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Anthelmintic Activity of *Artemisia monosperma* Methanol Extracts against *Eisenia fetida in vitro* Study

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ABSTRACT

Background: The present investigation was designed to investigate the protective effect of a natural product to control Anthelmintic in earthworm *Eisenia fetida*. We investigated the effectiveness of dried *Artemisia monosperma* leaves as an anthelmintic.

Methods: Five groups (50, 100, 200 and mg/mL) of the AMLE extract were utilized in this study. Negative and positive controls consisted of distilled water and mebendazole (10 mg/ml). Five worms that were around the same size were placed to each Petri plate along with the test solution (20 mL) of the extract. *Artemisia monosperma* leaves methanolic extract was prepared and evaluated its ability in vitro as anthelmintic activity against earthworms (*Eisenia fetida*). The ABTS, FRPA and DPPH techniques were used to calculate the antioxidant activity. Also, the phytochemical investigation of methanolic extract was performed on GC-MS equipment.

Result: Fourteen different biomolecules are predicted using GC-MS spectrum. Also, the AMLE had a greater percentage value of ABTS, FRPA and DPPH radical inhibition (94.5±0.03 trolox/gFW, 0.8±0.005 µmol/ gFW and 92.6±0.3**%**) respectively. Moreover, IC_{50} of AMLE was obtained at 20.5±0.9 µg/mL for the A549 and 24.3±0.9 µg/mL for MCF-7 cell lines. In comparison to AMLE, Mebendazole (10 mg/mL) produced paralysis and earthworm death by 13.91±0.007 and 18.2±0.980 min, respectively, while, AMLE (200 mg/mL) showed 3.860±0.320 and 5.464±0.422 min, respectively. The treated worms' surface architecture also had noticeable abnormalities, according to the histological study. The result of present study revealed that AMLE leaves can serve as a good natural source of potent antioxidants and anthelminthiasis medicines. Which promotes the conduct of numerous in vivo researches to discover an efficient treatment.

Key words: Anthelmintic, *Artemisia monosperma*, *Eisenia fetida*.

INTRODUCTION

Many nations experience significant death and economic loss as a result of parasitic illnesses caused by protozoa and helminths (Mehlhorn, 2001). For the vast majority of people living in developing nations, helminthiasis continues to pose a serious health danger (El-Badry *et al*., 2019). Anemia and weakness brought on by starvation are the main symptoms of worm infestation (Jones and Berkley, 2014).

Also, due to the lack of sufficient sanitary systems and drinking water sources, helminth infections rank among the most common human diseases worldwide and mostly afflict huge populations in underdeveloped nations (WHO, 2012). Although these infections seldom result in death, they do cause impairments in children's physical and mental development that have a negative impact on their academic achievement and can make them more susceptible to subsequent illnesses (Crompton *et al*., 2003).

The majority of helminth infections are treated with albendazole, a broad-spectrum antiparasitic medication that is also effective against some protozoa. but, the main downsides of this medication are its side effects, which include headache, nausea, vomiting, diarrhea, abdominal discomfort, dizziness and vertigo (Njomo *et al.,* 2010). Also, anthelmintic medications have some negative effects on the human body, especially the liver and kidney (Tripathi, 2008; Hong, 2018). Additionally, due to the high cost of pharmaceuticals, interest in medicinal plants as a potential source of anthelmintic drugs has increased (Dkhil *et al*., 2019).

The Arabian Peninsula is home to a large range of plants and there have long been widespread traditional medical practices in this area based on foods, spices and plants (Maideen, 2020). Due to its vast size and varied climatic and geographical conditions, the Kingdom of Saudi Arabia represents one of the most biodiverse regions on the Arabian Peninsula (Almehdar *et al*., 2012). There are numerous significant crops and medicinal plants that are traditionally used for medical purposes among Saudi Arabia's flora, such

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as *Artemisia monosperma, Artemisia Judaica, Acacia arabica, Pulicaria glutinosa, Calligonum comosum, Lantana camara etc*. (Ullah *et al*., 2020).

