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Antifungal activity of volatile components extracted from leaves, stems and flowers of four plants growing in Tunisia

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Summary. Volatile components extracted from the leaves, stems and flowers of *Lantana camara, Malvaviscus arboreus, Hibiscus rosa-sinensis* cv. red flowers and white flowers were tested against the fungi *Alternaria solani*, *Botrytis cinerea*, *Fusarium solani* f. sp. *cucurbitae*, *F. oxysporum* f. sp. *niveum*, *Pythium ultimum*, *Rhizoctonia solani* and *Verticillium dahliae*. The strongest inhibitory effect of the extracts was found with volatile components extracted from the stems and the flowers. Complete inhibition was achieved against *V. dahliae*. The weakest effect was against *P. ultimum*. Volatile components extracted from the leaves were not effective.

Key words: Malvaceae, Verbenaceae, antimicrobial components.

Introduction

Wild plants are widespread in Tunisia, where they represent a very rich and characteristic flora (Pottier-Alapetite, 1979, 1981). Many of these plants are used as natural medicines.

In order to discover new biologically active compounds that can be used to control disease, many wild plants have been in the past, and still are today, the subject of chemical investigations. For example, a number of species that are used in folk medicine, such as *Euphorbia macroclada*, *E. bougheii*, *E. striatella*, *E. serrata*, *E. virgata*, *E. fortissimo* and *E. cooperi*, have also been found to cause marked suppression of root infecting fungi including *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani* and *Verticillium dahliae* (Gundidza *et al*., 1992; Gundidza and Kufa, 1993; Shaudat and Siddiqui, 2002; Al-Mughrabi, 2003). *Chrysanthemum coronarium* has been found to be active against *Aspergillus* spp., *Pythium ultimum*, *Fusarium moniliforme*, *Sclerotinia sclerotiorum* and *Botrytis cinerea*. Other species, such as *Lantana camara*, have been reported to have antibacterial activity (Khan *et al*. 1988; Vijaya *et al*. 1995; Junior and Zani, 2000; Sutthivaiyakit *et al*. 2000). *Lantana camara*, *Hibiscus rosa-sinensis* (cv. red and white flowers) and *Malvaviscus arboreus* are widely distributed in Tunisia and are commonly used as ornamental plants all over the country. These species have not hitherto been the subject of biological

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and chemical investigation in Tunisia. However these plants when collected from other countries were the source of several metabolites, including some that were biologically active. Previous studies on *L. camara* reported on the chemical composition of the essential oil of this plant. This oil contains 25 components, the most important of which are sabinene (16.5–7.3%), β-caryophyllene (14.0–22.5%), 1,8-cineole (10.0–6.0%), bicyclogermacrene (8.1–8.4%) and α-humulene (6.0–10.8%). These volatiles have been found to possess antimicrobial and antimutagenic properties as well as being biologically active against tics (*Amblyoma variegatum*) and some fungi (Deena and Thoppil, 2000; Alitonou *et al*., 2004). The effect of hydroalcoholic extract of *L. camara* leaves on fertility, general reproductive performance and teratology in rats have also been studied (Mollo *et al*., 2005). This plant was further reported to be a source of some bioactive pentacyclic triterpenoids: lantadenes A-D, of which lantaden C was found to be a hepatotoxicant (Sharma *et al*., 1992; Sharma *et al*., 2000).

For the genus *Hibiscus*, the essential oil of *H. cannabinus* was rich in (*E*)-phytol (28.16%), (*Z*)-phytol (8.02%), n-nonanal (5.7%), benzene acetaldehyde (4.39%), (*E*)-2-hexanal (3.10%) and 5-methylfurfural (3%) and had antifungal activity against *Colletotrichum fragaria*, *C. gloeosporioides* and *C. acutatum* (Kobaisy *et al*., 2001).

