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EVALUATION OF OLIBANUM EXTRACT ACTIVITY AGAINST *TRICHINELLA SPIRALIS* ADULTS AND LARVAE: *IN VITRO* STUDY.

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Abstract

Trichinellosis is a globally re-emerging parasitic disease. The available current treatments are more or less not effective especially for larvae with many drawbacks. Consequently, appropriate natural treatment is needed. The present study evaluated the *in-vitro* anthelminthic effects of *Olibanum* (OL) extract against *Trichinella spiralis* adults and larvae. Adults and muscle larvae were incubated with OL extract at concentrations of 10, 25, 50, 100, & 150µg/ml on culture medium. The histopathological changes and mortality both stages were assessed by using scanning electron microscopy (SEM). The OL extract showed significant anthelminthic activity against adult and larval stages. The maximum and the earliest inhibitory effect were with 150µg/ml (100% mortality at 36hr incubation) for both stages. However, a concentration of 100µg/ml showed prolonged incubation time (48hr). Difference between both concentrations was significant ($P \leq 0.5$). Besides, *T. spiralis* adults and larvae incubated with OL extract at concentrations of 100 &150µg/ml exhibited various histopathological changes in the form of cracks, blebs and areas of degenerative changes with loss of normal annulations.

Key words: *T. spiralis*, Olibanum, *In-vitro*, Anthelminthic, Scanning electron microscopy.

Introduction

Trichinella spiralis, a zoonotic nematode can infect more than 150 mammalian species worldwide distribution (Yang *et al*, 2019). Trichinellosis is a resurgent disease that became a public health concern with recent reported epidemics in 55 countries (Barruet *et al*, 2020; Rózycki *et al*, 2022).

In Egypt, trichinosis (trichinellosis) was reported in man and animals (Morsy *et al,* 2022) as well as in rodents (Lotfy *et al,* 1999) and in the slaughtered pigs in Cairo Governmental Abattoir (Morsy *et al,* 2000). Trichinosis is foodborne disease, and human acquires infection through the ingestion of improperly cooked pork, horses or bear meat containing the active encysted larvae (Rostami *et al,* 2017). The common treatment for zoonotic trichinosis is Benzimidazole derivatives such as Thiabendazole®, Flubendazole®, Mebendazole®, and Albendazole® (Rawla and Sharma, 2022). However, the benzimidazole 2-carbamates, such as albendazole (ABZ) and mebendazole (MBZ), used for the treatment of helminthic infections, have low aqueous solubility and poor bioavailability, both lead to high inter-individual variability when used for human systemic helminthiasis, but none of them is totally effective in killing newborn larvae (NBL) and encapsulated larvae due to the limited bioavailability (Rivera *et al*, 2007), added to the emergence of the drug-resistant parasites (Abou-Shady *et al*, 2016). The development of alternative treatment by medicinal plants and herbs against helminthes was indicated (Caner *et al*, 2008). Botanicals possess a prime target for innovative therapeutic materials, as evidenced by the voluminous research on medicinal plants documented in scientific databases (Ibrahim *et al*, 2014). Aboul-Nour *et al.* (2016) in Egypt successfully used garlic, ginger and *Commiphora molmol* in treating cryptosporidiosis. Also, Kaiaty *et al.* (2021) *in-vivo* study successfully used *Punica granatum* and synthetic anthelmintics against gastrointestinal nematodes infecting cattle, sheep, goats, and buffalos. Even plant extracts were used in controlling *Biomphalaria alexandria* snails (Bakry and Hamdi, 2006) and also medicinal plants containing phenolic were used to ecological friendly in controlling the mosquito-vectors (Abdel-Hady *et al,* 2014).

The oleo-gum resin known as frankincense or *Olibanum* (OL) is made from the *Boswellia* species has a distinctive role among the remedies used in treating many diseases as gastric, dermatological, hepatic, rheumatoid arthritis and others (Al-Yasiry and Kiczorowska, 2016). It is natural, triterpenoid gum-resin obtained from *Boswellia caterii* trees native to Africa and Arabia. Olibanum, or frankincense, contains 3-8% volatiles oils (pinene, Dipentene), 56%-60% resins (mainly triterpenoids), 30%-36%gums, and 6-8% Bassorin. Olibanum is in hard yellow grains, and used in incense and perfumes (Brapdy, 1971). The resin majority is composed of a resin (olibanoresin) and a resin boswellic acid in equal proportions (Chowdary *et al*, 2006). Similar to other pentacyclic triterpenes, the chemical structure of *Boswellia* resin closely resembles that of anti-inflammatory steroids (Chevrier *et al*, 2005). The OL anti-inflammatory activity improved gut diseases by enhancing motility, inhibiting diarrhea without constipation, inhibiting intestinal muscles contraction and barium chloride-induced diarrhea (Borrelli *et al*, 2006).

