Environmental Engineering and Management Journal September 2019, Vol.18, No. 9, 2063-2072
http://www.eemj.icpm.tuiasi.ro/; http://www.eemj.eu

"Gheorghe Asachi" Technical University of Iasi, Romania

ANTIMICROBIAL ACTIVITY, ANTIOXIDANT POTENTIAL AND TOTAL PHENOLIC CONTENT OF TRANSGENIC ATCKX1 CENTAURY (*Centaurium erythraea* Rafn.) PLANTS GROWN IN VITRO

Milana Trifunović-Momčilov[1]*, Dijana Krstić-Milošević[1], Snežana Trifunović[2], Ana Ćirić[1], Jasmina Glamočlija[1], Slađana Jevremović[1], Angelina Subotić[1]

[1]*Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Bulevar despota Stefana 142, 11060 Belgrade, Serbia*
[2]*Faculty of Chemistry, University of Belgrade, Studentskitrg 16, 11000 Belgrade, Serbia*

Abstract

Common centaury, *Centaurium erythraea* Rafn., represent the best known and the most investigated medicinal plant species of genus *Centaurium*. Centaury has been used for centuries in traditional medicine. Secondary metabolites such as bitter secoiridoid glucosides (gentiopicrin, swertiamarin and sweroside), xanthones (eustomin and demethyleustomin), and phenolic acids are the main constituents responsible for the therapeutic properties of centaury. Previous investigation showed that overexpression of the *AtCKX* genes in transgenic centaury plants did not result in a decrease of total cytokinin (CK) content, but in an altered CK profile leading to a decline of bioactive, the most important physiologically active group of CKs.The aim of this study was to investigate antibacterial and antifungal activity of transgenic centaury methanol extracts as well as pure secoiridoid and xanthone compounds on four Gram positive, four Gram negative bacteria and eight species of microfungi. All tested methanol extracts of control and transgenic *AtCKX1* centaury shoots and roots showed better antibacterial activity, while pure compounds (gentiopicrin, swertiamarin, eustomin and demethyleustomin) showed better antifungal activity. The results obtained in this work suggest that centaury methanol extracts and pure compounds represent potential antimicrobials confirming the possibility of using these compounds in agronomy, veterinary, medicine or food industry.

Key words: antibacterial activity, antifungal activity, *AtCKX* genes, *Centaurium erythraea* Rafn., secondary metabolites

Received: January, 2019; Revised final: February, 2019; Accepted: March, 2019; Published in final edited form: September, 2019

1. Introduction

Microbes cause numerous and the most diverse infections in humans worldwide. The increasing in use of antibiotics and antifungal drugs has led to the development of multidrug-resistant pathogens (van de Sande-Brunisma et al., 2008). Thus, there is a need for discovering novel and effective tools for controlling these pathogenic microorganisms. More than one hundred secondary plant metabolites are being used as drugs. It is already known that medicinal plants produce a numerous physiologically active compounds used in the treatment of various chronic and infectious diseases (Dhama et al., 2015). It was discovered that plants producing terpenoids, alkaloids and polyphenols represent a very useful source of bioactive compounds with high antimicrobial activities (Silva and Fernandez, 2010).

Reactive oxygen species (ROS) including superoxide radical (O_2^{-}), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2) represent an important mediators of cell injuries such as membrane damage, lipid peroxidation, carbohydrate damage, protein oxidation and fragmentation, mutagenesis and

* Author to whom all correspondence should be addressed: e-mail: milanag@ibiss.bg.ac.rs

carcinogenesis (Valko et al., 2007). Thus, ROS are directly or indirectly involved in numerous chronic diseases including cancer and heart diseases. Antioxidants reduce the risk of chronic diseases and at the same time play a very important role as health protecting factors (Cui et al., 2004). Endogenous and exogenous antioxidants have an equally important role in the protection of the organism against oxidative stress. Most of the antioxidant compounds with a number of chemical properties have been detected in numerous agricultural and horticultural crops and medicinal plant species (Ksouri et al., 2009). Primary sources of naturally occurring antioxidants, such as vitamins C and E, carotenes, phenolic acids etc., are fruits and vegetables (Prior and Cao, 2000). The antioxidant activity of plant extracts is primarily due to phenolic compounds. Structurally, phenols are molecules that have one or more hydroxyl substituents attached to the carbon atom of an aromatic ring (Bravo, 1998). The antioxidant activity is based on the redox properties which allow phenolic compounds to serve as reducing agents or hydrogen atom donors. Thus, natural antioxidants act as free-radical scavengers (Tosun et al., 2011).

