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Effectiveness Evaluation of Leaves *Nerium oleander* Extract on the Viability of Protoscolices: *In vitro*

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ABSTRACT

Background: *Echinococcus granulosus* is a type of taeniid tapeworm, present in the small intestine of its primary hosts, including dogs and occasionally in other carnivores. The larval stage of *E. granulosus* causes cystic hydatidosis in humans and livestock. Although most hydatid cysts are found in the liver and lungs, the disease can occur anywhere in the body. Therefore, Hydatidosis remains a problem and causes significant economic losses in animals.

Methods: A study was performed to evaluate the activity of the leaves *Nerium oleander* extract on the viability of protoscolices. We used three graduated concentrations of extract (10, 30 and 50 mg/ml). 2 ml of the protoscolices suspension (containing at least 2700 protoscolices) was placed in test tubes using a Pasteur pipette. Then, 2 ml of various concentrations of the *Nerium oleander* extract were added to each test tube and mixed gently, incubated at 37°C for 5, 10, 20 and 30 min. One control group was kept that contain normal saline. The viability of protoscolices was evaluated by eosin method where, protoscolices suspension was mixed with 0.1% eosin for 5 minutes. The dead protoscolices absorb eosin and becomes red, whereas the live protoscolices remains colorless. Before drawing the conclusion, each experiment was performed thrice.

Result: The analysis of phytochemicals by FT-IR for alcoholic extracts of *Nerium oleander* extracts revealed the presence of 12 effective chemical ingredients. *Nerium oleander* extract showed the strongest scolicidal effect (85.6, 92.3%) after 20 and 30 min at a concentration of 50 mg/ml, respectively moderately (69.3%) after 30 min at 30 mg/ml and lowest (41.9%) after 30 min at 10 mg/ml. Microscopic examinations of dead protoscolices showed distortion of their morphology and degenerative effects. These effects were characterized by loss of hooks, or presence of free hooks and dissolution and rupture of the protoscolices wall. It can be concluded from this study that ethanol extract of *Nerium oleander* is effective against Protoscolies *in vitro*.

Key words: Carnivores, Nerium oleander, Protoscoleces, Tapeworm.

INTRODUCTION

Cystic echinococcosis is one of the most serious epidemiological health problems in most parts of the world (Eckert and Deplazes, 2004; Roberts and Janovy, 2000). Disease in humans and other intermediate hosts (sheep, cattle, buffalo, camels, horses and other animals) is caused by the larval stage of the parasitic tapeworm *Echinococcus*, which includes many species, the most important of which are *E. granulosus* and *E. multilocularis* and this stage can attack any organ of the intermediate host's body (Gholami *et al*., 2013). It is more common in rural areas where farming animals and carnivores are common, which completes the life cycle of this parasite requiring intermediate and final hosts like dogs, wolves, hyenas, leopards and other wild animals (Marquardt *et al*., 2000). Disease or epidemic in humans and other intermediate hosts is caused by ingestion of food and water contaminated with tapeworm eggs produced by adult parasitic worms in dogs (Torgerson and Budke, 2003). The pet dog is the only reservoir for the adult tapeworm and thus plays a major role in the infection. The released embryo penetrates the intestinal wall and travels through the portal system, primarily to the liver, lungs, or other organs where hydatid cysts grow (Eckert and Deplazes, 2004). In many Countries of the world, surgery remains the most effective treatment for hydatid disease (Brunetti *et al*., 2010). Many pesticides inactivate cystic contents, including hypertonic saline, Ag-nitrate, cetrimid, formalin, povidone iodine and ethanol. However, the use of these agents has been reported to be most often associated with toxicity and liver necrosis and methemoglobinemia (Karaoğlanoğlu *et al*., 2011). Recent studies on plant extracts and compounds of plant origin due to their lower side effects, low cost and wide availability have demonstrated a successful approach to treatment many diseases (Rocha *et al*., 2005). *Nerium oleander* is an ornamental evergreen shrub or small tree native to the Mediterranean region and Southeast Asia (Lans, 2007). *Oleander* has been used as a

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medicinal plant for the treatment of a variety of ailments. Traditional medicine uses oleander to treat epilepsy and cancerous tumors (Abbas *et al*., 2012). *Nerium* is employed in the practice of traditional medicine. The flower makes a green dye that is beneficial for treating skin conditions. Moreover, this dye has the power to treat wounds and lessen skin inflammation (Lans, 2007). In Kenya, an extract made by boiling the leaves and seeds in water is used to treat upper respiratory tract and gastrointestinal conditions þ (Nanyingi *et al*., 2008). The juice prepared from the stem bark of *Nerium oleander* is used as an earache remedy in the local traditional medical systems in the Kancheepuram region of Tamil Nadu, India (Nanyingi *et al*., 2008). Since *Nerium oleander* extract has been shown to have several medicinal properties, the present study aimed to evaluate the in vitro scolicidal effects of the ethanol extract of *Nerium oleander* on the vitality of the Protoscolies.

