RESEARCH ARTICLE

Sokoto Journal of Veterinary Sciences

(P-ISSN 1595-093X: E-ISSN 2315-6201)

http://dx.doi.org/10.4314/sokjvs.v21i2.1

Biu *et al.*/*Sokoto Journal of Veterinary Sciences*, **21**(2): 51 - 57.

Anthelmintic effects of aqueous extract *Balanites aegyptiaca* stem bark on strongyle larvae and the earthworm *Pheretima posthuma*

AA Biu[1], M Abdulkadir[2], TE Onyiche[1]*, ZA Muhammad[2] & J Musa[1]

1. *Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Maiduguri, P. M. B. 1069, Maiduguri, Nigeria*
2. *Department of Biological Sciences, Faculty of Science, University of Maiduguri, Nigeria*

*Correspondence: Tel.: +2348037035135; E-mail: et.onyiche@unimaid.edu.ng

Publication History:
Received: 09-09-2022
Revised: 24-11-2022
Accepted: 17-12-2022

Keywords: Anthelmintic Effects, Aqueous Extract, *Balanites aegyptiaca*, *Pheretima posthuma*, *Strongyle* Larvae

Abstract

The development of anthelmintic resistance and the high cost of conventional anthelmintics have led to the evaluation of medicinal plants as an alternative source of anthelmintics. In the current study, *in vitro* experiments were conducted to determine the possible anthelmintic effects of *Balanites aegyptiaca* stem bark aqueous extract on *Strongyle* larvae and the earthworm *Pheretima posthuma*. The *in vitro* studies revealed that the extract at graded concentrations of 300 mg/ml, 400 mg/ml, 500 mg/ml and 600 mg/ml exhibited larvicidal activity ($p<0.05$) against earthworms with 600 mg/ml having the highest activity causing paralysis and death. In a similar vein, a graded concentration response was observed against *Strongyle* larvae. The highest effective concentration on larvae was 250 mg/ml which had a larval mortality rate of 100% and a mortality index of one (1) comparable with that of albendazole. The IC_{50} and IC_{99} values for the time of paralysis were 442.7mg/ml and 767.80mg/ml respectively. The anthelmintic activity exhibited by the extract could be linked to the presence of phytochemicals present in the plant. These findings support the folkloric use of this plant in the control and management of gastrointestinal nematodes in humans and animals.

Introduction

One of the most common infections that affect livestock is gastro-intestinal parasitism and the clinical signs and sequelae are dependent on the parasite fauna present and the intensity of infection. In sheep, these can range from sub-clinical weight loss to lethal pathologies such as anaemia, diarrhoea and severe protein loss (Pugh & Baird, 2012). In the last six decades, anthelmintics have become an important strategy to control nematode infections in livestock for increased production (Kaplan, 2004). However, the development of resistance to synthetic chemical anthelmintics has led to the need to search for alternative biomolecules and hence medicinal plants are been explored for potential lead compounds (Lateef *et al.*, 2013), that could be developed further for new pharmaceutical products with potent anthelmintic activity (Diehl *et al*., 2004).

Sheep represent an important source of income in many countries including Nigeria and the effects of parasitism on their production have been well documented (Charlier *et al*., 2014). Anthelmintic resistance and climate change are likely to alter the geographical distribution of parasites and their impact on production animals, thus increasing the need for a clear understanding of the cost of parasitism to develop sustainable control strategies (FAOSTAT, 2013).

Desert date commonly referred to as *Balanites aegyptiaca*, (L.) Del. belongs to the family *Zygophyllaceae*. The plant is commonly distributed in the Sudano-Sahelian region of Africa, the Middle East and South Asia and the fruit is a rich source of nutrients and has a wide range of nutraceutical applications (Elfeel, 2010; Chothani & Vaghasiya, 2011). The anthelmintic activity of methanolic extract of *B. aegyptiaca* fruits has been evaluated against different parasites including *Toxocara vitulorum*, *Paramphistomum microbothrium*, *Trichinella spiralis* (Shalaby *et al*., 2010; Shalaby *et al*., 2012; Shalaby *et al*., 2016), *Schistosoma japonicum* and *Fasciola gigantica* (Koko *et al*., 2000). Several methods have been developed for evaluating *in vitro* nematodicidal activity of plant extracts. These assays are based on the assumption that a nematodicidal activity observed *in vitro* is indicative of potential *in vivo* activity. Therefore, this current study was carried out to assess the *in vitro* anthelmintic effects of *B. aegyptiaca* stem bark aqueous extract on the earthworm *Pheretima posthuma* and *Strongyle* larvae compared with Albendazole (Albavet®).

