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Short communication

Acaricidal activity of four fractions and octadecanoic acid-tetrahydrofuran-3,4-diyl ester isolated from chloroform extracts of neem (*Azadirachta indica*) oil against *Sarcoptes scabiei* var. *cuniculi* larvae *in vitro*

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ABSTRACT

Four fractions obtained from chloroform extracts of neem (*Azadirachta indica*) oil by column chromatography were investigated for acaricidal activity against *Sarcoptes scabiei* var. *cuniculi* larvae *in vitro*. Octadecanoic acid-tetrahydrofuran-3,4-diyl ester was isolated from an active fraction of the chloroform extract and its toxicity against *S. scabiei* larvae was tested *in vitro*. A complementary log–log model was used to analyse the toxicity data. Activity was found in the third fraction, with 100% corrected mortality after 4.5 h of exposure at a concentration of 200 mg ml^{-1}. This fraction was repeatedly re-crystallised in acetone to yield a white amorphous powder, identified as octadecanoic acid-tetrahydrofuran-3,4-diyl ester, with a median lethal concentration (LC_{50}) of 0.1 mg ml^{-1} 1 at 24 h post-treatment. The median lethal time (LT_{50}) for this compound was 15.3 h at a concentration of 7.5 mg ml^{-1}.

1. Introduction

Sarcoptes scabiei is an important veterinary ectoparasite in rabbits because of the possibility of zoonotic infection (Harrenstien et al., 1995) and causes considerable losses in weight, productivity, wool and fibre quality (Aiello et al., 1998). *S. scabiei* mites can be difficult to eliminate in rabbits compared to other domestic animals (Aiello et al., 1998). Despite the availability of chemotherapy, many side effects of chemical acaricides have prompted a search for new alternatives.

Neem oil extracted from the seeds of *Azadirachta indica* has versatile medicinal properties, including antifertility, antifungal, antibacterial, immunostimulant, antipyretic (Biswas et al., 2002) and acaricidal activities (Mulla and Su, 1999). As an acaricide, neem oil is effective against ticks (Williams and Mansingh, 1996; Ndumu et al., 1999; Kalakumar et al., 2000; Handule et al., 2002; Abdel-Shafy and Zayed, 2002; Al-Rajhy et al., 2003; Garboui et al., 2006), poultry red mites (Lundh et al., 2005) and *S. scabiei* (Dimri and Sharma, 2003; Sinha et al., 2004; Du et al., 2007). However, there are no reports on the isolation of compounds with acaricidal activity from neem oil. In a previous study, we found that chloroform extracts of neem oil exhibited potent acaricidal activity against *S. scabiei* var. *cuniculi* larvae (Du et al., 2008). Here we describe the acaricidal activity of four fractions and octadecanoic

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acid-tetrahydrofuran-3,4-diyl ester isolated from chloroform extracts of neem oil against *S. scabiei* var. *cuniculi* larvae *in vitro*.

2. Materials and methods

2.1. Plant material

Neem oil extracted from the seeds of *A. indica* A. Juss using CO_2 supercritical fluid was supplied by Green Gold Biological Science & Technology (Chengdu, P.R. China).

2.2. Extraction and isolation

Neem oil was successively extracted with petroleum ether, chloroform and ethyl acetate. Portions of each extract were dried under vacuum and assayed for acaricidal activity *in vitro*. Results revealed activity in the chloroform extract. Thus, this extract was fractionated by column chromatography over silica gel G (100–200 mesh) eluted with a hexane/ethyl acetate acid (8:2, v/v) mixture to afford four fractions with extraction yields of 2.6%, 46.4%, 19.2% and 4.6%, respectively. Each fraction was tested for its acaricidal activity. Fraction 3 was then repeatedly re-crystallised in acetone to yield a white amorphous powder.

The structure of the compound was identified by spectroscopic techniques including infrared (IR) spectroscopy, electron ionisation mass spectrometry (EI-MS), distortionless enhancement by polarisation transfer (DEPT), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and two-dimensional (2D) NMR spectroscopy.