There are more than 500 species of shrubs and tiny plants in the genus Artemisia, which can be found in Asia, Europe and North America (Bora and Sharma, 2011). In Saudi Arabian traditional medicine, *A. monosperma*, a common desert plant, is widely used to treat muscle spasms, hypertension and parasitic worms (Amin *et al*., 2019). According to reports, the extracts and essential oils of this plant offer a variety of biological activities, such as insecticidal, antibacterial and antimalarial effects (Stavri *et al*., 2004; Stavri *et al*., 2005 and Guetat *et al*., 2017). Also, the medicinal importance of *Artemisia monosperma* is attributed to its anticancer, antispasmodic and antihypertensive (Abu-Niaaj *et al*., 1996; Hijazi and Salhab, 2010 and Al-Soqeer, 2011).

Our study's main objective is to assess the anthelmintic activity of *Artemisia monosperma* leaf extract (AMLE) *in vitro*.

MATERIALS AND METHODS

This experiment was completed in the zoology Department of the College of Sciences-King Saud University, during the duration from (6/3/2023 - 6/6/2023).

The wild plant *A. monosperma's* leaves were collected in Tabuk (Saudi Arabia) and the botanical identity of the plant was validated by a taxonomist from the Botany and Microbiology Department at the College of Science, King Saud University (Saudi Arabia). A collection of plant samples was made and they were air-dried at room temperature. It was then sieved, ground in an electric mill and stored for extraction.

The crude extract was created using the approach recommended by Dkhil (2013). Samples (100 mg) were drenched in 1000 L of 70% methanol for 24 hours at 4°C, maintained in a laminated container with a capacity of 1500 L and shaken overnight. In a Japanese Yamato RE300 rotary vacuum evaporator, the resultant extract was dried and concentrated. The resulting crude extract was stored in a deep freezer until various studies could be carried out.

The phytochemical investigation of methanolic extract was performed on GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II (Kanthal *et al.,* 2014) Experimental conditions of the GC-MS system were as follows: TR 5-MS capillary standard non-polar column, dimension: 30 Mts, ID: 0.25 mm, Film thickness: 0.25 µm. The flow rate of the mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, the temperature program (oven temperature) was 40°C raised to 250°C at 5°C/min and the injection volume was 1 ìl. Samples dissolved in chloroform were run fully at a range of 50-650 m/z and the results were compared by using the Wiley Spectral library search program.

The free radical scavenging assay, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), was used to determine the antioxidant activities of AMLE (Liyana-Pathirana *et al*., 2005). Briefly, 1 mL of the extract was mixed with 1 mL of DPPH (0.135 mM) at different concentrations (31.25-1000 g/mL). The combination was kept at room temperature and in the dark for 40 minutes, while being gently stirred. At 517 nm, the absorbance of the samples and control solutions (Which included ascorbic acid as a positive control) was measured and the percentage of DPPH scavenging activity of the extracts was estimated using the formula:

DPPH scavenging activity (%) =

$$\frac{\text{Abs control - Abs sample}}{\text{Abs control}} \times 100$$

Where:

The absorbance of DPPH + methanol (Abs control). The absorbance of the DPPH radical + sample (Abs sample). According to the described procedure by Almuhayawi *et al*. (2021), ferric-reducing antioxidant power (FRAP) and 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were used to determine antioxidant activity. In the FRAP procedure, Canavalia samples weighing around 0.2 g were first extracted in 70% methanol and being centrifuged at 14,000 rpm for 20 min. After that, at room temperature, 0.1 mL of the evaluated extracts were added to 0.25 mL of the FRAP reagent (20 mM $FeCl_3$ in 0.25 M acetate buffer, pH 3.6). The absorbance was then measured at 517 nm and the antioxidant activity was reported as µmol/gFW. The ABTS method involved combining ABTS and potassium persulfate (2.4 mM), performing the reaction for 12 hours in the dark, measuring the reaction's absorbance at 734 nm and calculating the antioxidant activity as trolox/gFW.

The DMEM medium (Gibco, USA) was routinely supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, USA) for the routine cultivation of lung (A549) and breast (MCF-7) cancer cell lines. In an incubator with a humidified environment of 5% CO_2, all cells were maintained at 37°C.

