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In vitro anti-eimeriosis and anthelmintic activities for *Achillea fragrantissima*

[*Atividades antieimeriose e anti-helmínticas in vitro da Achillea fragrantissima*]

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ABSTRACT

The effectiveness of many plants has been reported as anthelmintic and anticoccidial because they possess active compounds. Excessive use of drugs has led to the emergence of drug-resistant *Eimeria* species. This study was designed to evaluate anticoccidial and anthelmintic activity of *Achillea fragrantissima* flower extract (AFFE) and leaves extract (AFLE). Infrared spectroscopy showed nine phytochemical compounds. Chemical examination revealed AF rich in phenols, flavonoids, and tannins. Flower extract showed the highest percentage inhibition of DPPH radical at 500 µg/mL (82.2%) compared to leaves extract (74.5%). AFFE and AFLE (100 mg/ml) caused paralysis and earthworm death by 13.67±1.96 and 15.25±2.48 min and 4.19±0.05 and 4.70±0.28 min, respectively, compared to mebendazole. In histological study, a clear defect was found in surface architecture of treated groups of worms with extract. At 96 h, significant inhibition (100%) of process sporulation for *E. papillata* oocyst was observed when exposed to AFFE (300 and 200 mg/mL), while AFLE was 98.4 and 96%, respectively. Additionally, amprolium, Dettol™, phenol, and formalin 5% showed different levels of inhibition. Results revealed anticoccidial and anthelmintic activities of AFFE and AFLE, which encourages conducting many *in vivo* studies to find an effective and cheap treatment.

Keywords: *Achillea fragrantissima*, extract, earthworms, sporulation

RESUMO

A eficácia de muitas plantas foi relatada como anti-helmíntica e anticoccidiana porque elas possuem compostos ativos. O uso excessivo de medicamentos levou ao surgimento de espécies de Eimeria resistentes a medicamentos. Esse estudo foi concebido para avaliar a atividade anticoccidiana e anti-helmíntica do extrato da flor de Achillea fragrantissima (AFFE) e do extrato das folhas (AFLE). A espectroscopia de infravermelho mostrou nove compostos fitoquímicos. O exame químico revelou que o AF é rico em fenóis, flavonoides e taninos. O extrato da flor mostrou a maior porcentagem de inibição do radical DPPH a 500 µg/mL (82,2%) em comparação com o extrato das folhas (74,5%). O AFFE e o AFLE (100mg/ml) causaram paralisia e morte das minhocas em 13,67±1,96 e 15,25±2,48 min e 4,19±0,05 e 4,70±0,28 min, respectivamente, em comparação com o mebendazol. No estudo histológico, foi encontrado um claro defeito na arquitetura da superfície dos grupos de vermes tratados com o extrato. Após 96 horas, foi observada uma inibição significativa (100%) do processo de esporulação do oocisto de E. papillata quando exposto ao AFFE (300 e 200mg/mL), enquanto o AFLE foi de 98,4 e 96%, respectivamente. Além disso, amprólio, DettolTM, fenol e formalina 5% apresentaram diferentes níveis de inibição. Os resultados revelaram atividades anticoccidianas e anti-helmínticas de AFFE e AFLE, o que incentiva a realização de muitos estudos in vivo para encontrar um tratamento eficaz e barato.

Palavras-chave: Achillea fragrantissima, extrato, minhocas, esporulação

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INTRODUCTION

Coccidiosis is one of the main parasitic diseases that infect several animals, and it leads to significant economic losses (Györke *et al*., 2013). The etiological agent of this illness, *Eimeria* spp., belongs to the genus *Eimeria*, family Eimeriidae, and phylum Apicomplexa (Mehlhorn, 2014). They are protozoan parasites that reproduce inside the enterocytes of the host, producing severe diarrhea, weight loss, and, in some cases, premature death. *Eimeria* infection can also help other opportunistic pathogens proliferate, worsening the animal's clinical condition (Quiroz-Castañeda and Dantán-González, 2015). The infectivity potential of oocysts is dependent upon the sporulation rate and oocyst wall structure for *Eimeria* spp. which provides a strong defense and resilience against chemical, mechanical, and physically damaging stimuli including anti-coccidia as well as other antimicrobial agents (Fatoba and Adeleke, 2018). Therefore, to effectively control this parasite, it is essential to inhibit the sporulation process (Mai *et al*., 2009).