Materials and Methods

Plant collection and identification

A fresh sample of *Balanites aegyptiaca* stem bark were collected in the evening from University of Maiduguri campus, Borno State, Nigeria. It was identified and authenticated at the herbarium of the department of Biological Sciences, University of Maiduguri. The fresh stem bark was air dried under shade for 4 days and they were ground into fine powder using a pestle and mortar.

Balanites aegyptiaca stem bark processing and extraction

Six hundred grams (600g) of the pulverized stem bark was exhaustively extracted in three litres of distilled water using an Ace Soxhlet Extractor 6730 and Condenser 6740 (Quick Fit, England) at 60^{o}C for 10 hrs. The extract was concentrated on an aluminium tray, placed into an oven and maintained overnight at 60^{o}C as a drying process to remove water. A yield of 56.92 g was obtained as dry powder using the formula as described by Sasongko *et al*. (2011)

$$\text{Percentage extraction yield} = \frac{\text{Amount of extract yield (g) X 100}}{\text{Amount of dried plant used (g)}}$$

The extract was stored as a stock solution in a refrigerator until used.

Evaluation of anthelmintic activity of the aqueous extract on Pheretima posthuma

Pheretima posthuma were collected from moist compost soils within the premises of the Departments of Fisheries and the Animal Farm, University of Maiduguri, Nigeria. They were rinsed with distilled water to remove all debris. The anthelmintic activity was performed using a standard protocol as described by Ajaiyeoba *et al.* (2001). *Balanites aegyptiaca* stem bark aqueous extract concentrations of 300, 400, 500 and 600 mg/ml were used for the *in vitro* assay. For each concentration, two petri dishes were prepared to make a total of eight petri dishes. A further duplicate was also prepared with distilled water as normal control. Albendazole (Albavet®) at concentrations 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml a standard commercial anthelmintic drug were also prepared in duplicates to serve as positive controls. Each of these test Petri dishes was exposed to 5 earthworms and were observed for time to paralyze noticed by movement failure unless plagued by touch or immersed in warm water (50^{o}C) and time to death noticed by a complete absence of movement upon stimulation or immersion in warm water coupled with white secretion and fading away of their body colour. Either the time to paralyze or time to death indicated anthelmintic activity (Husori *et al.*, 2018). The mean time for paralysis and death was noted and the number of dead and alive was recorded.

Collection and processing of sheep faeces

About 60 grams of faeces were collected from the rectum of 20 sheep at the Maiduguri metropolitan abattoir into fully labeled polythene bags and brought on ice to the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for analysis. Each faecal sample was first subjected to a simple floatation technique to ascertain the presence of helminth eggs or ova as described by Phiri *et al*. (2007). Positive samples were further subjected to the Modified McMasters Technique to determine the number of eggs present per gram of faeces (epg).

Only positive samples with at least 500 eggs per gram were used for the faecal culture.

Faecal culture and harvest of larvae

The test tube paper technique as described by Ngwese *et al*. (2020) was used for the faecal culture and harvest of larvae. 0.5g of each positive faecal sample was smeared on a filter paper strip and the lower end was each dipped into a test tube containing 2mls of distilled water. The test tubes were covered with a ball of clean cotton wool to stop evaporation and the set-up was allowed to stabilize for 10 days at room temperature. After 10 days, the cotton wool covering the test tubes was removed and the filter paper strip was carefully pulled out of the culture medium individually. The culture medium was then poured into a Petri dish and *Strongyle* larvae were identified as described by Phiri *et al*. (2007).

