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Evaluation of anthelminthic and antiprotozoal activity of myrrh (*Commiphora myrrha*) methanolic extract

S. ALBASYOUNI[1], S. AL-QURAISHY[1], N. AL-HOSHANI[2], T. AL-OTAIBI[3], E. M. AL-SHAEBI[1], R. ABDEL-GABER[1,*]

[1]Department of Zoology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia, *E-mail: *rabdelgaber@ksu.edu.sa*; [2]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; [3]Department of Science and Technology, Al-Nairiyah University College, University of Hafr Al-Batin, Hafr Al-Batin 31991, Saudi Arabia

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Summary

To treat and control parasitic infections, traditional medical remedies using plant products are utilized as antiparasitic agents rather than standard synthetic chemicals due to drug resistance. Myrrh, a resinous exudate of *Commiphora myrrha* (Burseraceae), is a powerful antioxidant with a variety of medicinal uses. This study aimed to investigate the effect of the myrrh methanolic extract (MyE) of three concentrations (100, 50, and 25 mg/ml) on the sporulation of oocysts and as an anthelminthic effector via *in vitro* study. Characterization of the plant was done by Fourier-transform infrared spectroscopy (FT-IR). The earthworm, *Eisenia fetida*, is used as a model worm to evaluate the anthelminthic activity of MyE. *Eimeria labbeana*-like oocysts are used as a model protozoan parasite in anticoccidial assays. The sporulation and inhibition (%) of *E. labbeana*-like were assessed by MyE compared to other chemical substances. FT-IR revealed the presence of twelve active compounds. Our results showed that paralysis and death of earthworms at MyE (100 mg/ml) were 7.88 ± 0.37 and 9.24 ± 0.60 min, respectively, which is more potency when compared to mebendazole (reference drug). In all treated worms, microscopic examinations revealed obvious surface architecture abnormality. This study shows that MyE affects oocysts sporulation in a dose-dependent manner. At 24 and 36 hr, a high concentration of MyE (100 mg/ml) inhibits sporulation by 90.95 and 87.17 %. At 36 hr, other concentrations of MyE (50 and 25 mg/ml), as well as amprolium, Dettol™, and phenol inhibits oocyst sporulation by 40.17 %, 29.34 %, 45.09 %, 85.11 %, and 61.58 %, respectively. According to our research, the MyE extract had powerful anthelmintic and anticoccidial properties.
Keywords: Parasitic infections; *Eimeria* species; *Eisenia fetida*; Standard drugs; Medicinal plants

Introduction

Parasitic infections caused by protozoans and helminths induce considerable health problems in various animal species (Mehlhorn, 2014). Helminth infections are among the most common form of gastrointestinal parasites in birds that leads to economic losses (Newbold *et al*., 2017; Al-Quraishy *et al*., 2020). Weakness is considered a major complaint of helminth infections resulting from malnutrition, anemia, and eosinophilia (Jones & Berkley, 2014). Anthelminthic drugs are used for expelling parasitic worms from the body, however, they induce side effects, especially for host tissue (Hong, 2018). Coccidiosis is a protozoal disease caused by *Eimeria* species (Kommu *et al*., 2016). *Eimeria labbeana*-like is a coccidian parasite that was first reported in domesticated pigeons (Yang *et al*., 2016). Infection of pigeons with coccidian parasites causes changes in physical appearance (Sood *et al*., 2018). Coc-

* – corresponding author

cidiosis is mostly treated with synthetic anticoccidial drugs, but this approach is facing a serious threat of the development of resistance in *Eimeria* strains (Grandi *et al*., 2016).

To control coccidiosis and helminthiasis in various animal species, different alternative options and protocols were effectively used worldwide (Liaqat *et al*., 2016). The use of herbs as medicine is becoming increasingly common, either as home remedies or as complementary and alternative medicines (Satyavati, 1990). Plant therapy is frequently considered to be less toxic with the least side effects than synthetic ones. The plant-derived medicines are based on the premise that they contain natural substances that can promote health and alleviate disease status (Swayamjot *et al*., 2005).

