Biotechnology Journal International

Volume 27, Issue 6, Page 43-51, 2023; Article no.BJI.108164
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Anthelmintic Potential of *Vernonia amygdalina* on *Toxocara canis* in Domesticated Dogs

Adams, Eka [a] and Ekott, Emmanuel [a*]

[a] Department of Science Laboratory Technology (Environmental Biology), Heritage Polytechnic, Eket. Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2023/v27i6705

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/108164

Original Research Article

Received: 20/08/2023
Accepted: 28/10/2023
Published: 06/11/2023

ABSTRACT

Toxocariasis is a worldwide zoonotic parasitic infection and one route of preventing the disease in dogs and subsequent transmission to humans, especially children, is through treatment with anthelmintic. A study aimed at assessing the anthelmintic efficacy of *Vernonia amygdalina leaves extract* on *Toxocara canis* in domesticated dogs was carried out where the proximate and phytochemical components of the leaf extract were determined using the standard method and Gas Chromatography-Mass Spectrometry, the anthelmintic efficacy was assessed *in vitro* and *in vivo* respectively. The study revealed that this plant contained alkaloids, saponins, tannins, glycosides, flavonoids, terpenoids, phenol and steroids. Lethal concentration (LC50) of 14.83ml/mg at 12 hours and a percentage egg reduction of 84% after 2 weeks was recorded, which revealed that the plant leaves have anthelmintic potential and this could be attributed to the presence of flavonoids which are associated with the treatment of gastric infections and also n-Hexadecanoic acid which accounts for it anthelmintic (nematicidal) effect, alongside Octadecanoic acid which is an anti-inflammatory compound.

**Corresponding author: E-mail: eekott@yahoo.com;*

Keywords: Anthelmintic; toxocariasis; Vernonia amygdalina; dog; efficacy.

1. INTRODUCTION

Zoonoses are those diseases that can be transmitted between animals and humans, in this regard dogs are considered a public health concern as they harbor various pathogens such as zoonotic helminths including *Toxocara canis* [1,2]. Toxocariasis is a worldwide parasitic infection of dogs and other carnivores, where the adult worms are found in the intestine and eggs are passed with feces into the environment which become embryonated under optimum climate condition and infection is established upon ingestion by another host [3]. The shedding of eggs by adult worm through the host is considered a means of infection dissemination, where human can become an accidental host by ingesting the eggs via pica and devouring on paratenic host such as chicken, cattle, pig, etc and vegetables. [4,5,6]. Although, in humans the development to adult is hindered, but the larvae migrate through various organs resulting in syndromes such as Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM) and Neuron Larva Migrans (NLM) as well as covert infection and asymptomatic toxocariasis [7,8].

The preventive measures required against this infection include environmental intervention strategies that limit where dogs are allowed in our houses and surroundings but unfortunately, dogs have been identified as one of man's closest companion since the ages of non-civilization where they act as emotional support, security etc., and limiting their access to man will reduce its effectiveness in fulfilling its role to man [9]. As such, treatment with anthelmintic becomes one of the major measures of preventing the disease in dogs and subsequent transmission to man especially children [10].

In recent times, reports have emerged on the potential reduction in the effectiveness or even emergence of drug resistance through widespread and frequent use of this anthelmintic chemotherapy [11]. These have created an urgent need for newer, inexpensive and accessible drugs, and traditional herbal medicine based largely on medicinal plants which offers a major and accessible source of health care to man and animals in most developing countries [12]. Therefore, there is need for proper scientific documentation of the efficacy of some of the plants extracts used as anthelmintic as various plants contain bioactive compounds that have shown potential in combating helminth infections as reported in folklore [13,14,15]. One of such plant is the bitter leaf scientifically known as *Vernonia amygdalina* which is a shrub that grows in tropical Africa and its parasitic use was first reported among chimpanzees in the wild and has been used by humans [16,17], it's important to note that the scientific evidence supporting its effectiveness as an anthelmintic is limited and this work is aimed at validating its use.