Anticoccidial medications are used in prevention and treatment of the disease (Abbas *et al*., 2011). Concerns have been reported about therapies due to the emergence of bacterial and coccidial resistance because of their widespread use, feed contamination, and poorly elucidated interactions with other drugs (Abu Hawsah *et al*., 2023). Therefore, developing new drugs from medicinal plants is a potentially sustainable alternative because they have anti-parasitic properties and anti-bacterial (Cobaxin-Cardenas, 2018), as well as a rich source of bioactive phytochemicals that have been used in traditional medicine for centuries, also, many of which have anti-inflammatory and antioxidant functions (Shahat *et al*., 2013).

One of the most well-known genera in the Asteraceae family, *Achillea* plant, contains more than 115 species (Saeidnia *et al*., 2015). *Achillea fragrantissima* is the most well-known species within Asteraceae. It is found in Saudi Arabia and known locally as Qaysūm (Arabic) (Barel *et al*., 1991; Al-Qarawi *et al*., 1996). The plant is used in traditional medicine to treat many ailments such as respiratory diseases, digestive problems (Saeidnia *et al*., 2015), as well as high blood pressure, skin diseases, stomach aches, and diabetes (Shabana *et al*., 1990; Hamdan and Afifi, 2004).

Achillea fragrantissima contains highly bio-logically active ingredients, including flavonoids, lignans, alkamides, and terpenic lactones (achillolid A) (Tarawneh *et al*., 2010; Patocka and Navratilova, 2019), tannins (El-Ashmawy *et al*., 2016). Recent studies showed that *A. fragrantissima* extracts have anti-inflammatory, antiproliferative capacities and antioxidants (Al-Mustafa and Al-Thunibat, 2008; Elmann *et al*., 2011; Akbar *et al*., 2023). Also, the plant has anthelmintic and carminative activity (Aboutable *et al*., 1986; Sincich, 2002), antiviral (Soltan and Zaki, 2009), antimicrobial and anticancer (Alshuail *et al*., 2022; Break *et al*., 2022), and antifungal (Alsohaili, 2018). Additionally, studies showed its antiparasitic activity such as *Trypanosoma evansi* (El-Ashmawy *et al*., 2016), *Leishmania infantum* (Ayrom *et al*., 2021), and *Blastocystis* (Mokhtar *et al*., 2019).

This study aimed (*in vitro*) to investigate the protective effect of *A. fragrantissima* flowers extract (AFFE) and leaves extract (AFLE) against oocyst sporulation (*Eimeria papillata*), also, to its anthelmintic activity.

MATERIALS AND METHODS

Leaves and flowers of *Achillea fragrantissima* were collected from Tabuk (Saudi Arabia), and a taxonomist at the Department of Botany, King Saud University, confirmed the botanical identity of the plant. According to the method of Dkhil (2013), 200g of leaves and 200g of flowers were air-dried at 40 °C, powdered, and then extracted with 70% methanol for 24 hr at 4°C. Resulting extract was concentrated and dried in a rotary vacuum evaporator (Yamato RE300, Japan). Distilled H_2O was used to dissolve powder to perform various experiments.

Plant (leaves or flowers) extract was analyzed using the KBr pellet method on a NICOLET 6700 (Thermo Scientific, Waltham, USA) FT-IR spectrometer with a range of 400–4000 cm^{-1} (Abu Hawsah *et al*., 2023).

The phenolic contents of AFFE and AFLE were determined using the technique of Singleton *et al*. (1999), with some modifications. Briefly, 0.1mL of Folin-Ciocalteu reagent, 1.5mL of

ultrapure water (Milli-Q), and 0.1mL of plant (flower or leaves) extract (1mg/mL) or gallic acid were mixed and left for 8 min, then, 0.3 mL of sodium carbonate solution (20%) was added and mixed by a vortex. Mixture was incubated in dark for 2 hr. A UV-visible spectrophotometer was used to measure the absorbance at 765 nm. Phenolics were calculated as gallic acid equivalent (mg/g DW).

The total flavonoids in AFFE and AFLE were determined using a method reported by Ordoñez *et al*. (2006). Briefly, 1.0mL of 2% $AlCl_3$ water solution was mixed with 1.0mL of flower extract or leaf extract (1mg/mL). At 420 nm, absorbance was measured following an hour of incubation at room temperature. Flavonoids were expressed as quercetin (mg/g DW).

The total tannins in AFFE and AFLE were determined according to the method of Kavitha Chandran and Indira (2016).