Myrrh, as traditional natural medicine, is an aromatic gum resin, which was the plant stem resinous exudate of *Commiphora myrrha* which belongs to the family Burseraceae (Alyafei, 2020). Myrrh is widely used as a home remedy in Saudi Arabia (Al-Faris *et al*., 2008; Bakhotmah & Alzahrani, 2010). Chemical compounds present in myrrh resins include triterpenoids, diterpenoids, steroids, and lignans (Hanus *et al*., 2005; Tonkal & Morsy, 2008). Myrrh has been shown to have a wide variety of therapeutic uses as an antimicrobial (Romero *et al*., 2005; Rahman & Gibbons, 2007; Alzahrani *et al*., 2011; Kuete *et al*., 2012), anticancer (Shoemaker *et al*., 2005), anti-inflammatory (Qureshi *et al*., 1993; Akbar, 2020), lipid-lowering (Omer & Al-Dogmi, 2018), antirheumatic (Su *et al*., 2015), antioxidant (El-Ashmawy *et al*., 2006; Ashry *et al*., 2010), and hypotensive (Abdul-Ghani & Amin, 1997). It is also known to stimulate uterine tone and promote uterine blood flow (Michie & Cooper, 1991). Moreover, it is believed to be effective in treating various parasites including *Eimeria stiedae* (Baghdadi & Almathal, 2010), *Trichinella spiralis* (Basyoni & El-Sabaa, 2013; Attia *et al*., 2015; Abd-Elrahman *et al*., 2020; Abuelenain *et al*., 2021, 2022), *Fasciola gigantica* (Massoud *et al*., 2013), and *Schistosoma mansoni* (Osman *et al*., 2010).

This study aims to evaluate the potential role of *Commiphora myrrha* extract as an anthelminthic and anticoccidial effector against *E. labbeana*-like.

Materials and Methods

Plant material and preparation of extract

Myrrh resin (*Commiphora myrrha*) was purchased from a local market in Riyadh, Saudi Arabia. The taxonomic identification was carried out with the help of a taxonomist at the Herbarium of Botany and Microbiology Department (College of Science, King Saud University, Saudi Arabia).

Myrrh resin was crushed in an electric blender to obtain coarse powder. About 100 g of coarse powder was extracted by maceration with 1000 ml of 70 % methanol (MeOH) as solvent. The mixture was removed continuously and stirred in the dark at 4°C for 24 hr. Then it was centrifuged at 5000 rpm for 15 min. The supernatant was filtrated and concentrated using a Büchi® rotary evaporator (Model R-200) at low temperature (40-50°C) to obtain the crude extract, and then transferred to -20°C for further use.

Fourier-transform infrared spectroscopy (FT-IR)

For myrrh extract (MyE) analysis, a Nicolet 6700 Fourier-transform infrared spectroscopy (FT-IR) optical spectrometer from Thermo Scientific (Waltham, USA) was used. The powder of the extract (10 mg) was mixed with 100 mg of KBr pellet to obtain a translucent sample disk that we then loaded into an FT-IR spectroscope at ambient temperature with a spectra band range of 400 – 4000 cm^{-1} with a resolution of 4 cm^{-1}. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectra (Pakkirisamy *et al*., 2017).

Anthelmintic activity of myrrh extract (MyE)

The adult earthworms (*Eisenia fetida*) were used for the anthelmintic activity of myrrh extract. All worms were washed with distilled H_2O and acclimatized at an ambient temperature 30 min

Table 1. Infrared (IR) spectrum of myrrh methanolic extract by frequency range.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound class
3423.13	strong, broad	3.026618	O-H stretching	alcohol
2969.17	strong, broad	7.255632	N-H stretching	amine salt
2932.49	strong, broad	6.570219	N-H stretching	amine salt
1739.07	strong	3.770522	C+O stretching	esters
1614.29	strong	6.823951	C=C stretching	α,β-unsaturated ketone
1438.45	medium	7.900225	O-H bending	carboxylic acid
1381.21	strong	6.630713	S=O stretching	sulfonyl chloride
1245.04	medium	7.112334	C-N stretching	amine
1039.34	strong	5.623318	S=O stretching	sulfoxide
766.62	strong	15.41832	C-Cl stretching	halo compound
729.36	strong	16.05396	C=C bending	alkene
597.88	strong	13.81419	C-I stretching	halo compound

