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*RESEARCH PAPERS*

# Antimicrobial activity of extracts from leaves, stems and flowers of *Euphorbia macroclada* against plant pathogenic fungi

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**Summary.** Extracts drawn from dried and powdered flowers, stems and leaves of *Euphorbia macroclada* with some organic solvents were tested for antimicrobial effect against the fungi *Verticillium dahliae*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Penicillium italicum*, *Rhizoctonia solani*, *Alternaria solani*, *Stemphylium solani*, *Cladosporium* sp., *Mucor* sp., and *Pythium* sp. The strongest inhibitory effect of the extracts was observed against *R. solani*, *V. dahliae*, *F. oxysporum*, *Pythium* sp. and *R. stolonifer*. The weakest effect was against *A. solani*. Extracts from the stems had a stronger inhibitory effect than those from the flowers or leaves. Butanol was the best solvent to extract antimicrobial compounds from leaves, stems and flowers and was superior to chloroform, water and petroleum ether. Results clearly indicate that *E. macroclada* is a promising source of antimicrobial compounds.

**Key words:** antifungal activity, Euphorbiaceae, antimicrobial compounds.

## Introduction

The genus *Euphorbia* belongs to the Euphorbiaceae, the sixth largest family among flowering plants. This genus alone accounts for one sixth of the whole group of flowering plants, with about 1000 species ascribed to it (Mabberley, 1987). Many of these species have been the subject of chemical and pharmacological investigation to determine any biological activity they might have. Some species that are used in folk medicine, such as *E. bougheii*, *E. striatella*, *E. serrata*, *E. virgata*, *E. fortissima* and *E. cooperi*, were found to cause skin irritation and promote tumours (Gundidza *et al.*, 1992; Gundidza and Kufa, 1993; Kinghorn and Evans, 1975; Upadhyay *et al.*, 1976; Upadhyay *et al.*, 1981; Upadhyay, *et al.*, 1984). Other *Euphorbia* species were reported to have antibacterial activity (Khan *et al.*, 1988; Vijaya *et al.*, 1995; Suthivaiyakit *et al.*, 2000).

This is the first study on the antimicrobial activity of *E. macroclada* against plant pathogenic fungi. We investigated the antimicrobial effects of twelve extracts of *E. macroclada* on ten fungi frequently isolated from crops, fruits or soils in Jordan and worldwide (Table 1).

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## Materials and methods

### Plant material

*Euphorbia macroclada* plants were collected from Al-Salt province in Jordan. The taxonomic identity of the plants was confirmed by comparing collected voucher specimens with *E. macroclada* specimens of known identity in the Herbarium of the Department of Biological Sciences, Faculty of Sciences, University of Jordan. A voucher specimen was also deposited in the research laboratory of Talal A. Aburjai at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan.

### Extraction of active compounds

*Euphorbia macroclada* plants were divided into three parts: leaves, stems, and flowers. Dried plant parts were powdered and each plant part was extracted separately three times with methanol (1.5 l) at room temperature. The combined extracts of each plant part were evaporated using a rotary evaporator at 40°C to give a concentrated crude extract for the leaves, one for the stems, and one for the flowers. Each crude extract was dissolved in water (1.5 l) and partitioned against petroleum ether (1.5 l), chloroform (1.5 l) and butanol (1.5 l), in that order. Each extract was then concentrated. Table 2 shows the weight of the plant material and that of each extract. Twelve extracts (marked with an asterisk in Table 2) were used in this study (Tables 3, 4 and 5).

### Fungi, media and testing of extracts

Fungi were collected from various locations in Jordan. Table 1 lists each fungus, the host and plant part from which it was isolated, isolate identification number, location and date of collection. All fungal isolates were identified by the author, and samples of each fungus were deposited in the fungal collection bank at the Department of Biotechnology of Al-Balqa' Applied University, Al-Salt, Jordan.

