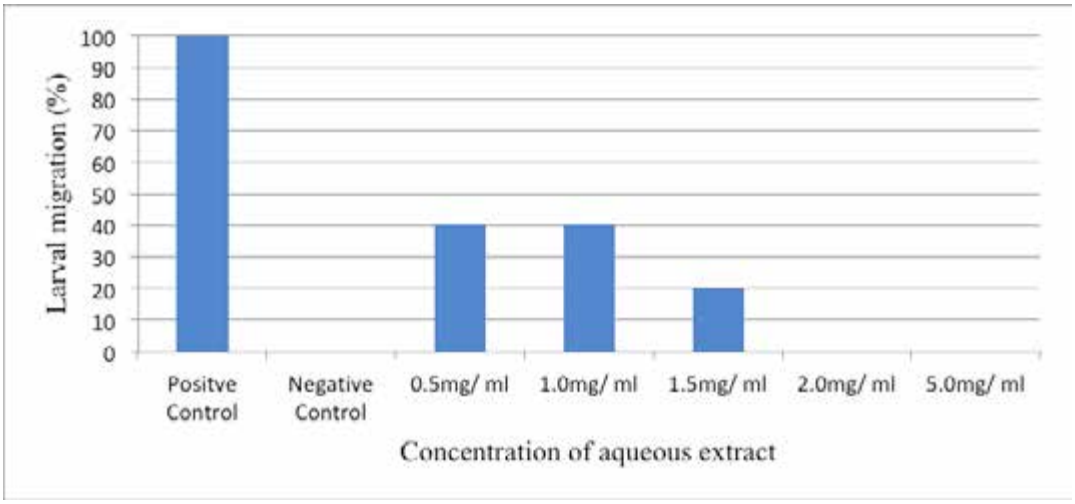

Figure 3. The percentage migration of nematode larvae against the 5 different concentrations of *Artocarpus heterophyllus* Lam (Jackfruit) leaves extract.

DISCUSSION

Haemonchus contortus is one of the most common nematode parasites that infect ruminants, especially sheep and goats. In Malaysia, there is nothing new about the occurrence of *Haemonchus contortus* in sheep and goats. Many studies have reported the presence of this species all over Malaysia (Mursyidah et al., 2017). Several studies have shown that the abundance of the H. contortus is related to the environmental factor and due to massive egg production (Santos et al., 2012). Field studies have shown that movement of larvae to pasture increases when rainfall occurs, and the increase seems to be proportional to the intensity and frequency of rainfall (Silva et al., 2008). The availability of the larvae to maintain the position on the pasture increases the chances for the animal to harbour the larvae in the grazing areas. Interestingly, the H. *contortus* larvae can survive for approximately 11 weeks in the pasture during the rainy season (Ndamukong & Ngone, 1996). This ability indirectly might influence the occurrence of H. *contortus* infection in an animal.

In motility assay, all L3 larvae were killed after being exposed to the aqueous leaves extract of *A. heterophyllus*. Based on the observation, the L3 was paralysed at the lower concentration and unable to move or stay active. This behaviour suggests that leaves extract affects the motility and movement of the L3 larvae. In the larval inhibition migration assay, the same behaviour of the larvae was observed. The explanation of the mechanism on how the extract affects the motility and the behaviour of the L3 larvae might depend on the phytoconstituents composition that presents in the extract (Ahmed *et al.,* 2014; Haladu *et al.,* 2020). The presence of saponins, tannins, flavonoids, and other phenolic compounds in the extracts of *A. heterophyllus* might have been responsible for its anthelmintic activity based on the previous reports on the anthelmintic roles of these compounds. For example, Al- Rofaai *et al.* (2012a) has suggested that the mechanism of saponin on the cell membrane contributes to the disintegration of the parasite teguments by changing the cell membrane permeability. Saponins have also been suggested to be associated with the formation of saponin cholesterol insoluble complex (Francis *et al.,* 2002). The presence of saponins was shown to reduce the level of cholesterol in nematode eggs and larvae (Ibrahim *et al.,* 2013). The reduction of cholesterol levels in nematodes affects the structural component of cell membranes and indirectly might affect the motility of the nematodes.

The mode of action of tannins as anthelmintic is attributed to their capacity to bind to some proteins of the metabolism or larva's organs and muscles causing a change in their functions and resulting in the paralysis or death of the L3 larvae (Al-Rofaai *et al.,* 2012a). Another study has suggested that tannins could bind to dietary protein and indirectly increase protein availability and enhance the immunological responses towards parasites (Coop & Kyriazakis, 1999). A study by Schultz (1989) suggested that tannins could bind to free protein in larvae which have resulted in larvae starvation and death. The death of the larvae has also been suggested due to the binding of tannins to the cuticle of the larvae (Thompson & Geary, 1995). Even with the promising results obtained from previous study of the plant extract against the nematodes, this technique is still lack of proper implementation in controlling the nematodes infection due to several challenges. According to WHO (2005), most common challenges include the regulatory status, assessment of safety and efficacy, quality control, safety monitoring and inadequate or poor knowledge about the herbal medicines within the national drug regulatory authorities. For example, the weak or absence

of regulation of herbal medicines in most countries and the occurrence of high-profile safety concerns had increased awareness of the need to monitor the safety associated with herbal medicines (Rodrigues & Barnes, 2013). It is also acknowledged that aspects such as the geographical origin of plant material, differing processing procedures, administration route, and compatibility with other drugs all complicate the determination of safety (Zhang et al., 2012). Because of that, the application of the control and treatment of the parasites is still largely depending on the current anthelmintic drugs.