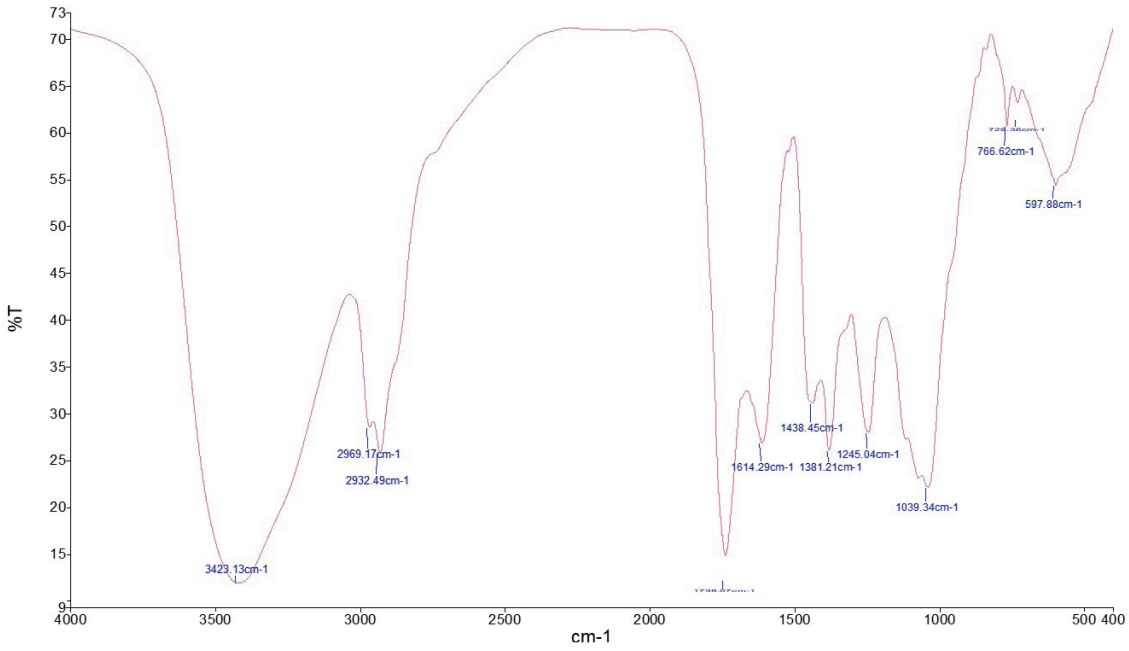

Fig. 1. FT-IR spectrum of myrrh (*Commiphora myrrha*) methanolic extract.

before the experiment. *E. fetida* worms were identified by a specialist in the College of Food and Agriculture Sciences (King Saud University). The experiment is carried out on *E. fetida* because they possess anatomical resemblance to intestinal roundworm parasites of human beings. Test samples of the extract were prepared at different concentrations including 25, 50, and 100 mg/ml. Mebendazole (Saudi Pharmaceutical Industries, Riyadh, Saudi Arabia) and distilled H_2O were used as a control. The earthworms were divided into five groups, each group consisted of 5 earthworms approximately of equal size (7 cm). The earthworms were placed in Petri dishes containing the different concentrations of extract solution as well as the standard drug and distilled H_2O. The chronological group arrangements are given as follows:

Group-1: Received distilled H_2O which served as the control.
Group-2: Received mebendazole suspension at a dose of 10 mg/ml which served as the standard.
Group-3: Received methanolic extract at a dose of 25 mg/ml.
Group-4: Received methanolic extract at a dose of 50 mg/ml.
Group-5: Received methanolic extract at a dose of 100 mg/ml.

Earthworms were kept under close observation, and the paralysis and death time for individual worms were recorded. Paralysis (movement was absent) was recorded (in minutes), except when

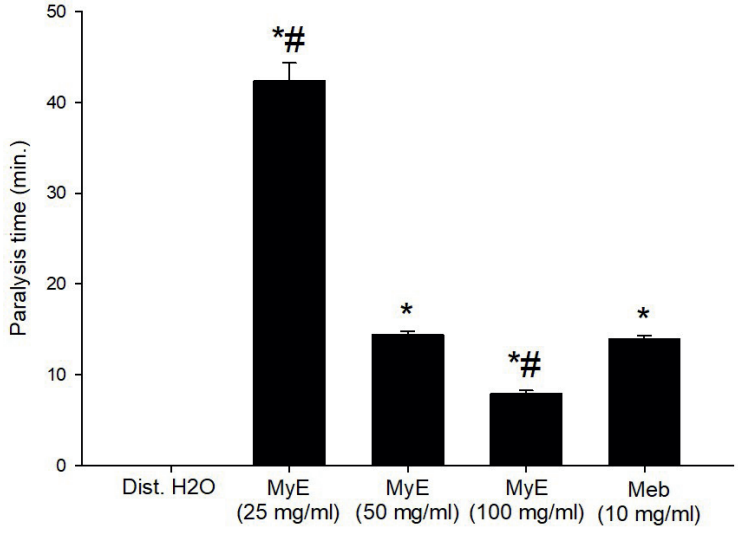

Fig. 2. Time taken for paralysis of the earthworms, *E. fetida*, in various treatments. * Significance change with respect to those treated with dist. H_2O, # Significance change with respect to those treated with mebendazole.

the worm was shaken vigorously, while the death of worms was recorded (in minutes) when the worms neither moved nor shaken when dipped in warm water (50°C) followed by the fading of the body colors (Parida *et al*., 2010).