According to Liyana-Pathirana and Shahidi (2005), the antioxidant activities of AFFE and AFLE were determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Briefly, 1mL of the extract was mixed with 1mL of 0.135mM DPPH at various concentrations (31.25–1000g/mL). Absorbance of the samples and the control solutions was measured at 517nm.

By oral gavage, 1×10^3 sporulated *Eimeria papillata* oocysts were administered to five laboratory mice *Mus musculus*. Feces were collected on the fifth day after infection, and oocysts were subsequently isolated using the flotation technique and used for an *in vitro* study.

Unsporulated oocysts (1×10^5) were incubated in all the groups and included: negative control group (5 mL of dist. H_2O), positive control group (5 mL of 2.5% potassium dichromate $K_2Cr_2O_7$), and the treatment groups (5 mL of 2.5% $K_2Cr_2O_7$) containing one of the following: AFFE (300, 200, 100 and 50mg/mL), AFLE (300, 200, 100 and 50mg/mL), Amprolium (8.3mg) (Veterinary Agriculture Products Company [VAPCO], Jordan), Dettol™ (109µL), phenol (25µL), and formalin (5%). Sporocysts were examined, and sporulation of the oocysts was tracked using an Olympus compound microscope (Olympus Co., Tokyo, Japan). For each treatment, we used three replicates, and all Petri dishes were incubated at 25 to 29°C for 72 and 96 hr (Gadelhaq *et al*., 2018). The sporulation percentage was calculated according to Daiba *et al*. (2022), and sporulation inhibition percentage was according to Cedric *et al*. (2018).

Adult earthworms, *Eisenia fetida*, were used to evaluate *in vitro* anthelmintic activity. Extract from AFFE and AFLE were prepared in dist. H_2O at concentrations of 100, 50, and 25mg/mL. Five worms of approximately the same size were placed in Petri dishes. Each petri dish contained 20 mL of test solution of extract. Mebendazole (10 mg/ml) was used as a positive control, and dist. H_2O was used as a negative control. The experiments were run in triplicate. Time for paralysis was recorded when no movement was observed except when shaken vigorously, while the time of death was recorded when the worms did not show any movement by vigorous shaking nor when dipped in warm water (50°C) (Parida *et al*., 2010).

Small parts of the *E. fetida* were taken and fixed in 10% buffered neutral formalin and processed for paraffin sections. Thin sections (4 µm) cut by means of a rotatory microtome were rehydrated and stained with hematoxylin and eosin (H&E) (Drury and Wallington, 1980). Light microscopy (Olympus BX61, Tokyo, Japan) was used to examine sections and photographed using a digital camera (DP 73) fitted on the microscope.

The data were analyzed using one-way analysis of variance (ANOVA) in SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA). At a p-value ≤ 0.05, differences between groups were deemed significant.

RESULTS

Analysis of AFFE using FT-IR showed major bands at 3417.82cm^{-1}, 2931.01cm^{-1}, 1739.16cm^{-1}, 1621.41cm^{-1}, 1514.11cm^{-1}, 1384.04cm^{-1}, 1255.48cm^{-1}, 1076.71cm^{-1}, and 571.82cm^{-1} (Figure 1 and Table 1). The O-H stretching was indicated by the band at 3417.82cm^{-1} confirming the presence of alcohol. The band at 2931.01 cm^{-1} implied C-H stretching for the presence of alkane. C=O stretching at 1739.16 cm^{-1} confirms the presence of esters. The band at 1621.41cm^{-1} corresponds to C=C stretching for the presence of α,β-unsaturated

ketone. N-O bending at the band 1514.11cm^{-1} confirmed the presence of nitro compound. The band of 1384.04 cm^{-1} (C-H bending), 1255.48 cm^{-1} (C-O stretching), 1076.71cm^{-1} (C-O stretching), and 571.82cm^{-1} (C-Br stretching) assigned to an alkane, alkyl aryl ether, primary alcohol, and halo compound, respectively (Table 1).