Fungal isolates were maintained on Potato Dextrose Agar (PDA, Difco Laboratories, Inc., Detroit, MI, USA), stored at room temperature and sub-

Table 1. Fungal isolates used to test the antifungal activity of extracts of *Euphorbia macroclada*.

| Fungus | Source | Plant part sampled | Isolate No. | Location in Jordan | Date collected |
|---|---|---|---|---|---|
| *Fusarium oxysporum* | Potato | Roots | 517 | Al-Balqa | June 1999 |
| *Rhizoctonia solani* | Cucumber | Roots and stem | 226 | Jerash | June 1999 |
| *Pythium* sp. | Thyme | Roots and stem | 580 | Yadoda | June 1999 |
| *Verticillium dahliae* | Tomato | Stems | 305 | Um Amad | August 1999 |
| *Alternaria solani* | Potato | Leaves | 070 | Jordan Valley | July 1999 |
| *Stemphylium solani* | Tomato | Stem and leaves | 245 | Jerash | June 1999 |
| *Rhizopus stolonifer* | Tomato | Fruit | 352 | Yadoda | June 1999 |
| *Penicillium italicum* | Bean | Stem and leaves | 234 | Al-Salt | June 1999 |
| *Cladosporium* sp. | Petunia | Stem | 250 | Jerash | August 1999 |
| *Mucor* sp. | Gerber | Roots | 568 | Baq'a | July 1999 |

Table 2. Plant parts, dry weight and weights of the extracts of *Euphorbia macroclada* used in this study.

| Plant part | Dry weight (g) | Crude methanol (g) | Petroleum ether extract (g) | Chloroform extract (g) | Butanol extract (g) | Water extract (g) |
|---|---|---|---|---|---|---|
| Leaves | 330 | 52.6 | 8.9* | 0.8* | 19.9* | 5.6* |
| Stems | 398 | 30.8 | 12.1* | 9.4* | 5.3* | 2.5* |
| Flowers | 410 | 39.5 | 10.7* | 15* | 7.2* | 4.4* |

The extracts tested for antifungal activity are marked with an asterisk.

cultured once a month (Deans and Svoboda, 1990). The medium (15 ml) was dispensed in sterile Petri dishes and allowed to cool down to ca. 50°C in a water bath before use. The isolates were grown for 7–10 days before use.

Testing of extracts was performed by the procedure described by Al-Mughrabi *et al*. (2001). Twelve extracts from *E. macroclada* (Table 2) were diluted with sterile distilled water (SDW) to give a final concentration of 1000 mg $l^{-1}$ each (Carter, 1968). The solution (2 ml) of each extract was evenly dispersed on PDA in the designated Petri dishes. Control dishes received 2 ml of SDW each. Plates were left overnight for the solutions to be absorbed through the media.

Using a 10-cm-long, 5-mm-diameter spring-loaded plunger, a plug of inoculum from the actively growing margin of a Petri culture of each fungal isolate (Table 1) was placed in the centre of each Petri dish with the mycelium face down. Each isolate for each extract was inoculated onto four dishes and incubated for 9 days at room temperature (ca. 22°C). Four control dishes receiving SDW only were run along with each fungal isolate and crude extract. The same procedure was followed as in the test samples.

Starting two days after inoculation, radial growth was marked every day for 7 days or until the plates were overgrown, whichever came first. The percentage of growth inhibition caused by each crude extract was calculated as follows: % inhibition = [(growth in control – growth in sample)/(growth in control) × 100]; where growth was expressed as colony diameter in mm (Daouk *et al*., 1995). The values shown for percent inhibition (Tables 3, 4 and 5) were the means of four determinations each. Pooled average percent inhibition values and standard errors were also calculated and are shown.

## Results and discussion

Extracts from the stems, flowers and leaves of *E. macroclada* had antimicrobial effects on all fungi under study. In general, extracts from stems exhibited stronger antimicrobial activity (mean pooled average value 39.7%) than extracts from flowers (mean pooled average value 35.3%). Extracts from leaves were less effective (mean pooled average value 2.7%).

Butanol extracts provided the strongest inhibition of all fungi tested: 55.1, 54 and 5% for flowers, stems and leaves respectively.

Stems extracted with butanol had the strongest antimicrobial effect (54%), followed by stems extracted with chloroform (51.4%), water (27.6%) and petroleum ether (25.8%). Flowers extracted with butanol showed the greatest antimicrobial activity (55.1%), compared to flowers extracted with chloroform (34.5%), water (30.7%) or petroleum ether (20.9%). Leaves extracted with butanol were the most effective in inhibiting fungal growth (59%), followed by leaves extracted with water (3.2%), petroleum ether (1.7%) and chloroform (0.8%).