Histological examinations

The treated and control worms were prepared for histological study immediately after the paralysis and death experiment, according to Drury and Wallington (1973). Briefly, specimens were fixed in formalin (10 %) for 24 hr, then dehydrated by graded ethanol series and embedded in paraffin. Tissues were then cut into thin sections using a microtome, stained with hematoxylin and eosin (H&E), and examined and photography using an Olympus B×61 microscope (Tokyo, Japan).

Scanning electron microscopic (SEM) study

Worms were fixed in 3 % buffered glutaraldehyde at 4°C for 2 h, then dehydrated with ascending grades of acetone, air-dried in tetramethylsilane (TMS), and mounted on metal stubs and coated with gold-palladium. Specimens were examined and photographed in Jeol JSM-6060LV at an accelerating voltage of 15 kV.

Anticoccidial activity of MyE

A coccidial avian parasite model was *Eimeria labbeana*-like. Five domesticated pigeons received 3×10^4 sporulated *E. labbeana*-like oocysts via oral gavage. On the 8th day following infection, feces were collected, and oocysts were then separated using the flotation method and employed in an *in vitro* study. The *in vitro* oocyst sporulation was carried out in small Petri dishes, as follows:

Plate dish-1: Received 5 ml 2.5 % $K_2Cr_2O_7$ (control)

Plate dish-2: Received methanolic extract at a dose of 25 mg/ml dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-3: Received methanolic extract at a dose of 50 mg/ml dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-4: Received methanolic extract at a dose of 100 mg/ml dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-5: Received 8.3 mg/ml amprolium dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-6: Received 109 µl Dettol ™ dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-7: Received 25 µl phenol dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Plate dish-8: Received 5 % formalin dissolved in 5 ml 2.5 % $K_2Cr_2O_7$

Each petri dish contained 1×10^4 unsporulated *E. labbeana*-like oocysts, which were incubated at 25 ºC for 24 and 36 hr. Sporocysts were examined under an Olympus compound microscope (Olympus Co., Tokyo, Japan) to track the oocysts' sporulation. Sporulation and inhibition (%) were calculated according to Thagfan *et al*. (2020).

Statistical analysis

Data were analyzed using SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA). All values were expressed as mean ± SD, at a significant level of *p*-value ≤ 0.05.

Ethical Approval and/or Informed Consent

This research was approved by the Research Ethics Committee (REC) at King Saud University (approval number KSU-SU-23-45).

Results

FT-IR of MyE showed major bands for the twelve compounds at 3423 cm^{-1}, 2969.17 cm^{-1}, 2932.49 cm^{-1}, 1739.07 cm^{-1}, 1614.29 cm^{-1}, 1438.45 cm^{-1}, 1381.21 cm^{-1}, 1245.04 cm^{-1}, 1039.34 cm^{-1}, 766.62 cm^{-1},

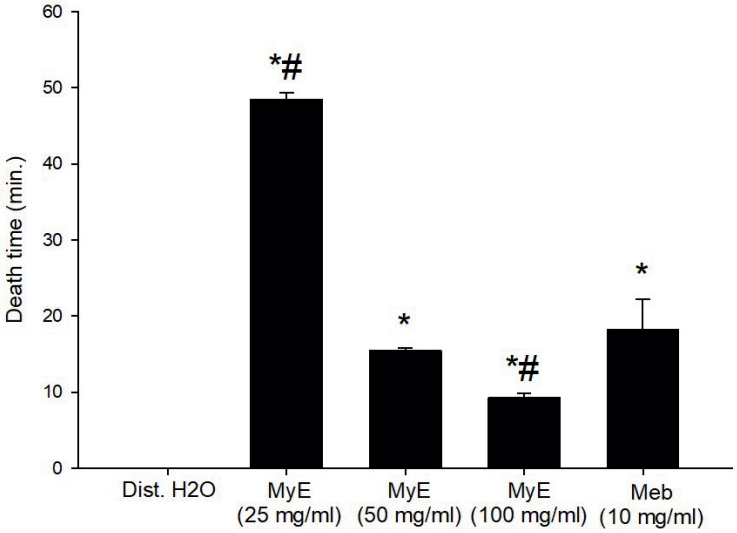

Fig. 3. Time taken for Death of the earthworms, *E. fetida*, in various treatments. * Significance change with respect to those treated with dist. H_2O, # Significance change with respect to those treated with mebendazole.