In vitro anthelminthic activity assay of the extract on Strongyle larvae

The anthelmintic activity was performed using a standard protocol as described by Ajaiyeoba *et al.* (2001). About 0.5ml each of the culture medium containing between 14 and 46 larvae was exposed to concentrations of 31.25, 62.5, 125 and 250 mg/ml of *B. aegyptiaca* stem bark aqueous extract as extract controls and Albendazole (Albavet®) concentrations 6.25mg/ml, 12.5 mg/ml and 25 mg/ml and distilled water as positive and normal controls. The Petri dishes were covered with filter paper to prevent evaporation and the experiment was observed for one (1) hour period. The number of dead and alive was recorded. The number of dead parasites were determined by subtracting the alive from the known total number of larvae in each Petri dish before the commencement of the assay. Larval mortality was calculated using the formula described by Fernandez *et al*. (2009):

$$\text{Larval mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae tested}}$$

The mortality index was also calculated for each concentration of the extract as well as the standard drug (Albendazole) using the formula as described previously (Nasai *et al*., 2016).

$$\text{Mortality index} = \frac{\text{Total number of immobile/dead larvae}}{\text{Total number of larvae per petri dish}} \times 100$$

Statistical analysis

Data collected were expressed as mean ± standard deviation (S.D.). Linear regression tests and analysis of variance (ANOVA) were performed using GraphPad Prism v.5 and $p < 0.05$ was considered significant. Also, the IC_{50} and IC_{99} values were created and calculated using the software GraphPad Prism v.5 (GraphPad Software, La Jolla California, USA).

Results

The results of the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract described as time taken for paralysis and death of the earthworm (*P. posthuma*) in relation to the negative and positive controls is presented in Table 1. There was a statistically significant difference ($p<0.05$) between the extract controls and the positive control in terms of the time taken for paralysis and death of the earthworms. The time taken for both paralysis and death of the earthworms became shorter with an increase in extract concentration.

Table 2 shows the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract on mortality

Table 1: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on time taken for paralysis and death of *Pheretima posthuma* within 60 minutes

Test Groups	Mean ± S. D (range) of minutes post exposure	
	Paralysis	Death
Balanites aegyptiaca		
300 mg/ml	0^a	0^a
400 mg/ml	22.00 ± 1.00^b (21-23)	23.66 ± 8.14^b (18-33)
500 mg/ml	14.28 ± 1.11^b (13-16)	17.57 ± 1.72^b (15-20)
600 mg/ml	12.60 ± 2.55^b (9-15)	12.00 ± 1.41^b (10-14)
Albendazole		
25.00 mg/ml	28.00 ± 1.00^c (27-29)	35.20 ± 1.48^c (33-37)
12.50 mg/ml	36.80 ± 1.64^c (35-39)	41.00 ± 1.58^c (39-43)
6.25 mg/ml	37.40 ± 1.40^c (36-39)	50.20 ± 1.30^c (49-52)
Distilled water	0 ± 0^a	0 ± 0^a

Mean ± SD values within columns with different superscripts are statistically significant ($p< 0.05$)

pattern of *Pheretima posthuma.* There was a positive correlation between graded extract concentrations and the mean number of earthworms alive and those dead 1hr post-exposure. The IC_{50} and IC_{99} values for the time taken for paralysis of the earthworms were recorded as 442.7 mg/ml and 767.80 mg/ml, respectively with a coefficient of determination (r^2) as 0.1814 (Figure 1). Finally, the IC_{50} and IC_{99} values for the time taken for death of the earthworms were 383.9 mg/ml and 755.0 mg/ml, respectively with a coefficient of determination (r^2) as 0.1471 (Figure 2). Table 3 shows the anthelmintic activity of *B. aegyptiaca* stem bark aqueous extract on *Strongyle* larvae. There was a significant difference ($p< 0.05$) between the extract treatment groups and the positive control (Albendazole) in terms of the time taken for paralysis and death of the *Strongyle* larvae. There was a positive correlation between the graded extract concentrations and both larval mortality and mortality index. The 250 mg/ml concentration of *B. aegyptiaca* stem bark aqueous extract compared favorably with the positive control (Albendazole) having a larval mortality and mortality index of 1 and 100 respectively.

