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Tables: 3, Fig : -

# Screening of native plants of central Argentina for antifungal activity

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## ABSTRACT

Ethanolic extracts from aerial parts of 71 native plants from Argentina were tested by bioautography on Thin Layer Chromatography (TLC) for their antifungal effects against pathogenic *Fusarium verticillioides*. The extracts of *Aristolochia argentina* (Aristolochiaceae), *Flourensia oolepis* (Asteraceae), *Gaillardia megapotamica* (Asteraceae), *Salvia cuspidata* (Lamiaceae) and *Trichocline reptans* (Asteraceae) at Minimum Inhibitory Concentrations (MICs) of 0.03 to 0.12 mg/spot were highly effective in inhibiting the fungal growth followed by extracts from *Baccharis artemisioides* (Asteraceae), *Baccharis salicifolia* (Asteraceae), *Dalea elegans* (Fabaceae), *Heterothalamus alienus* (Asteraceae), *Lepechinia floribunda* (Lamiaceae), *Vernonia nudiflora* (Asteraceae) and *Zanthoxylum coco* (Rutaceae) at MICs of 0.25 - 0.50 mg/spot. While, *F. oolepis*, *T. reptans* and *A. argentina* extracts proved most potent, with MICs of 0.03, 0.03 and 0.06 mg/spot, respectively. Further studies are required with extracts of the most active species in bioguided assay isolation to obtain new molecules with potent antifungal properties to control harmful fungi such as *F. verticillioides*.

**Key words**: Antifungal activity, Argentinian plants, *Aristolochia argentina, Flourensia oolepis, Fusarium verticillioides, Trichocline reptans,* TLC.

## INTRODUCTION

The antifungal resistance of fungal pathogens to plants, humans and animals is one of the best-documented cases of biological evolution (16). This resistance has rendered most commercially available antifungal agents ineffective (21,36) resulting in serious problems in agriculture. Intensive efforts are being made to discover new chemical structures, which are active against fungi with different modes of action than present ones and are also safe for humans and the environment. Plants synthesize a wide array of secondary metabolites, for self defence against microbial pathogens (39). Plants are an inexhaustible source of active compounds with remarkable antifungal properties. Although many plant extracts have shown their fungicidal activity (10,17,23), but only a small fraction of the known plant species have been investigated for this property (18). It is also true for the native flora of central Argentina.

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This study was done in the project to find the antifungal extracts of native/ naturalized plants in Argentina (5-8). Those extracts showing high activity could be suitable subjects for the subsequent isolation of compounds with antifungal activity. Córdoba Province, Argentina has large area under hills and mountains with a great diversity of native plants, which have been little investigated for their chemical composition or bioactivity. These little-explored plant species are potential source of antifungal compounds or leads for the synthesis of new molecules with fungicidal activity. Seventy-one extracts of these plants (native and naturalized to central Argentina) were screened to select those with highest antifungal activity. Extracts obtained from 71 plant species collected in Córdoba were screened for their growth inhibitory effect on *Fusarium verticillioides*, a pathogenic fungus responsible for ear, stalk and kernel rots in maize and for the production of toxigenic fumonisins (38). The presence of the fungus or its toxins is particularly damaging in developing countries where maize and maize-based products are the staple food for large populations. Available information about the biological activity, uses in traditional medicine and chemical composition of the most effective plants is also reported.

## MATERIALS AND METHODS

Plants (Table 1) were collected from the hills of Córdoba Province, Argentina, from November 2005 to March 2007. Voucher specimens have been deposited in the Herbarium "Marcelino Sayago" of the School of Agricultural Science, Catholic University of Córdoba and were identified by the botanist, Gustavo Ruiz. Plants were selected according to their availability, accessibility and in most cases the lack of scientific information about their activity and/or chemical pattern. Crushed dried aerial plant material was extracted for 48 h with ethanol. After solvent removal, a viscous extract was obtained from each plant.