CONCLUSION

The traditional practices of giving the livestock animals additional feed such as the leaves of jackfruit in Malaysia by farmers is not new. This practice was applied based on the farmers' observation of animals regaining health after being fed with the leaves of jackfruits. Results in this study suggest the potential use of *A. heterophyllus* Lam leaves extract as an alternative to anthelmintic for the treatment of parasitic nematodes. Further study should be carried out to investigate and validate the effectiveness of this plant in *vivo*. Extraction of the potential active compound should be carried out in order to isolate and identify the potential use of the compound in reducing the parasitic nematode infection in animals.

REFERENCES

1. Adesegun, S., Ayoola, G., Coker, H., Adepoju-Bello, A., Obaweya, K., Ezennia, E., and Atangbayila, T. (2008). Phytochemical Screening and Antioxidant Activities of Some Selected Medicinal Plants Used for Malaria Therapy in Southwestern Nigeria. Trop. J. Pharm. Res. 7(3): 1019-1024.
2. Ahmed AS, McGaw LJ, Moodley N, Naidoo N, Eloff, J.N. (2014). Cytotoxic, antimicrobial, antioxidant, antilipoxygenase activities and phenolic composition of Ozoroa and Searsia species (Anacardiaceae) used in South African traditional medicine for treating diarrhoea. S. Afr. J. Bot. S AFR J BOT. 95: 9-18.
3. Al-Rofaai, A., Rahman, W. A., Sulaiman, S. F., and Yahaya, Z. S. (2012a). *In vitro activity* of neem (*Azadirachta indica*) and cassava (*Manihot esculenta*) on three pre-parasitic stages of susceptible and resistant strains of *Teladorsagia* (Ostertagia) circumcincta. Vet. Parasitol. 188: 85-92.
4. Al-Rofaai, A., Rahman, W. A., Sulaiman, S. F., and Yahaya, Z. S. (2012b). *In vitro ovicidal* and larvicidal activity of methanolic leaf extract of *Manihot esculenta* (cassava) on susceptible and resistant strains of *Trichostrongylus colubriformis*. Vet. Parasitol, 190: 127-135.
5. Chandrawathani P., Adnan M. and Waller P.J. (1999). Anthelmintic resistance in sheep and goat farms in peninsular Malaysia. *Veterinary Parasitology*, 82: 305-310.
6. Chandrawathani P., Brelin, D., Nor Fasihah, S., Adnan, M., Jamnah, O., Sani, R. A., Hoglund, J., Waller, P.J. (2002). Evaluation of the neem tree (*Azadirachta indica*) as a herbal anthelmintic for nematode parasite control in small ruminants in Malaysia. Tropical Biomedicine, 19(1,2): 41-48.
7. Coop, R.L., Kyriazakis, I., (1999). Nutrition–parasite interaction. Vet. Parasitol. 84: 187–204.
8. Dorny P., Claerebout E., Vercruysse J., Sani R. and Jalila A. (1994). Anthelminthic resistance in goats in peninsular Malaysia. Vet. Parasitol, 55: 327-342.
9. Dikmans G. and Andrew J.S. (1933). A comparative morphological study of the infective larvae of the common nematodes parasitic in the alimentary tract of the sheep. Trans Am Microsc Soc. 52: 1-25.
10. Evans, W. C. & Trease, G. E. (2009). Pharmacognosy. Edinb. Med. J. 246-249.
11. Francis, G., Kerem. Z., Makkar. H.P., Becker. K. (2002). The biological action of saponins in animal systems: a review. Br. J. Nutr. 88: 587-605.
12. Gordon H.M. (1933). Differential diagnosis of the larvae of the *Ostertagia* spp. and the *Trischostrongylus* spp. of the sheep. Aust. Vet. J. 9: 223-237.
13. Haladu Ali Gagman, Nik Ahmad Irwan Izzauddin Nik Him, Hamdan Ahmad, Shaida Fariza Sulaiman, Rahmad Zakaria, Farah Haziqah Meor Termizi. (2020). In Vitro Efficacy of Aqueous and Methanol Extract of Cassia *siamea* Against the Motility of

