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In vitro Evaluation of *Nerium oleander* Leaf Extract against *Sarcoptes scabiei var. cuniculi* Mite Isolated from Naturally Infested Rabbits

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ABSTRACT

Background: Infestation of the mite *Sarcoptes scabiei var. cuniculi* is one of the most frequent skin diseases in rabbits all over the world. Disease is characterized by intense pruritus, alopecia, erythema, scales and crusts around the head, neck, trunk, feet, ears, nose and genitals, resulting in hypertensive reactions, considerable losses in body weight and productivity causing significant economic losses in rabbit production.

Methods: This study is aimed to evaluate the efficacy of *Nerium oleander* extract against *S. scabiei var. cuniculi* mite *in vitro*. Acaricidal bioassay,a dipping method was conducted on mites isolated from naturally infested rabbits. PBS and ivermectin were used as the negative and positive controls, respectively. In addition, three different concentrations of extract (100, 200 and 400 µg/ml) weretested at different periods and changes over time in the viability of mites were registered for (0.5, 1, 2, 4 and 8 hours). A chemical assay was performed *via* GC-MS to investigate the presence of several anticipated active chemical compounds in *Nerium oleander* leaf extract.

Result: The results showed that the extract had high efficacy in killing mites at 100%, 78% and 49% and ivermectin 91%, respectively, for 8 hours. The results indicate that Nerium oleander possesses effective compounds as a valuable therapeutic method for treating scabies. Further studies are needed to determine the precise active chemicals present in *Nerium oleander* leaf extract and their modes of action and application *in vivo*.

Key words: Alopecia, Arthropods, *Nerium oleander*, *Sarcoptesscabiei*.

INTRODUCTION

Mites of *Sarcoptes scabiei* (Family: Sarcoptidae) are usually regarded to be a single species. However, frequently are identified by a variety of names corresponding to the host species. The mite that causes scabies in rabbits is S. *scabiei var. cuniculi* and is an important species because of the possibility of zoonotic infection (Harrenstien *et al*., 1995). Diseases are characterized by intense pruritus, alopecia, erythema, scales and crusts around the head, neck, trunk, feet, ears, nose and genitals, resulting in hypertensive reactions, considerable losses in body weight, productivity, wool and death (Saha and Mukherjee, 1998) and is difficult to control in rabbits compared to other domestic animals (Aiello *et al*., 1998). All stages of the *S. scabiei* life cycle are found within the host and the entire life cycle lasts approximately 2 months (Suckow *et al*., 2002). Pregnant females of *S. scabiei* were approximately twice the size of males (Jofre *et al*., 2009) and lay eggs in tunnels in the stratum corneum of the skin, causing severe itchy rashes, hypersensitivity and inflammation. The six-legged larvae hatch within 3 to 10 days, move around in search of hair follicles on the skin and molt to the larval stage, where they mature into adult mites (Soulsby 1982). Adult mites live for 3-4 weeks within the skin of their host. Mites feed on lymphocytes and sloughed epithelial cells (Hofing and Kraus 1994). Demonstrating the presence of host IgG antibodies in the esophagus and midgut of fresh scabies mites removed from the host is evidence that these mites ingest host serum (Rapp *et al*., 2006). In general, acaricides such as pyrethroids and ivermectine are used for the control of infection (however, their efficacy is compromised. because of resistance, toxicity (O'Brien, 1999), environmental contamination and persistence (Halley *et al*., 1993) emphasizing the need for alternatives (Khater, 2011), medicinal plants. A study revealed the presence of mange mites through a microscopic examination of deep skin scrapings from infected sheep in the city of Riyadh (Murshed *et al*., 2022).

