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Isolation and Antifungal Evaluation of *Rumex cyprius* Murb Extracts

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Abstract: Ethanolic extract of dried *Rumex cyprius* Murb (polygonaceae) was tested against four pathogenic fungi: three dermatophytes, *Microsporum canis*, *Trichophyton mentagrophytes* and *T. rubrum*, and the causative agent of chalkbrood disease of bees, *Ascophaera apis*. The extract showed considerable activity against all these fungi. One of the main constituents of *Rumex cyprius* was isolated and tested for its antimycotic activity. It showed a significant activity against the test fungi and was identified as 1,3,8-trihydroxy-6-methylanthracene-9,10-dione based on its IR, UV-V and ^{1}H NMR spectra.

Key words: 1,3,8-trihydroxy-6-methylanthracene-9,10-dione, antifungal activity, medicinal plants, *Rumex cyprius* Murb.

1. Introduction

The use of medicinal herbs in the treatment of skin diseases including mycotic infections is an old-age practice in many parts of the world. Fungal infections remain a therapeutic problem despite the availability of a number of treatments. Being largely synthetic and non-biodegradable, these agents used in treating fungal infections can cause adverse effects and may have residual toxicity [1]. The evaluation of phytochemical constitution and their biological activities in medicinal plants is necessary for the development of new therapeutic agents. Novel chemical isolated from such plants with some biological activities may be used by the chemist as a guideline for the synthesis of useful drugs.

TAPHM (Traditional Arabic Palestinian Herbal Medicine) is widely practiced in the Palestinian Authority [2]. Cross-sectorial ethnopharmacological surveys conducted in this area revealed that a large number of indigenous plants are still used as sources of herbal therapies [3, 4]. Some of these herbal therapies, including *Rumex cyprius*, are used to treat skin diseases including dermatophytoses [5, 6]. At present, no laboratory data on the bioactivity of *Rumex cyprius* used to treat fungal skin diseases in TAPHM in Palestine exist. We hypothesized that the beneficial effect of this plant might be due to its antimycotic properties. Accordingly, we selected this plant used to treat these medical conditions and assessed its antifungal potential by measuring its ability to suppress the growth of selected fungi.

Rumex genus belongs to polygonaceae family and several species of this genus have indicated noteworthy therapeutic potentials. There are some reports in literature about evaluation of a few species of *Rumex* for various medicinal potential [7]. *Rumex cyprius* revealed antibacterial activity against methicillin resistant *Staphylococcus aureus*, which has shown relatively high resistant when exposed to reference antibiotics[8, 9]. It was also found to have maximum toxicity against *A. salina* [10], and antiviral activity

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against HIV through inhibition of reverse transcriptase [11].

This study was aimed at investigating the activity of *Rumex cyprius* extract against a selected number of dermatophytic fungi and the causative agent of chalkboard disease of bees. It was also aimed at the isolation and identification of any antifungal components that may be present in this plant.

2. Experiment

2.1 Plant Material

Whole mature plant was collected from Biet Lead village 15 km west Nablus during the summer 2009 and dried in the shadow. The plant was identified by Dr. M. S. Shtayeh in the BERC (Biodiversity and Environmental Research Center) and specimens of the plant were deposited at BERC herbarium.

2.2 Preparation

The authentic sample of 1,3,8-trihydroxy-6-methylanthracene-9,10-dione was purchased from Sigma Aldrich GmbH, Sternheim, Germany. All other chemicals and reagents employed in the present work were of analytical grade.

Melting point (mp) was taken on a BUCHI 530 apparatus. Column chromatography (cc) was carried out on a silica gel 60 (230-400 mesh; Merck). TLC (thin-layer chromatography) was performed on pre-coated silica gel F254 plates (Merck) using a 254-nm UV lamp to visualize the compounds. ^{1}H and ^{13}C NMR data were acquired at room temperature on a Varian 300 NMR spectrometer at 300 MHz and 75 MHz, respectively. Mass spectra were recorded on a JEOL JMS-300 spectrophotometer. IR spectra were recorded on a Shimadzu Forurier Transform Infrared Spectrophotometer FTIR-8700 using Nujol as mulling agent. UV-visible spectra were recorded on a Pye-Unicam SP-100 spectrometer with a quartz cell (1×1) cm. Elemental analysis was obtained using a Perkin-Elmer 2400 Elemental Analyzer.

2.2.1 Extraction and Isolation

The dried plant (100 g) was soaked in 95% ethanol for 5 days with continuous stirring. The extract was filtered. The solvent was removed from the extract under reduced pressure at 40 °C. The residue was denoted as "E1" and kept at 0 °C for further work. 1.5 g of the solid E1 was dissolved in 2 mL chloroform and subjected to column chromatography using silica gel chloroform–ethyl acetate (3:7) as eluent. A component "C" was obtained in a pure form as an orange long crystalline substance with an R_f value of 0.51 and melting point (mp) of 255-257 °C.

2.2.2 1,3,8-trihydroxy-6-methylanthracene-9, 10-dione [Component C]

IR spectrum: n (KBr) 3,420, 3,931, 2,857, 1,660, 1,473, 1,380, 1,229, 1,180, 1,127, 1,048, 932, 884, and 758 cm^{-1}.

^{1}H NMR spectra: ($CDCl_3$) δ 2.47 (3H, s, 3-Me), 6.67 (1H, d, J = 2.45, H-5), 7.15 (1H, br s, H-2), 7.26 (1H, d, J = 2.45, H-7), 7.56 (1H ,br s, H-4), 12.08 and 12.21 (2H, s, 1/8-OH).