2. MATERIALS AND METHODS

The study was carried out in Akwa Ibom State, Nigeria which lies between latitude 4^0 32' and 5^0 53' North and longitude 7^0 25' and 8^0 25' East with total land area of 7.249 km^2. The leaves of the *Vernonia amygdalina* plant (Fig.1) were collected from matured plants identified and authenticated in the herbarium unit, Department of Botany and Ecological Studies, University of Uyo. The ethanolic extraction of the leaves was carried out using maceration method [18]. Dogs (puppies) aged about 7 months weighing about 7 – 8 kg were bought from vendors and used for the studies. The crude leaf extract of *Vernonia amygdalina* commonly known in the area as bitter leaf (Etidot) was screened for anthelmintic effects *in vitro* using adult worms (*Toxocara canis*) and also *in vivo* using experimentally infected dogs (puppies infected with *Toxocara canis*) according to the method of Adams *et al*., [15].

Fig. 1. Picture of *Vernonica amygdalina Delile*

Qualitative phytochemical screening of the plant's leaves was carried out using standard screening tests as described by Adingra [19].

The proximate composition of the plant leaves samples was determined using the standard

methods of analysis of Association of Official Chemists [20]. Crude protein, crude lipid, carbohydrate, Moisture, crude fiber and ash contents in the samples were analyzed.

GC-MS analysis was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD). Ms model was agilent technology 5975 ms, the column used was HP-5MS agilent technology, length of the column 30 m, internal diameter 0.320 mm, thickness of 0.25 μm. Volume of sample injected was 1 μL. Oven temperature program with initial temperature of 80°C to hold for 2 minutes at 10°C/min to final temperature of 240°C to hold for 6 minutes with injector temperature of 250°C. The mobile phase was helium gas while the stationary phase was the column.

Detection of Components Analysis of mass spectrum GC-MS was carried out using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unidentified components was compared with the spectrum of the identified components stored in the NIST library. The name, molecular weight, structure of the components in the test material were ascertained [21].

In vitro analysis of the extract was carried out using the method of Adams *et al*. [15]. Prior to the experiment, all the puppies were examined coprologically and treated accordingly, except for few of those that had only *Toxocara canis* eggs present in their faeces and was kept as the reservoir host, to ascertain a worm free colony. Faeces containing *Toxocara canis* eggs was obtained from a naturally infected puppy (reservoir host). Dogs (worm free colony of puppies) were infected with *Toxocara canis* by contaminating their food and water with the infected faecal sample that was collected from the reservoir host and kept moist for some days at room temperature for embryonation to occur, and infection was confirmed through faecal analysis after three weeks. Some of the dogs that were passing eggs in faeces were sacrificed using appropriate humane method and the small and large intestine were carefully extracted from the body of the dogs. The intestines were opened with a scissor and the content washed with tap water into a clean plastic container, then the adult worms were collected carefully with the aid of the blunt head of a needle into petri dishes containing physiological saline (buffered saline).Two sets each of 5 petri dishes (groups) containing 10 adult worms each (total of 100 worms) was prepared and the plant extract was diluted at various concentrations 10%, 20%, 50% and 100% and a control with only physiological saline. 0.1ml of each diluted plant extracts (extract of *Vernonia amygdalina*) at various concentrations were pipetted into the different petri dishes (groups) leaving a control and then allowed for 12hours at room temperature. The groupings were as follows:

Group 1: adult worms treated with 10% extract
Group 2: adult worms treated with 20% extract
Group 3: adult worms treated with 50% extract
Group 4: adult worms treated with 100% extract (crude extract)
Group 5: adult worms which received only physiological saline

The non-motile worms were counted from the various plates (groups) after 30 mins, 1 hour, 6 hours and 12 hours respectively and then percentage mortality calculated.

$$\text{Percentage death} = \frac{\text{Number of dead parasites}}{\text{Total number of parasites}} \text{x } 100$$

Lethal concentration at 25 and 50% was calculated using probit analysis [22].