*Pythium* sp. was inhibited at various degrees (7–77.5%) by all extracts except extracts from flowers obtained with chloroform and petroleum ether, stem extracts obtained with petroleum ether, and leaf extracts produced with chloroform, butanol and water. The greatest inhibition of *Pythium* sp. was with flower extracts obtained with water (77.5%); and stem extracts obtained with butanol (77.3%) (Tables 3 and 4). *V. dahliae* was inhibited at various degrees (16.3–100%) by all extracts from flowers and stems, except those obtained from flowers with petroleum ether. Leaf extracts did not inhibit *V. dahliae*. Flower extracts obtained with chloroform, petroleum ether and butanol, and stem extracts obtained with chloroform gave complete inhibition of *F. oxysporum*. Leaf extracts were only moderately effective against *F. oxysporum* (Table 5), and flower and stem extracts were more effective (Tables 3 and 4). *P. italicum* growth was inhibited by all stem extracts except those extracted with water. Chloroform and petroleum ether flower extracts, and petroleum ether leaf extracts were not effective against *P. italicum*. The inhibition achieved by this fungus was greatest when it was extracted from flowers using butanol (75.7%) (Table 3).

The lowest percent inhibition overall was achieved against *A. solani* (Tables 3, 4 and 5). This fungus was not inhibited by flower extracts obtained with chloroform, petroleum ether or butanol, nor by stem extracts obtained with water, nor by leaf extracts obtained with chloroform or water. The greatest inhibition of *A. solani* (16.9%) was with stem extracts obtained with butanol.

Only extracts from flowers and leaves obtained

Table 3. Inhibition (%)[a] of fungal growth with extracts from flowers of *Euphorbia macroclada*.

| Fungus | Solvent | | | | Pooled average |
|---|---|---|---|---|---|
| | Chloroform | Petroleum ether | Butanol | Water | |
| *Verticillium dahliae* | 100 | 0.00 | 100 | 16.3±2.1 | 54.1 |
| *Fusarium oxysporum* | 100 | 100 | 100 | 0 | 75 |
| *Rhizoctonia solani* | 87.1±2.9 | 88.8±0.4 | 91.7±0.1 | 89.6±0.4 | 89.3 |
| *Penicillium italicum* | 0 | 0 | 75.7±2.8 | 40±3.9 | 28.9 |
| *Rhizopus stolonifer* | 47±2.1 | 1.6±0.1 | 100 | 64.5±1.6 | 53.3 |
| *Stemphylium solani* | 7.6±0.8 | 3.6±0.4 | 2±0.3 | 4.8±0.8 | 4.5 |
| *Alternaria solani* | 0 | 0 | 0 | 0.5±0.1 | 0.1 |
| *Cladosporium* sp. | 3.4±0.7 | 14.8±2.1 | 17±0.2 | 5.2±0.5 | 10.1 |
| *Mucor* sp. | 0 | 0 | 14.5±2.0 | 8.7±1.0 | 5.8 |
| *Pythium* sp. | 0 | 0 | 50.2±6.4 | 77.5±1.2 | 31.9 |
| Pooled average | 34.5 | 20.9 | 55.1 | 30.7 | |

[a] Percentage of inhibition is the mean ± SE of four determinations per fungus per extract.

Table 4. Inhibition (%)[a] of fungal growth with extracts from stems of *Euphorbia macroclada*.

| Fungus | Solvent | | | | Pooled average |
|---|---|---|---|---|---|
| | Chloroform | Petroleum ether | Butanol | Water | |
| *Verticillium dahliae* | 100 | 100 | 100 | 25.2±2.1 | 81.3 |
| *Fusarium oxysporum* | 100 | 0 | 0 | 0 | 25 |
| *Rhizoctonia solani* | 84.6±0.4 | 25±1.7 | 100 | 76.7±5.8 | 71.6 |
| *Penicillium italicum* | 62.9±4.4 | 56.1±0.4 | 59.2±2.9 | 0 | 44.6 |
| *Rhizopus stolonifer* | 91.7±8.3 | 31.3±4.8 | 91.1±0.4 | 10.4±0.1 | 56.1 |
| *Stemphylium solani* | 2.8±0.4 | 4.9±0.6 | 47.1±1.7 | 43.0±4.1 | 24. 5 |
| *Alternaria solani* | 0 | 7.5±0.9 | 16.9±3.1 | 3.6±0.8 | 7 |
| *Cladosporium* sp. | 3.3±0.9 | 33.4±1.7 | 48.3±3.4 | 4.8±0.6 | 22. 5 |
| *Mucor* sp. | 0 | 0 | 0 | 48.9±4.2 | 12.2 |
| *Pythium* sp. | 68.5±4.4 | 0 | 77.3±2.5 | 63.7±1.4 | 52.4 |
| Pooled average | 51.4 | 25.8 | 54 | 27.6 | |

[a] See Table 3.