2.3. Mite collection

S. scabiei mites were isolated from infected rabbits. Scabs collected from infested legs were placed in Petri dishes and transported to the laboratory within 2 h. The Petri dishes were then incubated at 35 °C for 30 min. Under a stereomicroscope, *S. scabiei* larvae have six legs that are easily distinguished from nymphs and from adults, which have eight legs, according to Walton and Currie (2007). Motile *S. scabiei* larvae were used in all experiments.

2.4. Assay of acaricidal activity in vitro

Acaricidal activity was assayed as previously reported (Fichi et al., 2007) with slight modification. Fractions 1–4 from the chloroform extract were diluted to 200 mg ml^{-1} with poly(ethylene glycol) (PEG) 400. Larval mites ($n = 20$) were placed in small polystyrene plates (8.5 mm in diameter and 0.3 mm deep) and 10 μl aliquots of fractions 1–4 were added to separate plates. Control larvae were treated with PEG 400 only. The study was performed in triplicate. All plates were placed in a humidity chamber (75% relative humidity) at 25 °C and observed under a stereomicroscope every 30 min. Each plate was observed for 5 min and then replaced in the test chamber. Persistent immobility of larval mites, even when stimulated with a needle, lack of reaction, was considered indicative of death (Macchioni et al., 2004).

2.5. Toxicity evaluation

The solution of the compound isolated from fraction 3 was diluted to concentrations of 7.5, 3.8, 1.9, 0.9 and 0.5 mg ml^{-1} with peanut oil. An aliquot of 10 μl of each solution was added to polystyrene plates containing 20 mites per plate. Peanut oil was used as the control. Each treatment was replicated three times. All plates were incubated at 25 °C and 75% relative humidity. The number of dead mites was counted every 4 h. Each plate was observed for 5 min and then replaced in the test chamber. The total incubation period was 32 h.

2.6. Statistical analyses

Mortality was corrected using the Abbott (1925) formula. The median lethal concentration (LC_{50}) and median lethal time (LT_{50}) values were calculated by the complementary log–log (CLL) model (Preisler and Robertson, 1989) using a special microcomputer program (Tang and Feng, 2002).

3. Results and discussion

Four fractions obtained from the chloroform extract of neem oil by column chromatography were tested for acaricidal activity against *S. scabiei* var. *cuniculi* larvae. Corrected mortality data for the four fractions are shown in Table 1. Compared to the control (PEG 400), fractions 1–4 from the chloroform extract were lethal to *S. scabiei* larvae. Both fractions 3 and 4 showed powerful acaricidal activity, with 100% corrected mortality after 4.5 h of exposure. However, fraction 3 exhibited higher activity than fraction 4 before 3.0 h of exposure. Fraction 3 was then repeatedly re-crystallised in acetone to yield a white amorphous powder.

The white powder was identified by spectroscopic techniques, including IR, EI-MS, DEPT, 1D and 2D NMR experiments, as octadecanoic acid-tetrahydrofuran-3,4-diyl ester (ODA-THF; Fig. 1). The structure of this acaricidally active compound is similar to that of a spermicidally active ester derivative (octadecanoic acid-4-palmitic acid-2,4-pentanediyl ester) isolated from neem oil (Yin et al., 2004).

Table 1
The corrected mortalities of the four fractions of the chloroform extract against *S. scabiei var. cuniculi* larvae *in vitro*.

Time (h)	Corrected mortality of the fractions of the chloroform extract (%)			
	Fraction 1	Fraction 2	Fraction 3	Fraction 4
1.0	0.0	0.0	62.5	32.5
1.5	2.4	5.4	67.6	40.5
2.0	5.2	8.6	71.4	37.1
2.5	5.3	14.7	76.5	50.0
3.0	5.5	15.6	84.4	71.9
3.5	19.2	29.6	88.9	92.6
4.0	23.8	42.3	92.3	96.2
4.5	36.6	61.5	100.0	100.0

Notice: The mortality of mites in PEG 400 was 11.7% at 4.5 h of exposure.