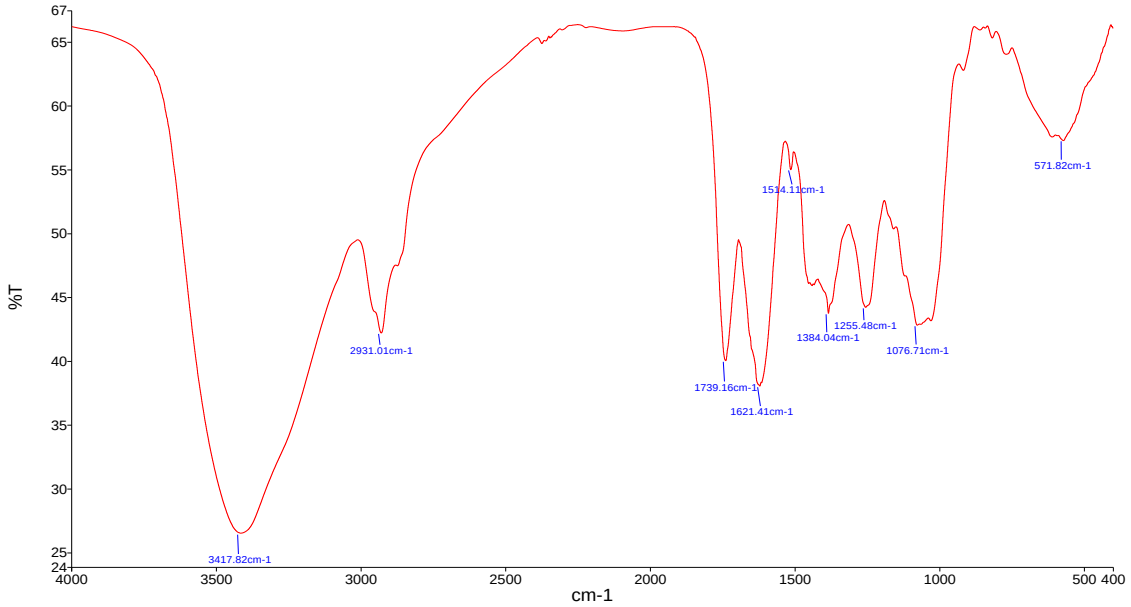

Figure 1. FT-IR of AFFE in a methanolic medium showing the functional characteristic of the material.

Table 1. FT-IR for *A. fragarntissima* flowers extract (AFFE)

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3417.82	6.797389	strong, broad	O-H stretching	alcohol
2931.01	10.82597	medium	C-H stretching	alkane
1739.16	10.26702	strong	C=O stretching	esters
1621.41	9.75912	strong	C=C stretching	α,β-unsaturated ketone
1514.11	14.11018	strong	N-O stretching	nitro compound
1384.04	11.21991	medium	C-H bending	alkane
1255.48	11.34165	strong	C-O stretching	alkyl aryl ether
1076.71	10.98301	strong	C-O stretching	primary alcohol
571.82	14.69575	strong	C-Br stretching	halo compound

The analysis of AFLE using FT-IR showed major bands at 3420.84cm^{-1}, 2928.06cm^{-1}, 1740.88cm^{-1}, 1624.27cm^{-1}, 1514.09 cm^{-1}, 1383.91cm^{-1}, 1255.78cm^{-1}, 1051.98cm^{-1}, and 572.27cm^{-1} (Figure 2 and Table 2). O-H stretching was indicated by the band at 3420.84cm^{-1} confirming the presence of alcohol. The band at 2928.06 cm^{-1} implied C-H stretching for presence of alkane. C=O stretching at 1740.88 cm^{-1} confirms the presence of esters. The band at 1624.27cm^{-1} corresponds to C=C stretching for the presence of conjugated alkene. N-O bending at the band 1514.09cm^{-1} confirmed the presence of nitro compound. The band of 1383.91 cm^{-1} (C-H bending), 1255.78cm^{-1} (C-O stretching), 1051.98 cm^{-1} (C-O stretching), and 571.82cm^{-1} (C-Br stretching) assigned to alkane, alkyl aryl ether, primary alcohol, and halo compound, respectively (Table 2).