Genus *Centaurium* (Gentianaceae) comprises about 50 species widespread in the northern hemisphere (Chevallier, 2000). In traditional medicine common centaury, *Centaurium erythraea* Rafn. (syn. *C. umbellatum* Gillib. and *C. minus* Moench., *Gentiana centaurium* L., *Erythraea centaurium*), has been used for centuries and represents a very important medicinal plant in the treatment of gastrointestinal tract diseases. Also, centaury represents the most investigated medicinal plant species of genus *Centaurium*. Because of the numerous biologically active pharmacological compounds this cosmopolitan plant species has been denoted as a plant with bitter digestive properties (Blumenthal et al., 1998; Hänsel et al., 1992), diuretic effect (Haloui, 2000), hepato-protective activity (Mroueh et al., 2004), antipyretic activity (Berkan et al., 1991), anti-inflammatory activity (Capasso et al., 1983; Hänsel et al., 1992; Newall et al., 1996) and antioxidant potential (Valentão et al., 2001; 2003). Phytochemical investigation of the genus *Centaurium* revealed the presence of bitter secoiridoid glucosides swertiamarin, gentiopicrin and sweroside (Jensen and Schripsema, 2002; Van der Sluis et al., 1983) and xanthones such as eustomin and demethyleustomin (Valentão et al., 2002; Van der Sluis, 1985a, 1985b). Kumarasamy et al. (2003a, 2003b) described the bioactivity of centaury secoiridoid glycosides sixteen years ago.

Cytokinin oxidase/dehydrogenase (CKX, EC 1.5.99.12) is the only known catabolic enzyme that catalyzes specific cytokinin (CK) degradation in plant tissues (Mok and Mok, 2001; Schmülling et al., 2003). Genetic transformation of plants using specific *AtCKX* genes is very useful tool for investigation of numerous physiological processes controlled by CKs. Obtained *AtCKX* plants are expected to be with increased CKX activity and decreased endogenous CKs content, accordingly. An efficient protocol for *A. tumefaciens*-mediated transformation of centaury and production of stable transformants overexpressing *AtCKX* genes were previously described (Trifunović et al., 2013). Transgenic *AtCKX* centaury plants showed changed CK content and altered CK homeostasis which resulted in reduced level of bioactive CKs and, at the same time, increased contents of storage, inactive CK forms and/or CK nucleotides (Trifunović et al., 2015).

Notwithstanding that centaury is widely used in traditional medicine there are no many literature data considering biological effects of pure xanthones isolated from centaury. Numerous biological activities of centaury have mainly been ascribed to whole plant extracts. Therefore, the aim of this study was to investigate antibacterial and antifungal activity of non-transformed and transgenic centaury methanol extracts as well as pure secoiridoid and xanthone compounds on four Gram positive, four Gram negative bacteria and eight species of microfungi. Also, the purpose of this work was to investigate the antioxidant capacity and the total phenolic content of centaury, very important medicinal plant, and to evaluate the its potential antioxidants for medicinal and food purposes.

2. Material and methods

2.1. Plant material and culture conditions

Gene coding for *Arabidopsis* CKX isoform, *AtCKX1*, was introduced into root explants of *C. erythraea* Rafn. as previously described (Trifunović et al., 2013). Four *AtCKX1* centaury lines were selected for further analyses. All selected lines showed increased expression of *AtCKX1* transgenes and at the same time increased level of CKX activity. Among all analysed transgenic centaury lines only one line, *AtCKX1*-29, produced increased content of xanthones compared to control (Trifunović et al., 2015). Control and only one transgenic centaury line, *AtCKX1*-29, were used in this study. Both centaury lines were cultured on solid, hormone free and half-strength MS medium (½MS, Murashige and Skoog, 1962) during four weeks. The used medium was supplemented with 3% sucrose and 100 mgL^{-1} *myo*-inositol. All *in vitro* cultured plants were grown at 25 ± 2°C and a 16h/8h photoperiod (“Tesla” white fluorescent lamps, 65W, 4500K; light flux of 47 47 μmols^{-1}m^{-2}).

2.2. HPLC analyses

Extraction and quantification of secondary metabolites from control and transgenic *AtCKX1*-29 centaury line was carried out as follows. The air-dried and powered *AtCKX* transgenic and control *C. erythraea* 4-weeks-old shoots and roots were extracted with methanol for 48h at room temperature in the dark. The ratio between plant material and solvent was 1:20, w/v. Analyses of extracts were carried out on an Agilent series 1100 HPLC instrument with DAD detector and a reverse phase

Zorbax SB-C18 analytical column (150 x 4.6 mm, 5 µm). Mobile phase consisted of 1%, v/v solution of orthophosphoric acid in water (solvent A) and acetonitrile (solvent B). The flow rate was 1 mL min^{-1}. Injection volume of sample was 5 µl and the gradient elution was as follows: 98-90% A, 0-5 min, 90-85% A, 5-10 min, 85% A, 10-13min, 85-70% A, 13-15 min, 70-10% A, 15-20 min, 10% A, 20-22 min, 10-0% A, 22-25 min. Detection wavelengths were set at 260 nm for secoiridoids and 320 nm for xanthones.