The *in vivo* screening was carried out according to the method of Adams et al. [15], the plant ethanolic extract was tested using 4 groups of experimentally infected puppies and one group of worm free colony of puppies which were made up of two males and two females respectively i.e. in duplicate, (making a total of 40 puppies).

Three of the experimentally infected groups were treated orally with LC50 and LC25 of the extract and Mebendazole, all at the dosage of 50 mg/kg body weight divided into three doses for 3 consecutive days (as it is the recommended dosage of Mebendazole for therapeutic use in Nigerian dogs) and one group was left untreated (positive control). The groupings were:

Group 1: Infected puppies treated with LC25 obtained from the *in vitro* studies
Group 2: Infected Puppies treated with LC50 obtained from the *in vitro* studies
Group 3: Infected Puppies treated with Mebendazole
Group 4: Infected puppies without treatment
Group 5: Non-infected puppies

The efficacy of the extract on the experimental animals was determined by faecal egg count from direct smear faecal analysis using the formula [23]:

$$\text{Faecal Egg Count Reduction (\%)} = \frac{\text{Pre- treatment egg count - post treatment egg count}}{\text{Pre- treatment egg count}} \text{ X } 100$$

Heamatological test which included white blood cell count (WBC), packed cell volume (PCV) and red blood cell count (RBC) was carried out on the puppies before and after the experiment. Packed cell volume (PCV) was assessed by the method of Nweze *et al.* [11]. Blood was collected from the tail veins of the dogs into heparinized capillary tubes. One end of the tube was sealed using sealer. The tubes were placed on a capillary tube rack and centrifuged with a micro-hematocrit centrifuge at 5000 revolutions/min for 5 min. The PCV was then read using a hematocrit reader. White and red blood cell count was carried out using the Neubauer haemocytometer. Behavioural changes observed from the experimental animals was noted.

All data obtained were subjected to analysis using Microsoft Excel and SPSS Version 21 data packages. Chi-square test was used to compare differences based on the level of statistical significance of p≥0.05 with 95% confidence interval and results are presented in Table 2.

Proximate analysis of *Vernonia amygdalina* presented in Table 1 revealed 22.0g of crude protein, 4.2g of fat, 31.5g of carbohydrate, 8.2g of moisture, 14.0g of ash and 20.1g of crude fiber.

The GC-MS Chromatogram of the ethanolic leaf extract *Vernonia amygdalina* revealed 15 peaks of compounds as presented in Fig 2 with their percentage peak area. The major phyto-compounds identified as shown in Table 2 were Oleic acid (29.52%), Octadecanoic acid (19.3%), n-Hexadecanioc acid (8.29%), Methyloctanoic acid (5.75%) and Tetradecanoic acid (4.80%) while the minor compounds encountered were Lupeol (2.38%), E-13-Decanoic acid(1.51%), Pentafluoropropanionic acid (1.40%), 3H-Byarazol-3-one and Decanoic acid (1.28%), 15-Tetracosenoic acid (1.21%), vitamin E (1.17%), Chiloscyphone (0.81%) and 4-Octenoic acid (0.73%).

Table 1. Proximate Analysis of *Vernonia amygdalina*

Vernonia amygdalina	
Parameter	**Value (mg/100g)**
Crude protein	22.0
Crude fat	4.2
Carbohydrate	31.5
Moisture	8.2
Ash	14.0
Crude fiber	20.1