Nerium oleander, or oleander (locally called Défla), belongs to the family Apocynaceae (Beasley, 1999). It is mainly found on alluvium and rocky terrain, along wadis in

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the northern Sahara and the Tassili and Hoggar mountains (Chopra *et al*., 1960) and is frequently cultivated as an ornamental plant (Delille, 2007) to form hedges in parks and gardens. All parts of the oleander plant contain highly toxic and lethal glycosides and alkaloids (Barbosa *et al*., 2008) with antibacterial (Derwich *et al*., 2010), antimicrobials (Hussain and Gorsi, 2004), insecticides (Dinan, 2001), inflammatory (Aggarwal and Shishodia, 2006) and cytotoxic (Mijatovic *et al*., 2007) properties. The toxicity is selectively exerted on the nervous system, the reproductive system, or the digestive system of pests (Kumar *et al*., 2013). Humans, animals and some insects have been the subject of several studies relating to the toxic effects of *N. oleander* (Adom *et al*., 2003). The object of this study was to study the acaricidal activity of *Nerium oleander* extract against *S. scabiei var. cuniculi* mite *in vitro*.

MATERIALS AND METHODS

Venue of the experiment

The study was carried out in July 2023 in Riyadh, Saudi Arabia the parasitological laboratory at the College of Science, King Saud University.

Preparation of extracts

The *Nerium oleander* extract is prepared from leaves obtained from one of the gardens in the city of Al-Kharj, Saudi Arabia. Leaves (100 g) were air-dried at 40°C, ground into powder and then extracted with 70% methanol for 24 h at 4°C. Then, the obtained extract was concentrated and dried using a vacuum rotary evaporator (Yamato RE300, Japan) at 40°C and reduced pressure. Once crude extracts were made, they were stored at -20°C until used in an experiment (Yang *et al*., 2014).

Phytochemical analysis of leaf extracts

GC-MS (gas chromatography-mass spectrometry) analysis of the extract was performed using the equipment 7000D Triple Quad GC-MS (Agilent Technologies, Thermo Scientific, Austin, Texas, United States). In Miami, California, in the United States, a Thermo Scientific Trace GC Ultra and an ISQ single quadruple MS were utilized. Both the Agilent 7890A and the Agilent 5975C inert XL EI/CI MSDs come equipped with a solitary quadrupole mass analyzer. The capillary employed was an HP-5MS UI (Ultra Inert) model with the following specifications: a length of 25 meters, an inner diameter of 0.25 millimeters, a film thickness of 0.25 millimeters, a stationary phase containing 5% phenyl and low-polar methyl polysiloxane. The split ratio was 50:50 and operated at 250°C using helium as the carrier gas at 1.0 mL/min. After running the GC-MS at 30°C for five minutes at a rate of five counts per minute (Co/min), the temperature was gradually raised to 250°C for 40 min. In the MS conditions, the ionization energy was measured to be 70 eV. We utilized the full scan detection mode and the mass range we looked at was from 50 to 500 Da. One minute passed during the solvent delay. Methanol was the liquid used to dissolve the sample. In order to identify the compounds, the Wiley 9 database, the answers and the libraries were utilized. Name, molecular weight, molecular formula and peak area were the metrics that were utilized in the analysis of the test substance's constituent parts.

Collection of mites

S. scabiei var. cuniculi mites were collected from the toe of naturally infested rabbits using a scalpel blade. Scabs collected were examined under a stereomicroscope for the presence of mites and were incubated at 35°C for 30 min in Petri dishes. Live adult mites (male and female) released from scabs were picked up with a needle and put in Petri dishes for further use. It's used in all experiments.

In vitro assay

The acaricidal activity of *Nerium oleander* leaf extract against adult mites was evaluated using a contact assay (immersion test). Mites (n=14) were immersed in different concentrations (100, 200 and 400ìg/ml), of extract at room temperature and examined under a stereomicroscope at 0.5, 1, 4, 6, 8 and 24 h post immersion. Mites immersedin PBS and ivermectin served as negative and positive controls, respectively. Immobility of adult mites and a lack of reactions or persistent immobility within 1 minute following stimulation with a needle were considered indications of death. The test was repeated twice independently and three replicates were run concurrently for all tested treatments.

RESULTS AND DISCUSSION

The GC-MS investigation uncovered a large number of bioactive compounds in the extract *i.e*., plant secondary metabolites with their retention time (RT) and peak area percentage. The components most available and potentially effective are as follows: 3-O-Methyl-d-glucose (69.8%), 5-Hydroxymethylfurfural (15.65%), Oleic Acid (5.85%), Linoleic acid (2.97%) and n-Hexadecanoic acid (1.14%) (Table 1 and Fig 1).

