Antimycotic activity of *Ruta chalepensis* L.

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Introduction

Ruta chalapensis L. (Rutaceae), a perennial herb, widely diffused in the Mediterranean area, with glabrous stem, alternate bi-pinnatisect leaves with narrowly oblong-lanceolate or obovate segments, inflorescence cymose, growing in dry, usually rocky areas, is an ancient medicinal plant still used in the traditional medicine of many countries as a laxative, anti-inflammatory, analgesic, antispasmodic, abortifacient, antiepileptic, emmenagogue and for dermatopathy treatment (Johnson T., 1999).

Pharmacological investigations clearly indicated that the ethanol extract of the aerial part of *R. chalepensis* shares the anti-inflammatory and antipyretic activities of the other common non steroïdal anti-inflammatory drugs and has significant dose-dependent central nervous system depressant activity (Mansour S. *et al.*, 1990), while toxicity studies have provided basic information about the possible safe use of this medicinal plant (Shah A.H. *et al.*, 1991).

Phytochemical screening showed that the aerial parts of *R. chalepensis* yielded the coumarins chalepensin, chalepin, rutamarin, bergapten, isopimpinellin and xanthotoxin (Ulubelen A. *et al.*, 1986), as well as alkaloids kokusaginine, skimmianine , arborinine, γ-fagarine, graveoline, 3'-hydroxygraveoline (Ulubelen A. *et al.*, 1986), taifine, isotaifine and 8-methoxytaifine (Mohr N. *et al.*, 1982).

As some secondary metabolites contained in plants often have good antibacterial or antimycotic activity against microorganisms that are resistant to drugs in common use, in a program of studies on the antimicrobic activity of vegetable drugs we devoted our attention to the aerial parts of this species; in fact, as some coumarins and alkaloids isolated from *R. chalepensis* have antimicrobial activity (Wolters B. *et al.*, 1981) and its extractive preparations are topically used in several skin diseases, we assayed the anti-mycotic activity of the ethanol extract of the aerial part of this traditional medicinal plant against fresh clinical isolates of hyphomycetes identified by conventional procedures (De Hoog G.S. *et al.*,1991).

Materials and methods

Plant material

R. chalepensis was collected in the Messina area in April 1999. A voucher specimen of the plant was deposited in the herbarium of the Pharmaco-biological Department of the University of Messina (Italy). The fresh material was immediately lyophilized and powdered.

Preparation of extract

Exhaustive extraction of 10 g of lyophilized and poxdered *R. chalepensis* aerial part was carried out in a Soxhlet using ethanol as solvent. The mixture was filtered and the organic solvent removed under vacuum.

Strains

Strains of hyphomycetes isolated from clinical specimens principally made up of dermatophytes: *Aspergillus fumigatus , Aspergillus terreus, Microsporum canis, Paecilomyces lilacinus, Scopulariopsis brevicaulis, Trichophyton mentagrophytes, Trichophyton rubrum.*

Assay medium

The composition of the assay medium was the following: case amino acids (Difco) 1.0% (p:v), dextrose (Oxoid) 1.0% (p:v), sodium b-glycerophosphate (Sigma) 0.35% (p:v), agar N.1 (Oxoid) 1.5% (p:v), glycine (Sigma) 0.1% (p:v), yeast nitrogen base (YNB) (Difco) 0.067%. All the components with the exception of glycine and YNB were solubilized in 100 ml of distilled water and sterilized in an autoclave. The two components, glycine and YNB, were prepared at a concentration of 10X, sterilized for filtration and added in aliquots of 1.0 ml to the medium maintained in a water bath at 50°C after sterilization. 12 ml of medium was poured into 90 mm Petri dishes to a thickness of 2 mm.