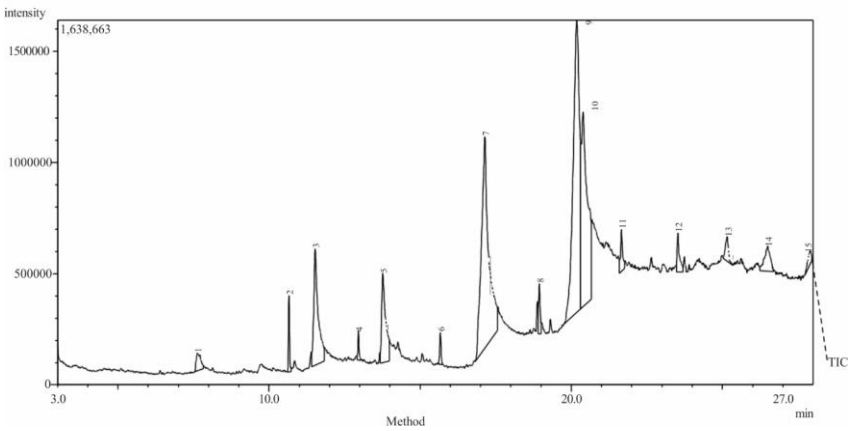

Fig. 2. GC-MS Chromotogram of Ethanolic Extract of *V. amygdalina* Leaf

Table 2. Phyto- components identified in the *Vernonia amygdalina*

Peak	Retention time	% Peak Area	Compound Analyzed	Molecular Weight	Molecular formular
1.	7.628	1.28	3H-Pyrazol-3-one	126	$C_6H_{10}N_2O$
2.	10.658	1.28	Decanoic acid	186	$C_{11}H_{22}O_2$
3.	11.526	8.29	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
4.	12.956	0.55	Pentanoic acid	130	$C_7H_{14}O_2$
5.	13.761	5.75	Methyloctanoic acid	158	$C_9H_{18}O_2$
6.	15.667	0.73	4-Octenoic acid	156	$C_9H_{16}O_2$
7.	17.139	4.80	Tetradecanoic acid	228	$C_{14}H_{28}O_2$
8.	18.941	1.21	15-Tetracosenoic acid	380	$C_{25}H_{48}O_2$
9.	20.178	29.52	Oleic acid	282	$C_{18}H_{34}O_2$
10.	20.389	19.31	Octadecanoic acid	284	$C_{18}H_{36}O_2$
11.	21.658	1.40	Pentafluoropropionic acid	332	$C_{15}H_{25}F_5O_2$
12.	23.524	1.51	(E)-13-Docosenoic acid	338	$C_{22}H_{42}O_2$
13.	25.156	1.17	Vitamin E	430	$C_{29}H_{50}O_2$
14.	26.499	2.38	Lupeol	426	$C_{30}H_{50}O$
15.	27.913	0.81	Chiloscyphone	218	$C_{15}H_{22}O$
Total		**100.0**			

Table 3. *In vitro* screening of the *Vernonia amygdalina* extracts on adult worms

Percentage (%) of Dead Adult Worms				
N=100				
Concentration (mg/ml)	**30mins**	**1hr**	**6hrs**	**12hrs**
10mg/ml (10%)	_	_	_	40
20mg/ml (20%)	_	_	10	60
50mg/ml (50%)	_	_	20	70
100mg/ml (100%)	_	_	20	80
Control	_	_	_	_