### Antifungal assay

Isolates of *Fusarium verticillioides* from maize (strain M-7075) were maintained as a monosporic culture in a V8 juice medium. Fungi was supplied by Dr. S. Chulze, Cátedra de Microbiología, Facultad de Ciencias Físicas, Exactas y Naturales, Universidad Nacional de Río Cuarto. To determine the antifungal activity of each extract, direct bioautography was done on thin layer chromatography (TLC) as per Carpinella *et al.* (6, 7). Each extract dissolved in ethanol was applied on TLC plates at the volume needed to reach a final dose of 0.01- 2.0 mg/spot. Controls with and without ethanol were also used. The fungicide mancozeb was used as positive control. Minimum Inhibitory Concentrations (MICs) were determined as the lowest extract concentration showing no visible fungal growth on spots.

**Chemical Composition:** The presence of chemicals (such as alkaloids, flavonoids and lignans) was assessed in the most effective extracts as per Wagner and Bladt, 1996 (42).

**Chemicals and materials:** Mancozeb [ethylenebis (dithiocarbamic acid) manganese zinc complex] technical grade was purchased from Riedel-de Haën Company. Silica gel plates (silicagel 60 F254) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade.

## RESULTS AND DISCUSSION

Extracts from *Aristolochia argentina, Flourensia oolepis, Gaillardia megapotamica, Salvia cuspidata* and *Trichocline reptans* showed maximum growth inhibition of *F. verticillioides* with MICs of 0.03 - 0.12 mg/spot (Table 1). The extracts of *F. oolepis*, *T. reptans A. argentina*, were most potent, with MIC values of 0.03, 0.03 and 0.06, mg/spot, respectively. Extracts from *Baccharis artemisioides, Baccharis salicifolia, Dalea elegans, Heterothalamus alienus, Lepechinia floribunda, Vernonia nudiflora* and *Zanthoxylum coco* inhibited *F. verticillioides* growth at MICs of 0.25 - 0.50 mg/spot (Table 1). About 8% of extracts showed some inhibition of pathogen with MIC of 1 mg/spot, while 75% plants tested showed little or no inhibition of *F. verticillioides* growth (Table 1).

Table 1. Antifungal activity of native plants from central Argentina

| Family | Specie | MIC (mg/spot) |
|---|---|---|
| Amaranthaceae | *Gomphrena pulchella* Mart. | > 2 |
| Anacardiaceae | *Lithraea molleoides* (Vell.) Engl. | > 2 |
| Apiaceae | *Eryngium horridum* Malme | > 2 |
| Apocynaceae | *Mandevilla laxa* (Ruiz & Pav.) Woodson | > 2 |
| | *Mandevilla pentlandiana* (A. DC.) Woodson | > 2 |
| Aristolochiaceae | *Aristolochia argentina* Griseb. | 0.06 |
| Asclepiadaceae | *Morrenia brachystephana* Griseb. | 2 |
| Asteraceae | *Achyrocline satureioides* (Lam.) DC. | 1 |
| | *Achyrocline tomentosa* Rusby | 2 |
| | *Baccharis artemisioides* Hook. & Arn. | 0.5 |
| | *Baccharis coridifolia* D.C. | > 2 |
| | *Baccharis flabellata* Hook. & Arn. | 1 |
| | *Baccharis salicifolia* (Ruiz et Pav.) Pers. | 0.25 |
| | *Baccharis sessiliflora* Vahl | > 2 |
| | *Eupatorium buniifolium* Hook. & Arn. var. *buniifolium* | > 2 |
| | *Eupatorium hookerianum* Griseb. | > 2 |
| | *Eupatorium viscidum* Hook. & Arn. | > 2 |
| | *Flourensia oolepis* S.L. Blake | 0.03 |
| | *Gaillardia megapotamica* (Spreng.) Baker | 0.12 |
| | *Grindelia pulchella* Dunal | 2 |
| | *Heterothalamus alienus* (Spreng.) Kuntze | 0.5 |
| | *Microliabum candidum* (Griseb.) H. Rob. | 2 |
| | *Senecio vira-vira* Hieron. | 2 |
| | *Tagetes minuta* L. | > 2 |
| | *Thelesperma megapotamicum* (Spreng.) Kuntze | > 2 |
| | *Trichocline reptans* (Wedd.) Hieron. | 0.03 |
| | *Vernonia mollisima* Hook & Arn | 1 |

