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Antimicrobial activity of *Epilobium* spp. extracts☆

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Abstract

The antimicrobial activity of the *Epilobium angustifolium*, *E. hirsutum*, *E. palustre*, *E. tetragonum* and *E. rosmarinifolium* ethanolic extracts was studied in vitro on Gram-positive and Gram-negative bacteria, yeasts and fungi. The cytotoxicity of the extracts was also evaluated using the *Artemia salina* test. All the extracts showed antimicrobial activity in a range of concentrations between 10 and 650 μg/ml of dry extract. *E. angustifolium* and *E. rosmarinifolium* had the most broad spectrum of action inhibiting bacteria, yeasts and fungi. The extracts were devoid of toxicity on *Artemia salina* within the range of antimicrobial concentrations, suggesting that the action is selective on microorganisms. © 2001 Éditions scientifiques et médicales Elsevier SAS

Keywords: *Epilobium*; Bacteria; Yeasts; Dermatophytes; *Artemia salina*

1. Introduction

The genus name *Epilobium* derives from the Greek words 'epi' (upon) and 'lobos' (a pod); the plant is so called because the flowers are arranged upon long, thin, pod-like seed cases. The genus *Epilobium* (Onagraceae) comprises more than 200 species, among which the most known are *E. angustifolium*, *E. hirsutum* and *E. parviflorum*, perennial herbs generally named Willow Herb, with reference to the willow-like nature of their leaves [1]. The medicinal parts are the herb and the roots that contain: flavonoids, in particular myricitrin, guaiaverin, quercitrin, quercetin-3-O-β-D-glucuronide); steroids, in particular beta-sitosterol and its esters; tannins [1]. Willow Herb preparations are traditionally used internally for prostate and gastrointestinal disorders and externally as antiphlogistic and antiseptic remedies, to treat mycoses and to improve the healing of wounds [1]. It has been shown that aqueous extracts of aerial parts of *E. angustifolium* have analgesic and anti-inflammatory activity and reduce the release of prostaglandins these effects being due, at least in part, to myricetin-3-*O*-β-D-glucuronide [2–4]. Moreover ethanolic extracts of *E. angustifolium* and alcoholic extracts of Willow Herb inhibited the growth of *Staphylococcus aureus*, *S. albus*, *Pseudomonas pyocyanea* and *Candida albicans* [1].

The aim of this work was to evaluate in vitro the antimicrobial activity of the extracts of some *Epilobium* species and to establish their selectivity of action by determining the cytotoxicity.

2. Materials and methods

2.1. Drugs

Ethanolic extracts of fresh aerial parts of five species of *Epilobium*: *E. angustifolium* L., *E. hirsutum* L., *E. palustre* L., *E. tetragonum* L., *E. rosmarinifolium* Haenke, kindly supplied by Boiron Laboratoires (Lyon, France), were used. For biological assays the extracts were diluted in order to obtain an alcohol concentration that did not interfere with the biological tests. The concentrations were expressed as dry extracts preliminarily determined by evaporating the extracts under vacuum and weighing the dry residues. Tetracycline, miconazole and podophyllotoxin, used as reference substances, were purchased from Sigma (Milan, Italy).

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2.2. Microorganisms

Standard microorganisms or clinically isolated strains were used. They were: Gram-positive bacteria (*Staphylococcus aureus* two strains, *Streptococcus pyogenes* ATCC 12345, *Streptococcus sanguis* CDC SS 910, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis*, *Listeria monocytogenes* ATCC 19111), Gram-negative bacteria (*Escherichia coli* ATCC 15221, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 7853, *Shigella flexneri* CDC 9767 IAL 1517 and *Salmonella enteritidis* IAL 1132), yeasts (*Candida albicans* two strains, *Candida glabrata* two strains and *Candida krusei*) and fungi (*Microsporum canis*, *Microsporum gypseum* two strains, *Tricophyton rubrum* and *Tricophyton mentagrophytes* four strains.