729.36 cm^{-1}, and 597.88 cm^{-1} (Fig. 1 and Table 1). O-H stretching was indicated by the band at 3423 cm^{-1} confirming the presence of an alcohol. The bands at 2969.17 and 2932.49 cm^{-1} implied N-H stretching for the presence of amine salt. C-O stretching at 1739.07 cm^{-1} confirms the presence of esters. The band at 1614.29 cm^{-1} corresponds to C=C stretching for the presence of the α,β-unsaturated ketone. The band 1438.45 cm^{-1} (O-H bending), 1381.21 cm^{-1} (S=O stretching), 1245.04 cm^{-1} (C-N stretching), 1039.34 cm^{-1} (S=O stretching), 766.62 cm^{-1} (C-Cl stretching), 729.36 cm^{-1} (C=C bending), and 597.88 cm^{-1} (C-I stretching) assigned to a carboxylic acid, sulfonyl chloride, amine, sulfoxide, halo compound, and alkene, respectively (Table 1).

MyE displays a relatively comparable anthelmintic activity with reference standard mebendazole against the adult *E. fetida* worms. Paralysis and death time of the worms were recorded, and the obtained results are shown in Figures 2 and 3. This experiment was carried out for up to 48 minutes. There was no paralysis recorded in the dist. H_2O (control group). From the observations achieved, a higher concentration of MyE (100 mg/ml) showed a paralytic effect much earlier (7.88 ± 0.37 min) and the time to death was shorter