*Scabies*area very common dermatological condition and representa major public health burden globally (Hay *et al*., 2014). Due to acaricidal resistance, significant effort is devoted to developing new, safe and effective drugs for scabies. After being submerged in concentrations, the mites, in general, exhibited excited behavior and moved in circles before slowing down and eventually passing away. In contact assay, *Nerium oleander* extract at different concentrations, exhibited potent acaricidal action against adult *Sarcoptes scabiei var* cuniculi (Fig 2). In fact, when the concentration of these extracts was increased to 400 mg/mL), the number of mortalities among adult mites was increased to (16.6±2) at 8 hrs (Table 2). It was very comparable to the standard medication, which included 1% ivermectin dosages, 200 and 100 mg/mL demonstrated a time-dependent acaricidal effect (Fig 3). At lower concentrations (100 mg/mL) the mortality of mites was 43% even after 8 h post-immersion. A low mortality rate was observed when adult mites were brought into contact at 43% for 8 h with a dose of 100 mg/mL. While

the extract dose of 200 mg/ml showed a death rate of mites at a rate of 78% after 8 h. At all of the time intervals studied, the control group did not exhibit any acaricidal activity; however, the tick moved more activity than usual for more than a day. The obtained results showed that there were significant differences between the mortality mean of 400 mg/ml concentration after 1hr and at 200 mg/ml concentration after 8hrs and the remaining mortality means (Table 2). Also, there were significant differences between control and at 400% concentration after 1 hr (Fig 4). The highest mortality was after 8 hours at 100 and 200% concentrations. The lowest mortality was observed after 0.5 hr at all concentrations (Table 2). Significant differences ($P<0.05$) were obtained in *S. scabiei var. cuniculi* adults mortality percentage among plant extract concentrations (100, 200 and 400 mg/mL) and control (Fig 3). While there are no significant differences between the 400% concentration and the reference treatment (1% ivermectin). These results indicated that *N. oleander* leaf extract possesses *in-vitro* mange activity against mites. A similar finding has been reported by several authors who estimated the *in-vitro N. oleander* activity against adult *S. mansoni* (Morsi *et al*., 2022) and developmental stages of insects (Rathi and Al-Zubaidi, 2011; Kumar *et al*., 2012). The observed acaricidal effect could be due to the presence of active metabolites; terpenoids, alkaloids, flavonoids, saponins, tannin and carbohydrates which were known to be present in *N. oleander* through phytochemical screening, (Barbosa *et al*., 2008; Bhuvaneshwari *et al*., 2007; Chaudhary *et al*., 2015).

Table 1: GC-Mass analysis to identification of phytochemical components biologically active in methanolic leaf extracts.

$t_{R\ (min)}$	Proposed compound	MW	Formula	Peak area%
6.49	3-Methoxycarbonylpyrazole	126	$C_5H_6N_2O_2$	0.94
7.48	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	0.86
8.98	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	15.65
13.47	2,5-Dimethoxy-4-ethylamphetamine	223	$C_{13}H_{21}NO_2$	0.815
14.08	Megastigmatrienone	190	$C_{13}H_{18}O$	0.205
14.23	Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one, 1,2,3b,6,7,8-hexahydro-6,6-dimethyl-	190	$C_{13}H_{18}O$	0.65
15.56	3-O-Methyl-d-glucose	194	$C_7H_{14}O_6$	69.8
17.88	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	1.14
18.03	Mandelic acid, 3,4-dimethoxy-, methyl ester	226	$C_{11}H_{14}O_5$	0.43
19.62	Linoleic acid	280	$C_{18}H_{32}O_2$	2.97
19.67	Oleic Acid	282	$C_{18}H_{34}O_2$	5.85
19.85	Octadecanoic acid	284	$C_{18}H_{36}O_2$	0.55
24.68	Pregn-5-en-20-one, 3-hydroxy-	316	$C_{21}H_{32}O_2$	0.18