2.3. Susceptibility tests

The antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and the minimum cytocidal concentration (MCC). The MIC values (minimum concentration that inhibits the inoculum growth) of *Epilobium* extracts against bacteria and yeasts was determined on 96-well culture plates by a microdilution method using Mueller–Hinton broth (Becton–Dickinson, Milan, Italy). The experiments on dermatophytes were carried out in tubes using Tryptone Soya broth (Oxoid, Unipath S.p.A., Milan, Italy) plus 5% Peptone (Sigma, Milan, Italy). Eight two-fold dilutions of the extracts were carried out starting from the concentration of 650 μg/ml (about 2% of ethanol). All preparations were sterilized with a 0.22-μm filter. The wells were inoculated with a microorganism suspension at a density of 10^5 cells/ml. The plates were incubated at 37°C for 24 h (bacteria) or 48 h (yeasts). The inoculum concentration of dermatophytes was approximately 4×10^4 spores/ml and the tubes were incubated for two weeks. After incubation the plates or tubes were observed in order to determine the MICs. The cultures that did not present growth were used to inoculate plates of solid medium (Mueller–Hinton Agar for bacteria and Sabouraud Agar for yeasts and fungi) in order to determine the MCC (minimum concentration that kills the inoculum). Tetracycline and miconazole, solubilized in 3% ethyl alcohol, were used as reference antibiotics. Proper blanks were prepared simultaneously; samples were tested in triplicate.

2.4. Artemia salina test

The cytotoxicity was evaluated on *Artemia salina* Leach according to the method of Mongelli et al. [5], slightly modified by Renzini et al. [6]. Brine shrimp eggs (Euroaquarium S.p.A., Bologna, Italy) were hatched in artificial sea water; after 48 h the phototrophic nauplii were collected and a suspension of 10–15 nauplii (100 μl) was placed into each well of a 48-well microplate (Kartell, Milan, Italy) containing 900 μl of extract in artificial sea water; control wells containing artificial sea water were also prepared. After 24 h of incubation the dead nauplii were counted using a Zeiss binocular microscope (10 ×), then 200 μl of methanol were added to each well and 60 min later the total number of nauplii were counted. *Epilobium* extracts were assayed starting from the concentration of 325 μg/ml; higher concentrations were not tested because some extracts, and particularly *E. rosmarinifolium*, after incubation produced a precipitate that hindered the count. Podophyllotoxin, dissolved in artificial sea water, was used as reference substance. The cytotoxicity, expressed as LC_{50} with 95% confidence limits, was calculated with the test of Lichtfield and Wilcoxon [7].

3. Results

The *Epilobium* extracts tested showed antimicrobial activity with a different spectrum of action (Table 1). All the extracts inhibited the growth of Gram-positive and Gram-negative bacteria with MIC values between 81 and 650 μg/ml; the action was mostly bactericidal. Only *E. rosmarinifolium* and *E. angustifolium* were active against yeasts; their MIC values were between 162 and 325 μg/ml. Finally, all the *Epilobium* extracts inhibited the growth of the fungi, generally between 81 and 650 μg/ml; *E. hirsutum* and *E. angustifolium*, instead, showed MIC values very low, corresponding to 10 μg/ml, against *Microsporum canis*. The action was always cytocidal.

In *Artemia salina* test none of the *Epilobium* extracts showed cytotoxicity at 325 μg/ml, the maximal concentration used (Table 2); LC_{50} value of podophyllotoxine, used as reference substance, was 9.5 μg/ml (C.L. 6.4–14.0).