Table 2: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on mortality pattern of *Pheretima posthuma*

The concentration of the extract	Mean ± S. D.	
	Alive	Dead
300 mg/ml	5.00±0	0±0
400 mg/ml	3.50 ±0.70	1.50±0.71
500 mg/ml	1.50±0.71	3.50±0.71
600 mg/ml	0±0	5.00±0

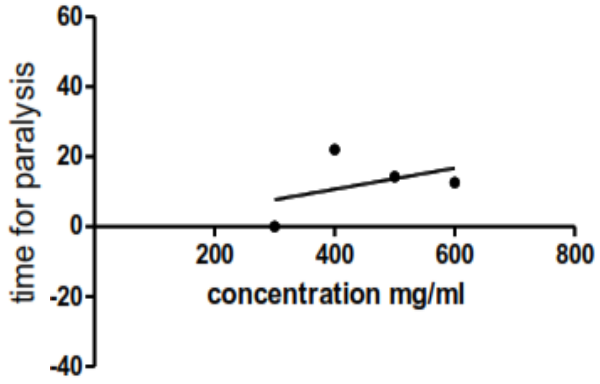

Figure 1: Regression equation of time of paralysis of earthworms against concentration

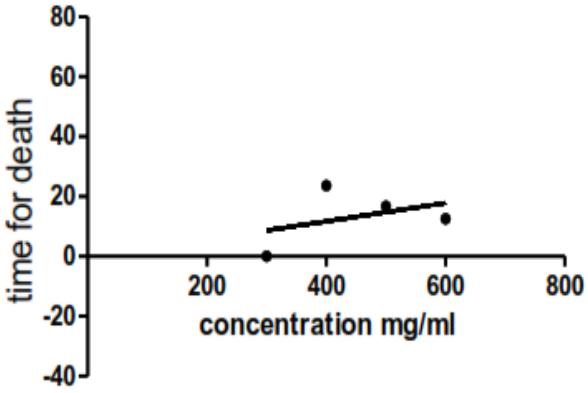

Figure 2: Regression equation of time of death of earthworms against concentration

Table 3: Anthelmintic activity of *Balanites aegyptiaca* stem bark aqueous extract on *Strongyle* larvae within 60 minutes

Test Groups	Mean ± S. D.		Larval mortality	Mortality index (%)
	Alive	Dead		
Balanites aegyptiaca				
31.25 mg/ml	25.5±4.95^a	4.5±0.71^a	0.15	15.0
62.5 mg/ml	17.5±12.02^b	14.5±7.78^b	0.45	45.3
125 mg/ml	17±12.73^b	6±0^a	0.26	26.09
250 mg/ml	0±0^c	17.5±3.54^b	1.00	100.0
Albendazole				
6.25 mg/ml	0±0^c	35±15.55^b	1.00	100.0
12.5 mg/ml	0±0^c	32.5±17.67^b	1.00	100.0
25 mg/ml	0±0^c	22.5±4.95^b	1.00	100.0
Distilled water	29±15.56^a	0±0^d	0	0

Mean ± SD values within column with different superscripts are statistically significantly ($p< 0.05$)

Discussion

Helminthosis is a serious disease in humans and in livestock farming. Although several commercial drugs are available in the market because of their side effects, medicinal plants have been appreciated as an alternative source of anthelmintic drugs. A handful of conducted studies have confirmed the effectiveness of many plants possessing anthelmintic activity.

Anthelmintic drugs are known to act by causing paralysis of worms or damaging cuticles, leading to partial digestion. They interfere with the metabolism of worms since the metabolic requirements of these parasites vary greatly from one species to another (Swargiary *et al.,* 2013).

In this study, *B. aegyptiaca* stem bark aqueous extract at graded concentrations tested *in vitro* showed significant antihelminthic activity against *Strongyle* larvae and the earthworm *P. posthuma* similar to albendazole. Studies on the therapeutic values of *B. aegyptiaca* have shown that the root bark has anthelmintic activity and it owes its medicinal values from the presence of phytochemicals (Dwivedi *et al.,* 2009). Helminthes or specifically nematodes are only one level lower than earthworms, the observed anthelminthic effects of the extracts on the earthworms is a mirror of what the extract will also exhibit on the stongyle larvae (Nasai *et al*., 2016). This was further confirmed with the *in vitro* experiment of the *B. aegyptiaca* stem bark extract on infective Strongyle larvae, giving credence to support the use of earthworms as a model to study the anthelminthic activity of plant extracts.