UV-vi is λ (EtOH) nm ε: 218, 250, 262, 287 and 436.

Mass spectrometry: m/z (rel. int.): 270 (M+, 100).

Elemental analysis: Found: C, 66.12; H, 3.81. Calc. for $C_{15}H_{10}O_5$: C, 66.63; H, 3.65.

2.3 Test Organisms

The following fungi were used in this study: *Microsporum canis* Bodin, *Trichophyton rubrum* (castellani) Sabouraud, *T. mentagrophyte*s (Robin) Blanchard, and *Ascosphaera apis* (Olive Spiltoir) Maassan ex Claussen. The first three fungi are human pathogens causing dermatophytoses. The fourth fungus is an animal pathogen that causes chalkboard disease of

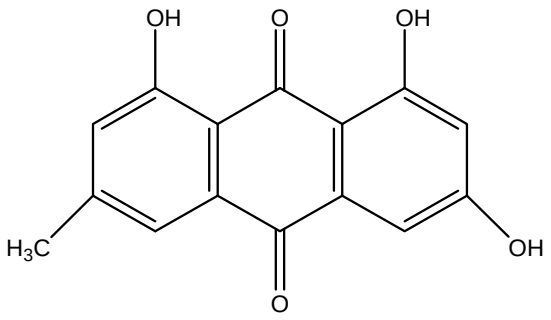

Fig. 1 Structure of 1,3,8-trihydroxy-6-methylanthracene-9, 10-dione.

bees. These fungal isolates were obtained from Prof. Ali-Shtayeh's fungal collection at the Department of Biology, An-Najah National University, Nablus. The isolates have been maintained under mineral oil in tubes on oat agar at room temperature.

2.3.1 Preparation of Samples for Testing

Each of extract E1 (100 mg) and component C (10 mg) were dissolved in 2 mL DMSO and the solution was sterilized using membrane filtration (0.45 μm millipore filters).

2.3.2 Antifungal Testing

The plant's extract E1 and component C were tested at different concentrations (Tables 1 and 2) for their antifungal activity against the test pathogens by a modified "poisoned food" technique [12]. The required amounts of each extract were mixed in requisite amount of pre-sterilized SDA (sabouraud dextrose agar) medium. A mycelial disk of 5 mm diameter, cut out from the periphery of 7 days old culture, was aseptically inoculated onto the medium. In controls, sterile DMSO was used in place of test extract. Three replicate agar plates were used for each treatment. The inoculated plates were used for each treatment. The inoculated plates were incubated at 24 °C and the observations were recorded after 7 days. Percentage of mycelial inhibition was calculated using the following formula [13]:

% of mycelial inhibition = [(colony diameter of control-colony diameter of treatment)/colony diameter of control] 100.

3. Results and Discussion

Only a few antifungal substances are known or available in the market as compared to antibacterial substances. Antimycotic substances are also relatively unsatisfactory in the control of dermatophytes. New, more powerful and specific antimycotic agents are needed to combat these infections. The discovery of active components exhibiting a broad spectrum antifungal activity may prove for the development of antifungal agents.

Tables 1 and 2 show the nature of fungi static

Fig. 2 Antimycotic activity of component C against *Ascosphaera apis*.

Table 1 Antifungal activity of the ethanolic extract (E1) of *Rumex cyprius*.

	% mycelial inhibition (mean ± SD)			
Fungi/concentration (mg/mL)	AsA*	MC	TR	TM
1.5	54 ± 1.48	67 ± 0.94	62 ± 2.17	73 ± 0.50
3.0	82 ± 0.82	100	100	100
6.0	100	100	100	100

*AsA: *A. apis*; MC: *M. canis*; TR: *T. rubrum*; TM: *T. mentagrophytes*.

Table 2 Antifungal activity of component "C".

	% mycelial inhibition (mean ± SD)			
Fungi/concentration (mg/mL)	Aa*	MC	TR	TM
0.05	42 ± 1.74	57 ± 2.48	45 ± 1.69	64 ± 3.62
0.10	55 ± 0.97	71 ± 1.59	59 ± 3.84	76 ± 2.94
0.20	81 ± 3.65	94 ± 2.79	78 ± 3.12	95 ± 1.66
0.40	100	100	100	100
Econazole	2.5-5 μg/mL			

*AsA: *A. apis*; MC: *M. canis*; TR: *T. rubrum*; TM: *T. mentagrophytes*.

activities encountered in *Rumex* extract and the pure component "C" compared with econazole as positive reference. The ethanolic extract "E1", and the pure component "C" all showed antimycotic activity against all tested fungi. Fungal toxicity (% mycelial inhibition) ranged from 82% in *Ascosphaera apis* (Fig. 2) to 100% inhibition in the other dematophytes at concentration of 3.0 mg/mL of extract "E1". Component "C" was clearly more efficient than extract "E1" inhibition test fungi; complete inhibition was achieved in all fungi at concentration of 0.4 mg/mL of component "C".

These results show that the use of *Rumex cyprius* Marb in combating fungal diseases of skin in folk medicine [4, 6] may be justified. Antifungal component(s) of *Rumex* may also prove useful for the control of the chalkboard disease, an economically important disease.

The chemical structure of the pure substance "C" was found to be 1,3,8-trihydroxy-6-methylanthracene-9, 10-dione based on its elemental analysis and IR, UV-V and ^{1}H NMR spectra .This was consistent with that of an authentic sample of 1,3,8-trihydroxy-6-methylanthracene-9,10-dione obtained from commercial source (Aldrich Chemical Company).

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