4. Discussion

In our experiments the extracts of *Epilobium* spp. tested possess antimicrobial activity. *E. angustifolium* and *E. rosmarinifolium* seem to have the most broad spectrum of action inhibiting bacteria, yeasts and fungi, generally between 81 and 650 μg/ml. These MIC values appear high in comparison with those of standard antibiotics; however, it has to be underlined that the raw extracts are constituted by a mixture of chemical compounds in which the active principles are generally contained in low percentage. The activity of *E. hirsutum* and *E. angustifolium* against *Microsporum canis* that is inhibited at a concentration as low as 10 μg/ml appears to be particularly interesting. The present results on one

Table 1
Minimum inhibitory concentration (MIC) and minimum cytocidal concentration (MCC) of *Epilobium* spp. extracts

Microorganism	MIC and (MCC) (μg/ml) [a]					
	Standard antibiotic [b]	*E. tetragonum*	*E. palustre*	*E. rosmarinifolium*	*E. hirsutum*	*E. angustifolium*
S. aureus ATCC 6538P	1.56	650 (>650)	650 (>650)	162 (>650)	650 (>650)	325 (325)
S. aureus	1.56	325 (325)	325 (325)	325 (>650)		325 (650)
S. pyogenes ATCC 12345	1.56	162 (162)	162 (162)	81 (81)	81 (81)	325 (325)
B. subtilis ATCC 6633	1.56	650 (>650)	325 (325)			325 (325)
E. faecalis	1.56					
L. monocytogenes ATCC 19111	<0.39					
S. sanguis CDC SS 910	1.56	325 (325)	650 (650)	650 (650)	650 (650)	325 (325)
E. coli ATCC 15221	1.56					
K. pneumoniae ATCC 10031	50	81 (325)	162 (650)	162 (162)	81 (>650)	81 (>650)
P. aeruginosa ATCC 27853	6.25	162 (162)	325 (325)	650 (650)	650 (650)	162 (162)
S. flexneri CDC 9767 IAL 1517	6.25					
S. enteriditis IAL 1132	1.56					
C. krusei	2			325 (325)		325 (650)
C. albicans (2 strains)	8–32			162 (650)		325 (650)
C. glabrata (2 strains)	1–4			162 (162)		
M. canis	0.5	650 (650)	325 (325)	325 (325)	10 (10)	10 (10)
M. gypseum (2 strains)	2–4	81–650 (81–650)	325–650 (325–650)	650 (650)	650 (650)	650 (650)
T. rubrum	0.5	650 (650)	650 (650)	650 (650)	650 (650)	650 (650)
T. mentagrophytes (4 strains)	0.5–4		650 (650)	325 (325)	650 (650)	162–650 (162–650)

[a] Concentrations of *Epilobium* extracts are referred to dry extract; blank cell denotes no effect; ATCC, American Type Colture Collection; CDC, Collecione de Coltura; IAL Istituto Alberto Luz.
[b] Tetracycline for bacteria, miconazole for yeasts and fungi.

Table 2
Cytotoxicity of *Epilobium* spp. extracts on *Artemia salina* Leach [a]

Concentration (μg/ml)	Dead nauplii %					
	E. tetragonum	*E. palustre*	*E. rosmarinifolium*	*E. hirsutum*	*E. angustifolium*	Podophyllotoxin
325	0	4 ± 0.3	4 ± 2.8	3 ± 1.4	0	
162	2 ± 1.0	1 ± 0.3	0	0	2 ± 1.0	
50						70 ± 3.6
12.5						55 ± 3.6
3.12						32 ± 8.2
Vehicle	4 ± 2.8	0	3 ± 1.5	2 ± 1.5	4 ± 2.2	0.3 ± 0.1

[a] Concentrations of *Epilobium* extracts are referred to the dry extract; values are expressed as M ± S.E. of at least three replications; blank cell denotes not tested.

hand confirm the antimicrobial activity observed in *E. angustifolium* and reveal a similar activity in other species of *Epilobium*, and on the other hand, they offer a scientific basis to the traditional use of Willow Herb preparations as antiseptic and antimycotic remedies to treat eczema, seborrhea, psoriasis and other skin conditions. Moreover, the antimicrobial activity appears to be selective on microorganisms because all the extracts are devoid of cytotoxicity within the range of antimicrobial concentrations. A deeper study in order to identify the active principles would be of interest.

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