Since the 11th Century, OL has been widely used treating microbial infections; particularly OL affects the urinary tract infections (Vuuren *et al*, 2010). Besides, it has antiparasitic activity against *Giardia lamblia* trophozoites (Abdalla *et al*, 2011), and antimicrobial efficacy against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Candida albicans* (Ljaljevi´c *et al*, 2018). Also, it has an anti-tumor activity by inhibiting topoisomerase I & II-alpha and stimulates apoptosis (Al-Yasiry and Kiczorowska, 2016).

Fahmy *et al*. (2020) in Egypt found that adults and muscle larvae of *T. spiralis* incubated with 100µg/ml of clove oil exhibited marked morphological changes, multiple vesicles, and blebs, sloughing of some areas of the cuticle with fissures, loss of normal annulation, and destruction of the cuticle. They suggested that the clove oil has the potential therapeutic agent and an alternative drug against *T. spiralis* adults and larvae.

The current study aimed to assess the *in-vitro* anti-helminthic efficacy of *Olibanum* species extract against *Trichinella spiralis* both adults and muscular encysted larvae.

Materials and methods

Parasite and animals: *T. spiralis* was initially recovered from infected pigs slaughtered in Cairo Abattoir and maintained by serial passage in male Swiss albino mice (6-8 weeks old and weighed 25-30g) in the animal house of Theodor Bilharz Research Institute (Giza). In accordance with Institutional and Governmental Regulations, the rodents were kept in appropriate cages and fed commercial diet. Each mouse was infected orally with 200 larvae of *T. spiralis* (Abou Rayia *et al*, 2017). To recover the adult stage, mice were sacrificed 48 hour (hr) post infection and the small intestine was removed, divided into pieces and maintained in phosphate-buffered saline (PBS) for 4hr at 37°C. At the 35th day post infection, muscular larvae were recovered from mice through digestion of the muscles in pepsin-HCL (Jiang *et al*, 2012).

Reference drug: Albendazole (ABZ) was supplied as a suspension from the Egyptian International Pharmaceutical Industries. The concentration of 20µg/ml used was prepared by dissolving albendazole tablet in PBS saline supplemented with 1% di-methylsulfoxide (DMSO) (Tomar and Preet, 2017).

Preparation of OL extract: *Olibanum* was purchased as solid, white-colored masses from the supermarket. The chemical identification was performed in accordance with Abdallah *et al*. (2011). First, OL gum was finely ground, and then 50g of the powder was dissolved in 200 mL of 70% ethanol for two days. After centrifuging the solution, the supernatant was evaporated to produce a sticky component. Subsequently, the final crude extract was recovered using a rotary evaporator (Jebelli *et al,* 2019). The extract was used in various concentrations (10, 25, 50, 100 & 150µg/ml).

In vitro experimental design: Adult worms and muscular larvae of *T. spiralis* collected

from infected mice were added to a 48-well micro-titer plate prepared with RPMI-1640 medium with antibiotics; 200U/ml penicillin and 200µg/ml streptomycin & 10% fetal calf serum). The dimethylsulfoxide (DMSO) was used to dissolve various concentrations of OL extract and diluted in RPMI-1640 medium (Abuelenain *et al*, 2022). Final OL extract concentrations were 10, 25, 50, 100, & 150µg/ml and ABZ at a concentration of 20µg/ml used against adults and muscle larvae at time periods of 1, 3, 6, 9, 12, 24, 36, & 48hr. Total wells for each concentration was calculated after each determination was made in triplicate. A microscope was used to examine the survival of *T. spiralis* stages on plates, which were incubated at 37°C & 5% CO_2 for 24hr. Parasites control was seen after incubation in RPMI-1640 media with 1% DMSO only.

Parasite viability rate was estimated using the following formula: number of viable parasites/total number of parasites ×100 (Abd-Elrahman *et al,* 2020).

The shape and mobility of adult parasites were examined to determine their viability; dead worms have a C-shaped body or a linear shape and are immobile. The muscle larvae were handled using the same procedure. After 24hr of incubation, the worm and larval samples were collected and prepared for SEM examination (Fahmy *et al*, 2020).

Scanning electron microscopic (SEM): *T. spiralis* adults and larvae were fixed in 2.5% glutaraldehyde solution and incubated at 4°C. After being post-fixed in sodium cacodylate buffer 2% (w/v) osmium tetroxide for an hour, the parasites were washed with sodium cacodylate buffer 0.1M at pH 7.2 for 5 minutes. The post-fixed specimens were dehydrated in increasing amounts of alcohol before being dried with carbon dioxide at a crucial point. Generated parasites were analyzed using scanning electron micros- copy (JEOL, JSM-5200, Japan). Electron image plates captured images (Bughdadi, 2010).

