EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

Original article:

IMMORTELLE (*XERANTHEMUM ANNUUM* L.) AS A NATURAL SOURCE OF BIOLOGICALLY ACTIVE SUBSTANCES

Milan S. Stanković*, Ivana D. Radojević, Olgica D. Stefanović, Marina D. Topuzović, Ljiljana R. Čomić, Snežana R. Branković

Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Radoja Domanovića 12, 34000 Kragujevac, Republic of Serbia

* corresponding author: Tel.: +381 34 336 223; Fax: +381 34 335 040
e-mail: mstankovic@kg.ac.rs

ABSTRACT

Antioxidant and antimicrobial effects, total phenolic content and flavonoid concentrations of methanolic, acetone and ethyl acetate extracts from *Xeranthemum annuum* L. were investigated in this study. The total phenolic content was determined using Folin-Ciocalteu reagent and ranged between 101.33 to 159.48 mg GA/g. The concentration of flavonoids in various *X. annuum* extracts was determined using spectrophotometric method with aluminum chloride and the results varied from 22.25 to 62.42 mg RU/g. Antioxidant activity was monitored spectrophotometrically using DPPH reagent and expressed in terms of IC_{50} (µg/ml), and it ranged from 59.25 to 956.81 µg/ml. The highest phenolic content and capacity to neutralize DPPH radicals were found in the acetone extract. *In vitro* antimicrobial activity was determined by microdilution method. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) have been determined. Testing was conducted against 24 microorganisms, including 15 strains of bacteria (standard and clinical strains) and 9 species of fungi. Statistically significant difference in activity between the extracts of *X. annuum* L. was observed and the acetone extract was found most active. The activity of acetone extract was in accordance with total phenol content and flavonoid concentration measured in this extract. The tested extracts showed significant antibacterial activity against G+ bacteria and weak to moderate activity against other microorganisms. Based on the obtained results, *X. annuum* can be considered as a rich natural source of polyphenolic compounds with very good antioxidant and antimicrobial activity.

Keywords: immortelle, *Xeranthemum annuum,* antimicrobial capacity, antioxidant, phenols, flavonoids

INTRODUCTION

Immortelle, *Xeranthemum annuum* L., belongs to the family Asteraceae (Compositae), subfamily Cichorioideae, tribe Cardueae, subtribe Carlininae. Four species belong to the genus *Xeranthemum* in the flora of Europe. *X. annuum* is an annual herb, growing to 50 cm, with a thin and spindle-shaped root and a tree with a small number of linear to oblong leaves that are white-tomentose beneath and about 30 mm long and 2-7 mm wide. Flowers are light-purple or pink forming individual capitula, up to 5 cm wide, having a hemispherical involucre. It is a thermophilic species and inhabits arid rocky meadows and dry, rocky, sunny and thermophilic habitats along roadsides, fields and vineyards in Southern Europe and Anatolia (Xeranthemum, 1975).

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

A large number of known medicinal species belonging to the family *Asteraceae* are used in phytomedicine and pharmacy. For very long time medicinal plants of this family have been used in the treatment of many diseases of the digestive, respiratory, and cardiovascular systems and skin diseases, as well as for the preparation of beverages, and as culinary spices. The species of the family *Asteraceae* are very rich in phenolic compounds with very strong biological activity and they exhibit strong antioxidant, antibacterial, antifungal, antiviral and antiproliferative effects (Özgen et al., 2004; Boussaada et al., 2008; Jayaraman et al., 2008; Muley et al., 2009; Kasim et al., 2011).

Literature data indicate that the *X. annuum* is a medicinal plant in traditional medicine and it is applied as a source of active substances (Vogl-Lukasser and Vogl, 2004; Watson and Preedy, 2008). However, there is very little data on laboratory phytochemical studies and biological activity of extracts and isolated components from *X. annuum*, which points to the fact that the plant has not been explored completely. The existing data often provide chemical properties of secondary metabolites from *X. annuum* and the description of the most common metabolites (Zemtsova and Molchanova, 1979; Skaltsa et al., 2000).