LC25= 3.32mg/ml, LC50=14.83mg/ml

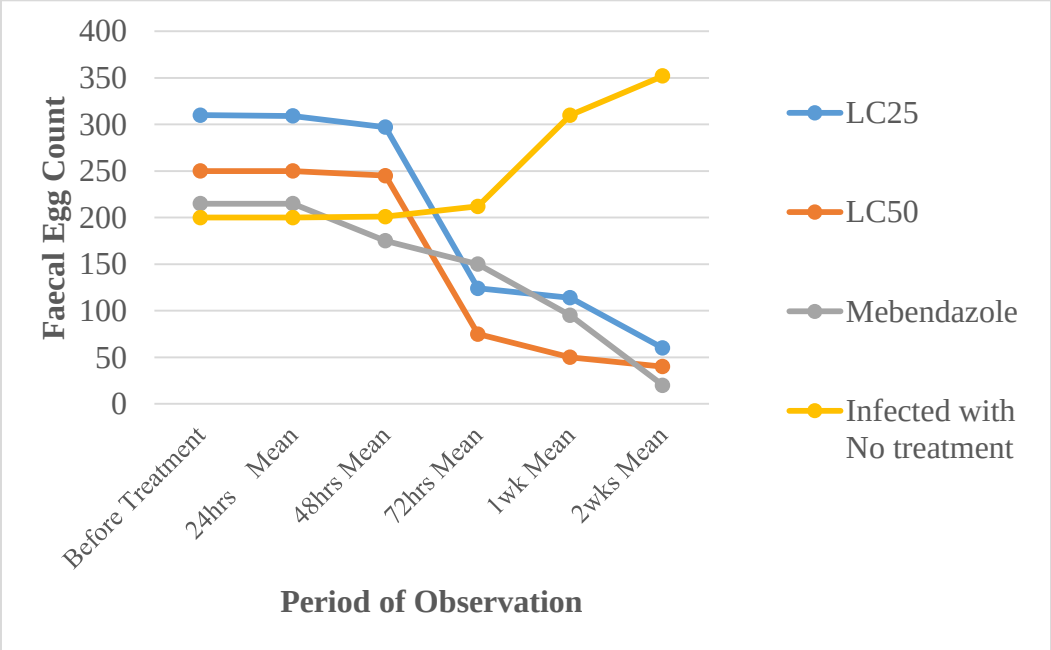

Fig. 3. *In vivo* screening of *Vernonia amygdalina* extracts on puppies (Fecal Egg Count)

Table 4. Heamatological analysis of the puppies used for the *In vivo* screening

Treatments	RBCs (millions/mL) Mean±SD	PCV (%) Mean±SD	WBCs (Thousands/ mL) Mean±SD
Non-infected	5.30±0.12a	42.00±1.73a	9.33±0.17cd
Infected untreated	3.70±0.12f	23.77±0.62c	14.17±0.88a
Infected treated with Mebendazole	4.40±0.06de	25.83±0.83c	11.63±0.52b
Infected treated with LC25 of *Vernonia amygdalina*	4.43±0.07cd	31.00±0.58b	9.00±0.76d
Infected treated with LC50 of *Vernonia amygdalina*	4.73±0.09bc	33.00±1.53b	10.00±0.29bcd
p Value	<0.001*	<0.001*	<0.001*

*Means along the same column with different Alphabet(s) are statistically significant at p≤0.05, ns – Not significant at p>0.05, * - significant at p≤0.05.*

In vitro administration of the ethanolic extract of *Vernonia amygdalina* to determine the lethal concentration shown in Table 3 revealed that the extract had no effect on the adult *Toxocara canis* within 30mins to one hour. Observable effect was seen within 6hrs and 12hrs, where ten percent (10%) and 60% deaths were recorded at 20% concentration, 20% and 70% deaths recorded at 50% concentration and the crude extract(100% concentration) recorded 20% and 80% death respectively. But with 10 % concentration, no death was observed in the first 6 hours although 40% deaths were recorded at 12hours. However, the lethal concentration was calculated at 12hrs to be 14.83mg/ml.

In vivo screening of *Vernonia amygdalina* extract on puppies revealed a high percentage egg reduction of 80.6% and 84% from the Faecal Egg Count (FEC) at LC25 and LC50 respectively, while the drug (Mebendazole) showed a 90.6% reduction as shown in Fig. 3.

A marked reduction was observed in the pack cell volume (PCV) and Red blood count (RBC) of the infected untreated puppies (23%, 3.70 $X10^6$ mL) and also those that were treated with Mebendazole (25.83%, 4.40 $X10^6$mL) while those treated with LC25 and LC50 extracts concentration showed a slight reduction of 31, 3.4.43 $X10^6$ mL and 33%, 4.73 $X10^6$ mL. The difference was statistically significant at p<0.001.