Statistical analysis: **D**ata were examined with SPSS version 22 (SPSS Inc., Chicago, IL, USA). All data sets were subjected to Shapiro–Wick tests to ensure normal distribution. This study included both quantitative and qualitative data. A P value of <0.05 was considered statistically significant.

Results

Olibanum extract revealed an inhibitory effect on viability of *T. spiralis* adult and muscular larvae. Olibanum extract decreased the viability of *T. spiralis* adults in a timely and dose-dependent manner, the maximum efficacy (100%) was achieved on 36h at concentration of 150µg/ml while at concentration of 100µg/ml; a 100% mortality rate was detected within 48hr of the incubation period. There was statistically significant difference ($P< 0.001$) in inhibitory effect in different concentrations of the OL extracts treated adults with reference to parasite contrl ones. With respect to ABZ, 100% adults' mortality occurred in 24hr incubation period at a concentration of 20µg/ml.

With regard to the inhibitory effect of OL extract on muscular larvae, a 100% mortality rate (0% viability percentage) was reached at 36hr with a concentration of 150µg/mL of OL extract. While 100µg/ml of OL extract resulted in complete reduction in viability percentage (0%) in the 48hr incubation period. Inhibitory effects of ABZ on larvae were completed (100% mortality) in a 24hr incubation period**.**

SEM didn't show degenerative changes were recorded in control worms until the end of the exposure period**.** Ultrastructure of *T. spiralis* adults cultured with different concentrations of OL extract showed whitish discoloration, multiple cracks and vesicles with loss of normal annulations of cuticle after incubation with 25 & 50µg/ml of OL extract respectively. Also, after incubation with 100µg/ml, serious changes were occurred, the cuticle was damaged with large outer masses and cavity depression**,** but the cuticle was severely destroyed and degenerated with sloughing, large bleb and erosions with completely disappeared lines and annulations of surface after incubation with 150

µg/ml OL extract.

SEM of *T. spiralis* larvae of control had normal cuticle with normal transverse creases and typical coiled appearance. But, larval treated with OL extracts cuticle showed deformities and loss of striations following incubation with 50µg/ml OL extract while larvae incubated with 100µg/ml of OL extract were flattened, with multiple small blebs and loss of the striations with opaque cuticle**.** The incubation with 150µg/ml of the OL extract caused marked destructive effects as the cuticle was disfigurement with desquamation and loss of striations.

Details were shown in tables (1 & 2) and figures (1 & 2)**.**

Table 1: *In vitro* effects of different concentrations of *Olibanum* extract on viability rates (%) of *T. spiralis* adult worms

Dose (µg/ml)	Time intervals (Mean ± SD)							
	1st hr	3rd hr	6th hr	9th hr	12th hr	24th hr	36th hr	48th hr
10	100.0±0.0	100.0±0.0	100.0± 0.0	100.0 ±0.0	100.0±0.0	82.56±5.33	73.51±10.54	63.30±10.76
25	100.0 ±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	90.11±4.08	70.31±7.62*	62.80 ± 8.45	50.55±5.44
50	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	76.26±6.11*	48.0±7.04**	29.26±3.53**	12.76 ±3.53*
100	100.0±0.0	100.0±0.0	94.23±0.86	77.60±2.50**	60.36±0.63**	36.10±3.48**	18.33±6.01**	0.0±0.0**
150	100.0±0.0	93.65±0.80**	76.81±4.99*	60.30±9.00**	41.06±7.78**	22.36±7.12**	0.0 ±0.0**	0.0±0.0**
ABZ (20)	100.0±0.0	69.36±2.41**	48.46±16.00**	35.40±6.70**	18.43±0.0**	0.0±0.0**	0.0±0.0**	0.0 ±0.0**
Control	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	88.80±1.25	73.53±9.36	62.23±10.77

* P <0.05 and ** P <0.001 compared to parasite control

Table 2: *In vitro* effects of different concentrations of *Olibanum* extract on viability rates (%) of *T. spiralis* larvae

