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Studies on antimicrobial activity of *Inula helenium* L Romanian cultivar

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Abstract

This study was carried out to evaluate the *in vitro* antimicrobial activities of dried roots of a Romanian indigenous population of *Inula helenium* L. The powdered dried root of the plant was extracted in ethanol (using 30%, 50% and 70% v/v). The antimicrobial activity has been tested on five potential pathogenic bacterial species (*Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus*) and four fungal species (*Candida albicans, C. parapsilosis, C. lipolytica* and *Aspergillus niger*), all of veterinary interest. Antimicrobial activity was investigated by the drop-diffusion test method, measuring the inhibition zones. The preliminary results of our study indicated that ethanolic extracts (50% and 70%) from the roots of a Romanian cultivar of *I. helenium* L. showed significant antimicrobial activity against all tested microorganisms, except the pathogenic philamentous fungi *A. niger*. On the dermatophytic species (*Candida* sp.) the inhibitory effects of 50% and 70% extracts are very similar. Further investigation will be done to determine the minimal inhibitory concentrations for each susceptible microorganism. As final goal is the design of a new veterinary product with antimicrobial effects.

Keywords: in vitro, *Inula helenium*, ethanolic extracts, antibacterial, antifungal

1. Introduction

Since the beginning of the twentieth century, the discovery and development of antibiotics revolutionized human and veterinary medicine. However, due to excessive use of antibiotics to treat the infectious diseases, many microorganisms developed several mechanisms to resist conventional antibiotics, creating serious problems worldwide. For this reason, many research have been directed towards finding alternatives such as new antimicrobial compounds from natural sources in order to achieve the effective treatment. Since ancient times, medicinal and aromatic plants, or spices have been used as food and feed additives, food supplements and medicinal value. More recently, much attention has been paid to investigate weed plants as sources of new bioactive compounds. The presence of antimicrobial and other biological activities have been already demonstrated in extracts of *Eupatorium* spp. (L.-L. JI & al. [1]; N. ARVIND and S. AMIT [2]), *Helleborus* spp. (S. PUGLISI & al. [3]), *Inula* spp. (T. KONISHI & al. [4]; A. STOJAKOWSKA & al. [5, 6, 7]; P.D. LOKHANDE & al. [8]; A. DERIU & al. [9]; Z. STOJANOVIĆ-RADIĆ & al. [10]; H. YAN & al. [11]).

The genus *Inula (Asteraceae; tribe Inuleae)* consists of approximately 100 species mainly distributed in Europe, Africa and Asia. Recently, several species (*Inula britannica* L., *I. ensifolia I. helenium* L, *I. racemosa* L., and *I. viscosa* L.) have been investigated for their pharmacological benefit including antioxidant and anti-inflammatory activities, hepatoprotective characteristics, cytotoxicity, and antimicrobial properties (Y.M. ZHAO & al. [12]; W. H. TALIB & al. [13]; S. AMIN & al. [14]).

Among these species*, Inula helenium* L. also known as elecampane is a perennial herbaceous plant, with tuberous, thick roots. Chemical analysis of the rhizome and roots showed that *I. helenium* contains many bioactive compounds including polysaccharide inulin (up to 44%), essential oil with eudesmane –type (up to 5%), sesquiterpene lactones (mainly alantolactone and isoalantolactone), thymol derivatives, terpenes, and sterols (T. KONISHI & al. [4]; A. STOJAKOWSKA & al. [5, 6]; A. DERIU & al. [9]; Z. STOJANOVIĆ-RADIĆ & al. [10]; H. YAN & al. [11]; J. WANG & al. [15]). It is known that a number of these compounds are responsible for its anti-inflammatory, anti-microbial and anti-helminthic properties. Sesquiterpene lactones (Z. STOJANOVIĆ-RADIĆ & al. [10]) and thymol derivatives (A. STOJAKOWSKA & al. [5, 6]) from elecampane have been demonstrated to be main antimicrobial agents in the roots.