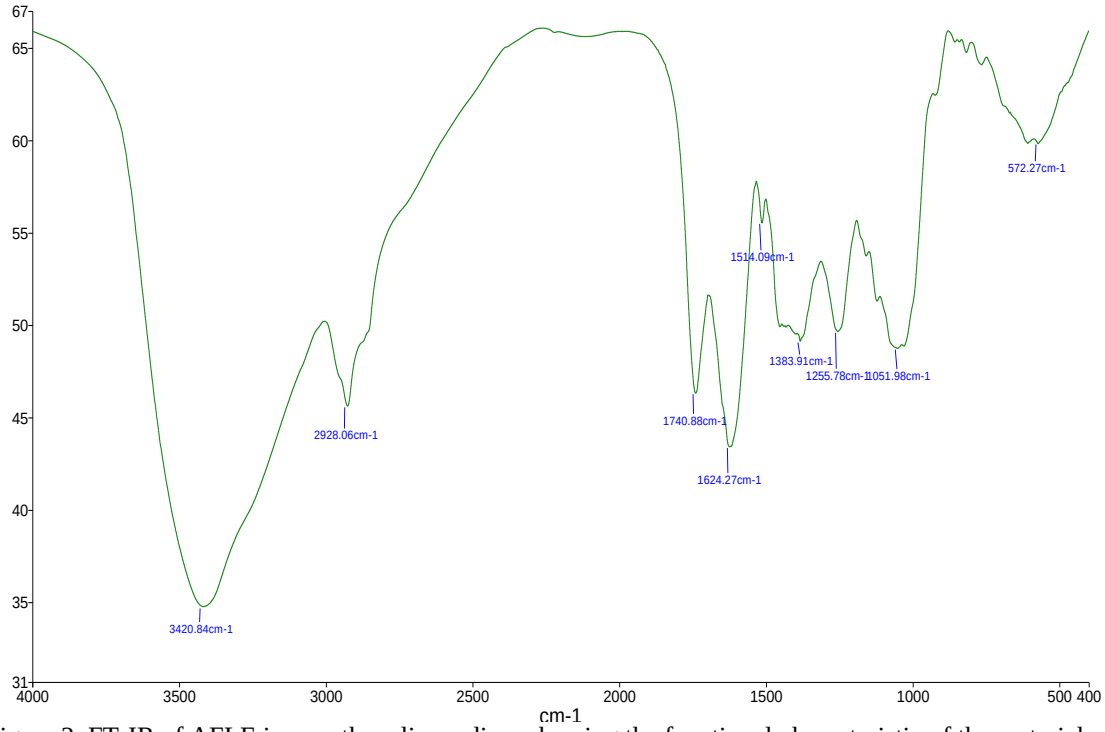

Figure 2. FT-IR of AFLE in a methanolic medium showing the functional characteristic of the material.

Table 2. FT-IR for *A. fragarntissima* leaves extract (AFLE)

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3420.84	8.023352	strong, broad	O-H stretching	alcohol
2928.06	10.5347	medium	C-H stretching	alkane
1740.88	10.69856	strong	C=O stretching	esters
1624.27	10.02569	medium	C=C stretching	conjugated alkene
1514.09	12.8267	strong	N-O stretching	nitro compound
1383.91	11.34973	medium	C-H bending	alkane
1255.78	11.46696	strong	C-O stretching	alkyl aryl ether
1051.98	11.25579	medium	C-O stretching	primary alcohol
572.27	13.81852	strong	C-Br stretching	halo compound

The contents of some secondary metabolites, like phenolics, flavonoids, and tannins in AFFE and AFLE, were determined. Amounts of phenols in flower extract (127.3±0.66) were less than in leaves extract 148.51 ± 0.66 (Figure 3). Also, the methanolic extract of the flowers showed higher values for flavonoids compared to leaves extract 43.42±0.21 and 24.87±0.12, respectively (Figure 3). In addition, the number of tannins in AFFE was 55.6±3.4 (mg TAE/g DW), while in AFLE it was 44.7±11.4 (mg TAE/g DW) (Figure 3).

The antioxidant activities of AFFE and AFLE were determined using free radical scavenging activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Overall, the scavenging activity against the DPPH radical increased with concentration increases in extracts, peaking at 500 g/mL, and after that started to decline (Table 3). The results indicated that AFFE showed the highest percentage inhibition value of DPPH radical at 500 µg/mL (83.95%) compared to the leaves extract (82.2%) (Table 3).