| | | |
|---|---|---|
| | *Vernonia nudiflora* Less. | 0.25 |
| | *Viguiera tucumanensis* (Hook. et Arn.) Griseb. | > 2 |
| | *Wedelia glauca* (Ortega) Hicken | > 2 |
| | *Zexmenia buphtalmiflora* (Lorentz) Ariza | > 2 |
| Bignoniaceae | *Amphilophium cynanchoides* (DC.) L.G. Lohmann | > 2 |
| | *Dolichandra cynanchoides* Cham. | > 2 |
| | *Dolichandra unguis-cati* (L.) L.G. Lohmann | > 2 |
| | *Podranea ricasoliana* (Tanfani) Sprague | > 2 |
| | *Pyrostegia venusta* (Ker Gawl.) Miers | > 2 |
| Buddlejaceae | *Buddleja mendozensis* Benth. | > 2 |
| Capparaceae | *Capparis atamisquea* Kuntze | > 2 |
| Chenopodiaceae | *Chenopodium ambrosioides* L. | > 2 |
| Dipsacaceae | *Dipsacus fullonum* L. | > 2 |
| Euphorbiaceae | *Acalypha communis* Müll. Arg. | > 2 |
| | *Croton lachnostachyus* Baill. | > 2 |
| Fabaceae | *Adesmia cordobensis* Burkart | > 2 |
| | *Astragalus distinens* Macloskie | > 2 |
| | *Dalea elegans* Hook. & Arn. | 0.25 |
| | *Otholobium higuerilla* (Gillies ex Hook.) Grimes | 2 |
| | *Senna aphylla* (Cav.) H.S. Irwin et Barneby | > 2 |
| Lamiaceae | *Lepechinia floribunda* (Benth.) Epling | 0.5 |
| | *Melissa officinalis* L. | > 2 |
| | *Minthostachys verticillata* (Griseb.) Epling | 2 |
| | *Salvia cuspidata* Ruiz & Pav. | 0.12 |
| Malvaceae | *Pavonia aurigloba* Krapov. & Cristóbal | > 2 |
| | *Sida rhombifolia* L. | > 2 |
| | *Sphaeralcea bonariensis* (Cav.) Griseb. | > 2 |
| | *Sphaeralceae cordobensis* Krapov. | > 2 |
| Papaveraceae | *Argemone subfusiformis* G. B. Ownbey | > 2 |
| Polygalaceae | *Monnina dyctiocarpa* Griseb. | > 2 |
| Ranunculaceae | *Thalictrum decipiens* Boivin | 1 |
| Rhamnaceae | *Condalia microphylla* Cav. | > 2 |
| Rosaceae | *Cotoneaster glaucophylla* Franch. | > 2 |
| | *Kageneckia lanceolata* Ruiz & Pav. | 1 |
| Rutaceae | *Zanthoxylum coco* Hook. f. & Arn. | 0.25 |
| Santalaceae | *Jodina rhombifolia* (Hook. & Arn.) Reissek | > 2 |
| Solanaceae | *Solanum argentinum* Bitter & Lillo | > 2 |
| | *Solanum palinacanthum* Dunal | > 2 |
| | *Solanum sisymbriifolium* Lam. | > 2 |
| Verbenaceae | *Aloysia citriodora* Palau | 1 |
| | *Aloysia gratissima* (Gill. & Hook.) Tronc. | > 2 |
| | *Lantana grisebachii* Seckt | > 2 |
| | *Lippia turbinata* Griseb. | 2 |
| Zygophyllaceae | *Porlieria microphylla* (Baill.) Descole, O´Donell | > 2 |
| | Mancozeb | 0.0006 |