To screen the anthelmintic activity of plant compounds, *in vitro* testing of L3 is regarded as the best approach (Hernández-Villegas *et al*., 2011). The larvicidal activity of the extract was directly dependent on the concentration dose of the extract with high activity at the 250 mg/mL treatment group. Our study observed that the highest larvicidal activity of *B. aegyptiaca* stem bark was at 250 mg/mL producing an efficacy of 100.0% for the aqueous extract. The treated larvae were immobile and the findings suggest that the phytochemicals present caused severe weakening of the larvae that eventually resulted in their death. Our observation from this study confirms the traditional applications of *B. aegyptiaca* against internal parasites, as plants containing phytochemicals like alkaloids and saponins, possess antiparasitic activity. These bioactive compounds work separately or jointly to alter the membrane permeability of the parasite (Chothani & Vaghasiya, 2011) or binding to a specific glycoprotein of the cuticle of the parasite (Kumar *et al*., 2011).

The most important are steroidal saponins, which yield diosgenin, a source of steroidal drugs, such as corticosteriods, contraceptives and sex hormones as described by Farid *et al.* (2002). Others include cardiac glycosides, tannins and alkaloid salts. Specific compounds that have been isolated from the stem bark of *B. aegyptiaca* are furanocoumarin bergapten and dihydrofuranocumarin D- marmesin (Murthy *et al*., 2020), as well as three common metabolites, vanillic acid, syringic acid; and 3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone and two specific alkaloids namely N-trans-feruloyltyramine and N-cis-feruloyltyramine (Ansari *et al*., 2006; Breimer *et al*., 2007). Additionally, a long-chain aliphatic compound and a novel sugar, di-glucosyl-di-rhamnoside and 10-methyl-n-heptacosane, have also been isolated from the stem barks (Kapseu *et al*., 1997; Hardman *et al*., 2001; Ansari *et al*., 2006; Breimer *et al*., 2007; Al-Thobaiti & Abu Zeid, 2018). Saponins exhibit their effects by causing damage to the membrane of the parasite as well as vacuolization and disintegration of their integument (Wang *et al*., 2010) while tannins are known to hinder energy production by uncoupling oxidative phosphorylation by binding to glycoproteins on the cuticle of helminths thereby leading to the death of the parasite. For instance, Balantin-7, a steroidal saponin isolated from *B. aegyptiaca* was shown to exhibit strong anthelmintic activity against *Caenorhabditis elegans* adult worm viability (Gnoula *et al*., 2007). Furthermore, alkaloids can intercalate with the protein synthesis of the parasite (Al-Shaibani *et al*., 2009). Koko *et al.* (2005) pointed out that the plant is used as a purgative to remove intestinal parasites with the root, branches, bark, fruit and kernel extracts shown to be lethal to the miracidia and cercariae of *Schistosoma mansoni* and to *Fasciola gigantica*.

Albendazole, a proprietary antihelmitic belongs to the benzimidazole group and acts by uncoupling mitochondrial function associated with electronic transport to enable ATP generation. The molecular mode of action of all benzimidazoles, including albendazole, consists in binding to tubulin, a structural protein of microtubules. The blocking of microtubules in the worm perturbs the uptake of glucose leading to the exhaustion of glycogen reserves. This blocks the whole energy management mechanism of the worms that are paralyzed and they die and are subsequently are expelled. Albendazole also inhibits fumarate reductase, an enzyme involved

in the energy management of the worm cells as well (Adedapo *et al.,* 2007).

In conclusion, the results of this study showed that the aqueous extract of *B. aegyptiaca* stem bark produced immobilization of earthworms (100.0%) and *Strongyle* larvae (100.0%) under *in vitro* conditions. Also, the promising anthelmintic activity of the extract supports its folkoric use by locals in the treatment of helminth infections in both humans and animals.

Funding

No funding was received.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Adedapo AA, Otesile AT & Soetan KO (2007). Assessment of the anthelmintic efficacy of an aqueous crude extract of *Vernonia amygdalina*. *Pharmaceutical Biology*, **45**(7): 564-568.

Ajaiyeoba EO, Onacha PA & Olarenwaju OT (2001). *In vitro* anthelmintic properties of *Buchhlozia coriaeae* and *Gynandropsis gynamdra* extracts. *Pharm*aceutical *Biol*ogy, **39**(3): 217-220.