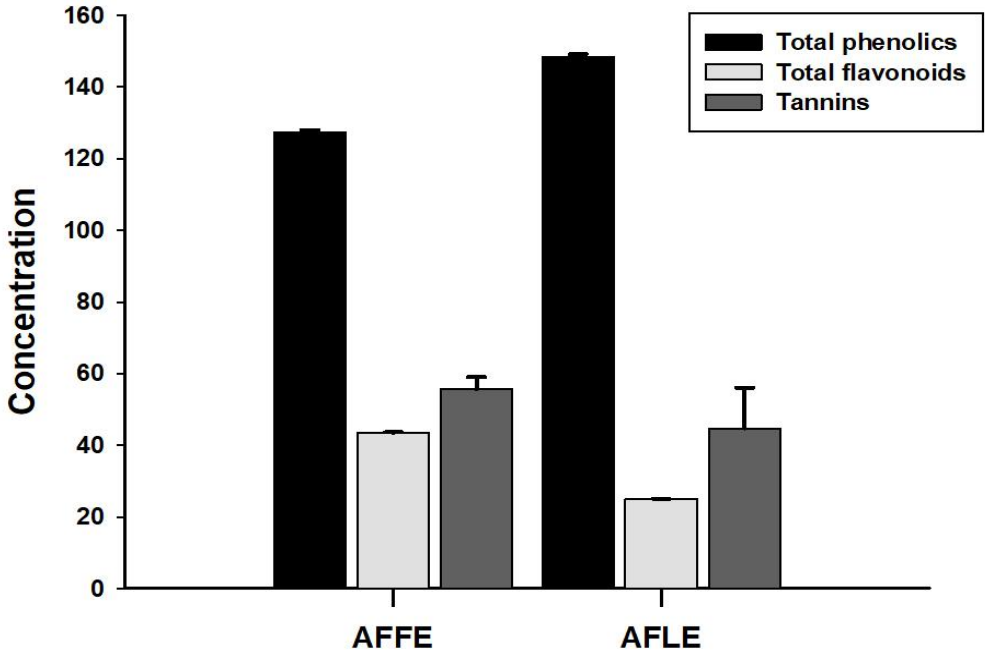

Figure 3. The concentration of phenolics (mg GAE/g DW), flavonoids (mg QE/g DW) ,and tannins (mg TAE/g DW) in AFFE and AFLE

Table 3. Radical scavenging activity (%) of flower extract (AFFE) and leaves extract (AFLE) for the *A. fragrantissima* plant

Concentrations (µg/mL)	DPPH Radical Scavenging Activity (%)	
	AFFE	AFLE
31.25	22.5±0.96	33.1 ± 1.1
62.5	44.7±0.8	47.4 ± 0.6
125	63.5±0.7	65.7 ± 0.5
250	80.98±0.5	80.5 ± 0.4
500	83.95±0.3	82.2 ± 0.1
1000	76.3± 0.7	79.01 ± 0.4

Values are means ± SEM, n = 3 per treatment group

AFFE, AFLE, and some materials *in vitro* were assessed on process of the sporulation for *E. papillata* oocyst and identified sporulation of oocyst (%) and inhibition of sporulation (%) at different intervals of 72 and 96 hr. Considerable level of oocysts sporulation (%) in dist. H_2O was observed to be 79.78% at 96 hr. At 96 hr, AFFE showed a high level of inhibition of sporulation (100%, 100%, 98.4%, 92.2%) at concentrations of 300, 200, 100, and 50 mg/mL, respectively, compared to AFLE, was 98.4%, 96.3%, 94.1% and 67.04%, respectively (Table 4). While, amprolium, Dettol™, phenol, and formalin 5% showed different inhibition of sporulation at 96 hr which were 37.33%, 81.33, 89.33, and 100%, respectively (Table 4).

Both AFFE and AFLE were observed to have anthelmintic activity against *E. fetida*. Where the most effective dose, AFFE (100 mg/mL) showed the time to paralysis and death was 13.67±1.96 and 15.25±2.48 min, respectively, while AFLE (100mg/mL) showed the time to paralysis and death was 4.19±0.05 and 4.70±0.28 min, respectively. Mebendazole showed 13.91±0.37 and 18.20±0.98 min for paralysis and death time, respectively) (Table 5). There are no changes in the uppermost layer of the cuticle for worms in the control group, while reduction in the segment length for worms in plant extracts as well as complete destruction of the upper layer with drug treatment (Figure 4).

Table 4. *In vitro*, anti-coccidial effects of *Achillea fragarntissima* flowers (AFFE) and leaves (AFLE) extract on the sporulation percentage of *Eimeria papillata* oocysts