Dose (µg/ml)	Time intervals (Mean ± SD)							
	1st hr	3rd hr	6th hr	9th hr	12th hr	24th hr	36th hr	48th hr
10	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	82.46±6.48	61.40±10.43*	52.23±8.65**	35.16±11.89**
25	100.0±0.0	100.0±0.0	100.0±0.0	87.10±2.72	68.26±6.08*	45.70±13.59**	30.23±10.00**	17.80±8.40**
50	100.0±0.0	100.0±0.0	100.0±0.0	85.50±2.10	74.43±0.98*	41.75±12.61**	23.11±7.42**	13.11±5.24**
100	100.0±0.0	100.0±0.0	96.05±3.43	86.75±6.83	33.40±10.35**	20.65±6.41**	12.30±4.50**	0.0±0.0**
150	100.0±0.0	93.65±0.80**	87.33±2.25**	53.80±10.65**	27.93±16.31**	17.45±9.89**	0.0±0.0**	0.0±0.0**
ABZ 20	100.0±0.0	81.70±1.65**	72.60±2.50**	23.83±8.24**	17.73±7.15**	0.0±0.0**	0.0±0.0**	0.0±0.0**
Control	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	92.96 ±6.11	85.46±4.78	70.10±7.65

* P <0.05 and ** P <0.001 compared to parasite control

Discussion

Generally, medical plants and herbs volatile oils (and/or active components) were used as alternatives or adjuncts to current antiparasitic therapies. The garlic oil has broad-spectrum activity against *Trypanosoma*, *Plasmodium, Giardia* and *Leishmania*, and *Cochlospermum planchonii* and *Croton cajucara* oils specifically inhibited *P. falciparum* and *Leishmania* amazonensis, respectively. Other plant oils have immuno-modulatory effects that modified host-parasite immunobiology, and the lipid solubility of plant oils offered alternative, transcutaneous delivery routes (Anthony *et al*, 2005). Moreover, Bezabh *et al.* (2022) in Australia assessed the preclinical and clinical studies exploring the antiparasitic activity of tea tree oil (TTO) & its' components against clinically significant zoonotic ecto-parasites, such as bed bugs, chiggers, demodicosis, fleas, house dust mites, pediculosis and scabiasis. Also, Capasso *et al.* (2022) in Italy reported that TTO can be used for lid scrubs, facial cleanser, eyelid patch, eyelid gel, eyelash shampoo or, more commonly, as TTO impregnated eyelid wipes. No doubt, the plant products have acted as a best source for pharmaceutical re-search (Wink, 2012). Abd-Elrahman *et al*. (2020) in Egypt who reported that extracts and volatile oil of myrrh had in vitro larvicidal activities against muscular larvae of *T. spiralis*. Also, El-Kady *et al.* (2022) reported that the *Artemisia annua* was effective in treating experimentally induced trichinellosis.

In the present study, a significant reduction of viability rates of incubated *T. spiralis* adults with 50µg/ml concentration of OL extract and significant complete inhibition effect with 100µg/ml concentration after 48 hr and after 36hr for 150µg/ml concentration. This agreed with Shelke *et al*. (2020) in India who reported significant anti-helminthic activity of crude extract of *Boswellia serrata* at 150mg/ml against *Pheretima posthuma* earth worm. Khan *et al*. (2016) reported that the oleo-gum resin of *B. serrata* tree (is one such folk medicine, which has been traditionally used for religious, cosmetic as well as medical purposes since ages without

any side effect. Abdallah *et al.* (2011) in Egypt reported that OL extract had fatal activity against giardiasis infective stages that led to a significant decrease when compared to infected control ones in concentration and exposure-time-dependent manner**.**

In the current study, the full larvicide effect of OL extract occurred at 36hr at a concentration of 150µg/ml & at 48hr at a 100µg /ml with significant difference. This agreed with Schmidt *et al*. (2011) showed that there is an *in vitro* antiprotozoal activity of serratol (Oleoresin of *B. serrata*) against *Trypanosoma brucei rhodesiense* (African sleeping sickness) and *Plasmodium falciparum* (tropical malignant malaria).

In the current study, SEM showed that OL extract caused many structural changes in *T. spiralis* adults as well as larval stages. These changes were in the form of blobbing, sloughing and cuticle erosion, which were more prominent and more destructive at the higher OL extract concentrations. There was a direct relationship between the degenerative changes level recorded and the increase in dose and time of exposure. This agreed with Abdalla *et al*. (2011) reported that *in-vitro* cultivation of *G. lamblia* trophozoites on TYI-S-33 medium followed by inoculation of OL extracts caused severe ultrastructural changes of *Giardia* trophozoites 48hr after exposure. Besides, Attallah *et al*. (2021) who demonstrated a significant reduction in cell aggregation of *Prophyromonus gingivalis* after *in-vitro* incubation with frankincense extract by SEM examination.