Bearing in mind that the family *Asteraceae* comprises species that are well known in phytomedicine and pharmaceutical industry, it is obvious that the evaluation of *X. annuum* as a new source of natural medicinal substances may contribute to the knowledge about the use and importance of the family.

Therefore, the purpose of this study was to explore *X. annuum* as a new potential natural source of effective antioxidant and antimicrobial agents, as well as its total phenolic content and flavonoid concentrations. Antioxidant activity, phenolic content and flavonoid concentrations are determined by spectrophotometric methods and *in vitro* antimicrobial activity was investigated on bacterial and fungal strains by microdilution method.

MATERIAL AND METHODS

Plant material

In August 2009 aerial parts of *X. annuum* were collected from natural populations on Plackovica hill in the region of Vranje sity in south Serbia: (position: 42°34′51.94″N, 21°53′53.90″E, altitude: 1075.00 m, exposition: E, habitat: arid thermophilic rocky meadows). Plants identified were confirmed and voucher specimens deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The collected plant material was air-dried in darkness at ambient temperature (20 °C). The dried plant material was cut up and stored in tightly sealed dark containers.

Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from „Zorka pharma“ Šabac, Serbia. Gallic acid, rutin hydrate, chlorogenic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St Louis, MO, USA. Folin-Ciocalteu phenol reagent, and aluminium chloride hexahydrate ($AlCl_3$) were purchased from Fluka Chemie AG, Buchs, Switzerland. Nutrient liquid medium, a Mueller–Hinton broth was purchased from Liofilchem, Italy, while a Sabouraud dextrose broth was obtained from Torlak, Belgrade. An antibiotic, doxycycline, was purchased from Galenika A.D., Belgrade, and antimycotic, fluconazole was from Pfizer Inc., USA. All other solvents and chemicals were of analytical grade.

Preparation of plant extracts

Prepared plant material (10 g) was transferred to dark-coloured flasks with 200 ml of solvent (methanol, ethyl acetate, acetone) and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents.

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 °C using rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 °C.

Determination of total phenolic contents in the plant extracts

The total phenolic content was determined using spectrophotometric method (Singleton et al., 1999). The reaction mixture was prepared by mixing 0.5 ml of methanolic solution (1 mg/ml) of extract, 2.5 ml of 10 % Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5 % $NaHCO_3$. The samples were incubated at 45 °C for 15 min. The absorbance was determined at λ_{max} = 765 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. Blank was concomitantly prepared with methanol instead of extract solution. The same procedure was repeated for the gallic acid and the calibration line was construed. The total phenolic content was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of flavonoid concentrations in the plant extracts

The concentrations of flavonoids was determined using spectrophotometric method (Quettier et al., 2000). The sample contained 1 ml of methanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2 % $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined at λ_{max} = 415 nm. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for the rutin and the calibration line was construed. The concentration of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

Evaluation of DPPH scavenging activity

The ability of the plant extract to scavenge DPPH free radicals was assessed using the method described by Tekao et al. (1994), adopted with suitable modifications from Kumarasamy et al. (2007). The stock solution of the plant extract was prepared in methanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, 0.97 µg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of DPPH methanolic solution (80 µg/ml). After 30 min in darkness at room temperature (23 °C) the absorbance was recorded at 517 nm. The control samples contained all the reagents except the extract. The percentage inhibition was calculated using the equation: % inhibition = 100 x (A *control* – A *sample*)/A *control*), whilst IC_{50} values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis. The data were presented as mean values ± standard deviation (n = 3).

Test microorganisms

Antimicrobial activity of acetone, ethyl acetate and methanolic extract was tested against 24 microorganisms including fifteen strains of bacteria (standard strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Bacillus pumilus* NCTC 8241 and clinical strains: *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Proteus mirabilis, Sarcina lutea, Salmonella enterica, Bacillus subtilis* and *Bacillus cereus*) and nine species of fungi: *Penicillium italicum* PMFKG-F29, *Penicillium digitatum* PMFKG-F30, *Penicillium chrysogenum* PMFKG-F31, *Trichothecium roseum* PMFKG-F32, *Botrytis cinerea* PMFKG-F33; *Aspergillus niger* ATCC 16404; *Candida albicans* (clinical isolate); *Rhodotorula sp.* PMFKG-F27 and *Saccharomyces boulardii* PMFKG-P34. All clinical isolates were a generous gift from the Institute of Public Health, Kragujevac. The other microorganisms were provided from a collection held

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

by the Microbiology Laboratory, Faculty of Science, University of Kragujevac.