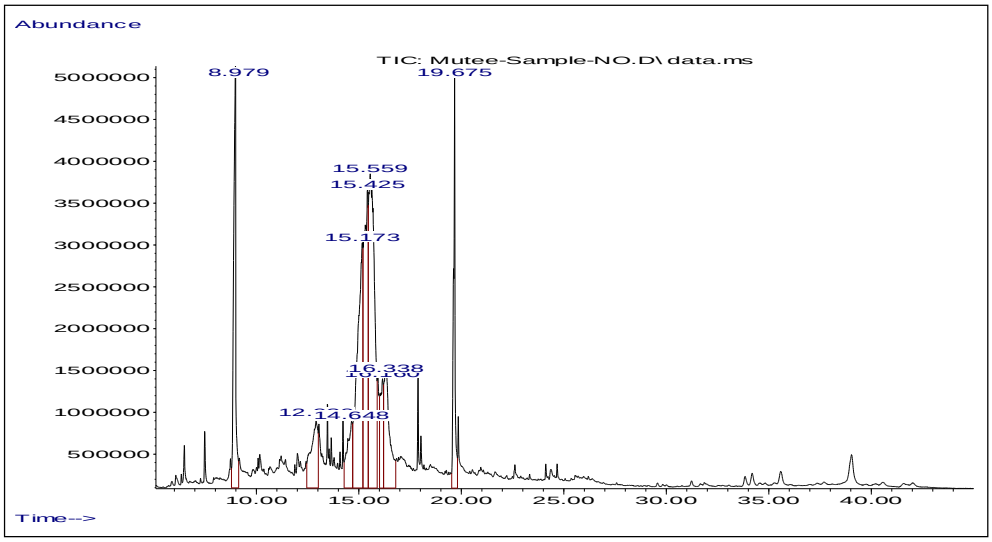

Fig 1: Infrared spectroscopy of *Nerium oleander* extract leaf methanolic.

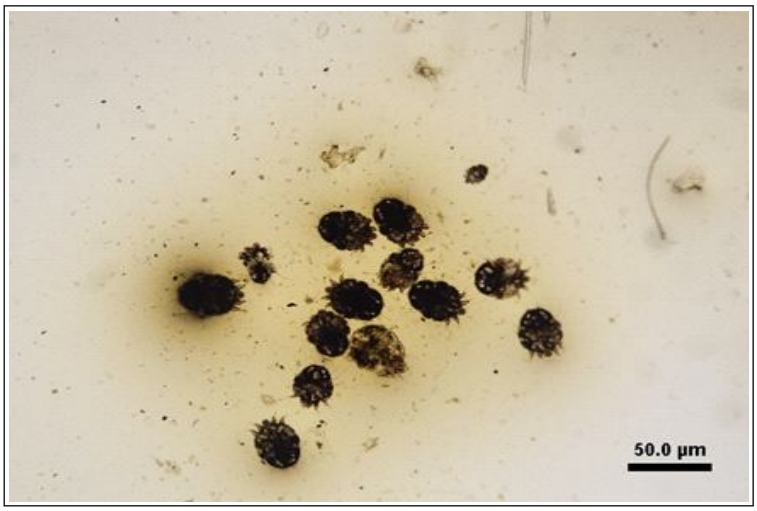

Fig 2: An illustrative image of the effect of the extract on *S. scabiei var. cuniculi* at a concentration of 400%.