Generally, the nematodes cuticle is active and in charge of selective nutrients absorption and regulation of osmosis, which was known to be the primary entry channel and primary site of action of nematicidal drugs (Abdel-Wahab *et al*, 2017). So, the erosive changes and deformities could be attributed to the passive diffusion of the drugs through the cuticle. This also agreed with Greve *et al*. (2017) in Germany reported that the dichloromethane extract of Indian Frankincense (OL), the oleo-gum-resin obtained from *Boswellia serrata*, showed *in-vitro* activity against *Plasmodium falciparum*. Abers *et al*. (2021) in USA who reported that frankincense extract had antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Propionibacterium acnes* Di Stefano *et al*. (2020) in Italy found that OL available in the fight against pathogens and to combat antibiotic resistance phenomenon, encouraging the use of alternative resources, especially in farms, food processing…etc.

Conclusion

The present outcome postulated that, OL extract was more effective at concentration of 150µg/ml. *In-vitro* anthelminthic action against trichinosis adults and muscular larvae was in a dose dependent manner. Olibanum extract proved to be an effective and safe alternative treatment for *T. spiralis.* Studies are going to confirm its efficacy and safety in-vivo, and will be published in due time elsewhere.

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References

Abdalla, SF, Mohamed, AA, El-Deeb, HK, Al-Khadrawy, FM, Badawy, AF, 2011: A study on the effect of *Myrtus communis* and *Olibanum* on *Giardia lamblia* infection in Egypt. PUJ 4, 1:89-100.

Abdel-Hady, NM, El-Hela, AA, Morsy, TA, 2014: Phenolic content of some selected Lamiaceous Egyptian medicinal plants: Antioxidant potential and ecological friend mosquito-larvicidal. J. Egypt. Soc. Parasitol. 44, 1:21-4.

Abd-Elrahman, SM, Dyab, AK, Mahmoud, A E, Mostafa, SM, Elossily, NA, 2020: Anti-parasitic activity of myrrh crude extract and myrrh volatile oil compered to albendazole against *Trichinella spiralis* muscular larvae in vitro. JESP 50, 2:307-14.

Abdel-Wahab, A, Maogood, S, Mahdy, O, 2017: Scanning electron microscopic changes in *Habronema muscae* after in vitro exposure to plant extract (*Verbesina alternifolia*). J. Egypt. Soc. Parasitol. 47, 1:47-54.

Abers, M, Schroeder, S, Goelz, L, Sulser, A, Rose, TS, *et al,* 2021: Antimicrobial activity of the volatile substances from essential oils. BMC

Complement. Med. Ther. 21:1-14.

Abou Rayia, DM, Saad, AE, Ashour, DS, Oreiby, RM, 2017: Implication of artemisinin nematocidal activity on experimental trichinellosis: In vitro and in vivo studies. Parasitol. Int. 66, 2: 56-63

Abouel-Nour, MF, El-Shewehy, DMM, Hamada, SF, Morsy, TA, 2016: The efficacy of three medicinal plants; garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum*: ii- Histological changes. J. Egypt. Soc. Parasitol. 46, 1:185-200

Abou-shady, OM, Mohammed, SS, Attia, SS, Yusuf, HAS, Helmy, DO, 2016: Therapeutic effect of mefloquine on *Schistosoma mansoni* in experimental infection in mice. J. Parasit. Dis. 40, 2:259-67.

Abuelenain, GL, Fahmy, ZH, Elshennawy, A M, Selim, EHA, Elhakeem, M, *et al,* 2022: Phenotypic changes of *Trichinella Spiralis* treated by *Commiphora molmol, Lepidium sativum*, and Albendazole: In *vitro* study. Helminthologia 59, 1:37-45.

Al-Yasiry, ARM, Kiczorowska, B, 2016: Frankincense: Therapeutic properties. Postepy. Hig. Med. Dosw.70:380-391.

Anthony, JP, Fyfe, L, Smith, H, 2005: Plant active components; a resource for antiparasitic agents? Trends Parasitol. 21:462-8.

Attallah, NGM, Negm, WA, Elekhnawy, E, Altwaijry, N, Elmongy, EI, *et al,* 2021: Antibacterial activity of *Boswellia sacra* Flueck. Oleoresin extract against *Porphyromonas gingivalis* periodontal pathogen. Antibiotics 10:859-62.

Bakry, FA, Hamdi, SAH, 2006: The molluscicidal activity of some plant extracts against *Biomphalaria alexandrina* snails. Egypt. J. Exp. Biol. (Zool.), 2: 99-106.

Barruet, R, Devez, A, Dupouy-Camet, J, Karadjian, G, Plavsa, D, *et al,* 2020: Outbreaks a common source for a trichinellosis outbreak reported in France and Serbia in 2017. Eurosurveillance 25:1-7.

Bezabh, SA, Tesfaye, W, Christenson, JK, Carson, CF, Thomas, J, 2022: Antiparasitic activity of tea tree oil (TTO) and its components against medically important ectoparasites: A systematic review. Pharmaceutics Jul 29; 14(8): 1587. doi: 10.3390/pharmaceutics14081587.