There are few reports concerning the antimicrobial activity of others members of the *Inula* spp. against several microorganisms, including bacteria and fungi. P.D. LOKHANDE & al. [8] reported that alantolactone isolated from root of the plant *I. racemosa* have been shown maximum antibacterial activity as compared to other constituents and ethyl acetate extract of the roots. Y.M. ZHAO & al. [12] isolated and identified five thymol derivatives from the roots of *I. hupehensis*. One of these compounds was the most active, not only displaying moderate antibacterial activities against *S.aureus*, Methicillin-resistant *S. aureus* and *E. coli*, but also exhibiting inhibitory activities against three plant pathogenic fungi: *Rhizoctonia solani, Phytophthora melonis* and *P. litchi*, and its content accounted for 0.033% of the roots of *I. hupehensis* (Y.M. ZHAO & al. [12]). W. H. TALIB & al. [13] investigated the antiproliferative and antimicrobial effects of thirteen compounds isolated from *Inula viscosa* L. and demonstrated that two flavonoids (3-*O*-methylquercetin and 3,3′-di-*O*-methylquercetin) inhibit *Salmonella typhimurium* (at MIC of 125 μg/mL) and *Bacillus cereus* (at MIC of 125 μg/mL and 62.5 μg/mL, respectively).

In the present study, we have proceeded to a qualitative screening regarding the antimicrobial activity of a Romanian cultivar of *Inula helenium* L root alcoholic extracts, in order to future use of this plant as a source of new antimicrobial products for veterinary use. In Romania, *Inula helenium* grows on plane and hills area, very close to the villages and it has been used as old medicinal plant. As a general remark, it has been noticed that Romanian indigenous *Inula* plants are shorter than the other Southern European cultivars

2. Materials and methods

Collection of plant material

Dried roots from a Romanian cultivar of *Inula helenium*L. have been harvested from the experimental field of Dacia Plant S.A., Bod, Romania. The plants have been cultivated under certified ecological conditions. The cultivar has been introduced in the collection from a local *Inula* population growing in South-Eastern side of Romania (Brăila) in a plane area. Herbarium specimen was preserved at the manufacturer.

Preparation of plant extracts

Roots of *Inula helenium*L. have been dried and chopped in small pieces of 0.2-1 cm and extracted in ethanol with different concentrations: 30%v/v (I30^o), 50% v/v (I50^o) and 70%v/v

(I70°). After preliminary tests (data not shown) the optimal extraction time in hydro-alcoholic solutions has been established at 6 hours at room temperature.

Test microorganisms

The *Inula* extracts have been tested on six potential pathogen bacterial strains and four fungal strains which can affect the animals (Table 1).

Table 1 - Microorganisms tested for their susceptibility to *Inula helenium* alcoholic extracts

No.	Strain	Characteristics	Origin
1.	*Bacillus subtilis* ICCF 276	*Gram*-positive, catalase-positive	Collection ICCF, Bucharest, Romania
2.	*Bacillus cereus* CP1	*Gram*-positive, beta hemolytic bacterium	Collection of Faculty of Biotechnologies, Bucharest, Romania
3.	*Escherichia coli* MI 57	*Gram*-negative, non-toxinogenic serotype	Collection of Faculty of Biotechnologies, Bucharest, Romania
4.	*Escherichia coli* CP2	*Gram*-negative, toxinogenic serotype	Collection of Faculty of Biotechnologies, Bucharest, Romania
5.	*Staphylococcus aureus* CP 3	*Gram*-positive, catalase-positive	Collection of Faculty of Biotechnologies, Bucharest, Romania
6.	*Enterococcus faecalis* CP 4	*Gram*-positive, sensible to β-lactam-based antibiotics	Collection of Faculty of Biotechnologies, Bucharest, Romania
7.	*Candida albicans* ATCC10231	Serotype A, sensible to nystatin	Collection of Microbial Genetics and Biotechnology, Faculty of Biology, Bucharest, Romania
8.	*Candida parapsilosis* CBS604	Sensible to amphotericin B, nystatine, essential oils of *Coriander sativum*	Collection of Microbial Genetics and Biotechnology, Faculty of Biology, Bucharest, Romania
9.	*Candida lypolytica* MI 2	non-conventional yeast, lipase production	Collection of Faculty of Biotechnologies, Bucharest, Romania
10.	*Aspergillus niger* F2T	Responsible for pulmonary infections in dogs	Collection CBM Biotehgen Bucharest, Romania