To further research on *L. camara*, *H. rosa-sinensis* and *M. arboreus* and if possible to encourage cultivation of these species in Tunisia, in the present work the antifungal activity of volatile extracts from various air organs of these plants was studied. As target organisms 7 fungal species were used that are frequently isolated from crops, fruits or soil in Tunisia.

Material and methods

Plant material

Four plants were tested in the study: *Lantana camara, Malvaviscus arboreus, Hibiscus rosa-sinensis* cv. red flower and *Hibiscus rosa-sinensis* cv. white flower. The plants were collected and identified according to their morphological characteristics. Identification was confirmed by comparing collected voucher species with species of known identity.

Extraction of volatile components

Each plant was divided into three parts: leaves, stems and flowers. An appropriate amount (Table 1) of each plant sample was subjected to steam-distillation for 3–5 h. Five hundred ml of each aqueous distillate was subjected to two successive extractions with chloroform ($CHCl_3$) (2×100ml). After decantation and separation, the recuperated organic layer containing volatiles was dried over anhydrous sodium sulfate, then filtered and desiccated under reduced pressure.

Fungal isolates and testing of extracts

The fungi used in the biological assays were collected from various locations and hosts in Tunisia (Table 2). All fungal isolates were identified and samples of each fungus were deposited in the collection bank at the Plant Pathology Laboratory (Ecole Supérieure d'Horticulture et d'Elevage d'Horticulture, Sousse, Tunisia).

Fungal isolates were maintained on potato dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI, USA), stored at room temperature and subcultured once a month (Deans and Svoboda, 1990) when needed. The isolates were grown for 7–10 days on PDA at 25°C.

Extracts were tested as in Al-Mughrabi *et al*. (2001). Twelve volatile components extracted from *L. camara, M. arboreus, H. rosa-sinensis* cv. red flower and white flower (Table 1) were diluted with sterile distilled water (SDW) to give a final concentration of 1000 mg l^{-1} for each component (Carter, 1968). Two milliliters of each solution of each extract was evenly dispersed on PDA in the appropriate Petri dishes. Control dishes received 2 ml of SDW each. Plates were left overnight for the solutions to be absorbed through the medium.

A 6-mm-diameter plug of inoculum taken from the actively growing margin of a colony of each isolate was placed in the centre of each Petri dish with the mycelium face down. Each isolate for each volatile component was inoculated on five dishes and incubated at 22°C for 8 days. Five control dishes were run along with each fungal isolate and volatile fraction and tested in the same way.

Radial growth was marked every day, starting two days after incubation, for 7–9 days or until the dishes were overgrown. The percent growth inhibition caused by each volatile component 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 percent inhibition values were the means of five replications. Pooled average percent inhibition values and standard errors were also calculated.

Results and discussion

Volatile components extracted from the leaves, stems and flowers of the four plants tested in this work had antifungal effects on the majority of fungi under study. In general, extracts from flowers exhibited stronger antifungal activity (pooled average from 38 to 41.2%) than extracts from the stems (pooled average between 23.1 and 29.8%) or the leaves (15.7 to 27.1%).

Volatile components extracted from the flowers of *L. camara* had the strongest antifungal effect (38%), followed by extracts from the leaves (27.1%) and stems (26.6%) (Table 3). This antifungal activity could be due to the presence of some terpenic components such as sabinene, β-caryophyllene, 1,8-cineole, bicyclogemacrene and α-humilene, which are the main constituents of the oils (Sefidkon, 2002).

Table 1. Plant parts, dry weight, weights and yield of the extracts of the four plants used in this study.

Plant part and species	Dry weight (g)	Weight of volatile fraction (mg)	Yield (%)
Lantana camara			
Leaves	611	41	6.7×10^{-3}
Stems	635	20	3.1×10^{-3}
Flowers	505	21	4.1×10^{-3}
Hibiscus rosa-sinensis cv. *red flower*			
Leaves	1908	34	1.7×10^{-3}
Stems	625	13	2.0×10^{-3}
Flowers	630	36	5.7×10^{-3}
Hibiscus rosa-sinensis cv. *white flower*			
Leaves	523	10	1.9×10^{-3}
Stems	525	23	4.3×10^{-3}
Flowers	2572	5	0.8×10^{-3}
Malvaviscus arboreus			
Leaves	860	43	5.0×10^{-3}
Stems	1255	10	0.7×10^{-3}
Flowers	260	15	5.9×10^{-3}

Table 2. Fungal isolates used to test the antifungal activity of extract plants.