The cytotoxic activity of plant extract was evaluated using an MTT test. In brief, cells were plated in a 96-well culture plate at 5×10^4 cells per ml and permitted to grow for 24 h. Next, with different concentrations of plant extract (500, 250, 125, 62.5, 31.125 and 15.625 µg/mL) cells were treated while doxorubicin was utilized as a positive control. After 48 h of the incubation period, each well received 10 µL of MTT solution (5 mg/mL in PBS). and further incubated for 4 h. Afterward, 100 µl of acidified isopropanol was added to each well to solubilize the formazan product and the plate was kept in a shaker for 10 min. The absorbance was measured at 570 nm using a microplate reader (Bio Tek, USA). The cell viability % was calculated as follows:

$$\text{Cell viability (\%)} = \frac{\text{Mean absorbance treated cells}}{\text{Untreated cells}} \times 100$$

The IC_{50} values (concentration of extract that triggered 50% inhibition) were specified from the dose-response curve of cell viability per cent using OriginPro software.

Utilizing adult *E. fetida*, the *in vitro* anthelmintic activity was assessed. 50, 100 and 200 and mg/mL of the extract

from AMLE was prepared in distilled H_2O. The test solution (20 mL) of the extract was added to each Petri plate along with five worms that were roughly the same size. Negative and positive controls were distilled H_2O and mebendazole (10 mg/ml), respectively. Three duplicates of each test were carried out. According to Parida *et al*. (2010), recorded was the time of paralysis when the worm was shaken forcefully while dipping its tip in warm water (50°C) and the time of death was recorded when there was no movement of the worm.

The *E. fetida* was cut up into small pieces, fixed in 10% neutral formalin buffered and then prepared for paraffin embedding. Hematoxylin and eosin (H and E) were utilized to stain thin slices (4 µm) cut using a rotatory microtome (Drury and Wallington, 1973). A digital camera (DP 73) mounted on the microscope was used to take pictures while components were being examined under light microscopy (Olympus BX61, Tokyo, Japan) at a magnification of ×400.

RESULTS AND DISCUSSION

Numerous studies have revealed that different herbal extracts contain anthelmintic effects (Mehlhorn *et al*., 2011; Dkhil *et al*., 2019). In this study, earthworms were used as a model to evaluate the anthelmintic activity of AMLE due to the physiological similarity between *E. fetida* worms and several intestinal worms that infect humans (Cáceres *et al*., 2017; Abu Hawsah *et al*., 2023).

In this work, the properties of several compounds were identified by using GC-MS analysis of the methanolic extract of *A. monosperma*. The results showed the presence of 14 different biomolecules (Fig 1 and Table 1), such as, p-Cymenene, p-Cymen-8-ol, Acetophenone, 2′-hydroxy-, (2S,3S,6S)-6-Isopropyl-3-methyl-2-(prop-1-en-2-yl)-3-vinylcyclohexanone, Citronellyl iso-valerate, Spathulenol, Geranyl isovalerate, Isoelemicin, Shyobunol, Tremetone, Isocalamenediol, Ethanone, 1-[2,3-dihydro-2-(1-methy lethenyl)-5-benzofuranyl]- androst-5-en-17â-ol-3-one, Pectolinaringenin.

Also, the antioxidant activity of AMLE was determined by employing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique. Overall, the DPPH radical scavenging activity rose as extract concentration increased, peaked at 500 g/mL and then began to decline (Table 2). According to the findings, the AMLE had a higher percentage value of DPPH

Table 1: The phytochemical composition of *A. monosperma* was determined by using GC-MS.

$t_{R\ (min)}$	Proposed compound	MW	Formula	Peak area
6.58	p-Cymenene	132	$C_{10}H_{12}$	13040515
8.16	p-Cymen-8-ol	150	$C_{10}H_{14}O$	11570673
12.03	Acetophenone, 2′ -hydroxy-	136	$C_8H_8O_2$	30616290
12.84	(2S,3S,6S)-6-Isopropyl-3-methyl-2- (prop-1-en-2-yl) -3-vinylcyclohexanone	220	$C_{15}H_{24}O$	30035003
13.56	Citronellyl iso-valerate	240	$C_{15}H_{28}O_2$	24042447
13.67	Spathulenol	220	$C_{15}H_{24}O$	45259314
13.97	Geranyl isovalerate	238	$C_{15}H_{26}O_2$	15175318
14.51	Isoelemicin	208	$C_{12}H_{16}O_3$	28187618
15.04	Shyobunol	222	$C_{15}H_{26}O$	20143752
15.52	Tremetone	202	$C_{13}H_{14}O_2$	1.22E+08
15.68	Isocalamenediol	238	$C_{15}H_{26}O_2$	37671592
20.24	Ethanone, 1- [2,3-dihydro-2- (1-methylethenyl)-5-benzofuranyl]-	234	$C_{13}H_{14}O_4$	1.7E+09
22.25	Androst-5-en-17β-ol-3-one	288	$C_{19}H_{28}O_2$	48202049
27.02	Pectolinaringenin	314	$C_{17}H_{14}O_6$	18216520