Borrelli, F, *et al*, 2006: Effect of *Boswellia serrata* on intestinal motility in rodents: inhibition of diarrhoea without constipation. Br. J. Pharmacol. 148, 4:553-60.

Brady, GS, 1971: Materials Handbook, McGraw-Hill Book Co., New York.

Bughdadi, FA, 2010: Ultrastractural studies on the parasitic worm *Trichinella spiralis.* Tibah. J. Univ. Sci. 3:33-8.

Caner, A, Döskaya, M, Degirmenci, A, Can, H, Baykan, S, Uner, A, *et al*, 2008: Comparison of the effects of *Artemisia vulgaris* and *Artemisia absinthium* growing in western Anatolia against trichinellosis (*Trichinella spiralis*) in rats. Exp. Parasitol. 119:173-9.

Capasso, L, **Abbinante, G, Coppola, A, Salerno, G, De Bernardo, M, 2022:** Recent evidence of tea tree oil effectiveness in blepharitis treatment. Biomed Res Int. 2022 Jul 30;2022:9204251. doi: 10.1155/2022/9204251.

Chevrier, MR, Ryan, AE, DYW, Zhongze, M, Wu-Yan, Z, *et al,* 2005: *Boswellia carterii* extract inhibits TH1 cytokines and promotes TH2 cytokines in vitro. Clin. Diag. Lab. Immunol. 12, 5:575-80.

Chowdary, KPR, Mohapatra, P, Murali, M, 2006: Evaluation of olibanum resin as micro-encapsulating agent for controlled drug delivery. Indian J. Pharm. Sci. 68, 4:461-4.

Di Stefano, V, Schillaci, D, Cusimano, MG, Rishan, M, Rashan, L, 2020: In vitro antimicrobial activity of frankincense oils from *Boswellia sacra* grown in different locations of the Dhofar Region (Oman). Antibiotics.9:195.

El-Kady, AM, **Abdel-Rahman, IAM, Sayed, E, Wakid, MH, Alobaid, H, *et al,* 2022**: A potential herbal therapeutic for trichinellosis. Front Vet. Sci. Aug 23;9:970327. doi:10.3389/fvets.

Fahmy, A, Zalat, RA, Rabei, A, 2020: In-vitro evaluation of the antiparasitic activity of *Syzygium aromaticum* against adult and larval stages of *Trichinella spiralis*. Sci. Parasitol. 21:94-101.

Greve, HL, Kaiser, M, Brun, R, Schmidt, TJ, 2017: Terpenoids from the oleo-gum-resin of *Boswellia serrata* and their antiplasmodial effects in vitro. Planta. Med. 83:1214-26.

Ibrahim, MA, Mohammed, A, Isah, MB, Aliyu, AB, 2014: Anti-trypanosomal activity of African Medicinal Plants: A review update. J. Ethnopharmacol. 154:26-54.

Izumi, E, Ueda-Nakamura, ET, Dias Filho, B P, Júnior, VF, Nakamura, CV, 2011: Natural products and Chagas' disease: A review of plant compounds studied for activity against *Trypanosoma cruzi*. Nat. Prod. Rept. 28:809-23.

Jebelli, A, Khalaj-Kondori, M, Bonyadi, M, Hosseinpour Feizi MA, *et al,* 2019: Betabosw-

ellic acid and ethanolic extract of olibanum regulating the expression levels of CREB-1 and CREB-2 genes. Iran. J. Pharm. Res. 18:877-86.

Jiang, P, Wang, ZQ, Cui, J, Zhang, X, 2012: Comparison of artificial digestion and Baermann's methods for detection of *Trichinella spiralis* pre-encapsulated larvae in muscles with low-level infections. Foodborne Pathog. Dis. 9, 1: 27-31.

Kaiaty, AM, Salib, FA, El-Gameel, SM, Hussien, AM, Kamel, MS, 2021: Anthelmintic activity of pomegranate peel extract (*Punica granatum*) and synthetic anthelmintics against gastrointestinal nematodes in cattle, sheep, goats, and buffalos: In-vivo study. Parasitol. Res. 120, 11:3883-93

Khan, MA, Ali, R, Parveen, R, Najmi, AK, Ahmad, S, 2016: Pharmacological evidences for cytotoxic and antitumor properties of *Boswellic acids* from *Boswellia serrata*. J. Ethnopharmacol. 15, 191:315-23.

Kim, CW, Myron, CL, 1980: Surface morphology of *Trichinella spiralis* by scanning electron microscopy. J. Parasitol. 66, 1:75-81.