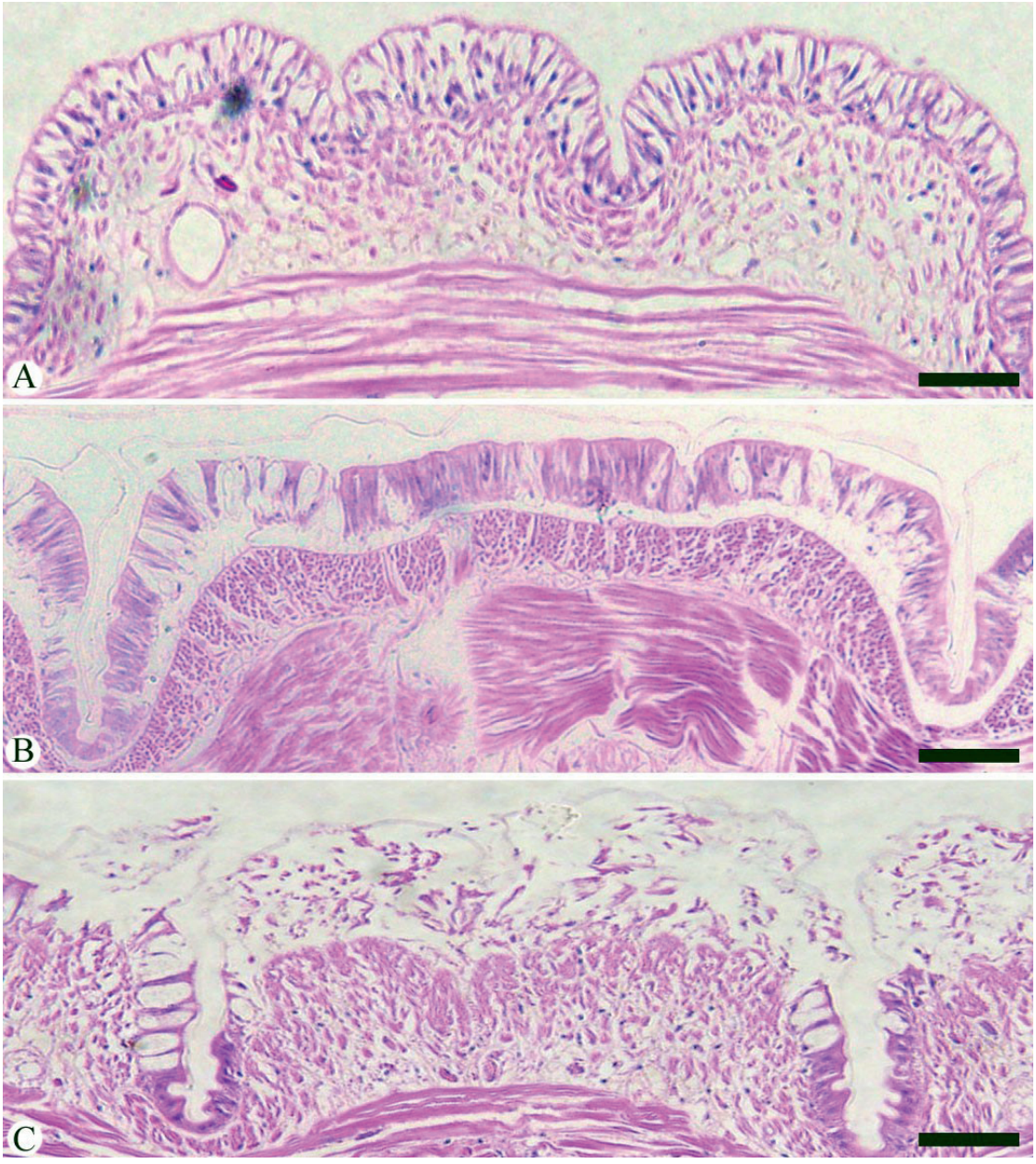

Fig. 4. Cuticle thickness of *E. fetida* with various treatments. (A) earthworms in dist. H_2O. (B) earthworms in MyE (100 mg/ml). (C) earthworms in the reference drugs of mebendazole (10 mg/ml). (Scale bar = 25 µm).

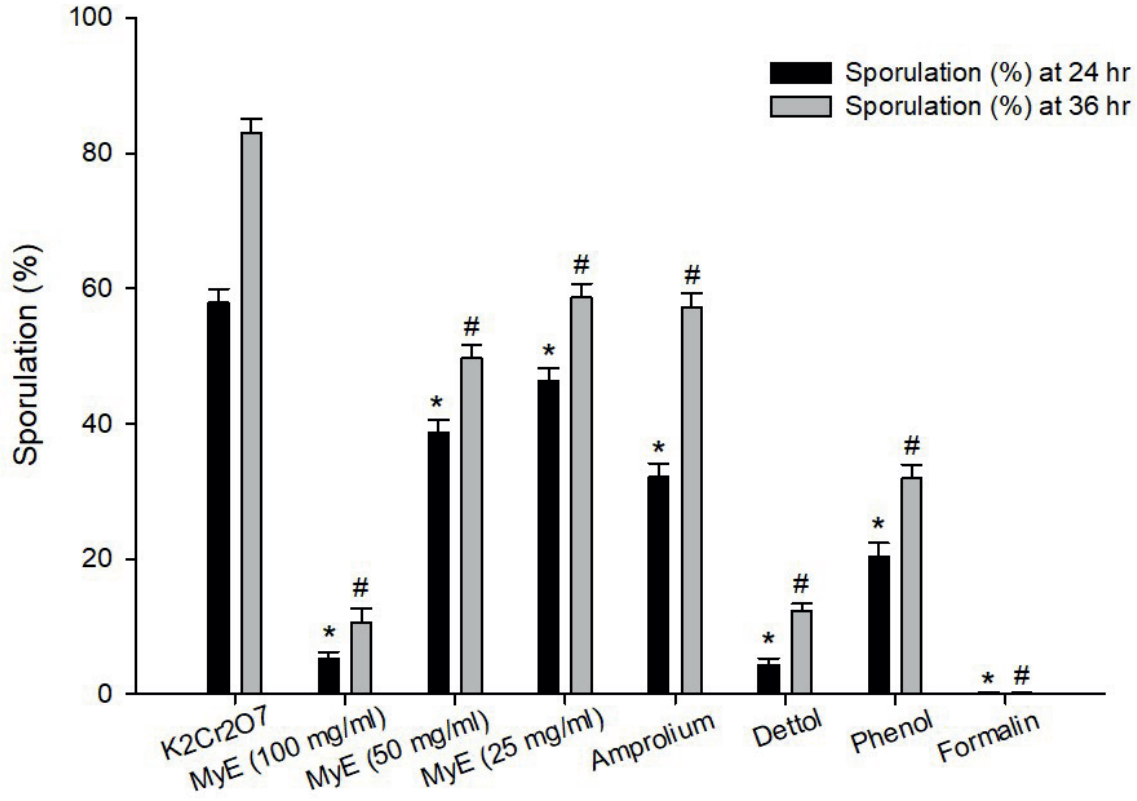

Fig. 5. Sporulation percentage at 24 and 36 hrs for different treatments. * Significance change at 24 hr with respect to those treated with $K_2Cr_2O_7$, # Significance change at 36 hr with respect to those treated with $K_2Cr_2O_7$.

(9.24 ± 0.60 min) for almost all the worms. Mebendazole at 10 mg/ml showed paralysis and death times after 13.91 ± 0.37 and 18.20 ± 3.98 min, respectively. The other MyE concentrations showed a marked degree of anthelmintic activity.