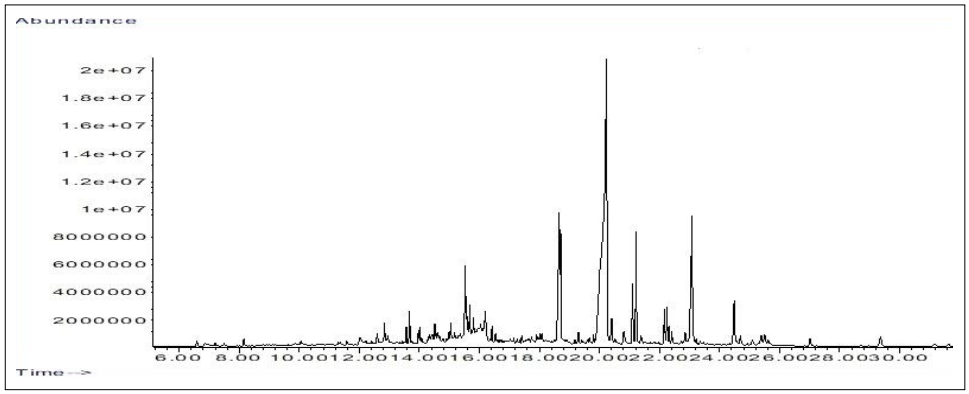


Fig 1: GC-MS of the methanolic extract of *A. monosperma* showing 14 peaks with retention times ranging from 6.58 min to 27.02 min.

radical inhibition at 500 µg/mL (92.6±0.3) (Table 2). Antioxidants' effect on DPPH radicals is thought to be due to their capacity to donate hydrogen. As a result, DPPH is commonly used as a substrate to assess free radical scavenging activity or antioxidant agents' antioxidative. Mustafa *et al*. (2010) confirmed phenolic compounds' antioxidant role by referring to their redox properties, which are considered free radical scavengers. The current findings show that the AMLE has strong to moderate DPPH radical scavenging activity. This result is consistent with prior research on the antioxidant activity of *A. monosperma* is Eos (Romeilah *et al*., 2021 and Abd-ElGawad *et al*., 2021). The hydroxyl groups' reactivity is thought to be the cause of oxygenated terpenes' higher antioxidant activity (Zuo *et al*., 2018).

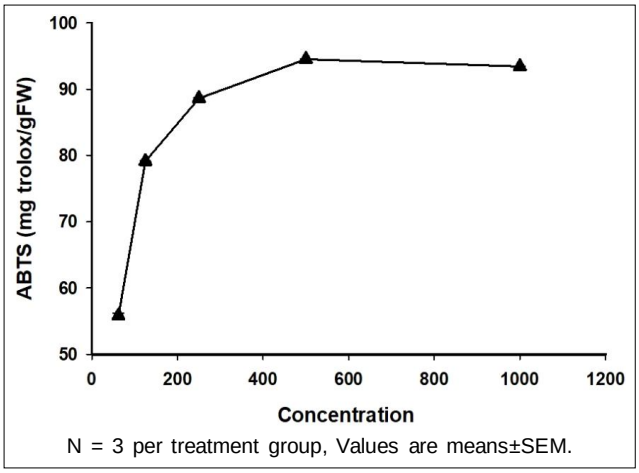
Moreover, our results showed the highest ABTS radical scavenging ability was show in concentration 500 µg/mL (94.5±0.03 mg trolox/gFW), while FRAP was (0.8±0.005 µmol/gFW) in concentration 1000 µg/mL (Fig 2 and 3).

