ANTHELMINTIC AND ANTIMICROBIAL PROPERTIES OF PEELS OF *CITRUS SINENSIS*

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Summary

The objective of the present investigation was to determine the anthelmintic and antimicrobial activity of petroleum ether extract of the peels of *Citrus sinensis*. Anthelmintic activity of this extract was evaluated on Indian adult earthworms, *Pheretima posthuma,* and exhibited a dose dependent inhibition of spontaneous motility (paralysis), and evoked responses to pin-prick, and the effects were comparable with that of piperazine citrate. The extract were also assayed for antimicrobial activity against various Gram positive organisms such as *Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis,* and Gram negative organisms such as *Escherichia coli, Pseudomonas vulgaris, Salmonella typhi,* and fungal strains *Aspergillus niger, and Candida albicans*. Antimicrobial activity was conducted by the agar well diffusion method. The extract showed varies levels of antimicrobial activity on different test microorganisms. Future studies are in process to isolate the active principles responsible for the activity.

Keywords: Petroleum ether extract, *Citrus sinensis* (Rutaceae), antimicrobial activity, anthelmintic activity, peels.

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Introduction

Citrus sinensis (*C. sinensis*) is a plant of the Rutaceae family native to Asia and India (1-3), which is widely cultivated all over the world. Its fruit, rich in vitamin C, is consumed fresh or industrialized as juice and is of great economical importance. Citrus fruits are commonly consumed because they contain a high amount of vitamins, minerals and antioxidant compounds, such as flavonoids. Flavonoids are a family of phenolic compounds that have many biological properties, including hepatoprotective, antithrombotic, antibacterial, antiviral and anticancer activity. These physiological benefits of flavonoids are generally thought to be due to their antioxidant and free radical scavenging properties (4).

Studies of the antimicrobial activities of flavonoids have become important because of the increasing occurrence of opportunistic systemic mycosis, as well as the rising prevalence of drug resistance in human pathogenic bacteria (5). Prevalence of human helminthiasis in India is as high as 70 to 80 % of which ascariasis is most common, and it exacts a heavy toll on human health and productivity. Although effective anthelmintics are currently available for most infections, the search of novel compounds is essential in view of the development of resistance as noted in animals (6,7). The objective of this work was to explore the anthelmintic and antimicrobial properties of peels of *C. sinensis.*

Materials and Methods

Plant material

Fruits were collected in the month of April from *C. sinensis* trees located in the Pavaikulam, Tamilnadu, India. They were immediately transferred to the laboratory and washed in tap water containing 0.01% detergent using a soft brush, rinsed with tap water, and finally rinsed with distilled water. Peels were excised from 50 fruits using a razor blade, which left the white spongy portion (albedo) on the fruit. The peels are authenticated by Prof. S. Srinivasan, Professor, Department of Botany, Madurai Kamaraj University. A Voucher Specimens deposited at the Museum of Department of Zoology.

Extraction and isolation

Fresh peels (300 g) were homogenized with 600 mL of petroleum ether for 5 min in a blender. The homogenate was placed in a 2 L Erlenmeyer flask. Three hundred milliliters of petroleum ether were added in the flask and further extracted three times for 10 min each in an ultrasonic bath at room temperature. The extract was filtered and evaporated under vacuum at 35 °C to dryness. The residue (2.22 g) was collected and stored at -20°C until use.

Test microorganism

The microorganisms used for the antimicrobial activity evaluation were obtained from the Microbiology Laboratory, Vel's Deemed University, Pallavaram, Chennai, India. They were *Staphylococcus epidermidis* SMC 65, *Micrococcus luteus* MLM 541, *Bacillus subtilis* BSCC 87, *Escherichia coli* ECM 453, *Pseudomonas vulgaris* PVS 01, *Salmonella typhi* TSP 501, *Candida albicans* CAS 22 and *Aspergillus niger* ANG 432.