Assay method

The activity of the extract was evaluated by a method designed by the Department of Microbiological Sciences and Gynaecological Sciences at the University of Catania. It allows the evaluation of the growth of hyphomycetes strains under the action of a concentration gradient of the substance under examination, obtained by the diffusion of three quantities deposited around an inoculum point. In particular, in solid media some pits were created according to the plan in figure 1. Around each pit of 2 mm diameter, into which the inoculum of conidia suspended in sterile distilled water is added, three pits of 3 mm diameter are made: the first along the axis that joins the inoculum pit to the center of the dish, the second and the third on the axis that is perpendicular, passing through the center of the inoculum pit. The distance between the edges of the pits along the two axes is 10 mm. The amounts of both the control chemotherapeutic and the assayed extract were used in the following ratio 1: 0.1: 0.01. For each test the three amounts of the substance were applied in a clockwise direction starting from the pit nearest the center of the dish. The activity of the extract, solubilized in dimethyl sulfoxide (DMSO) at 20%, was evaluated against that from miconazole, used as a standard, this was also solubilized in dimethyl sulfoxide at 20 % . Into the 2 mm diameter pit, 5 µl of the inoculum was poured, for each strain, from a suspension of spores kept in sterile distilled water having a concentration of between 5×10^4 and 1×10^5 ml^{-1}. In the 3 mm diameter pits 10 µl of the *Ruta* extract and miconazole were poured. For the *Ruta* extract doses of 2000, 200 and 20 µg were used, for miconazole doses of 10, 1, 0.1 µg. In each dish two assays were carried out for the *Ruta* extract, one for miconazole and one for the solvent (DMSO at 20%). The dishes were incubated at 30° C, from 48 to 120 h, depending on the growth rates of the different species.

Evaluation of antifungal activity

The anti-fungal activity was evaluated by means of the appearance on the surface growth area determined by the image acquisition system Foto/Analyst by Fotodyne Incorporated (Hartland U.S.A.) and the software Collage (Image Analysis Software). For the estimation of the surface growth area in the presence of *Ruta* extract the mean values of the two tests on each dish were used.

The evaluation of the activity of the extract of *Ruta* was carried out with the parameter found from the following formula: $I_r = (S_r - S_d) / S_d . S_r$, where I is the value of the surface growth area in the presence of the extract, S_d is the value of the surface growth area in the presence of only the solvent. As regards miconazole an analogous formula was used. The value of I varies from −1 to ≥ 0. The value of -1 indicates the maximum activity while a value tending to 0 or just above indicates the absence of activity of the substance in examination. The interval from -1 to 0 was divided into three classes correlated to three levels of activity: low or null ($-0.20 < I$), intermediate ($0.6 \leq I \leq -0.2$), elevated ($-1 \leq I < -0.6$).

Results and discussion

The activity of *Ruta* extract is characterized by a marked variability between the strains of the same species. In fact, the range of the parameter I (Fig. 2) varies for *M. canis* from -0.20 to -0.74, for *T. mentagrophytes* from 0 to -0.65 and for *T. rubrum* from 0 to -0.64. From table 1, where the number of strains for each class of parameter I is shown, it can be seen that for *M. canis* the ethanol extract of *Ruta* has an elevated activity in 8 strains (57.1 %) and intermediate in 6 strains (42.9 %); miconazole has an elevated activity in 12 strains (87.5%) and intermediate in 2 strains (12.5 %). Against *T. mentagrophytes* the extract of *Ruta* has a low activity: in fact, it is elevated in 4 strains (40 %), intermediate in 4 strains (40 %) and inactive in 2 strains (20 %). Miconazole has an elevated activity in 6 strains (60%) and intermediate in 4 strains (40%). Against *T. rubrum* the extract of *Ruta* has an elevated activity in 1 strain (12.5 %), discreet in 6 strains (75 %) and is inactive in 1 strain (12.5 %). Miconazole, instead, has an elevated activity in 8 strains (100 %).

For all the other hyphomycetes (Fig. 3), *Ruta* has an intermediate activity while miconazole has an elevated activity with the exception of one strain of *Scopulariopsis brevicaulis*. The intervals of the value of the parameter I relative to the extract of *Ruta* are: from -0.21 to -0.35 for *A. fumigatus*, from -0.21 to -0.32 for *A. terreus*, from -0.28 to -0.29 for *P. lilacinus*, from -0.33 to -0.44 for *S. brevicaulis*.

Conclusion

The ethanol extract of *Ruta* aerial parts has, *in vitro*, a good antifungal activity against the strains of dermatophytes assayed and in particular against *M. canis* even if the doses of the extract assayed in our model are greater than those of miconazole used as an antifungal standard because in this study we used crude extract; this activity seems probably due to the presence of alkaloids in the aerial parts of *Ruta chalepensis*.

The results obtained, as a whole, justify the use in traditional medicine of several regions in the world of this medicinal plant for the treatment of cutaneous pathologies in which dermatophytes could be involved.

References

DE HOOG G.S., GUARRO J. (1991) *Atlas of Clinical Fungi*, CBS, Baarn, Netherlands, 91-120.