The WBC revealed an increase in the infected untreated (positive control) (14 x 10^3 mL) and also those treated with Mebendazole (11.63 x 10^3 mL) while those treated with LC_{25} and LC_{50} extract showed a slight increase of 9.0 and 10 $x10^3$ mL. The difference was statistically significant at P<0.001. *Vernonia amygdalina* revealed a minimal alteration of the three heamatological parameters (WBC, PCV and RBC) analyzed compared to the uninfected control group and group treated with Mebendazole.

3. DISCUSSION

The study revealed that *Vernonia amygdalina* has an anthelmintic efficacy on *Toxocara canis* in dogs which agrees with the works of Danquah et al. [24], Nweze et al. [11], Ejiofor et al. [25]. *Vernonia amygdalina*, also known as bitter leaf, is a plant that is widely used in Africa to treat a multiplicity of diseases including schistosomiasis, amoebic dysentery, and gastrointestinal problems [26]. Research has reported that any part of the plant can be used, but there seems to be a preference for the leaves, which are quite bitter, the bitterness is seen as a sign of potency [26,27]. It is found to contain two classes of bitter compounds namely sigmastane-type steroid glucoside and four known sesquiterpene lactones, vernodalin, vernolide, hydroxyvernolide, and vernodalol. All the pharmacological activity observed for this plant has been attributed to these bioactive compounds [28].

The reduction in fecal egg count by the leaf extract of *Vernonia amygdalina* in this study which was also reported by Adeolu et al. [29] is a positive and welcomed development in our local helminths struggle because the plants are available year-round in Nigeria. The easy access to this plant and its availability might mean reduced cost of veterinary attention which in most cases are neglected.

The study showed that the plant leaves contained alkaloids, saponins, tannin, glycosides,

flavonoids, terpenoids, phenol and steroid which agrees with the report of Ekekere et al. [30]; Kaur et al.[31], Ujah, et al. [32]. The presence of these phytochemical compounds especially flavonoids indicate that the plants could be useful in the treatment of helminthiasis alongside other diseases. Also, compounds like Alkaloids are implicated with antimalarial and analgesic value and can equally be used as stimulants [33].

GC-MS analysis revealed that it contained n-Hexadecanioc acid as one of its major compounds which is a nematicidal compound that accounts for its anthelmintic effect and it also has anti-inflammatory compounds such as Octadecanoic acid and Decanoic acid. This agrees with the findings of Oshiobugie *et al*., [21] and Nuryanto [34].

The leaf extract used in *in vivo* treatment revealed a minimal alteration of the three hematological parameters (WBC, PCV and RBC) examined in the study which agrees with the report of Adeolu et al. [3].

The activities shown by *Vernonia amygdalina* leaf extract are of considerable importance and justify its use as anthelmintic in folklore traditional medicine as the use of medicinal herbs in the treatment of infectious diseases has attracted the attention of scientists worldwide [14,35].

4. CONCLUSION

Besides the use of prescribed drugs for the treatment of toxocariasis in dogs, various plant extracts (both water and ethanolic extract) have been used by traditional/herbal medicine in the treatment of gastrointestinal helminthiasis in both humans and animals. The plant leaves of bitter leaf (*Vernonia amygdalina*) which is one of the most commonly used plants were collected, identified and anthelmintic efficacy on *Toxocara canis* was tested *in vivo* and *in vitro*. LC50 of 14.83ml/mg at 12 hours and a percentage egg reduction of 84% after 2 weeks was recorded which revealed that the plant leaves have anthelmintic potentials with minimal alteration of hematological parameters and this could be attributed to the presence of flavonoids which is associated with the treatment of gastric infections and also n-Hexadecanioc acid which accounts for it anthelmintic effect, alongside Octadecanoic acid which is an anti-inflammatory compound.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the authors