Suspension preparation

Bacterial and yeast suspensions were prepared by the direct colony method. The turbidity of initial suspension was adjusted by comparing with 0.5 McFarland's standard (Andrews, 2005). Initial bacterial suspension contains about 10^8 colony forming units (CFU)/mL and yeast suspension contains 10^6 CFU/mL. 1:100 dilutions of initial suspension were additionally prepared into sterile 0.85 % saline. The suspensions of fungal spores were prepared by gentle stripping of spores from slopes with growing aspergilli. The resulting suspensions were 1:1000 diluted in sterile 0.85 % saline.

Microdilution method

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) using microdilution method with resazurin, an indicator of microbial growth (Sarker et al., 2007). The 96-well plates were prepared by dispensing 100 µL of nutrient broth, Mueller–Hinton broth for bacteria and Sabouraud dextrose broth for fungi, into each well. A 100 µL from the stock solution of tested extracts (concentration of 80 mg/mL) was added into the first row of the plate. Then, twofold, serial dilutions were performed by using a multichannel pipette. The obtained concentration range was from 40 to 0.156 mg/mL. MIC was defined as the lowest concentration of tested extracts that prevented resazurin color change from blue to pink (Vasić et al., 2010).

Minimum microbicidal concentration (MMC) was determined by plating 10 µL of samples from wells, where no indicator color change was recorded, on nutrient agar medium. At the end of the incubation period the lowest concentration with no growth (no colony) was defined as minimum microbicidal concentration.

Doxycycline and fluconazole were used as a positive control. Solvent control test was performed to study the effects of 10 % DMSO on the growth of microorganism. It was observed that 10 % DMSO did not inhibit the growth of microorganism. Also, in the experiment, the concentration of DMSO was additionally decreased because of the twofold serial dilution assay (the working concentration was 5 % and lower). Each test included growth control and sterility control. All tests were performed in duplicate and MICs were constant.

Statistical analysis

Data are presented as means ± standard deviations where appropriate. All statistical analyses were performed using SPSS package. Mean differences were established by Student's *t*-test. Data were analyzed using one-way analysis of variance (ANOVA). In all cases P values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Total phenol content and flavonoid concentrations

Three extracts from *X. annuum* were prepared using different solvents (methanol, acetone, ethyl acetate) in order to examine the total phenolic content, flavonoid concentrations and free radical scavenging activity. Different solvents were used to extract active ingredients of diverse polarities. The choice of solvents proved to be very effective in earlier studies (Stanković et al., 2010).

The results of the total phenolic content determination of the examined plant extracts, using Folin-Ciocalteu method, are presented in Table 1. The total phenolic content in extracts was expressed as gallic acid equivalents ranged between 101.33 to 159.48 mg GA/g. Total phenolic content was the highest in all extracts from *X. annuum*, among which the acetone extract (159.48 mg GA/g) contained the highest concentracion of phenolic compounds. Analyzing the results of total phenolic content in all extracts, it was noticed that the highest concentration of phenolic compounds in the extracts were obtained using the solvents of moderate polarity. Other au-

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

thors reported that high dissolubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar or moderately polar solvents for the extraction (Zhou and Yu, 2004).

Table 1: Total phenolic contents[1] and concentrations of flavonoids in *X. annuum* extracts

Type of extract	Total phenolic content (mg GA/g of extract)	Flavonoid concentration (mg RU/g of extract)
methanolic	101.33 ± 0.99	36.52 ± 0.98
acetone	159.48 ± 1.12	62.42 ± 1.01
ethyl acetate	105.32 ± 1.24	22.25 ± 0.85

[1]Each value in the table was obtained by calculating the average of three analyses ± standard deviation

The results of the analysis of different extracts indicate that acetone is the most effective solvent for extraction of phenolic compounds from *X. annuum*, and that moderately polar solvents should be used for the purpose.