MATERIALS AND METHODS

Preparation of extracts

The *Nerium oleander* leaves were collected from Botanical Gardens in Riyadh city, Saudi Arabia. After air drying 1000 g at 44°C, the dried leaves were ground into a fine powder with an electric mixer. after which 500 g leaves powder was extracted with 70% ethanol as follows: 100 g of dry powder was added to 400 ml of 70% ethanol and mixed gently for 1 h using a magnetic stirrer. The obtained solution was left at room temperature for 24 h, then stirred again and filtered. The solvent was then evaporated on a rotary evaporator (Inter world highway, LLC). After obtaining the crude extract, it was lyophilized and kept at a temperature of -20 degrees Celsius until usage (Chen *et al*., 2006).

Infrared spectroscopy

After the completion of the processing steps, a minute portion of the material was homogenized by mixing it with an excessive quantity of potassium bromide powder (1: 99 wt%). After that, the material went through a coarse crushing process before being loaded into a pellet-forming die. The infrared spectrum was analyzed using an optical spectrometer from Thermo Scientific called the NICOLET 6700 Fourier-transform infrared spectroscopy (FT-IR). This allowed for the prediction of the most probable constituent classes. The greatest number of waves absorbed is denoted by the expression “a number of waves” (cm^{-1}). Spectra were recorded at 25°C, with a resolution of 4 cm and the range of the spectrum was from 4000 cm^{-1} to 400.

Collection of protoscolices

Protoscolices were obtained from infected organs like liver and lungs of sheep slaughtered at Riyadh slaughterhouse and transported to the parasitology laboratory of the zoology department of the University King Saud. The hydatid liquid from the cysts was transferred aseptically into glass bottles and left to stand for 30 min, the protoscolices were deposited at the bottom of the bottles. The supernatant becomes then removed and the yielded protoscolices have been washed three instances the usage of ordinary saline. A fertility test and viability turned into assessed via a means of 0.1% eosin staining check under light microscopy. Finally, the live protoscolices were transferred to a dark container of ordinary and stored at 4°C for later use.

Determination of *in vitro* effects

To investigate the scolicidal effects of *Nerium oleander* against protoscolices of *E. granulosus*, we used various concentrations (10, 30 and 50 mg/ml) of the extract for 5, 10, 20 and 30 min. In order to prepare the ones different concentrations, respectively, 0.05, 0.1, 0.3 and 0.5 g of dried extract modified into dissolved in 10 ml of distilled water. In each experiment, 2 ml of the protoscolices solution (containing at least 2700 protoscolices) was placed in test tubes using a Pasteur pipette. Then 2 ml of various concentrations of the *Nerium oleander* extract were added to each test tube and mixed gently, then incubated at 37°C for 5, 10, 20 and 30 min. At the end of each incubation period, the upper phase was carefully removed, taking care to avoid disturbing the settled protoscolices. Then, 100 µl of 0.1% eosin dye was added to the remaining precipitated protoscolices and mixed gently. After 5 min, the top part of the solution changed into once more discarded. The closing settled protoscolices had been smeared on a glass slide, included with a cover glass and tested microscopically for viability. In addition, at least 2700 protoscolices in 2 ml normal saline were used as control groups each experiment was performed 3 times.

Viability test

To evaluate the viability of protoscolices, eosin solution with a concentration of 0.1% (1 g of eosin powder in 1000 ml distilled water) was used (Smyth and Barrett, 1980). After 5 minutes of dye exposure, the dead protoscolices absorb eosin and becomes red, whereas the live protoscolices remains colorless (Fig 1). The survival index of protoscolices was determined as the percentage of viable protoscolices to the total number of protoscolices (Haghani *et al*., 2014).

Statistical analysis

One-way ANOVA was performed using a statistical software package (Sigma Plot version 11.0). All p values were two-sided and p 0.001 was considered significant.