Microscopic examination revealed uniform normal body architecture for *E. fetida* worms in water (Fig. 4, Supplementary Fig. 1). On the other hand, all *E. fetida* worms exposed to MyE had alterations in the topography including a decrease in the length of body segments accompanied by cuticular thickness (Fig. 4, Supplementary Fig. 1). All *E. fetida* worms treated with mebendazole showed observable destruction of the cuticle layer (Fig. 4, Supplementary Fig. 1).

Oocyst incubation with $K_2Cr_2O_7$ (2.5 %), MyE (100, 50, and 25 mg/ml), amprolium, phenol, and Dettol™ showed different levels of sporulation (Fig. 5). The lowest rate of sporulation recorded for the higher concentration (100 mg/ml) of MyE is 5.23 % (at 24 hr) and 10.65 (at 36 hr). After incubation with formalin, the unsporulated *E. labbeana*-like oocysts showed no rate of sporulation. Incubation with MyE (100 mg/ml) for 24 and 36 hr inhibited oocysts sporulation by 90.95 and 87.17 %, respectively. MyE (50 and 25 mg/ml), amprolium, Dettol™, and phenol induced variable inhibition levels at 36 hr of 40.17 %, 29.34 %, 45.09 %, 85.11 %, and 61.58 %, respectively (Fig. 6).

Discussion

In our environment, there are different pathogens affecting various animal species causing parasitic diseases (including coccidiosis and helminthiasis) that lead to severe economic losses. Many therapeutic agents are available to control and management of these diseases, but these agents are also now adopting serious side effects and development of resistance and therefore, are no more effective in the management of infections (Chartier *et al*., 2001). These factors paved the way for herbal remedies as alternative agents (Coles, 1997). This study aimed to evaluate the effectiveness of one of the most famous herbal remedies in Saudi Arabia, myrrh, as an anthelmintic and anti-coccidial effector.

Anthelmintic treatments are known to act by causing irritation resulting in restriction of movement and further leading to paralysis and/or death of worms (Mackenstedt *et al*., 1993; Kopp *et al*., 2008; Lalthanpuii & Lalchandama, 2020). In this study, in earthworms *E. fetida*, the methanolic extract of MyE showed anthelmintic activity in a dose-dependent manner. The activity of MyE at 100 mg/ml was found to be inversely proportional to the time taken for paralysis/death of the earthworms. This result agreed with previous studies which reported that the presence of many classes of phytoconstituents of myrrh, especially terpenoids, propose an endogenous action via interaction with the polysaccharides of the worm cuticle