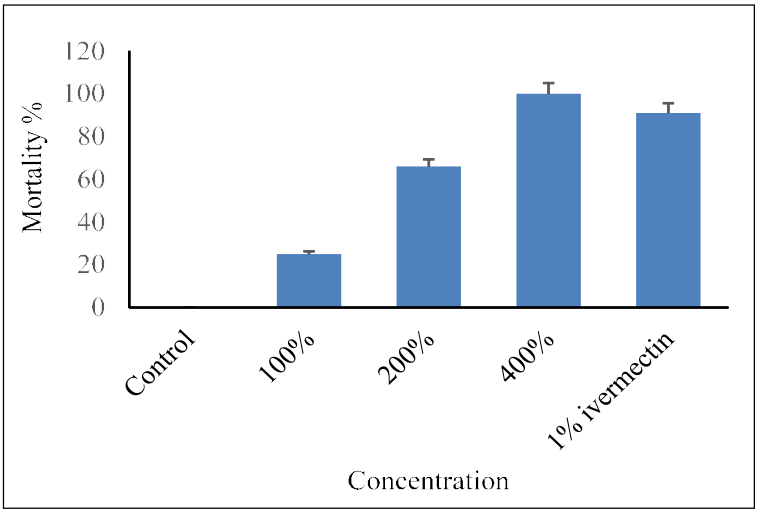

Fig 3: Mortality % of *S. scabiei var. cuniculi.*assayed with leaf oleander extracts at various exposure periods to concentrations (100, 200 and 400 mg/mL), compared with the reference drug.

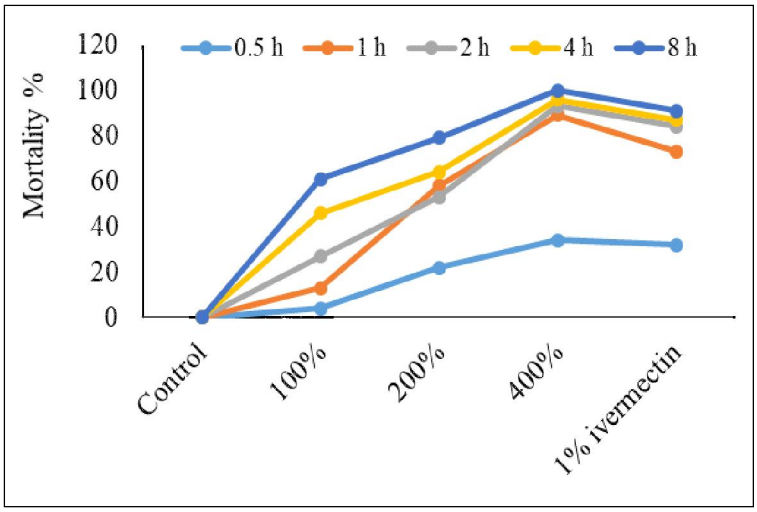

Fig 4: Mortality % of *Sarcoptes scabiei* assayed with leaf oleander extracts at various exposure periods (0.5, 1, 2, 4 and 8h).

Table 2: *In vitro* mite lethal effect of leaf oleander extract against *S. scabiei var. cuniculi.*

Dose	Average number of mites dead an hour after exposure				
mg/mL	0.5 hrs	1 hrs	2 hrs	4 hrs	8 hrs
Control	0.0±0.0^{c}	0.0±0.0^{c}	0±0.0^{c}	0±0.0^{c}	0±0.0^{c}
100 mg/mL	0.0±0.0^{c}	1.1±0.5^{c}	11±3.8^{abc}	3.3±2.7^{bc}	4±1.2^{c}
200 mg/mL	0.0±0.0^{c}	12.3±2.8^{abc}	10±12.5^{a}	2.3±4.4^{abc}	12±2.7^{bc}
400 mg/mL	2.2±1.1^{bc}	4.7±8.3^{abc}	5.3±14.4^{ab}	7±6.2^{abc}	10±1.8^{bc}
1% ivermectin	1.9±1.1^{bc}	3.5±4.7^{a}	4.3±1.6^{ab}	23.3±10.3^{abc}	16.6±2^{bc}

Following an analysis of variance (ANOVA), Duncan's multiple range test reveals a significant difference between the means with letters in columns or rows ($p<0.05$). Each value is expressed as mean±standard error.

CONCLUSION

The current study concluded that the *Nerium oleander* showed a promising lethal effect against *Sarcoptes_scabiei var* cuniculi mite. Therefore, this extract could be used in pest management applications against ticks. More in vivo studies are needed to better evaluate the possibility of this extract, or some of their pure components, as useful alternatives for the treatment of external parasites.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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