The concentration of flavonoids in various extracts of *X. annuum* was determined using spectrophotometric method with $AlCl_3$. The content of flavonoids was expressed in terms of rutin equivalents. The summary of quantities of flavonoids identified in the tested extracts is shown in Table 1. The concentrations of flavonoids in plant extracts ranged from 22.25 to 62.42 mg RU/g. High concentrations of flavonoids were measured in acetone extracts. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005). Based on the obtained values of the concentration of flavonoids in the examined extracts of *X. annuum*, it was found that the highest concentration of these compounds was in the extracts obtained using solvents of moderate polarity.

Antioxidant activity

The antioxidant activity of different plant extracts of *X. annuum* was determined using methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent. DPPH method has also been used to quantify antioxidants in complex biological systems in recent years and it's based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The scavenging activity was measured as the decrease in absorbance of the samples versus DPPH standard solution.

The antioxidant activity of three different extracts from *X. annuum* is expressed in terms of IC_{50} (µg/ml) values. Along with the examination of the antioxidant activity of the plant extracts, the values for chlorogenic acid as reference substance were obtained and compared to the values of the antioxidant activity of *X. annuum*. IC_{50} values for antioxidant activity of the extracts are given in Table 2. The antioxidant activity values examined by DPPH radical scavenging activity ranged from 59.25 to 956.81 µg/ml. The largest capacity to neutralize DPPH radicals was measured in the acetone extract from *X. annuum*, which neutralized 50 % of free radicals at the very small concentrations of 59.25 µg/ml. In the measuring of total phenolic content and flavonoid concentrations acetone extract showed the highest activity from the tested extracts.

Table 2: Antioxidant (DPPH scavenging) activity[1] of investigated plant extracts and standard substance presented as IC_{50} values (µg/ml)

Type of extract	IC_{50} values (µg/ml)
methanolic	91.31 ± 1.32
acetone	59.25 ± 0.93
ethyl acetate	956.81 ± 1.84
chlorogenic acid	11.65 ± 0.52

[1]Each value in the table was obtained by calculating the average of three analyses ± standard deviation

Based on these results, each extract of *X. annuum* showed a phenol concentration-dependent scavenging effect. Numerous investigations of the antioxidant activity of plant extracts have confirmed significant linear correlation between total phenolic content and antioxidant activity (Katalinić et al., 2004).

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

In previous studies of quantitative and qualitative composition of the main biological active ingredients of *X. annuum*, luteolin (3',4',5,7-tetrahydroxyflavone), **1**, and quercetin (3,3',4',5,7-pentahydroxyflavone), **2**, were identifed as the predominant flavonoids and ursolic acid - pentacyclic triterpene acid, **3**, as dominant triterpenoid compound (Zemtsova and Molchanova, 1979). A number of pharmacological studies showed that luteolin and quercetin exhibit antioxidant, antibacterial, antifungal, anti-inflammatory and anticancer activities (López-Lázaro, 2009; Gusdinar et al., 2011). Ursolic acid exist widely in most of the active components of medicinal herbs, spices, fruits, and vegetables. Several stydies demonstrated the strong antioxidant, antimicrobial and antitumor activity of this important compound (Assimopoulou et al., 2005; Feng et al., 2009).

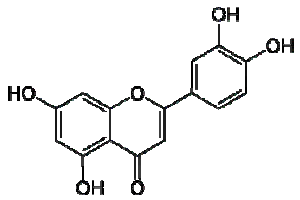

Figure 1: Structure of luteolin (**1**)

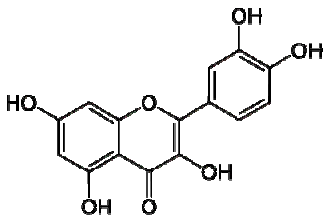

Figure 2: Structure of quercetin (**2**)

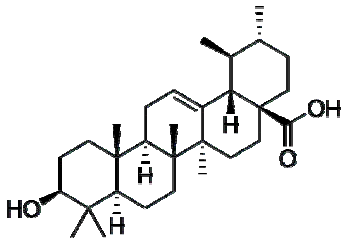

Figure 3: Structure of ursolic acid (**3**)

In addition, the phenolic contents of extracts depend on the solvent used for extraction, and not only the phenolic content but also the properties of these compounds do contribute to the activites of different extracts.