Groups	Time	Sporulation of oocyst (%)	Inhibition of sporulation (%)
Distilled H_2O	72 hr	66.6 %	24.2 %
	96 hr	79.78 %	15.7 %
Potassium dichromate (2.5%)	72 hr	88.03 %	0 %
	96 hr	94.67 %	0 %
AFFE (300 mg/mL)	72 hr	0 %	100 %
	96 hr	0 %	100 %
AFFE (200 mg/mL)	72 hr	0 %	100 %
	96 hr	0 %	100 %
AFFE (100 mg/mL)	72 hr	0 %	100 %
	96 hr	1.52 %	98.4 %
AFFE (50 mg/mL)	72 hr	0 %	100 %
	96 hr	7.4 %	92.2 %
AFLE (300 mg/mL)	72 hr	0 %	100 %
	96 hr	1.5 %	98.4 %
AFLE (200 mg/mL)	72 hr	0 %	100 %
	96 hr	3.5 %	96.3 %
AFLE (100 mg/mL)	72 hr	0 %	100 %
	96 hr	5.6 %	94.1 %
AFLE (50 mg/mL)	72 hr	5.5 %	93.7 %
	96 hr	31.2 %	67.04 %
Amprolium	72 hr	65.39 %	34.61 %
	96 hr	62.67 %	37.33 %
Dettol™	72 hr	23.08 %	76.92 %
	96 hr	18.67 %	81.33 %
Phenol	72 hr	7 %	92.30 %
	96 hr	10.67 %	89.33 %
Formalin	72 hr	0 %	100 %
	96 hr	0 %	100 %

Table 5. *In vitro* anthelmintic activity of AFFE and AFLE

Test samples	Concentration (mg/ml)	Time taken for paralysis (min.)	Time taken for death (min.)
Control (H_2O)	--	--	--
AFFE	25 mg/mL	$6.24 \pm 0.06^{*\#}$	$6.70 \pm 0.28^{*\#}$
	50 mg/mL	$5.44 \pm 0.17^{*\#}$	$6.08 \pm 0.05^{*\#}$
	100 mg/mL	$4.19 \pm 0.05^{*\#}$	$4.70 \pm 0.28^{*\#}$
AFLE	25 mg/mL	$50.31 \pm 9.15^{*\#}$	$54.85 \pm 8.10^{*\#}$
	50 mg/mL	$16.92 \pm 6.04^{*}$	$18.19 \pm 6.00^{*}$
	100 mg/mL	$13.67 \pm 1.96^{*}$	$15.25 \pm 2.48^{*\#}$
Mebendazole	10 mg/mL	$13.91 \pm 0.37^{*}$	$18.20 \pm 0.980^{*}$

Values are mean ± SD. All superscripts indicate significance at $p \leq 0.05$, * compared to untreated (H2O), # compared to mebendazole.

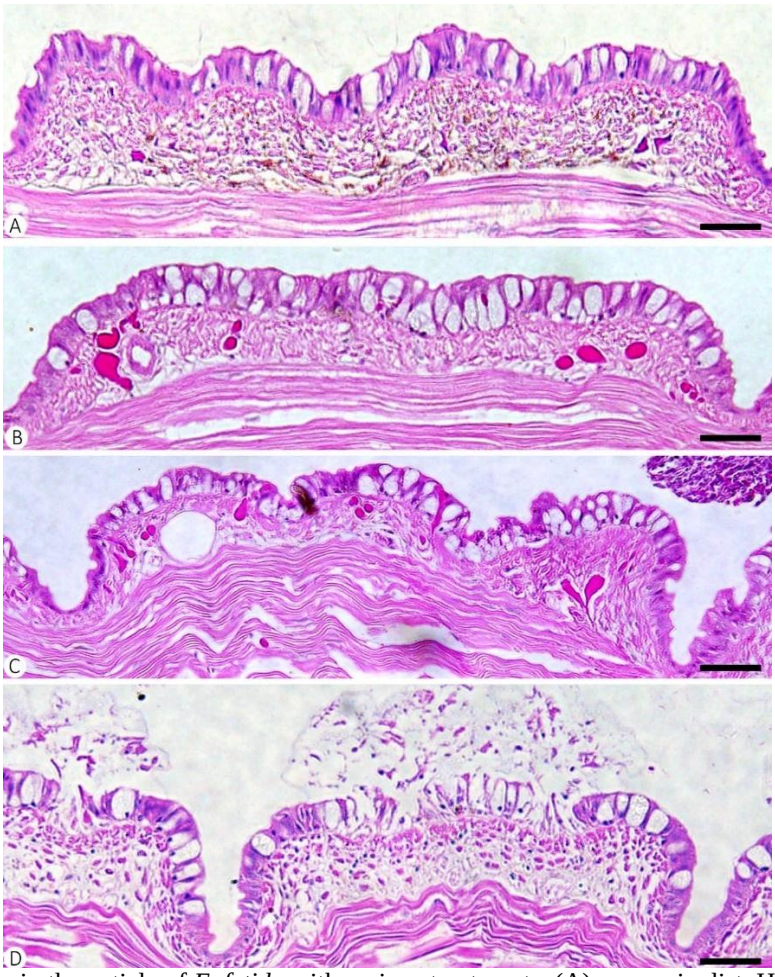

Figure 4. Changes in the cuticle of *E. fetida* with various treatments. (A) worms in dist. H_2O (control). (B) worms in AFLE (100 mg/ml). (C) worms in AFEE (100 mg/ml). (D) worms in mebendazole. Scale bar = 25µm