Fungus	Source	Plant-part sampled	Location	Collection date
Alternaria solani	Tomato	Leaves	Chott mariem	12/11/2002
Botrytis cinerea	Vine	Fruit	Bou Argoub	11/05/2000
Fusarium oxysporum f. sp. *niveum*	Watermelon	Roots	Skhira	23/05/2001
F. solani f. sp. *cucurbitae*	Watermelon	Roots	Skhira	23/05/2001
Pythium ultimum	Watermelon	Roots and stem	Regueb	23/05/2001
Rhizoctonia solani	Watermelon	Roots and stem	Jebeniena	05/07/2001
Verticillium dahliae	Potato	Roots	Boufícha	03/11/1999

Hibiscus rosa-sinensis cv. red flower and white flower showed respectively the highest pooled inhibition average for volatile component extracts from flowers (38.8 and 41.2%), with lower values for extracts from stems (29.8 and 25.4%) and leaves (18.4 and 19.9%) (Tables 4 and 5). The present study is the first to examine the chemical composition of the volatile components of the different organs of this plant. However, essential oil of the leaves of *H. cannabinus* L. has been investigated: it had significant fungitoxic activity, probably due to its major constituents such as (*Z*) and (*E*)-phytol, n-nonanal, benzene acetaldehyde, 2-hexenal and 5-methylfurfural (Kobaisy *et al*. 2001).

Table 3. Inhibition (%) of fungal growth with different parts of *Lantana camara*.

Fungus	Leaves	Stem	Flowers	Pooled average
Alternaria solani	28.0±2.5[a]	12.0±0.8	28.9±3.0	23.0
Botrytis cinerea	31.3±3.0	33.0±2.8	44.0±.2	36.1
Fusarium oxysporum f.sp. *niveum*	27.5±1.9	12.4±0.8	32.4±3.0	24.1
F. solani f.sp. *cucurbitae*	16.0±2.1	13.5±2.0	26.3±3.0	18.6
Pythium ultimum	0	0	0	0
Rhizoctonia solani	23.0±2.3	15.0±1.1	34.0±2.0	24.0
Verticillium dahliae	64.0±3.4	100	100	88.0
Pooled average	27.1	26.6	38.0	

[a] Inhibition percent is the mean ± standard error of five determinations per fungus and per volatile component.

Table 4. Inhibition (%) of fungal growth with different parts of *Hibiscus rosa-sinensis* cv. red flower.

Fungus	Leaves	Stem	Flowers	Pooled average
Alternaria solani	37.0±1.0[a]	33.5±0.8	39.3±2.4	36.6
Botrytis cinerea	28.3±0.7	23.2±0.9	44.8±3.0	32.1
Fusarium oxysporum f. sp. *niveum*	24.6±2.0	29.8±3.1	32.4±3.0	28.9
F. solani f. sp. *cucurbitae*	15.4±0.5	16.8±0.9	26.5±3.2	19.6
Pythium ultimum	0	0	0	0
Rhizoctonia solani	0	19.8±0.5	26.0±1.4	15.3
Verticillium dahliae	23.4±0.8	75.3±2.0	100	66.2
Pooled average	18.4	29.8	38.4	

[a] See Table 3.

Table 5. Inhibition (%) of fungal growth with different parts of *Hibiscus rosa-sinensis* cv. white flower.