The bacterial strains were maintained and tested on nutrient agar (0.5% peptone; 0.3% yeast extract; 1.5% agar; 0.5% NaCl) at 37ºC, using 24 hours old inoculums. Yeasts and fungal strains were maintained and tested on Yeast Extract Glucose Agar (1% yeast extract; 2% peptone; 2% glucose; 2% agar) at 30ºC, using 24 hours old inoculums for yeasts or mycelium block (5 mm) for filamentous fungi.

Antimicrobial Analysis

In vitro, the antimicrobial activity was assessed by the "drop agar diffusion" method (fig.1). The microorganisms were spread using 100µl of suspension containing 10^8 CFU/ml on nutrient agar or on Yeast Extract Glucose Agar respectively.

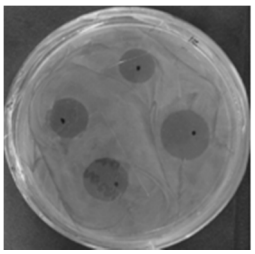

Fig.1 - Aspects from the "drop agar diffusion" screening method

Concerning *A. niger* F2T, the mycelium block (5 mm) was placed in

the centre of the medium in Petri dishes. After 1 hour, 10 μL of each sterile extract have been added in the agar plates pre- inoculated with the tested microorganisms. Non diluted ampicillin (for bacteria) and fluconazole (for fungi) have been used as positive control and the *Inula* extracts solvent (hydro ethanolic solutions of 30%, 50% and 70%) have been used in the master plates. All plates were incubated according to the itemed test microorganisms. The diameter of growth inhibition zones (halos) was measured in millimetres on two axes and the mean value reported. The results are the mean of two separate experiments with three repetitions for each sample.

3. Results and discussion

On international levels main reports on antimicrobial activity of *Inula* sp. are linked to extracts made of other species that *Inula* hellenium, like *Inula racemosa* (P.D. LOKHANDE & al. [8]), *Inula hupehensis* (J. ZHAO & al [16]) or *Inula viscosa* (W. H. TALIB & al. [13]). The present study was conducted on ethanol extracts (30%, 50%, 70%) from dried roots of a Romanian cultivar of *Inula helenium* L. Antimicrobial activity was determined by drop agar diffusion method, measuring the diameter of zone of inhibition. All the measurements have been done in triplicate. As control, solutions of ethanol of same concentrations as the plant extracts have been used. As general remark, the results indicated that the antimicrobial activity increases while increasing the solvent concentration from 30% to 50%, while from 50% to 70% concentration no significant activity increase has been noticed (tables 2 and 3) comparing to the control.

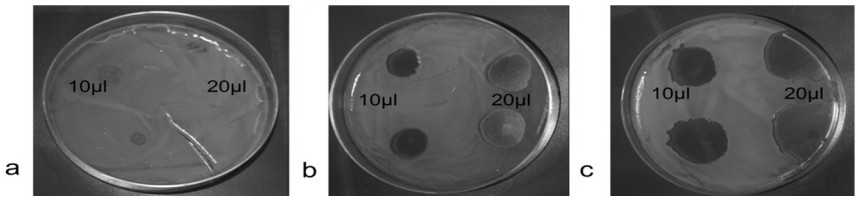

Fig.2 - *Inula*' s extract antimicrobial activity on non-toxinogenic serotype *Escherichia coli* MI 57 (a- I30, b- I50, c- I70).

Against the bacterial strains, the ethanol extracts (I50˚ and I70˚) exhibited moderate to high inhibitory activity, while the ethanol extract I30˚ proved not significant inhibitory activity only on non-toxinogenic serotype *Escherichia coli* MI 57 (fig. 2, table 2), close to the control.

Table 2- Antibacterial activity of dried root extracts from *I. helenium* L.