Antimicrobial activity

The agar diffusion method was used for the antimicrobial activity evaluations (8). Wells of 8 mm diameter were punched into the Mueller-Hinton Agar (MHA, Merck), having the test microorganism and filled with 100 mg/ml of petroleum ether extract. The plates were incubated for 18 h at 37 ºC. Antimicrobial activity was evaluated by measuring the inhibition zone (including 8 mm diameter wells) against the test microorganisms. Standard antibiotic discs Ciprofloxacin (25 mg) and Griseofulvin (25 mg) was used as a reference.

Anthelmintic activity

The anthelmintic activity was assessed using adult indian earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being (9). The method of Dash et al. (10) was followed for anthelmintic screening. Groups are divided into seven, each group consisting of six earthworms of approximately equal size. Each group was treated with one of the following: vehicle (1% gum acacia in normal saline), piperazine citrate (10, 20, 50 mg/ml) and petroleum ether extract of *C. sinensis* (10, 20, 50 mg/ml) in normal saline containing 1% gum acacia. Observation was made

for the time taken to paralysis and/or death of individual worms up to four hours of test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.

Results and Discussion

In the search for compounds with anthelmintic activity, a number of substances have been screened using different species of worms, for example, earthworms, *Ascaris, Nippostrongylus* and *heterakis*. Of all these species, earthworms have been widely for the initial evaluation of anthelmintic compounds in vitro because they resemble intestinal worms in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic (11). In this study we have evaluated the effect petroleum ether extract of *C. sinensis* on earthworms. The results obtained are summarized in Table 1. It has been noted that petroleum ether extract showed comparable activity with that of standard piperazine citrate, a drug now widely used as anthelmintic, and in which the activity increased with concentration.

Table 1: Anthelmintic activity of petroleum ether extract of *Citrus sinensis*

Treatment	Time taken for paralysis (min)	Time taken for death (min)
Vehicle	-	-
Petroleum ether extract 10 mg/ml	88.50 ± 1.23	216.83 ± 2.76
20 mg/ml	52.16 ± 1.10	181.16 ± 1.02
50mg/ml	20.33 ± 1.41	54.43 ± 1.10
Piperazine citrate 10 mg/ml	99.10 ± 1.12	-
20 mg/ml	72.30 ± 1.40	-
50mg/ml	34.20 ± 0.49	-

Values represent the mean ± SD from six observations

Furthermore the antimicrobial activity of petroleum ether extract of *C. sinensis* was examined and found to exhibit good antibacterial activity against most of the Gram positive and Gram negative organisms which has been depicted in the Table 2. Among the test organisms the extract showed good antimicrobial activity against *Staphylococcus epidermis*, *Micrococcus luteus* and *Pseudomonas vulgaris*, and moderate activity against *Escherichia coli* and *Salmonella typhi*, and no activity against *Bacillus substilis*. The extract showed good antifungal activity against *Candida albicans* and no activity against *Aspergillus niger*. The result of the antimicrobial activity expressed in terms of diameter of zone of inhibition in millimeter. The performance of *C. sinensis* extract against sensitive bacteria isolates did not show difference when compared with established commercial antibiotics prepared with amoxicillin and ciprofloxacin (Table 2).

Table 2: Antimicrobial activity of petroleum ether extract of *Citrus sinensis*

Organisms	**Mean zone of inhibition (mm)**			
	Petroleum extract	Control	Ciprofloxacin (25 µg)	Griseofulvin (25 µg)
Staphylococcus epidermidis	26	-	30	NT
Micrococcus luteus	20	-	24	NT
Bacillus subtilis	-	-	27	NT
Escherichia coli	14	-	29	NT
Pseudomonas vulgaris	27	-	31	NT
Salmonella typhi	16	-	27	NT
Candida albicans	14	-	NT	17
Aspergillus niger	-	-	NT	12

NT – not tested

These results suggests the need for further studies on this extract to identify, isolate, characterize and elucidate the structure of the active ingredient(s) using some spectroscopic techniques such as infrared spectrometry, nuclear magnetic resonance spectroscopy and mass spectrometry.

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