Antimicrobial activity

The results of *in vitro* antibacterial and antifungal activities of acetone, ethyl acetate and methanolic extracts of *X. annuum* are shown in Table 3 and Table 4. For comparison, MIC and MMC values for doxycycline and fluconazole are also listed in Table 3 and Table 4. The solvent (10 % DMSO) did not inhibit the growth of the tested microorganisms.

Antimicrobial activity of tested extracts was evaluated by determining MICs and MMCs in relation to the 24 species of microorganisms. MICs and MMCs values were in range from 0.625 mg/ml to 40 mg/ml. The tested extracts showed different degree of antimicrobial activity in relation to the tested species. The intensity of antimicrobial action varied depending on the groups of microorganisms and on the type of the extracts.

In general, the tested extracts demonstrated selective and moderate antimicrobial activity, while showing more potent inhibitory effects on the growth of G+ bacteria than to the other tested microorganisms ($p < 0.05$). Statistically significant difference in activity between the extracts of X. *annuum* was observed ($p < 0.05$). The acetone extract showed the strongest activity, and ethyl acetate appeared the weakest. At the acetone extract statistically significant difference can be seen in the activity on G+ bacteria compared to other microorganisms. In relation to the tested standard and clinical strains of bacteria, all tested extracts demonstrated approximately similar activity. An exception was observed in *S. aureus*, where the clear difference can be seen at acetone and methanolic extract.

EXCLI Journal 2011;10:230-239 – ISSN 1611-2156
Received: September 14, 2011, accepted: November 20, 2011, published: November 24, 2011

Table 3: Antibacterial activities of acetone, ethyl acetate and methanolic extracts of *X. annuum* against tested microorganisms based on microdilution method

Species	Acetone		Ethyl acetate		Methanolic		Doxycycline	
	MIC[1]	MMC[2]	MIC	MMC	MIC	MMC	MIC	MMC
Escherichia coli ATCC 25922	10	10	5	20	20	20	15.625	31.25
Escherichia coli	20	40	40	40	40	40	7.81	15.625
Pseud. aeruginosa ATCC 27853	5	5	10	20	5	10	62.5	125
Pseud. aeruginosa	2.5	10	10	20	5	10	250	> 250
Salmonella enterica	10	10	40	40	20	20	15.625	31.25
Proteus mirabilis	1.25	2.5	20	40	2.5	5	250	> 250
Staph. aureus ATCC 25923	10	10	20	20	20	20	0.224	3.75
Staph. aureus	0.625	1.25	5	10	2.5	2.5	0.448	7.81
Enter. faecalis ATCC 29212	10	10	20	40	20	20	7.81	62.5
Enter. faecalis	20	40	20	40	20	40	7.81	62.5
Bacillus subtilis ATCC 6633	1.25	2.5	5	5	2.5	5	1.953	31.25
Bacillus subtilis	1.25	2.5	10	10	1.25	5	0.112	1.953
Bacillus cereus	0.625	1.25	1.25	5	1.25	2.5	0.977	7.81
Bacillus pumilus NCTC 8241	0.625	1.25	1.25	5	1.25	2.5	0.112	7.81
Sarcina lutea	2.5	2.5	10	20	5	5	< 0.448	3.75

[1]Minimum inhibitory concentration (MIC) and [2]minimum microbicidal concentration (MMC) values are given as mg/ml for plant extract and μg/ml for antibiotics. Antibiotic: doxycycline

Table 4: Antifungal activities of acetone, ethyl acetate and methanolic extracts of *X. annuum* against tested microorganisms based on microdilution method