Fungus	Leaves	Stem	Flowers	Pooled average
Alternaria solani	28.0±1.3[a]	25.2±0.9	30.3±2.1	27.8
Botrytis cinerea	35.0±3.1	27.0±0.9	29.8±1.2	30.6
Fusarium oxysporum f. sp. *niveum*	30.8±2.0	24.0±1.3	56.6±3.2	37.1
F. solani f. sp. *cucurbitae*	25.0±1.0	12.3±0.3	28.0±1.5	21.8
Pythium ultimum	0	0	11.4±1.1	3.8
Rhizoctonia solani	23.5±1.3	18.5±0.7	27.0±1.2	23.0
Verticillium dahliae	0	68.0±4.6	100	56.0
Pooled average	19.9	25.4	41.2	

[a] See Table 3.

Table 6. Inhibition (%) of fungal growth with different parts of *Malvaviscus arboreus*.

Fungus	Leaves	Stem	Flowers	Pooled average
Alternaria solani	35.2±3.4[a]	36.4±4.5	28.3±1.2	33.3
Botrytis cinerea	25.0±1.4	34.8±3.5	0	20.0
Fusarium oxysporum f. sp. *niveum*	31.0±2.0	31.0±1.8	13.5±0.7	25.2
F. solani f. sp. *cucurbitae*	11.0±0.3	30.2±3.1	12.0±1.5	17.7
Pythium ultimum	0	0	0	0
Rhizoctonia solani	23.0±2.0	29.6±2.4	0	17.5
Verticillium dahliae	15.8±0.6	0	0	5.3
Pooled average	15.7	23.1	7.9	

[a] See Table 3.

As regards *Malvaviscus arboreus*, the pooled average of volatile components extracted from the stems of this plant was more strongly antifungal than the extract from the leaves. The extract from the flowers was least effective (pooled average 7.9%) (Table 6).

The metabolites responsible for such activity are not known at present because the chemical composition of the volatile extracts of the different organs of this plant was not studied before. However, certain fatty acids have been identified by GC-MS and these acids could be ingredients of the volatile fractions mentioned above (Carballeira and Cruz, 1997).

Complete inhibition of *V. dahliae* was achieved with stem and flower components extracted from *L. camara* and with flower components from *H. rosa-sinensis* cv. red flower and white flower. A high level of inhibition (75.3%) against *V. dahliae* was also obtained with the volatile component from the stems of *H. rosa-sinensis* cv. red flower, and with leaves of *Lantana camara* (64.0%). The volatile component extracted from *M. arboreus* leaves exhibited a low inhibition percentage (15.8%), while the components from the stems and flowers were ineffective against *V. dahliae*.

The lowest percent inhibition overall was achieved against *Pythium ultimum* (Tables 3, 4, 5 and 6). This fungus was not inhibited by the leaves, stems or flower extracts of any of the four plants tested, with the exception of flower extract from *H. rosa-sinensis* cv. white flower, which had a low inhibitory effect (11.4%).

Fusarium solani f. sp. *cucurbitae* was strongly inhibited by volatile components from the flowers of *L. camara* (26.3%) and *Malvaviscus arboreus* (30.2%), by the stem components of *H. rosa-sinensis* cv. white flower (28%) and of *H. rosa-sinensis* cv. red flower (26.5%) (Tables 3, 4, 5 and 6).

The strongest inhibition of *F. oxysporum* f. sp. *niveum* was obtained with flower components extracted from *L. camara* (32.4%) and *Malvaviscus arboreus* (31%), and with stem components of *H. rosa-sinensis* cv. white flower (56.6%) and *H. rosa-sinensis* cv. red flower (32.4%) (Tables 3, 4, 5 and 6).

Botrytis cinerea was strongly inhibited by all extracts (44%) except flower extract from *H. rosa-sinensis* cv. white flower. *Alternaria solani* and *R. solani* were moderately inhibited by all extracts, with inhibition varying from 26 to 39.33% (Tables 3, 4, 5 and 6).

The results of the study show that *L. camara*, *H. rosa-sinensis* and *M. arboreus* may be promising sources of antimicrobial compounds to be used as alternative pesticides in the control of plant diseases.

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