Xanthones, eustomin and demethyleustomin, were isolated from aerial parts of *C. erythreae* plants collected from nature. Their structures were confirmed by spectroscopic techniques: UV, 1D and 2D NMR spectroscopy and MS spectrometry. Secoiridoids, swertiamarin and gentiopicrin, were supplied by Cfm Oscar Tropitzsch (Germany). These compounds were used as standards. Quantification was performed using HPLC and the amounts of these compounds were calculated using calibration curves. The results are presented as mg/g of dry weight (DW).

2.3. Antibacterial and antifungal activity

Antibacterial and antifungal activities were determined using the microdilution method as described previously (Božunović et al., 2018). The antibacterial activity of investigated extracts was evaluated using eight bacterial strains: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Enterobacter cloacae* (human isolate), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) and *Staphylococcus aureus* (ATCC 6538). The antibacterial assay was carried out by a microdilution method (CLSI, 2009; Tsukatani et al., 2012). The centaury extracts (10 mg/mL) and pure compounds (1 mg/mL) were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v) and added in Tryptic Soy broth (TSB) medium (100 µl) with bacterial inoculum (1.0×10^4 CFU per well). The results were expressed as minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

The antifungal activity of the extracts used in this study was evaluated using eight fungal species such as *Aspergillus fumigatus* (human isolate), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Penicillium verrucosum* var. *cyclopium* (food isolate). A modified microdilution technique was used (Espinel-Ingroff, 2001; Hanel and Raether, 1988). Antifungal results were expressed by MICs and minimum fungicidal concentrations (MFCs). A serial dilution technique using microtiter plates was used for determination of MICs. Investigated centaury extracts (10 mg/mL) and pure compounds (1 mg/mL) were dissolved in 5% DMSO solution containing 0.1% Tween 80 (v/v). Further, centaury extracts were added in broth Malt medium supplemented with inoculum. The microtiter plates were further incubated in a rotary shaker (160 rpm) at 28°C during 72h. Minimum inhibitory concentrations were determined as lowest concentrations without visible growth. Also, a serial dilution technique using microtiter plates was used for determination of MFCs. The serial subcultivation of tested compounds (2 µL) were dissolved in medium and inoculated for 72h. Microtiter plates containing 100 µL of broth per well were further incubated at 28°C during 72h. Minimum fungicidal concentrations, indicating 99.5% killing of the original inoculum, were determined as lowest concentrations with no visible growth. Solution of 5% DMSO was used as a negative control, commercial fungicides, bifonazole (Srbolek, Belgrade, Serbia) and ketoconazole (Zorkapharma, Šabac, Serbia), were used as positive controls (1–3500 µg/mL). All experiments were performed in duplicate and repeated three times.

2.4. DPPH radical scavenging activity

The DPPH assay is a rapid and simple method for measuring antioxidant capacity, based on the reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical. The effect of centaury methanol extracts on DPPH free radicals was estimated according to the procedure described by Brand-Williams et al. 1995, with some modifications. Centaury extracts were dissolved in methanol (dilution series from 0.125 – 2 mg/mL) and mixed with methanol solution of DPPH 150 µM). The resulting mixture was shaken and incubated for 20 minutes at room temperature in the dark. Thereafter, absorbance (A) of the samples was measured spectrophotometrically at 517 nm. Radical scavenging activity was calculated by following formula: % Inhibition=[(A blank sample-A centaury extract)/A blank sample] x 100. The IC_{50} values were calculated by linear regression of plots where the abscissa represented the concentration of tested samples and ordinate the average percent of inhibition activity from three separate tests.

2.5. Determination of total phenolic content

The level of total phenols in centaury methanol extracts was determined by using Folin–Ciocalteu reagent and external calibration with gallic acid. Determination of total phenolic content was carried out according to the procedure described by (Singelton et al., 1999) with slight modification. To 100 µL of each extract (5 mg/mL), 500 µL of Folin-Ciocalteu reagent (previously tenfold diluted with distilled water) was added and mixed. After 5 minutes, 400 µL of sodium carbonate (7.5 g/mL) was added, and the mixture was incubated at room temperature in the dark for 2 hours. The absorbance was measured spectrophotometrically at 760 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight of plant extract (mg GAE/ g DW).

Triplicate measurements were taken and mean values were calculated.

2.6. Statistical analysis

All statistical analyses were performed using StatGrafics software version 4.2 (STSC Inc. and Statistical Graphics Corporation, 1985–1989, USA). The data were subjected to analysis of variance (ANOVA) and comparisons between the mean values were made using the least significant difference (LSD) test calculated at a confidence level of $p \leq 0.05$.