REFERENCES

1. Lescano SA, Nakhle MC, Ribeiro MC, Chieffi PP. IgG antibody responses in mice coinfected with *Toxocara canis* and other helminths or protozoan parasites. Rev Inst Med Trop Sao Paulo. 2012;54:145–152.
2. World Health Organization (WHO). Zoonoses;2020. Available:https://www.who.int/topics/zoonoses/en/ accessed on 20 July 2020.
3. Habluetzel A. Traidi C, Ruggieri S, Attili AR, Scuppa P, Marchetti G, Esposito F. An Estimation of *Toxocara canis* Prevalence in Dogs, Environmental Egg Contamination and Risk of Human Infection in the Marche Region of Italy. Vet Parasitol 2003;113: 243-252.
4. Anunobi TJ, Okeye IC, Godwin N. Toxocariasis and public health: an epidemiology Review; 2017.
5. Merigueti YFFB, Santarem VA, Ramires LM, Da silveira BA, Da Costa B. Eserra LV, Nuci AL, De Paula Esposte TM. protective and risk factors associated with the presence of Toxocara spp eggs in dog fur. Vet parasitol. 2017;244:39-43.
6. Healy SR, Morgan ER, Prada JM, Betson M. Brain food: rethinking food-borne toxocariasis. Parasitology. 2021;16:20-26.
7. Eslahi AV, Badri M, Nahavandi KH, Houshmand E, Dalvand S, Riahi SM. Occurrence of *Dictophyme renela* in Road Killed Canids of Iran and its Public Health Implication. Vet Parasitol. 2021;24(1):568-587.
8. Morsy TA. Toxocariasis: Visceral and Ocular Larva Migrans. Journal of the Egyptian Society of Parasitology. 2020; 50(10):41-18
9. Udoidung NI, Adams EG, Ekpo EJ, Opara KN. A Survey on Intestinal Nematodes of Dogs in Uyo, Akwa Ibom State, Nigeria. Journal of Parasitology and Vector Biology 2018;10(3):39
10. El-Menyawe SM, Mohammed A.The Review of Toxocara in Man and Animal. Vet Parasitol. 2015;12(3):231-242.
11. Nweze NE, Arine O, Ngongeh LA. Anthelminthic Potential of Three Plants Used in Nigeria Ethnoveterinary Medicine.

Pharmaceutical Biology. 2013;5(3):311-315.