RESULTS AND DISCUSSION

The FT-IR analysis and qualitative phytochemical investigation of alcoholic extracts of the leaves of *Nerium oleander* showed the presence of 12 compounds of active chemical constituents. The analysis of *Nerium oleander* leaf extracts using FT-IR explained main bands at 3383.10 cm^{-1}, N-H stretching, 2934.35 cm^{-1}, C-H stretching, 2124.09 cm^{-1}, N=C=N stretching, 1633.45 cm^{-1}, C=C stretching, 1515.92 cm^{-1}, N-O stretching, 1423.03 cm^{-1}, O-H bending, 1273.79 cm^{-1}, C-O stretching, 1117.70 cm^{-1}, C-O stretching, 1048.87 cm^{-1}, CO-O-CO stretching, 926.30 cm^{-1}, bending, 828.28 cm^{-1}, C=C

bending and 717.43 cm^{-1}, C=C bending (Fig 1 and Table 1).

The scolicidal effects of different concentrations of *Nerium oleander* extracts are summarized in (Table 2). *Nerium oleander*, given a concentration of 10 mg/ml, mortality rates of 15.7%, 25%, 33% and 41.9% were observed following treatment periods of 5, 10, 20 and 30 min, respectively. When the concentration of *Nerium oleander* was increased to 30 mg/ml, mortality rates of 36.6%, 46.6%, 57% and 69.3% were observed at the same time intervals. *Nerium oleander* concentration of 50 mg/ml, meanwhile, led to mortality rates of 69%, 80.6%, 85.6% and 92.3% after 5, 10, 20 and 30 min, respectively. Compared to the control group, the difference between the mortality rates due to effects of *Nerium oleander* extracts was statistically highly significant (P<0.001) for all three concentrations of and at each of the various application times. Microscopic examinations of dead protoscolices showed distortion of their morphology and degenerative effects. These effects were characterized by loss of hooks, or presence of free hooks and dissolution and rupture of the protoscolices wall (Fig 2).

The current study agrees with what was done by researcher (Hamad, 2021) in that the oleander plant has an effect on the vitality of Protoscolies *in vivo*. The effective activity of the *Nerium oleander* extract is due to the chemical content of the active substances such as alkaloids, phenols, flavonoids, tannins and actins (Al-Rabei, 1999). The inhibition of these compounds is explained by the fact that they interfere in the chain of protein metabolism reactions necessary for the continuity of the micro-organism's viability and its ability to break down the cell wall and what it contains of proteins and fats and then the parasite's destruction (Cowan, 1999). And prove that aqueous leaf extract of *N. oleander* has ovicidal and larvicidal activity against Culex tritaeniorhynchus and Culex gelidus (Kumar *et al*., 2012). Rathi and Al- Zubaidi (2011) showed that *N. oleander* has toxic effects on the development of larval and pupal stages for *Bemisia tabaci* species. The ethanolic extract of *Nerium*

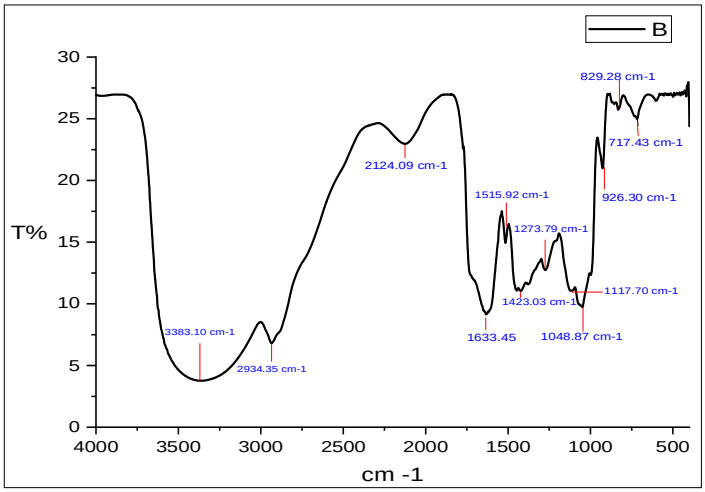

Fig 1: FT-IR chromatogram of *Nerium oleander* leaf extracts in methanolic medium showing the functional characteristic of the active chemical compounds.

Table 1: Analyze NOLE to identify potential active chemical compounds using FT-IR.