Al-Shaibani IRM, Phulan MS & Shiekh M (2009). Anthelmintic activity of *Fumaria parviflora* (Fumariaceae) against gastrointestinal nematodes of sheep. *International Journal of Agricultural Biology*, **11**(4): 431-436.

Al-Thobaiti SA & Abu Zeid IM (2018). Phytochemistry and pharmaceutical evaluation of *Balanites aegyptiaca*: An overview. *Journal of Experimental Biology and Agricultural Sciences*, **6**(3): 453-465.

Ansari MM, Ahmad J & Ali M (2006). 10-Methyl-n-heptacosane and diglucosyldirhamnoside from the stem bark of *Balanites aegyptiaca* Delile. *Indian Journal of Chemistry,* **45**(2): 2154–2156.

Breimer L, ElSheikh SH & Furu P (2007). Preliminary investigation of the disposition of the molluscicidal saponin deltonin from *Balanites aegyptiaca* in a snail species (*Biomphalaria glabrata*) and in mice. *Journal of Pesticide Science,* doi.10.1584/jpestics.G06-19.

Cabardo Jr DE & Portugaliza HP (2017). Anthelmintic activity of Moringa oleifera seed aqueous and ethanolic extracts against Haemonchus contortus eggs and third stage larvae. *International Journal of Veterinary Science and Medicine*, **5**(1): 30-34.

Charlier J, van der Voort M, Kenyon F, Skuce P & Vercruysse J (2014). Chasing helminths and their economic impact on farmed ruminants. *Trends* in *Parasitology,* **30**(7): 361-367.

Chothani H & Vaghasiya U (2011). A review on *Balanites aegyptiaca Del* (desert date): Phytochemical constituents, traditional uses and pharmacological activity. *Pharmacognosy Review,* **5**(9): 55-62.

Diehl MS, Atindehou KK, Tere H & Betschart B (2004). Prospects for anthelmintic plants in the lvory Coast using ethnobotanical criteria. *Journal of Ethnopharmacology,* **95**(2-3): 277-284.

Dwivedi A, Joshi V, Barpete PK, Akhtar AK, Kaur, A & Kumar S. (2009). Anthelmintic activity of root bark of *Balanites aegyptiaca* (L.) Del. *Ethnobotanical Leaflets*, **13**(5): 564–567.

Elfeel AA (2010). Variability in *Balanites aegyptiaca* var. *aegyptiaca* seed kernel oil, protein and minerals contents between and within locations. *Agricultural and Biological Journal of North America*, **1**(2): 170-174.

Food and Agricultural Organization Statistics (FAOSTAT), (2013). Online statistical series (Live Animal and Livestock Primary Datasets. http://www.fao.org/faostat/en/#home, retrieved 05-04-2022

Farid H, Haslinger E, Kunert O, Wegner C & Hamburger M (2002). New steroidal glycosides from *Balanites aegyptiaca*. *Helvetica Chimica Acta*, **85**(4): 1019-1026.

Gnoula C, Guissou P, Duez P, Frederich M & Dubois J (2007). Nematocidal compounds from the seeds of *Balanites aegyptiaca* isolation and structure elucidation. *International Journal of Pharmacology*, **3**(3): 280–284.

Hardman JG, Limbird LE & Gilman AG (2001). The Pharmacological Basis of Therapeutics. In: Goodman and Gilman's, tenth edition. New York, NY. Pp 1126.

Hernández-Villegas MM, Borges-Argáez R, Rodriguez-Vivas RI, Torres-Acosta JFJ, Méndez-González M & Caceres-Farfan M (2011). Ovicidal and larvicidal activity of the crude extracts from *Phytolacca icosandra* against *Haemonchus contortus*. *Veterinary Parasitology*, **179(**1-3): 100-106.

Husori DI, Sumardi TH, Gemasih S & Ningsih SR (2018). *In vitro* anthelmintic activity of *Acanthus illcifolius* leaves extracts on *Ascaridia galli* and *Pheretima posthuma*. *Journal of Applied Pharmaceutical Science,* **8**(2): 164-167.