12. Tandon V, Yadav AK, Roy B, Das B. Emerging Trends in Zoology: Phytochemicals as Cure for Worm Infections in Traditional Medicine System. Narendra Publication House, New Delhi. 2011;351-378
13. Eguale T, Giday M. *In vitro* Anthelminthic Activity of Three Medicinal Plants against *Haemonchus contortus*. International Journal of Green Pharmacy. 2009;29-34.
14. Oyeyemi IT, Akinlabi AA. Adewumi A, Aleshinloye AO. *Vernonia amygdalina:* A Folkloric herb with anthelminthic properties. International journal of adavance res. 2018;9(4):477-483
15. Adams EG, Ekott EJ, Usip LP, Opara KN. Efficacy of *Azadirachta indica* in the Treatment of Gastrointestinal Helminthiasis. Biotechnology Journal International. 2023;27(2):47-55.
16. Huffman MA and Seifu, M. Observation on the Illness and Consumption of a Possibly Medicinal Plant *Vernonia amygdalina (Del)* by Wild Chimpanzee in the Mahale Mountains National park, Tanzania. Primates. 1989;30:51-63.
17. Al-Shaibani IRM, Phulan MS, Shiekh M. Anthelminthic Activity of *Fumaria parviflora* (Fumariaceae) against Gastrointestinal Nematodes of Sheep. Int. J. Agric. Biol. 2009;11(4):431–436.
18. Sujon MA, Mostofa M, Jahan MS, Das AR, Rob S. Studies on Medicinal Plants against Gastroinstestinal Nematodes of Goats. Bangl. J. Vet. Med. 2008;6(2):179–183
19. Adingra KMD, Konan KH, Eugene JP, Tano K. Phytochemical Properties and Proximate Analysis of Papaya (*Carica papaya*) Pee, Olaniyi, A. M. and Raphael, A. O. AAS and GC-MS Analysis of Phytocomponents in the Leaf, Stem and Root of *Azadirachta indica A*. Juss (Dongoyaro). British Journal of Pharmaceutical Research. 2017;15(4):1-12
20. AAT Bioquest Inc. Quest Graph LC50 Calculator; 2022 Available:http://www.aatbiio.com/tools/LC50-Calculator
21. Salien AM, Kassuku AA, Magwisha H, Edward C, Marisa J, Ngovi C. Determination of Anthelminthic Resistance in Goats and Sheep Using Faecal Egg Count Reduction Test At Luguruni Farm, Dares Salaam, Tanzania. Tazania Veterinary Association Proceedings. 2019; 34:87-90
22. Danquah CA, Koffuor GA, Annan K, Ketor EC. The anthelminthic activity of *Vernonia amygdalina* (*Asteraceae*) and *Alstonia boonei* de wild (*Apocynaceae*). J Med Biomed Sci .2012;1(1):21–27
23. Ejiofor II, Zaman K, Das A. Effect of Extracts of *Vernonia amygdalina in Helminthiasis:* A Troical Negelected Disease. Open Access Journal of Pharmaceutical Research. 2017;1(8):147-151
24. Masaba S. The Anthelminthic Activity of *Vernonica amygdalina Del* (Compositae). Transaction of the Royal Society of Tropical Medicine and Hygiene. 2000; 94(6):694-670
25. Ijeh II, Ejike CEC. Current perspectives on the medicinal potentials of *Vernonia amygdalina Del.* Journal of Medicinal Plant Resource. 2011;5:1051-1061.
26. Alawa CD, Adamu AM, Gefu JO, Ajanusi OJ, Adadu PA, Chiezey NP, Alawa JN, Bowman DD. Invitro Screening of two Nigerian Medicinal Plants *Vernonia amygdalina* and *Annna senegalensis* for Anthelminthic Activity. Veterinary Parasitology. 2003;113:73-81.
27. Adeolu A, Adedapo A, Otesile T, Soetan KO. Assessment of the anthelminthic Eficacy of an Aqueous Crude Extract of *Vernonia amygdalina.* Pharmaceutical Biology. 2007;45(7):546-568
28. Ekekere GJ, Jeyachandran R. phytochemical and antimicrobial efficacy of *Vernonia amygdalina.* International journal of research in applied science and engineering technology. 2000;7(4):91-93
29. Kaur D, Kaur N, Chopra A. A comprehensive review on phytochemistry and pharmacological activities of Vernonia amygdalina. Journal of Pharmacognosy and Phytochemistry. 2019;8:2629-2636.
30. Ujah II, Nsude CA, Ani ON, Alozieuwa UB, Okpako IO, Okuwur AE. Phytochemical of Neem plant (*Azadirachta indica*) explains it use in traditional medicine and pest control. GCS Biological and Pharmaceutical science. 2021;14(2):163-171
31. Aron C, Tamakar V. *Gmelina arborea*: Chemical Constituents, Pharmacological Activities and Applications. Journal of Phytomedicine. 2017;9:528-542.

32. Nuryanto MK, Paramita S, Iskandar A. Membrane Stabilization Activity As Anti-Inflammatory Mechanisms of *Vernonia Amygdalina* Leaves Extracts. IOP Conf. Ser.: Earth Environ. Sci. 2018;144.

33. Aswathi PV, Dhivya R. Quality Phytochemical Screening and Mosquito Replelency of *Chromolaena odonrata* leaf extract against adult *Culex sp*. Indo American Journal of Pharmaceutical Science. 2017;4(3): 698-705.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/108164