Test bacteria	Inhibitory effect		
	Extract I30˚	Extract I50˚	Extract I70˚
Escherichia coli MI 57	+	+ +	+ + +
Escherichia coli CP2	-	+	+ +
Bacillus spp. ICFF 276	-	+ +	+ +
Bacillus cereus CP1	-	+ +	+ +
Staphylococcus aureus CP3	-	+	+ +
Enterococcus faecalis CP4	-	+ +	+ +

Legend:
-: non inhibitory halo
+: low inhibitory activity
++: moderate inhibitory activity
+++: high inhibitory activity

The susceptibility of the fungal strains inhibition is shown in Table 3. Ethanol extract (I50˚) showed moderate antifungal activity against *Candida albicans* and good activity against *C.parapsilosis* and *C. lipolytica*. No antifungal activity was found against *A. niger* (table 3). It has been noticed that on the testes yeast (*Candida* spp.) the inhibitory effects of I50˚ and I70˚ are very similar.

Table 3- Antifungal activity of dried root extracts from *I. helenium* L.

Test microorganism	Inhibitory effect		
	Extract I30˚	Extract I50˚	Extract I70˚
Candida albicans ATCC10231	-	+	+ +
C. parapsilosis CBS604	-	+ +	+ +
C. lipolytica MI2	-	+ +	+ +
Aspergillus niger F2T	-	-	-

Legend:
-: no inhibitory halo
+: low inhibitory activity
++: moderate inhibitory activity
+++: high inhibitory activity

These results are in agreement with the data reported by A. DERIU & al. [9], assaying root essential oil of *I. helenium* against some Gram-positive and Gram-negative bacteria and *Candida* species.
Regarding the *Inula* effects on the filamentous fungi, few reports have been issued. Some recent data (TE ZHAO & [17]) reported the inhibitory effect of *Inula britannica* extracts on most important plant pathogenic fungi (*Fusarium, Phytophthora, Colletotrichum, etc)* and the potential to produce botanical fungicide to protect crops. In our study, the *Inula helenium* ethanolic extracts did not show any inhibitory effect on animal pathogenic *Aspergillus niger* which is the main responsible for pulmonary infections in dogs, while other authors (M.A. NAN & [18]) reported some inhibitory activity on 50 mg/l (MIC).

4. Conclusions

The preliminary results of our study, evaluated by the diameter of the inhibition zone of microbial growth, indicated that both ethanol extracts (I50˚ and I70˚) from the roots of a Romanian cultivar of *I. helenium* L. have significant activity against the pathogenic bacteria and dermatophytic fungi, while on filamentous fungi *A. niger* there is no inhibitory activity. On *Candida sp.* the inhibitory effects of I50˚ and I70˚ are very similar. In order to develop a veterinary product with anti-aspergillosis effect some other plant extract (*Helleborus, Eupatorium*) should be taken into account.
Further research is required to identify and analyze the bioactive compounds present in the extracts and to establish the minimal inhibitory concentration for each type of pathogenic microorganisms. Considering the bioactive potential of roots extracts from indigenous population of *I. helenium* could be of great interest for the development of new pharmaceuticals for veterinary uses.

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References

1. L.-L. JI, M.-Y. LUO, GUI-LONG YAN. Studies on the antimicrobial activities of extracts from *Eupatorium lindleyanum* DC against food spoilage and food-borne pathogens. *Food Control*, **19**, 995, 1001 (2008).
2. N. ARVIND, S. AMIT. Antimicrobial Potential of *Eupatorium adenophorum* Spreng. *Phcog. J.*, **2**(18), 61, 64 (2011).
3. S. PUGLISI, A. SPECIALE, R. ACQUAVIVA, G. FERLITO, S. RAGUSA, R. DE PASQUALE, L. IAUK Antibacterial activity of *Helleborus bocconei* Ten. subsp. *siculus* root extracts - Ethnopharmacological communication. *J. Ethnopharmacol.,* **125**, 175, 177 (2009).
4. T. KONISHI, Y. SHIMADA, T. NAGAO, H. OKABE, T. KONOSHIMA. Antiproliferative sesquiterpene lactones from the roots of *Inula helenium*. *Biol Pharm Bull.*, **25**(10), 1370, 1372 (2002).