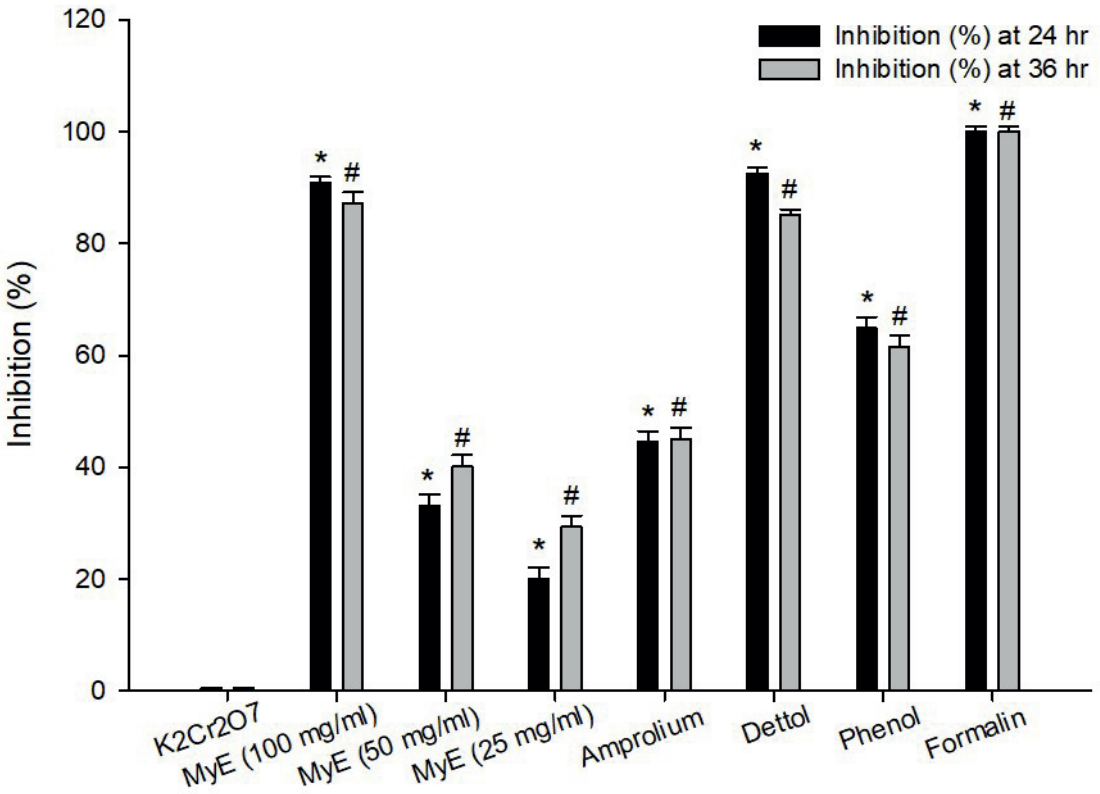

Fig. 6. Inhibition percentage at 24 and 36 hrs for different treatments. * Significance change at 24 hr with respect to those treated with $K_2Cr_2O_7$, # Significance change at 36 hr with respect to those treated with $K_2Cr_2O_7$.

and leads to paralysis and death in the worm (Hanus *et al*., 2005; Tonkal & Morsy, 2008). Borgesa and de Borgesa (2016) showed that terpenoids have antiparasitic properties since they disrupt the fluidity and permeability of the membrane of the parasite.

Histopathology has validated the *in vitro* study and examined the topographical effects of MyE in comparison to the standard drug on the worms to assess anthelmintic activity. The cuticular surface of the worms treated with MyE showed extraordinary modifications, including significant shrinking. This agreed with Abu Hawsah *et al*. (2023) who described how anthelmintic treatments caused modifications to the worm's body surfaces. However, mebendazole has been shown to affect worms by destroying the cytoskeletal structure of the worm thereby causing paralysis, which agreed with the previous study of Kern (2003) that mebendazole is known to block microtubule functions of parasites through inhibition of polymerization of β-tubulin and glucose uptake which eventually lead parasites to be in shortage of glycogen. Wang (2010) stated also that therapeutic drugs have been reported to affect the permeability of the cell membrane of worms, causing vacuolization and disintegration of the upmost layer.

Coccidiostats are a group of analogs to thiamine (vitamin B1) that act by inhibiting the uptake of thiamine which is required for many essential metabolic reactions for the *Eimeria* parasites (Kart & Bilgili, 2008). The excessive use and misuse of these drugs have led to the emergence of drug-resistant strains of *Eimeria* species (Noack *et al*., 2019). In this study, amprolium has been reported to inhibit sporulation at 36 hr by 45.09 %. As a result, developing new drugs from medicinal plants is a potentially sustainable alternative to conventional chemical agents. In this study, MyE exhibited anticoccidial effect in the *in vitro* experiment by inhibiting sporulation of *E. labbeana*-like oocysts in a dose-dependent manner, which is attributable to phytoconstituents studied by Mohamed *et al*. (2014), Ahamad *et al*. (2017), Alasady *et al*. (2021), Koriem (2022) which interrupt the metabolism of *Eimeria* parasites. This finding agreed with the data presented by Baghdadi and Almathal (2010) and Massoud *et al*. (2010) for the efficacy of myrrh in controlling coccidiosis. In this study, phenol and Dettol™ have been shown significant degrees of sporulation inhibition at 36 hr reached to 85.11 %, and 61.58 %, respectively. These compounds act as chemical substances that elevate the impermeability of the oocyst wall to water-soluble substances and become more resistant to proteolysis, which agreed with Gadelhaq *et al*. (2018), Thagfan *et al*. (2020), and Al-Otaibi *et al*. (2023). Moreover, 5 % formalin showed 100 % sporulation inhibition of *E. labbeana*-like oocysts, which agreed with Kasem *et al*. (2019), Felici *et al*. (2021), and Abu Hawsah *et al*. (2023) reported that this highly reactive chemical interacts with proteins of the oocyst wall *in vitro* and inhibits oocysts sporulation.

Conclusion

The findings of this study have shown promising anthelmintic and anticoccidial activities suggesting the possible use of myrrh in intestinal parasite control. Future studies are needed to know the mechanism of myrrh's action on both parasite and the host tissues, as well as further fractionation of the herb to many molecules and to select the most potent one for the antiparasitic effect.

Conflict of Interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

Acknowledgments

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Data Availability Statement

All the datasets generated or analyzed during this study are included in this published article.

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