In vitro, cytotoxicity of methanolic plant extract (AMLE) was tested against the breast (MCF-7) cancer and Lung (A549) cell lines at different concentrations (15.625, 31.125, 62.5, 125, 250 and 500 µg/mL) for 48 h. The IC_{50} of AMLE was obtained at 20. 5±0.9 µg/ml for the Lung (A549) cell line while was 24.3±0.9 µg/ml for the MCF-7 cell line (Fig 4). Our findings supported the notion that cell viability is directly dose-dependent. Data showed that the incubation of (A549) and (MCF-7) with different concentrations for 48 h of *A. monosperma* significantly decreased the viability of those cells when compared to untreated cells. These findings imply that the antiproliferative effect of artemisia extracts is mediated by an ERa-related mechanism. Era is a member of the steroid receptor superfamily that regulates transcription to control processes such as growth and differentiation in a variety of target cells (Björnström and Sjöberg, 2005).

The findings of the present study indicated the anthelmintic efficacy of AMLE against earthworms compared to mebendazole, Where the most effective dose of AMLE (200 mg/mL) showed the time to death and paralysis was 5.464±0.422 and 3.860±0.320 min, respectively, while mebendazole showed less effect (13.91±0.373 and 18.2±0.980 min for paralysis and death time, respectively) (Table 3). Changes in the integrity of the sheath were observed, in addition to serious morphological damage such as destruction and peeling of the sheath observed through microscopic examination of tissue sections. There are no changes in the uppermost layer of the cuticle for worms in the control group (Fig 5).

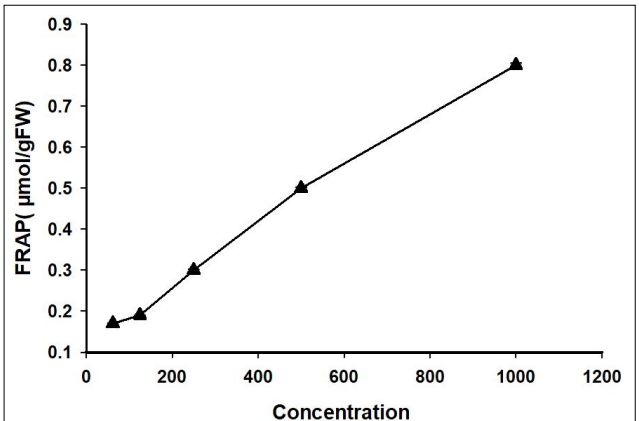
The cuticle is an essential part of annelids and Nematodes worms because it protects and covers the worm's body and supports its internal organs (Meyer *et al*., 2021). The tegument of the parasite is the primary pathway for nutritional uptake, metabolite secretion and defense against the host immune system (Xiao *et al*., 2000). Benzimidazole and mebendazole have been shown to have an impact on parasites' ability to energy metabolism (Kern, 2003).

The efficacy of *A. monosperma* is attributed to the presence of several bioactive phytochemical components such as artemisinin (El Maggar, 2012). Toxic effects of artemisinins on *S. mansoni* in vitro were seen at concentrations above 100 µg/mL (Xiao and Catto, 1989) and *Trichinella spiralis* (Abou Rayia *et al*., 2017). The reductive cleavage of artemisinin's highly reactive endoperoxide bridges by iron complexes or iron-producing free radicals can result in a state of oxidative stress by creating a cascade of extremely reactive free oxygen radicals (Levander Liyana-Pathirana *et al*., 1989) and the capability of



N = 3 per treatment group, Values are means±SEM.

Fig 2: (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) of *A. monosperma*.



N = 3 per treatment group, Values are means±SEM.

Fig 3: Ferric-reducing antioxidant power (FRAP) of *A. monosperma*.

Table 2: Radical scavenging activity (%) of *A. monosperma*.

