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Search for active metabolites of *Erythrina crista-galli* and its endophyte *Phomopsis* sp.

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ABSTRACT
In the search for bioactive compounds from Argentine Medicinal Plants and their endophytic fungii, extracts from *Erythrina crista-galli* and its endophyte (*Phomopsis* sp) were assayed for antimicrobial activity.
Three isoflavonoids isolated from the plant extract showed activity against *Bacillus subtilis* and *B. brevis*. In order to find any possible metabolic interaction between plant and endophyte, HPLC profile of their extracts were compared and two common compounds were detected.

Key Words: *Erythrina crista-galli*; *Phomopsis* sp; antimicrobial isoflavonoids.

INTRODUCTION

The close relationship between endophyte microorganisms and their hosts involves co-evolution processes, which can influence the physiologic plant mechanisms. Endophytes colonize an ecological niche similar to that of the phytopathogenes, specially vascular pathogenes. This could favor endophytes as agents for control of pathogenic microbes (Araújo *et al.*, 2001). Furthermore, as in the case of *Taxus brevifolia*, endophytic fungii can produce the same metabolites as those of the host plant. This offers the attractive possibility to obtain metabolites for bioproduction even from either protected plant species or in plants in way of extinction.

Erythrina crista-galli L. is a tree that grows naturally in South America in riverside areas. In Argentina it is known as 'ceibo'. The infusion of its leaves, bark and flowers is used as analgesic, anti-inflammatory, for healing wounds, antimicrobial, astringent, anticoagulant, narcotic, and sedative (Toursarkissian, 1980).

From different *E. crista-galli* collections endophyte fungii of the genus *Phomopsis* were isolated (Weber *et al.*, 2005). *Phomopsis/Diaporthe* is a frequent culture host of sunflower, soy, citrus, mint, tea. The aim of this work was to study the plant biological activities, and its endophyte fungus. A screening of antimicrobial, anti-inflammatory and cytotoxic activity of plant and fungus extracts was carried out. Extracts were fractioned guided by the antimicrobial activity in order to isolate and identify active compounds. Common compounds from both plant and endophyte were looked for.

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METHODOLOGY

Plant collection.
Samples of young and old twigs of *Erythrina crista-galli* were collected in Buenos Aires surroundings, from December to March.

Isolation, fermentation and extraction of *Phomopsis* sp. were carried out according to Weber *et al.*, 2005.

Extraction Procedure.
Young twigs were successively extracted with acetone and methanol. Mycellium and fungus culture broth were extracted with acetone-methanol (1:1), and ethyl acetate, respectively.

Screening of antimicrobial activity.
Agar diffusion method
Plant and fungus extracts were analysed using 100 µg of extract/paper disc (6 mm). Fungii were cultured in the YMG medium with (g/l): malt extract, 10; glucose, 4; yeast extract, 4; agar, 20. *Mucor miehei* was cultured at 37° C; *Penicillum notatum* and *Nematospora coryli* at 27°C. Bacteria were cultured at 37°C in nutrient broth culture (Difco) containing 2 % agar.

Tested microorganisms.
Bacillus brevis ATCC 9999; *B. subtilis* ATCC 6633; *Enterobacter dissolvens* LMG 2683; *Paecilomyces variotti* ETH 114646; *Micrococcus luteus* ATCC 381; *Nematospora coryli* ATCC 10647; *Penicillium notatum* collection IBWF.

Bioassay-guided fractionation of the active plant extract.
The acetone extract of young twigs was chromatographed (CC) on Silicagel 60/ using cyclohexane, ethyl acetate, acetone, methanol, and their mixtures. Eight fractions were obtained (F_I-F_{VIII}).

Purification of the active fraction F_{IV}
Fraction F_{IV} was subjected to CC on Sephadex LH-20/Cl_2CH_2 and MeOH. Subfractions were bioassayed against susceptible microorganisms.

Isolation of compounds from the active fraction $F_{IV\text{-}35/40}$ by semipreparative HPLC.
The active fraction $F_{IV\text{-}35/40}$ was subdivided in 5 fractions (F_A-F_E) by semipreparative HPLC-DAD; C_{18} (250 mm x 10 mm, Nucleosil 100-7, Macherey-Nagel)/ H_2O:ACN (65:35) up to ACN 100% for 25 min; UV detection at 210 nm, 280 nm and 330 nm; flow 4 ml/min. Subfraction 35/40-D was subjected to preparative chromatography under the same conditions, modifying the mobile phase: H_2O:MeOH (70:30) to MeOH 100% for 20 min. Three compounds were isolated from this fraction.

Bioautography.
The active fraction $\mathbf{F_{IV\text{-}35/40}}$ was analysed by TLC Silicagel 60 F_{254}/ Cyclohexane:AcOEt (3:7) against *B. brevis*.

The antimicrobial activity of fractions F_I- F_{VIII}, subfractions of F_{IV}, and pure compounds was determined using the agar diffusion method which was used in the screening.

HPLC-DAD. The extracts of young and old twigs of *E. crista-galli* as well as those extracts of the mycellium and culture of *Phomopsis* were analysed in a C_{18} column (LiChrospher 5 microns, 125 x 4 mm, Merck/H_2O:MeOH 0-70% for 20 min, 70 - 100% for 10 min; flow 1.5 ml/min.

RESULTS AND DISCUSSION

The acetone extract of young twigs of *Erythrina crista-galli* inhibited the growth of *Bacillus brevis* and *B. subtilis*. The extracts of *Phomopsis* sp. culture were also active against these microorganisms, besides *M. luteus*, *E. dissolvens*, *N. coryli*, *M. miehei*, *P. notatum* and *P. variotii*.

The bioassay-guided fractionation of the active plant extract showed that F_{III} and F_{IV} inhibited the development of *B. subtilis* and *B. brevis*. F_{IV} also inhibited *S. lutea*.

Fractionation of F_{IV} led to the isolation and characterization (UV analysis) of three isoflavonoids, which exhibited antimicrobial activity against *B. brevis* (inhibition zones of 8.5, 20 and 17 mm, respectively). The identification of these compounds (^{1}HRMN, MS) is in progress.

The presence of *Phomopsis* sp was detected in young and old twigs of *E. crista- galli*. Compounds mellein, mevinic acid and nectriapyrone with antimicrobial activity were identified from mycellium and fungus culture extract (Weber *et al.*, 2005).

Two common compounds were observed in the HPLC profile of both extracts of old twigs of the plant and of the endophyte mycellium.

Molecular Medicinal Chemistry

IDECEFYN

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CONCLUSIONS

The investigation performed and reported herein allowed to determine the antimicrobial activity of extracts from both *Erythrina crista-galli* and its endophyte *Phomopsis* sp.

Bioassay-guided fractionation of the active plant extract led to the isolation of three isoflavonoids active against *B. brevis.*

From the extracts of the fungus culture three antimicrobial compounds were isolated.

Two common compounds were detected in the plant and in the endophyte extracts. Isolation, identification and determination of the biological activity of these compounds is being carried out.

Results indicate that both the plant and its endophyte possess antimicrobial compounds. The occurrence of common compounds in both plant and endophyte suggests a possible interaction between these organisms in the production and/or transference of metabolites, -and probably also in the biological activity- which will be the aim of next studies.

Note: This study was presented at the 'I Reunión de Biotecnología Aplicada a Plantas Medicinales y Aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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