Species	Acetone		Ethyl acetate		Methanolic		Fluconazole	
	MIC[1]	MMC[2]	MIC	MMC	MIC	MMC	MIC	MMC
Candida albicans	20	40	20	40	20	20	62.5	1000
Rhodotorula sp.	5	10	5	10	5	10	62.5	1000
Saccharomyces boulardii	20	20	20	20	20	40	31.25	1000
Aspergillus niger ATCC 16404	10	40	1.25	5	0.625	0.625	62.5	62.5
Penicillium italicum	2.5	2.5	20	40	1.25	20	1000	1000
Penicillium digitatum	40	40	20	40	20	20	31.25	31.25
Penicillium chrysogenum	40	40	20	40	10	20	62.5	500
Trichothecium roseum	10	20	20	40	20	20	500	500
Botrytis cinerea	10	40	20	40	10	20	31.25	500

[1]Minimum inhibitory concentration (MIC) and [2]minimum microbicidal concentration (MMC) values are given as mg/ml for plant extract and μg/ml for antibiotics. Antibiotic: fluconazole

The tested extracts showed high anti-bacterial activity against G+ bacteria, especially for species of the genus *Bacillus* (clinical isolates and standard strains). MICs values were in range from 0.625 mg/mL to 10 mg/mL, and MMCs values were from 1.25 mg/mL to 20 mg/mL. The acetone extract showed a significant effect against *B. pumilus* NCTC 8241 and *B. cereus*. Clinical isolate of *S. aureus* also showed a promising sensitivity to acetone extract, MIC was at 0.625 mg/mL and MMC at 1.25 mg/mL.

The tested extracts did not affect the growth of clinical isolates and standard strains of G- bacteria or their activities were very low (MIC and MMC ranged from 2.5 mg/mL to 40 mg/mL). The exception is in the activity of the acetone extract against *P. mirabilis*, where MIC was at 1.25 mg/mL and MMC at 2.5 mg/mL.

The tested extracts showed low to moderate antifungal activity. Methanol extract showed a significant effect on *A. niger* ATCC 16404 with MIC and MMC at 0.625 mg/mL.

Stamatis et al. (2003) have investigated the activity of different plants of Greek herbal medicines on clinical isolates of *Helicobacter pylori* and have determined that *X. annuum* has no effect on these bacteria. Although it is mentioned in the papers as a wild medicinal herb of these areas (Jaric et al., 2007; Dogan et al., 2008; Radanova, 2009), with the exception from Stamatis et al. (2003), there are no other data about antimicrobial activity of *X. annuum*. In this study antimicrobial activity of acetone, ethyl acetate and methanolic extracts of *X. annuum* against wide range of G+, G- bacteria and fungi was investigated for the first time. The results of our research indicate good antimicrobial potential of *X. annuum*. Thus, *X. annuum* can be considered as a source of potential antimicrobial substances.

CONCLUSION

The results of our study suggest the great value of *X. annuum* in phytotherapy, pharmacy and food industry. Therefore, the aerial parts of this plant are potential natural source of biologically active substances with strong antioxidant and antimicrobial effects. For the extraction of active components from *X. annuum* acetone is an effective solvent.

ACKNOWLEDGEMENTS

This investigation was supported by the Ministry of Education and Science of the Republic of Serbia, grants No. III41010 and OI173032.

REFERENCES

Andews JM. BSAC standardized disc susceptibility testing method (version 4). J Antimicrob Chemother 2005;56:60-76.

Assimopoulou AN, Zlatanos SN, Papageorgiou VP. Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food Chem 2005;92:721-7.

Boussaada O, Ben Halima Kamel M, Ammar S, Haouas D, Mighri Z, Helal NA. Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum.* B Insectol 2008;61:283-9.

Dogan Y, Nedelcheva AM, Yarci C. Plant taxa used as brooms in several southeast European and west Asian countries. Nat Croat 2008;17:193-206.

Feng J, Chen W, Zhao Y, Ju X. Anti-tumor activity of oleanolic, ursolic and glycyrrhetinic acid. The Open Nat Prod J 2009;2:48-52.

Gusdinar T, Herowati R, Kartasasmita RE, Adnyana IK. Anti-inflammatory and antioxidant activity of quercetin-3,3',4'-triacetate. J Pharm Toxicol 2011;6:182-8.

Jaric S, Popovic Z, Macukanovic-Jocic M, Djurdjevic L, Mijatovic M, Karadzic B et al. An ethnobotanical study on the usage of wild medicinal herbs from Kopaonik Mountain (Central Serbia). J Ethnopharmacol 2007;111:160-75.