Caenorhabditis elegans. Trop. Life Sci. Res. 31(3): 145.
14. Harada, Y. & Mori, O. (1955). A new method for culturing hook-worm. Yonago Acta Med. 1(3): 177 – 179.
15. Ibrahim, M, A. R. and Srour, Hany A.M. (2013). Saponins Suppress Nematode Cholesterol Biosynthesis and Inhibit Root Knot Nematode Development in Tomato Seedlings. Nat. Prod. Res: 2(1).
16. Kaushik, S., Pushker, A. K., Lakhanpaul, S., Krishansharma, K., & Ramani, R. (2012). Investigations on some of the important host plants of Kerria lacca with reference to phloem distance. EurAsian J. Biosci. 32-38.
17. Khadijah, S., Rahman, W. A., Chandrawathani, P., Waller, P. J., Vasuge, M., Nurulaini, R., Adnan, M., Jannah, O., & Vincent, N. (2006). Nematode anthelmintic resistance in government small ruminant farms in Peninsular Malaysia. Malays J Vet Res. 18(1): 1-5.
18. McGaw, L. J and Eloff, J. N. (2010). Methods for Evaluating Efficacy of Ethnoveterinary Medicinal Plants. *Ethnoveterinary Botanical Medicine Herbal Medicines for Animal Health*. 1-24.
19. Molan, A. L., Waghorn, G. C., Min, B. R., & McNabb, W. C. (2000). The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration in vitro. Folia Parasitol. 47: 39 – 44.
20. Mursyidah, A.K., Khadijah, S. and Rita, N. (2017). Nematode infection in small ruminants and the management of the farms in Terengganu, Peninsular Malaysia. Trop. Biomed. 34(1): 59-65.
21. Nor-Azlina, A.A., Sani, R.A., & Ariff, O.M. (2011). Management Practices Affecting Helminthiasis in Goats. *Pertanika* J. Trop. Agric. Sci. 34(2): 295-301.
22. Ndamukong, K.J., Ngone, M.M., (1996). Development and survival of *Haemonchus contortus* and *Trichostrongylus* sp. on pasture in Cameroon. Trop. Anim. Health Prod. 28: 193–198.
23. Pandey V.S. and Sivaraj S. (1994). Anthelmintic resistance in *Haemonchus contortus* from sheep in Malaysia. Vet. Parasitol. 53: 67-74.
24. Raaman, N. (2006). *Phytochemical Techniques*. New Delhi. NIPA. 19-24.
25. Rodrigues, E., & Barnes, J. (2013). Pharmacovigilance of herbal medicines: the potential contributions of ethnobotanical and ethnopharmacological studies. *Drug Saf*. 36, 1–12.
26. Santos, M. C., Silva, B. F., and Amarante, A. F. T. (2012). Environmental factors influencing the transmission of *Haemonchus contortus*. *Vet Parasitol.* 188: 277-284.
27. Schultz, J.C., (1989). Tannin–insect interactions. In: Hemingway, R.W., Karchesy, J.J. (Eds.), *Chemistry and Significance of Condensed Tannins*. Plenum Press, New York. 417–433.
28. Silva, B.F., Amarante, M.R.V., Kadri, S.M., Carrijo-Mauad, J.R., Amarante, A.F.T., (2008). Vertical migration of *Haemonchus contortus* third stage larvae on *Brachiaria decumbens* grass. Vet Parasitol. 158, 85–92.
29. Siti Futri Farahininajua Fikri, Nik Ahmad Irwan Izza Suhaila Ab, Rahmad Zakaria, Shaida Fariza Sulaiman. (2018). In vitro anti-parasitic activities of pomegranate, *Punica granatum* against parasitic nematodes of ruminants. MJVR. 9(1): 1-9.
30. Stepek, G., Buttle, D., Duce, I., Lowe, A., & Bahnke, J. (2005). Assessment of the anthelminthic effect of natural plant cysteine proteinases against the gastrointestinal nematode, *Heligmosomoides polygyrus*, in vitro. *Parasitology*. 130(02): 203-211.
31. Thompson, D.P., Geary, T.G., (1995). The structure and function of helminth surfaces. In: Marr, J.J., Muller, M. (Eds.), *Biochemistry and Molecular Biology of Parasites*, (1st ed). Academic Press, NewYork. 203–232.
32. World Health Organisation (WHO). (2005). National Policy on Traditional Medicine and Regulation of Herbal Medicines. Report of a World Health Organization Global Survey. Geneva, Switzerland: WHO.
33. Zajac, A. M. & Conboy, G. A. (2012). *Veterinary Clinical Parasitology,* (8th ed). United Kingdom John Wiley & Sons. 3-15.
34. Zhang, L., Yan, J., Liu ,X., Ye, Z., Yang, X., Meyboom, R.,et al. (2012). Pharma-covigilance practice and risk control of traditional Chinese medicine drugs in China: current status and future perspective. *J. Ethnopharmacol*, 140: 519–525.

ACKNOWLEDGEMENT. The authors express their sincere thanks to farmers for providing the animals for the samples in this project. The authors are grateful to Universiti Sains Malaysia (Research University Grant (1001/PBIOLOGI/811275) for financing the project and providing the laboratory facilities.