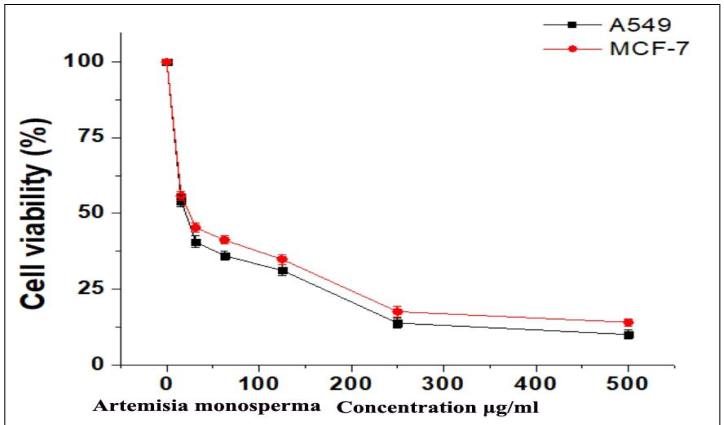
Concentrations (µg/mL)	DPPH radical scavenging activity (%)
62.5	31.3±0.6
125	72.4±0.2
250	90.9±0.1
500	92.6±0.3
1000	90.9±0.7

N = 3 per treatment group, Values are means±SEM.

Table 3: *In vitro* anthelmintic activity of *A. monosperma*.

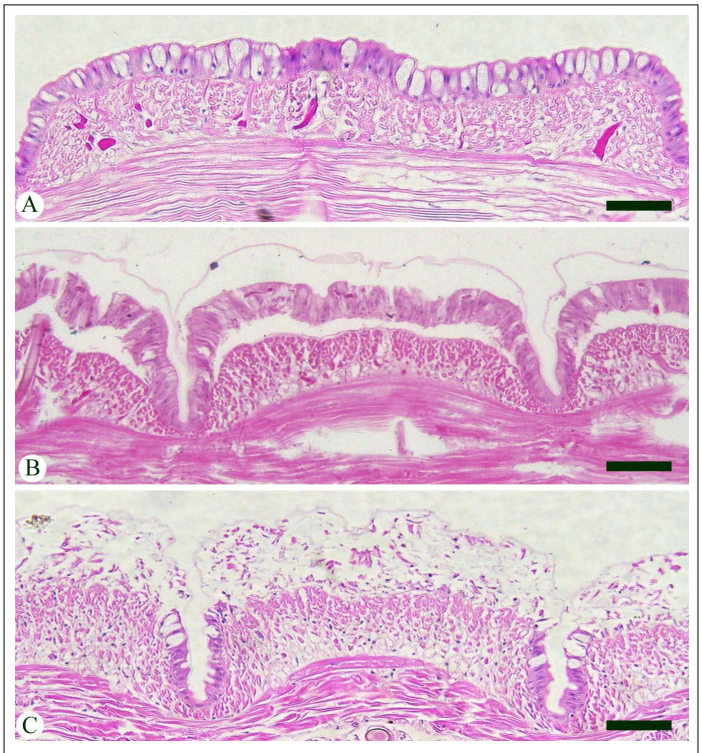
Test samples	Concentration (mg/ml)	Time is taken for paralysis (min.)	Time is taken for death (min.)
Control (H_2O)	-	-	-
AMLE	50 %	15.556±0.979*#	17.518±1.637*#
	100 %	10.642±0.857*#	13.920±0.347*#
	200 %	3.860±0.320*#	5.464±0.422*#
Mebendazole	10 mg/ml	13.91±0.373*	18.2±0.980*

Values are mean±SD. Compared to the untreated group, all superscripts indicate significance at p 0.05 (H_2O), #compared to mebendazole.



IC_{50} indicates the dose of tested plant extract, inducing 50% A549 (20. 5±0.9 µg/ml) and MCF-7 (24.3±0.9 µg/ml) cancer cell growth inhibition.

Fig 4: Cytotoxicity (MTT) assay for tested *A. monosperma* at different concentrations (µg/mL) against breast (MCF-7) cancer and Lung (A549) cell lines after 48 h of incubation.



(A) worms in distilled water (H_2O) (control). (B) worms in *A. monosperma* (200 mg/mL). (C) worms in mebendazole. Scale bar = 25 µm.

Fig 5: Histological changes in the cuticle of *E. fetida* with diverse treatments.

the free radical to alkylate protein causing the killing of the parasite (Galasso Guetat *et al*., 2007; Zhang and Gerhard, 2008; del Cacho *et al*., 2010).

CONCLUSION

It could be concluded that *A. monosperma* has anthelmintic efficacy, *in vitro*. More research should be done to determine the *in vivo* effectiveness of *A. monosperma*. This will help guide ongoing studies into the development of AMLE, as a novel medication that can be used to treat coccidian infections that affect animals.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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