Table 5. Inhibition (%)[a] of fungal growth with extracts from leaves of *Euphorbia macroclada*.

| Fungus | Solvent | | | | Pooled average |
|---|---|---|---|---|---|
| | Chloroform | Petroleum ether | Butanol | Water | |
| *Verticillium dahliae* | 0 | 0 | 0 | 0 | 0 |
| *Fusarium oxysporum* | 4.6±0.3 | 7.5±0.6 | 14.8±1.1 | 2.7±0.2 | 7.4 |
| *Rhizoctonia solani* | 0 | 0 | 0 | 0 | 0 |
| *Penicillium italicum* | 0.2±0.1 | 0 | 17.2±1.3 | 1.3±0.1 | 4.8 |
| *Rhizopus stolonifer* | 0 | 1.3±0.1 | 0.2±0.1 | 0 | 0.4 |
| *Stemphylium solani* | 2.9±0.2 | 0.4±0.1 | 10.2±0.5 | 18.8±0.4 | 8.1 |
| *Alternaria solani* | 0 | 0.7±0.1 | 5.9±0.2 | 0 | 1.6 |
| *Cladosporium* sp. | 0 | 0 | 0 | 0 | 0 |
| *Mucor* sp. | 0 | 0 | 1.7±0.1 | 9.6±0.3 | 2.8 |
| *Pythium* sp. | 0 | 7±0.2 | 0 | 0 | 1.8 |
| Pooled average | 0.8 | 1.7 | 5 | 3.2 | |

[a] See Table 3.

with butanol and water and from stems extracted with water were effective against *Mucor* sp. (Tables 3, 4 and 5). The strongest inhibition of this fungus (48.9%) was with stem extracts obtained with water.

Complete inhibition of *R. solani* was obtained with butanol stem extracts. A high level of inhibition (>87%) against this fungus was also obtained with flower extracts, but leaf extracts were ineffective.

Inhibition of *S. solani* was low with all extracts, the greatest inhibition being nevertheless achieved with stem extracts obtained with butanol (47.1%) and water (43%).

The greatest inhibition of *R. stolonifer* was achieved with stem extracts (pooled value 56.1%), followed by flower extracts (pooled value 53.3). Leaf extracts were not effective against this fungus (Tables 3, 4 and 5). Butanol stem and flower extracts were the most effective against *Cladosporium* sp. (48.3 and 17% respectively). All stem and flower extracts were effective against this fungus, but leaf extracts were not effective.

Butanol was the most effective solvent in extracting antimicrobial compounds from the leaves, stems and flowers of *E. macroclada*. Water was moderately effective, and petroleum ether the least effective, and therefore is not recommend as a solvent for obtaining extracts.

In this first study on the antimicrobial activity of *E. macroclada* against plant pathogenic fungi, flower and stem extracts proved a promising source of antimicrobial compounds. Variations in the antifungal effectiveness of different extracts against different organisms was most likely due to differences in the nature of the inhibitory materials they contained. Different compounds have been isolated and purified from different *Euphorbia* species. A lectin was purified from *E. neriifolia* latex (Seshagirirao and Prasad, 1995), diterpene esters from the roots of *E. prolifera* (Wu *et al*., 1995), macrocyclic diterpenoids from *E. semiperfoliata* (Appendino *et al*., 1998) and diterpenoids and jatrophane diterpenoids from *E. peplus* (Hohmann *et al*., 1999; Hohmann *et al*., 2000).

The higher plants may play an important role in controlling many plant diseases, including those of other higher plants (Fawcett and Spencer, 1970; Bhargava *et al*., 1981). Plants produce natural chemicals that are possible sources of non-phytotoxic, systemic and readily biodegradable alternative pesticides (Fawcett and Spencer, 1970) and the extracts of many plant species have antifungal activity (Osborn, 1943; Spencer *et al*., 1957; Dixit and Tripathi, 1975; Misra and Dixit, 1976; Lapis and Dumancas, 1978; Chaudhuri, 1982; Franje, 1984; Guesin and Reveillere, 1984; Mahmood, 1985; Akhtar, *et al*., 1986; Asthana, 1986; Al-Bana and Hijazi, 1987; Chaturvedi, 1987; Deans and Svoboda, 1990; Al-Abed *et al*., 1993). This is the first paper reporting the antifungal activity of *E. macroclada* crude extracts.

The findings suggest that *E. macroclada* is a potential source of compounds that are effective against many fungi (Table 1). Further studies on *E. macroclada* are recommended to identify the antifungal compounds. Identifying such compounds was beyond the scope and purpose of this study.

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