Absorption (cm^{-1})	Appearance	Transmittance (%)	Group	Compound class
3383.10	Medium	4	N-H stretching	Aliphatic primary amine
2934.35	medium	7	C-H stretching	Alkane
2124.09	Strong	23	N=C=N stretching	Carbodiimide
1633.45	Medium	9	C=C stretching	Alkene
1515.92	Strong	15	N-O stretching	Nitro compound
1423.03	Medium	11	O-H bending	Alcohol
1273.79	Strong	12	C-O stretching	Alkyl aryl ether
1117.70	Strong	10	C-O stretching	Secondary alcohol
1048.87	Strong, broad	9	CO-O-CO stretching	Anhydride
926.30	Strong	21	C=C bending	Alkene
829.28	Medium	26	C=C bending	Trisubstituted
717.43	Strong	25	C=C bending	Disubstituted (cis)

Table 2: Scolicidial effect of *Nerium oleander* extracts on the viability of protoscolices of *E. granulosus*.

Concentrations	Experiment	% of mortality rates after exposure			
		5 min	10 min	20 min	30 min
10 mg/ml	1	16	19	27	38
	2	14	25.1	39	42.7
	3	17.2	31	33	45
	Average	15.7	25	33	41.9
30 mg/ml	1	34	49	59	66
	2	38	52	48	73
	3	38	39	64	69
	Average	36.6	46.6	57	69.3
50 mg/ml	1	61	77	85	89
	2	76	84	89	92
	3	70	81	83	96
	Average	69	80.6	85.6	92.3
Control	1	5	8.6	11	14.9
	2	7	10	13	16.2
	3	7	12	13.5	18
	Average	6.3	10.2	12.5	16.3

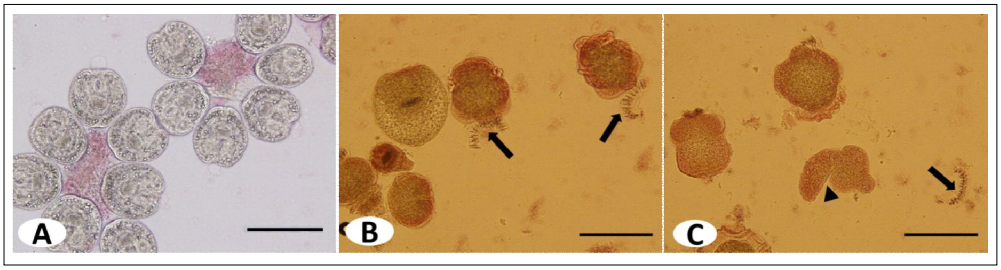

Fig 2: Live protoscolices after staining with 0.1% eosin (A), dead protoscolices after treat with the extract and staining with 0.1% eosin (B and C) and dissolution and rupture of the protoscolices wall (arrowhead) free hooks (arrows). Scale bar = 20 µm.

oleander leaves showed the highest antibacterial action against *Pseudomonas aeruginosa* at 900 mg/ml concentration (Malik *et al*., 2015). And Aqueous leaf extract of *Nerium oleander* showed significant activity against Indian earthworms *Pheretima posthuma* because presence of active chemical constituents such as Carbohydrates, Alkaloids, Flavonoids, Glycosides and Tannins, which are mainly responsible for anthelmintic activity (Kandagatla *et al*., 2019). The flower *Oleander* produces a green dye that is beneficial for treating skin conditions. In addition, this dye also has the ability to heal wounds and reduce skin inflammation (Lans, 2007). Morsi *et al*. (2022) found the effect of *Nerium oleander* leaf extract on the schistosomiasis parasite. It also explained the effectiveness of the components of *Nerium oleander* of alkaloids on the basis of inhibition of carbohydrate metabolism by affecting the mitochondria and then obstructing the breathing process Delorenzi *et al.* (2001) that the effect of alkaloids and phenols is due to the union of these active substances by affecting the metabolism of nitrogen and amino acids that are the basis for building the mitochondrial membrane, the nucleus and the Golgi body, which are important in the viability of micro-organisms, or the reason for their ability to combine with proteins, which leads to changes in the chemical properties of the cell wall or a change of the shape of the whole cell and this may result in its death.

CONCLUSION

It can be concluded from this study that ethanol extract of *Nerium oleander* is effective against Protoscolies *in vitro*. further studies are needed to isolate the pharmacologically active compounds responsible for these activities and a study conduct *in vivo* to evaluate *N. oleander* extract in the eradication of Hydatid Cysts and to determine the safe doses that will be used *in vivo*.

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Data availability statement

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

Conflict of interest statement

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

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