JOHNSON T. (1999) *Ethnobotany desk reference*, CRC Press Boca Raton London, New York, Washinghton, D.C., 730.

MANSOUR S. AL-SAID, TARIQ M.A., AL-YAHYA M.A., RAFATULLAH S., GINNAWI O.T., AGEEL A.M. (1990) Studies on *Ruta chalepensis*, an ancient medicinal herb still used in traditional medicine, *J. Ethnopharmacol.* 28, 305-312.

MOHR N., BUDZIKIEWICZ H., EL-TAWIL B.A.H., EL-BEIH F.K.A. (1982) Further furoquinolone alkaloids from *Ruta chalepensis*, *Phytochemistry* 7, 1838-1839

SHAH A.H., QURESHI S., AGEEL A.M. (1991) Toxicity studies in mice of ethanol extracts of *Foeniculum vulgare* fruit and *Ruta chalepensis* aerial parts, *J. Ethnopharmacol*, 34, 167-172.

ULUBELEN A., TEREM B., TUZLACI E., CHENG K.F., KONG Y.C. (1986) Alkaloids and coumarins from *Ruta chalepensis*, *Phytochemistry*, 25, 2692-2693

WOLTERS B., EILERT U. (1981) Antimicrobial substances in callus cultures of *Ruta graveolens*, *Planta med*, 43, 166-174.

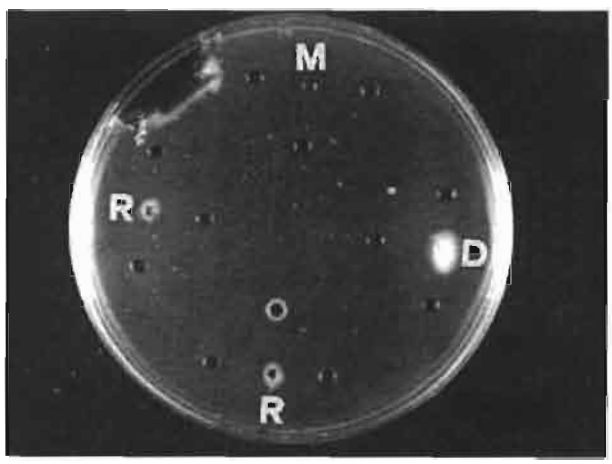

Figure 1. Surface growth area of *M. canis* under the action of a concentration gradient of *Ruta* extract (R), miconazole (M) and DMSO (D)

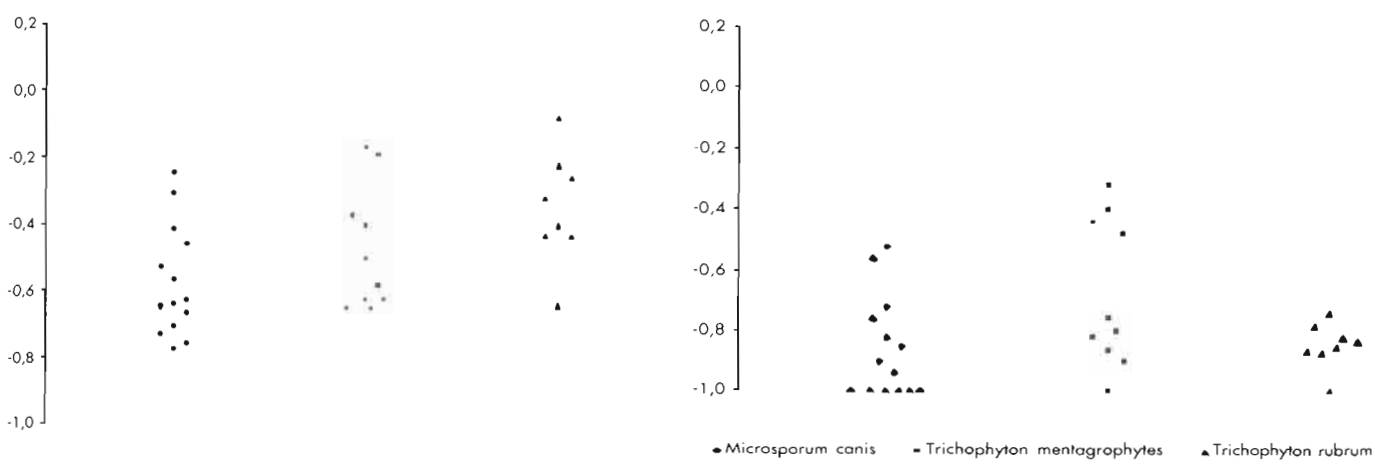

Figure 2. Distribution of I values for *Ruta* extract (A) and miconazole (B) against dermatophytes