Ljaljevi´c Grbi´c, M, Unkovi´c, N, Dimki´c, I, Jana´ckovi´c, P, Gavrilovi´c, M, 2018: Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients: Wisdom of the ancients? J. Ethnopharmacol. 219:1-14.

Loutfy, NF, Awad, OM, El-Masry, AG, Kandil, GM, 1999: Study on rodents' infestation in Alexandria and prevalence of *Trichinella spiralis* infection among them. J. Egypt. Soc. Parasitol. 29:897-909.

Morsy, TA, Ibrahim, BB, Haridy, FM, Rifaat, MMA, 2000: *Trichinella* encysted larvae in slaughtered pigs in Cairo (1995-1999). J. Egypt. Soc. Parasitol. 30, 3:753-60.

Morsy, TA, Sallam, TA, Hawam, SM, 2022: Trichinosis (trichinellosis) in man and animals with reference to Egypt: An overview JESP 52, 3:431-4

Rawla, P, Sharma, S, 2022: *Trichinella spiralis*. StatPearls [Internet]. Last Update: October 9.

Rivera, JC, Yépez-Mulia, L, Hernández-Campos, A, Moreno-Esparza, R, Castillo, R, *et al,* 2007: Biopharmaceutic evaluation of novel anthelmintic (1*H*-benzimidazol-5(6)-yl) carboxamide derivatives. Int. J. Pharm. 343:159-65.

Rostami, A, Gamble, HR, Dupouy-Camet, J, Khazan, H, Bruschi, F, 2017: Meat sources of infect-ion for outbreaks of human trichinellosis. Food Microbiol. 64:65-71.

Rózycki, M, Korpysa-Dzirba, W, Bełcik, A, Pelec, T, Mazurek, J, *et al,* 2022: Analysis of a trichinellosis outbreak in Poland after consumption of sausage made of wild boar meat. J. Clin. Med. 11:485-9.

Schmidt, TJ, Kaiser, M, Brun, R, 2011: Complete structural assignment of serratol, a cembrane-type diterpene from *Boswellia serrata*, and evaluation of its antiprotozoal activity. Planta Med.77, 8:849-50.

Shelke, PS, Jagtap, PN, Tanpure, PR *et al*, 2020: In-vitro anthelmintic activity of *Boswellia serrata* and *Aloe barbadensis* extracts on *Pheretima posthuma*: Indian earthworm. Int. J. Res. Med. Sci. 8, 5:1843-7.

Tomar, RS, Preet, S, 2017: Evaluation of anthelmintic activity of biologically synthesized of si lver nanoparticles against gastrointestinal nematode, *Haemonchus contortus*. J. Helminthol. 91, 4:454-61.

Vuuren, SFV, Kamatou, GPP, Viljoen, AM, 2010: Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples. S. Afr. J. Bot. 76:686-91.

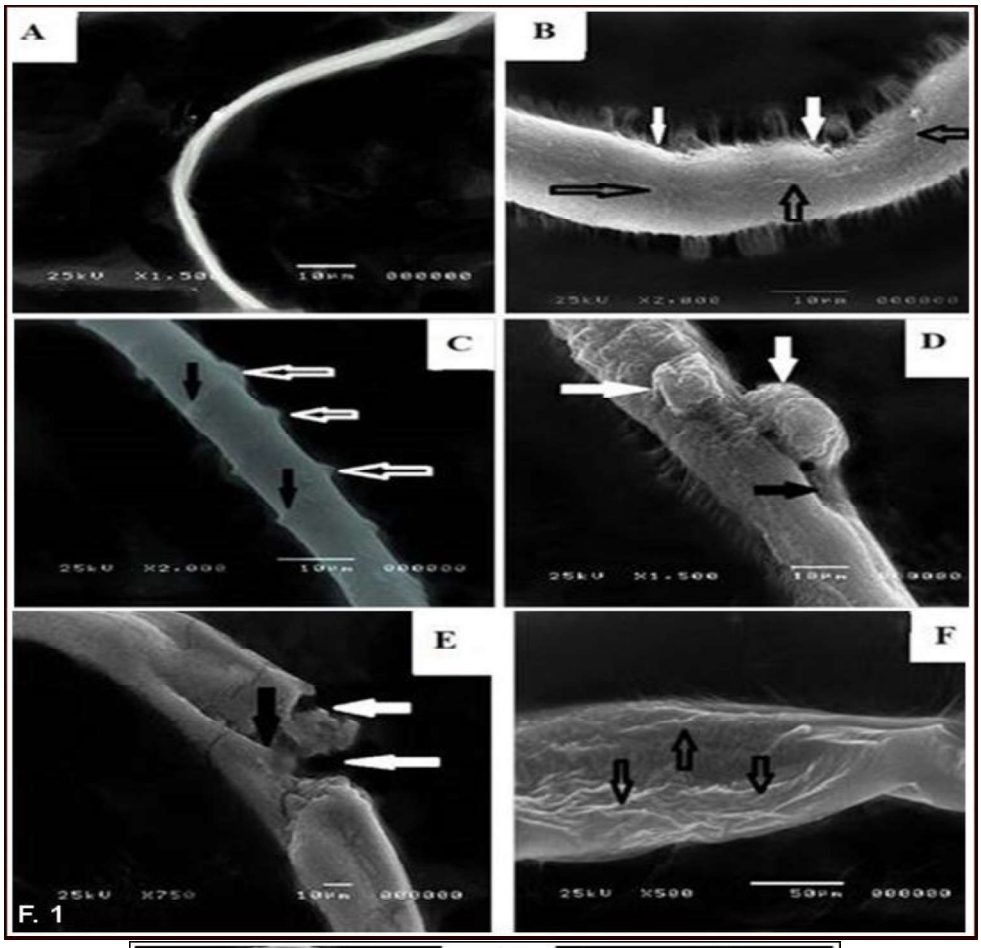
Wink, M, 2012: Medicinal plants: A source of anti-parasitic secondary metabolites. Molecules 17:34-9.