Kaplan, RM (2004). Drug resistance in nematodes of veterinary importance: A status report. *Trends in Parasitol*ogy, **20**(10): 477-481.

Kapseu C, Mbofung CMF & Kayem GJ (1997). Fatty acids and triglycerides of fruit oils from *Cyperus esculentus* and *Balanites aegyptiaca*. *Sciences des Aliments*, **17**(5): 531–537.

Koko WS, Abdalla HS, Galal M & Khalid HS (2005). Evaluation of oral therapy on mansonial schistosomiasis using single dose of *Balanites aegyptiaca* fruits and praziquantel. *Fitoterapia,* **76**(1): 30–34.

Koko WS, Galal M & Khalid HS (2000). Fasciolicidal efficacy of *Albizia anthelmintica* and *Balanites aegyptiaca* compared with albendazole. *Journal of Ethnopharmacology,* **71**(1-2): 247–252.

Kumar S, Chaudhary S & Jha KK (2011). Anthelminthic activity on the *Leptadenia pyrotechnica* (forsk.) decne. *Journal of Natural Product Plant Resource*, **1**(4): 56-59.

Lateef MZ, Iqbal MS, Sajid RZ, Abbas ZUD, Sindhu M, Akhtar MN, Khan MM, Awais A & Iqbal QU (2013). An account of botanical anthelmintics and methods used for their evaluation. *Revenue Veterinary Animal Sci*ence, **1**(1): 7-14.

Murthy HN, Yadav GG, Dewir YH & Ibrahim A (2020). Phytochemicals and biological activity of desert date (*Balanites aegyptiaca* (L.) Delile). *Plants*, **10**(1): 32.

Nasai NB, Abba Y, Abdullah FFJ, Marimuthu M, Tijjani A, Sadiq MA & Omar MAB (2016). *In vitro* larvicidal effects of ethanolic extract of *Curcuma longa* Linn. on *Haemonchus* larval stage. *Veterinary World*, **9**(4): 417-420.

Ngwese MM, Manouana GP, Moure PAN, Ramharter M, Esen M & Adégnika AA (2020). Diagnostic techniques of soil-transmitted helminths: Impact on control measures. *Tropical Medicine and Infectious Diseases*, doi.10.3390/tropicalmed5020093.

Phiri IK, Phiri AM, Ziela M, Chota A, Masuku M & Monrad J (2007). Prevalence and distribution of gastrointestinal helminths and their effects on weight gain in free-range chickens in Central Zambia. *Tropical Animal Health and Production*, **39**(4): 309-315.

Pugh DG & Baird (2012). *Sheep and Goat Medicine*. USA Elsevier Health Sciences. Pp 32.

Sasongko P, Laohankunjit N & Kerdchoechuen O (2011). Evaluation of physicochemical properties of plant extracts from *Persicaria odorata*. *Agricultural Science Journal*, **42**(2): 333-336.

Shalaby HA, El Namaky AH, Khalil FA & Kandil OM (2012). Efficacy of methanolic extract of *Balanites aegyptiaca* fruits on *Toxocara vitulorum*. *Veterinary Parasitology*, **183**(3-4): 386-392.

Shalaby H, Soad Nasr & Farag T (2016). Tegumental effects of methanolic extract of *Balanites aegyptiaca* fruits on adult *Paramphistomum microbothrium* (Fischoeder 1901) under laboratory conditions. *Iranian Journal of Parasitology*, **11**(3): 396.

Shalaby MA, Moghazy FM, Shalaby HA & Nasr SM (2010). Effect of methanolic extract of *Balanites aegyptiaca* fruits on enteral and parenteral stages of *Trichinella spiralis* in rats. *Parasitology Research*, **107**(1): 17-25.

Swargiary A, Roy B, Giri BK & Ronghang BA (2013). Comparative study on the anthelmintic efficacy of some medicinal plants of North-East India: Alteration in the glycolytic enzymes of *Fasciolopsis buski*, a giant intestinal fluke. *Asian Pacific Journal of Tropical Med*icine, **3**(6): 412–420.

Wang GX, Han J, Zhao LW, Jiang DX, Liu YT & Liu XL (2010). Anthelmintic activity of *steroidal saponins* from Paris polyphylla. *Phytomedicine*, **17**(14): 1102-1105.