5. A. STOJAKOWSKA, J. MALARZ, W. KISIEL. Thymol derivatives from a root culture of *Inula helenium*. *Z Naturforsch C.*, **59**, 606, 608 (2004).
6. A. STOJAKOWSKA, B. KEDZIA, W. KISIEL. Antimicrobial activity of 10- isobutyryloxy-8, 9-epoxythymol isobutyrate. *Fitoterapia*, **76**, 687, 690 (2005).
7. A. STOJAKOWSKA, J. MALARZ, S. ZUBEK, K. TURNAU, W. KISIEL. Terpenoids and phenolics from *Inula ensifolia. Biochem. Syst. Ecol.*, **38**, 232, 235 (2010).
8. P.D. LOKHANDE, K.R. GAWAI, K.M. KODAM, B.S. KUCHEKAR, A.R. CHABUKSWAR, S.C. JAGDALE. Antibacterial activity of isolated constituents and extract of roots of *Inula racemosa*. *J. Med. Plants Res.,* **1**(1), 7, 12 (2007).
9. A. DERIU, S. ZANETTI, L.A. SECHI, B. MARONGIU, A. PIRAS, S. PORCEDDA, E. TUVERI. Antimicrobial activity of *Inula helenium* L. essential oil against Gram-positive and Gram-negative bacteria and *Candida* spp. *International Journal of Antimicrobial Agents*, **31**, 581, 592 (2008).
10. Z. STOJANOVIĆ-RADIĆ, LJ. ČOMIĆ, N. RADULOVIĆ, P. BLAGOJEVIĆ, M. DENIĆ, A. MILTOJEVIĆ, J. RAJKOVIĆ, T. MIHAJILOV-KRSTEV. Antistaphylococcal activity of *Inula helenium* L. root essential oil: eudesmane sesquiterpene lactones induce cell membrane damage. *Eur. J. Clin. Microbiol. Infect. Dis*., **31**(6), 1015, 1025 (2012).
11. H. YAN, S. HAIMING, G. CHENG, L. XIAOBO. Chemical constituents of the roots of *Inula helenium. Chem. Nat. Compd.*, **48**(3), 522, 524 (2012).
12. Y.M. ZHAO, M.L. ZHANG, Q.W. SHI, H. KIYOTA. Chemical constituents of plants from the genus *Inula* (Review). *Chemistry & Biodiversity*, **3**(4), 371, 384 (2006).
13. W. H. TALIB, M. H. A. ZARGA, A. M. MAHASNEH. Antiproliferative, antimicrobial and apoptosis inducing effects of compounds isolated from *Inula viscosa*. *Molecules*, **17**, 3291, 3303 (2012).
14. S. AMIN, Z. A. KALOO, S. SINGH, T. ALTAF. Medicinal importance of genus *Inula-A Review. IJCRR*, **5**(2), 20, 26 (2013).
15. J. WANG, Y.M. ZHAO, C.Y. GUO, S.M. ZHANG, C.L. LIU, D.S. ZHANG, X.M. BAI. Ultrasound-assisted extraction of total flavonoids from *Inula helenium*. *Pharmacognosy Magazine*, **8,** 166, 170 (2012).
16. J. ZHAO, Y. LI, Q. LIU, K. GAO. Antimicrobial activities of some thymol derivatives from the roots of *Inula hupehensis*. *Food Chem.*, **120**, 512, 516 (2010).
17. TE ZHAO, F. GAO, L. ZHOU, T. SONG. Essential oil from *inula britannica* extraction with SF-CO_2 and its antifungal activity. *Journal of Integrative Agriculture* **12** (10), 1791, 1798 (2013).
18. M.A. NAN. Doctoral thesis, Pharmacobotanical study of *Inula helenium* L. (*Asteraceae*) species regarding its use in phytotherapy, *UMF Iuliu Haţieganu*, Cluj-Napoca (2011).