Jayaraman S, Manoharan MS, Illanchezian S. *In vitro* antimicrobial and antitumor activities of *Stevia rebaudiana* (Asteraceae) leaf extracts. Trop J Pharm Res 2008;7: 1143-9.

Kasim LS, Ferro VA, Odukoya OA, Ukpo GE, Seidel V, Gray AI et al. Evaluation of cytotoxic and antimicrobial activities of *Struchium sparganophora* (Linn.) Ktze Asteraceae. J Med Plant Res 2011;5:862-7.

Katalinić V, Miloš M, Kulišić T, Jukić M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 2004;94:550-7.

Kumarasamy Y, Byres M, Cox PJ, Jasapars M, Nahar L, Sarker SD. Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother Res 2007;21: 615-21.

López-Lázaro M. Distribution and biological activities of the flavonoid luteolin. Min-Rev in Med Chem 2009;9:31-59.

Min G, Chun-Zhao L. Comparison of techniques for the extraction of flavonoids from cultured cells of *Saussurea medusa* Maxim. World J Microb Biot 2005;21:1461-3.

Muley BP, Khadabadi SS, Banarase NB. Phytochemical constituents and pharmacological activities of *Calendula officinalis* L. (Asteraceae): A review. Trop J Pharm Res 2009;8:455-65.

Özgen U, Mavi A, Terzi Z, Coşkun M, Yildirim A. Antioxidant activities and total phenolic compounds of some Asteraceae species. Turkish J Pharm Sci 2004;3:203-16.

Quettier DC, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx C et al. Phenolic compounds and antioxidant activities of buckwheat (*F. esculentum* Moench) hulls and flour. J Ethnopharmacol 2000;72:35–42.

Radanova S. Variety of medicinal plants in a cultigenic ecosystem. XI Anniversary Scientific Conference. Biotechnol & Biotechnol Eq 2009;23:389-92.

Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007;42:321-4.

Singleton VL, Orthofer R, Lamuela RRM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth Enzymol 1999;299:152–78.

Skaltsa HD, Lazari DM, Constantinidis T. Composition of the essential oil of *Xeranthemum annuum* L. from Greece. J Essent Oil Res 2000;12:742-4.

Stamatis G, Kyriazopoulos P, Golegou S, Basayiannis A, Skaltsas S, Skaltsa H. *In vitro* anti-*Helicobacter pylori* activity of Greek herbal medicines. J Ethnopharmacol 2003;88:175-9.

Stanković MS, Topuzović M, Solujić S, Mihailović V. Antioxidant activity and concentration of phenols and flavonoids in the whole plant and plant parts of *Teucrium chamaedrys* L. var. *glanduliferum* Haussk. J Med Plant Res 2010;4:2092-8.

Tekao T, Watanabe N, Yagi I, Sakata K. A simple screening method for antioxidant and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Biosci Biotechnol Biochem 1994;58:1780-3.

Vasić GP, Glodjović VV, Radojević ID, Stefanović OD, Ćomić LjR, Djinović VM et al. Stereospecific ligands and their complexes. V. Synthesis, characterization and antimicrobial activity of palladium(II) complexes with some alkyl esters of (S,S)-ethylenediamine-N,N0-di-2-propanoic acid. Inorg Chim Acta 2010;363:3606-10.

Vogl-Lukasser B, Vogl CR. Ethnobotanical research in homegardens of small farmers in the alpine region of Osttirol (Austria): An example for bridges built and building bridges. Ethnobot Res Appl 2004;2:111-37.

Watson R, Preedy VR. Botanical medicine in clinical practice (p 421). Trowbridge, UK: CAB International, 2008.

Xeranthemum. In: *Flore de la Republique Socialiste de Serbie VII*, Acad. Serb. Sci. & Arts. Gajić M, Belgrade (in Serbian), 1975, (p 176).

Zemtsova GN, Molchanova LP. Flavonoids and triterpenoids of *Xeranthemum annuum* L. Chem Nat Comp 1979;15:762.

Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT-Food Sci Technol 2004;37:717-21.