MIC : Minimum inhibitory concentration

Table 2. Antifungal activity of native plants from central Argentina

| Plant species (Voucher Number UCCOR) | Family | Biological activities | Uses in traditional medicine | Previously isolated constituents (References) |
|---|---|---|---|---|
| *Aristolochia argentina* (191) | Aristolochiaceae | Insecticide (3). | Antiseptic (28). Emmenagogue; treatment of arthritis and pruritus (33) | Aristololactam alkaloids (32). Aristolochic acids (33) |
| *Baccharis artemisioides* (142) | Asteraceae | - | - | Macrocyclic trichothecenes (35). Clerodane-type diterpenes (40, 41). |
| *Baccharis salicifolia* (175) | Asteraceae | - | Against rheumatic pains, anti-inflammatory, anticancer; antisyphilitic (19) | Sterols (15) |
| *Dalea elegans* (254) | Fabaceae | - | Antirheumatic (28) | Prenylated flavanones (4) |
| *Flourensia oolepis* (135) | Asteraceae | Insecticide (14) | - | Flavonoid (14). |
| *Gaillardia megapotamica* (127) | Asteraceae | - | Antineuralgic; against headache; antialopecic; antiseborrhoeic (19) | Sesquiterpene lactone; pseudoguaianolides; flavonoids (31) |
| *Heterothalamus alienus* (194) | Asteraceae | Antifungal (29) | Renal affections (19) | - |
| *Lepechinia floribunda* (195) | Lamiaceae | - | - | - |
| *Salvia cuspidata* (266) | Lamiaceae | Trypanocidal (37) | Febrifuge; against palpitations (19) | Diterpenoids (27) |
| *Trichocline reptans* (244) | Asteraceae | - | Digestive; diaphoretic (19) | Benzofurane; coumarins; furanocoumarins (1). |
| *Vernonia nudiflora* (129) | Asteraceae | - | - | Glaucolides; sesquiterpene lactones; steroids; flavonoids (2) |
| *Zanthoxylum coco* (263) | Rutaceae | - | Diaphoretic; astringent (19) | Alkaloids (9,12,13,34). Coumarin (25) |

There were no reported studies for the antifungal activity for organic extracts for any studied plants except *A. argentina* (20), *H. alienus* (29), *Eupatorium buniifolium*, *Lithrea molleoides* (26) and *Sida rhombifolia* (24), and only the first two of these showed any effectiveness in our screening.

Table 2 presents bibliographic information about biological activities, uses in traditional medicine and the chemicals isolated from extracts obtained with different organic solvents from the active plants (MIC = 0.03 - 0.50 mg/spot). *A. argentina* showed strong potential to inhibit the *F. verticillioides* growth (MIC = 0.06 mg/spot). This level of effectiveness had not been observed against another fungus, *Aspergillus niger*, whose growth was not inhibited with ethanol extract of *A. argentina* at 0.75 mg/cylinder (20). The present work is thus the first report of fungal inhibition shown by *A. argentina*.

Phytochemical analysis of most potent extracts revealed that alkaloids were absent in all plants, except in *A. argentina*, while flavonoids were present in all active species except in *A. argentina* (Table 3). As many flavonoids have shown the ability to inhibit fungal growth (11), they could be responsible for the antifungal action exhibited by many studied plants, e.g. *D. elegans*, *F. oolepis* and *H. alienus*. Results regarding the activity of *D. elegans* obtained in this work were not surprising, with the compound 2’4’-dihidroxy-5’- (1’’’-dimethylallyl)-6-prenylpinocembrin, a pinocembrin derivative isolated from this plant (4), showing inhibitory activity on *Candida* sp., *Cryptococcus neoformans* and *Trichophyton mentagrophytes* at 2 mg/mL by the agar-well diffusion method (30). The flavanone pinocembrin has been previously reported as an antifungal compound against *Cladosporium cladosporioides* and *C. sphaerospermum* at 1 μg in a bioautographic TLC assay (22). This compound is present in aerial parts of *F. oolepis* (14) could be responsible for the plant antifungal action.

Table 3. Phytochemical composition of plants showing inhibitory effect on growth of *Fusarium verticillioides*

| Species | Alkaloids | Flavonoids | Lignans |
|---|---|---|---|
| *Aristolochia argentina* | + | - | + |
| *Baccharis artemisioides* | - | + | - |
| *Baccharis salicifolia* | - | + | - |
| *Dalea elegans* | - | + | + |
| *Flourensia oolepis* | - | + | - |
| *Gaillardia megapotamica* | - | + | + |
| *Heterothalamus alienus* | - | + | + |
| *Lepechinia floribunda* | - | ND | ND |
| *Salvia cuspidata* | - | + | + |
| *Trichocline reptans* | - | + | + |
| *Vernonia nudiflora* | - | + | - |
| *Zanthoxylum coco* | - | + | + |

+: Present. -: Absent. ND: Not determined.

Further studies are required to isolate the compounds responsible for the antifungal activity of extracts obtained from active plants and mainly from the *A. argentina* and *T. reptans*. Whole plant extracts and their active components could emerge as new strategies to control of harmful fungi such as *F. verticillioides*.

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