Yang, F, Yang, DQ, Song, YY, Guo, KX, Li, YL, *et al,* 2019: In vitro silencing of a serine protease inhibitor suppresses *Trichinella spiralis* invasion, development, and fecundity. Parasitol. Res. 118: 2247-55.

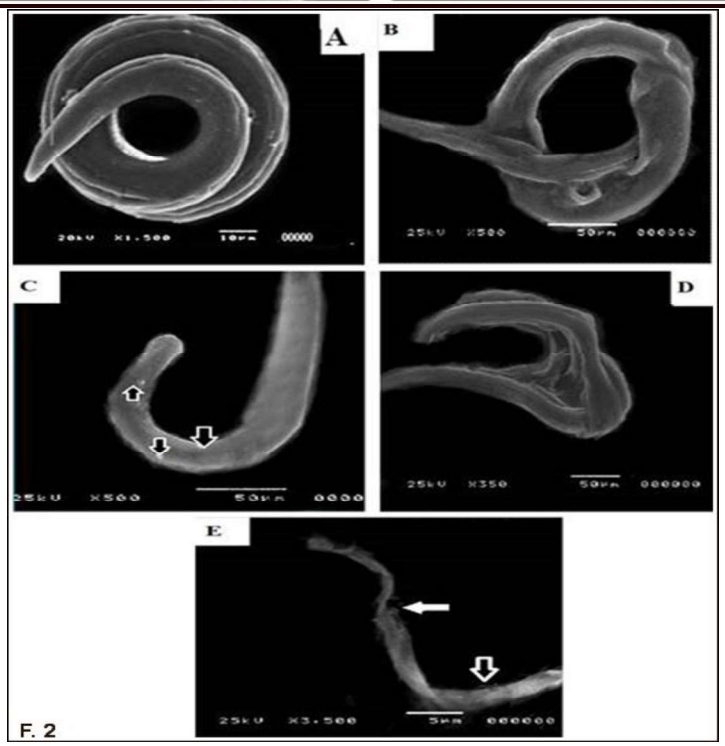
Explanation of figures

Fig.1: SEM of cultured *T. spiralis* adults: (A) after media incubation only revealing normal cuticular worm body; (B) after incubation with 25µg/ml OL extract showed whitish discoloration, multiple cracks (black arrows) and vesicles (white arrows) with loss of normal annulations of cuticle; (C) after incubation with 50µg/ml OL extract showed well developed vesicles (white arrows) with developed cracks (black arrows); (D) after incubation with 100µg/ml OL extract, cuticle damaged with large outer mass (white arrows) and cavity depression (black arrow) together with loss of normal annulations; (E&F) after incubation with 150µg/ml OL extract, cuticle became severely destroyed and degenerated with sloughing, large bleb (white arrows), cavity depression (black arrow) (E), and erosions (hollow black arrows) with completely disappeared lines and annulations of surface (F).

Fig.2: SEM of cultured *T. spiralis* larvae: (A) Isolated larvae after media incubation only showed typical coiled appearance with normal cuticle; (B) after incubation with 50 µg/ml OL extract showed cuticle deformity and loss of striations; (C) after incubation with 100µg/ml OL extract, larvae became flattened with multiple small blebs (black arrows) , loss of striations with opaque cuticle; (D&E) after incubation with 150µg/ml OL extract, sever destruction results in cuticular deformity with sloughing (white arrow) and loss of striations (black arrow with white outline)



F. 1



F. 2