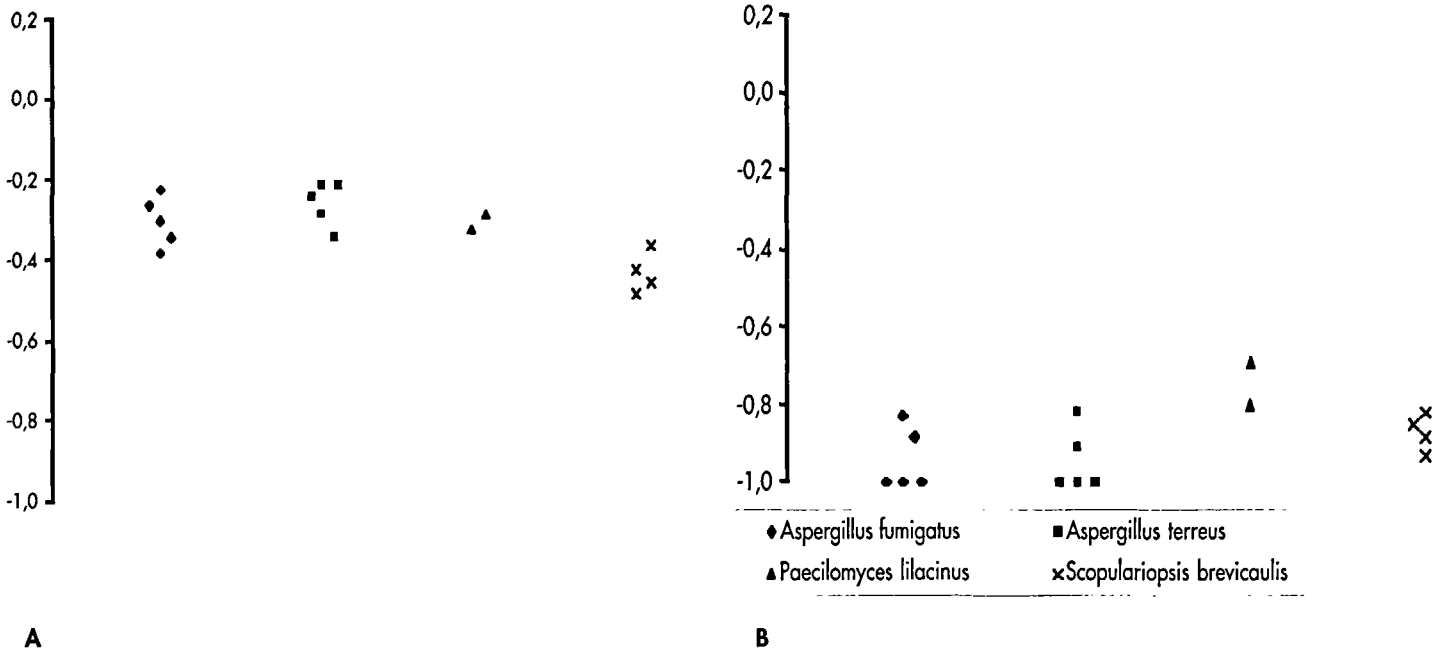

Figure 3. Distribution of I values for *Ruta* extract (A) and miconazole (B) against other hypomycetes

Table I. Distribution of I values for *Ruta* extract (A) and miconazole (B) against other hypomycetes

Species	n°strains	-0.20 < I		-0.6 ≤ I ≤ -0.2		-1 ≤ I < -0.6	
		R	M	R	M	R	M
Microsporum canis	14	0	0	6	2	8	12
Trichophyton mentagrophytes	10	2	0	4	4	4	6
Trichophyton rubrum	8	1	0	6	0	1	8
Aspergillus fumigatus	5	0	0	5	0	0	5
Aspergillus terreus	5	0	0	5	0	0	5
Paecilomyces lilacinus	2	0	0	2	0	0	2
Scopulariopsis brevicaulis	4	0	0	4	1	0	3

R = *Ruta chalepensis* ethanol extract M = Miconazole