DISCUSSION

Eimeria species cause severe infections in animals (coccidiosis), especially in chickens and cattle (Morgoglione *et al*., 2020). Anticoccidial medications when overused resulted in the development of drug resistance as well as the buildup of their byproducts in tissues and organs (Elmahallawy *et al*., 2021; Jamil *et al*., 2022). The development of treatment resistance in various harmful parasites and microbes results in enormous economic losses because there are few effective drugs available and the cost is high (Jamil *et al*., 2022). This will impose discovery of novel drug sources to overcome therapeutic failure. Medicinal herbs, Due to their well-known antibacterial and antiparasitic characteristics, they can be a successful treatment for infections (Cobaxin-Cardenas, 2018). Natural herbs' therapeutic value rests on their bioactive components, which come from crude plants (Jamil *et al*., 2022). The aim of this study (*in vitro*) was to investigate the protective effect of different concentrations of AFFE and AFLE against oocyst sporulation (*Eimeria papillata*), as well as its anthelminthic activity.

Our results, different concentrations of AFFE and AFLE showed high anthelmintic activity against earthworms in comparison to mebendazole, which agreed with result Aboutable *et al*. (1986) and Sincich (2002), which is attributed to the presence of many active phytochemical components. According to prior studies, Khaled *et al*. (2010), Elmann *et al*. (2011), and El-Ashmawy *et al*. (2016), the quantitative phytochemical analysis of the methanolic extract

of *A. fragrantissima* was revealed a high number of flavonoids, polyphenols, and tannins, which agreed with our findings. Flavonoids clearly have a part in illness prevention, whether directly or indirectly. Ferreira *et al*. (2010) showed that flavonoids have an impact on cancer, cardiovascular disease, and parasitic diseases like malaria. Tariq *et al*. (2008) reported that extract of *Achillea millifolium* showed anthelminthic activity as it inhibited the movement and led to death of *Haemonchus contortus* worm, which infect the gastrointestinal tract of sheep. Also, the extract of *A. wilhelmsii* showed anthelminthic activity against *Pheretima posthuma* and *Raillietina spiralis*, due to plant's containment of crude saponins (Ali *et al*., 2011). In addition, the extract of *A. millefolium* L. (flowers) showed nematicidal activity against *S. papillosus* larvae (Buza *et al*., 2020). In a related study, *A. fragrantissima* showed the acaricidal efficacy against the common camel tick *Hyalomma dromederi* in Saudi Arabia (Al-Harbi *et al*., 2015). Also, the essential oil of the plant showed considerable acaricidal activity against *Tyrophagus putrescentiae* (Al-Assiuty *et al*., 2019). Moreover, *A. fragrantissima* possesses anti-trypanosomiasis activity, due to the fact that the plant is rich in flavonoids and tannins (El-Ashmawy *et al*., 2016).

In addition, our results showed that AFFE and AFLE had a significant effect on the sporulation for *E. papillata* oocysts with dose dependence. Likewise, the methanolic extract for *A. fragrantissima* led significantly inhibited the growth of *Blastocystis in vitro*, and changes in *Blastocystis* shape under the influence of the extract were observed, with complete demolition of *Blastocystis* forms (after 72 hr) (Mokhtar *et al*., 2019). At least in part, the observed activity of *A. fragrantissima* extract against sporulation process of oocyst *E. papillata* can be explained by presence of several bioactive phytochemical components. In contrast, Dettol and phenol had few effects on oocyst sporulation, and these findings could be attributed to the oocyte wall's impermeability to water-soluble substances and its resistance to proteolysis (Kuticic and Wikerhauser, 1996; Mai *et al*., 2009; Abu Hawsah *et al*., 2023). Hazardous chemical formalin (5%) completely prevented sporulation process. Previous studies showed that different concentrations of formalin (2% and 10%) influence sporulation process (Chroustová and Pinka, 1987; Gadelhaq *et al*., 2018; Thagfan *et al*., 2020).

CONCLUSION

It could be concluded that AFFE and AFLE have anticoccidial and anthelmintic efficacy, *in vitro*. Further studies should be recommended to include *in vivo* effectiveness of AF.

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