3. Results and discussion

3.1. Secondary metabolite content

HPLC analysis of centaury methanolic extracts revealed that there were no differences in detected secondary metabolites from transgenic centaury line *AtCKX1*-29 grown *in vitro* and control centaury plants. The common peaks of secoiridoids and xanthones were detected in all tested extracts. Quantitative differences in secondary metabolites content from centaury shoots and roots were presented in Table 1. Increased xanthones content and decreased content of secoiridoids was determined in transgenic centaury line in comparison to control centaury extracts. It was also noticed that in control centaury shoots grown *in vitro* secoiridoid swertiamarin was dominated (Table 1). However, in shoots and roots of transgenic line *AtCKX1*-29, production of swertiamarin significantly decreased and the content of this bitter glucoside reduced to only 1.34 mg/g DW in the shoots. Also very low content of gentiopicrin was detected (0.47 mg/g DW). On the contrary, the amounts of xanthones were higher in both transgenic shoots and roots compared to control extracts. The highest amount of eustomin (4.71 mg/g DW) and demethyleustomin (1.70 mg/g DW) was determined in the roots of transgenic line *AtCKX1*-29. Typical chromatographic profile of centaury methanol extract is shown in Fig. 1.

Swertiamarin was the most abundant secoiridoid in control centaury shoots grown *in vitro* On the other hand, the roots of control centaury contained gentiopicrin as the dominant secoiridoid. The results of this work are corresponding with previous reports only partially. Earlier investigations showed that swertiamarin was the dominant secoiridoid in centaury shoots and roots collected from the natural habitat but secoiridoidgentiopicrin was dominated in centaury shoot and roots grown *in vitro* while swertiamarin was detected only in traces (Janković et al., 1997; Piatczak et al., 2005; Van der Sluis, 1985a, 1985b).

In *AtCKX1*-29 transgenic centaury shoots and roots significantly lower content of swertiamarin and gentiopicrin was detected compared to control shoots and roots. Based on the content of centaury swertiamarin and gentiopicrin Piatczak et al. (2005) concluded that *in vitro* culture conditions influenced on reduced production of gentiopicrin. However, the results presented in this work indicated that *in vitro* culture conditions stimulated the production of gentiopicrin but only in centaury roots.

Table 1. Content of secoiridoids (swertiamarin and gentiopicrin) and xanthones (eustomin and demethyleustomin) in shoots and roots of 4-week-old control and *AtCKX1*-29 transgenic *Centaurium erythraea* plants grown *in vitro*. Data represent mean ± standard error. Means marked with an asterisk are significantly different from the control according to the LSD test ($p \leq 0.05$)

Secondary metabolites (mg/g DW)	*Shoots*		*Roots*	
	control	*AtCKX1-29*	*control*	*AtCKX1-29*
swertiamarin	64.77 ± 3.92	1.34 ± 0.11*	6.36 ± 0.97	3.76 ± 0.33*
gentiopicrin	1.56 ± 0.19	0.47 ± 0.10*	12.75 ± 1.28	2.50 ± 0.46*
eustomin	1.18 ± 0.12	1.48 ± 0.02	3.31 ± 0.56	4.71 ± 0.04*
demethyleustomin	1.30 ± 0.15	1.17 ± 0.01	0.90 ± 0.01	1.70 ± 0.06*

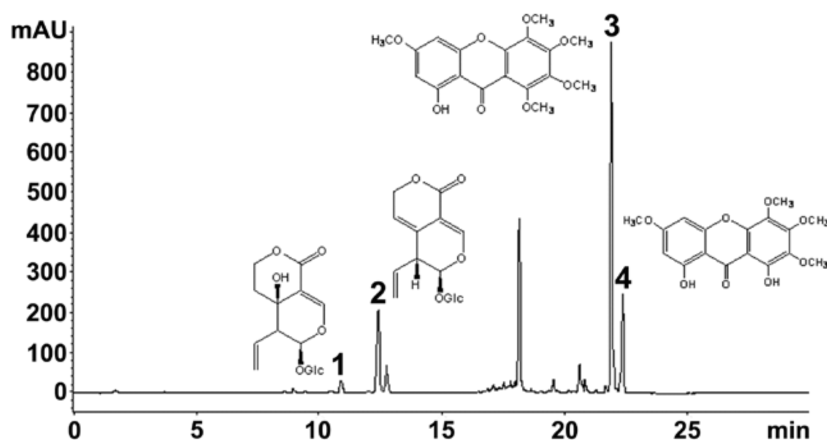

Fig. 1. HPLC profile of methanol extract of transgenic centaury roots (λ= 260 nm). Peaks: swertiamarin (1), gentiopicrin (2), eustomin (3), demethyleustomin (4)

Contrarily, Janković et al. (1997) showed increment of gentiopicrin content only in centaury shoots grown *in vitro* compared to the shoots from natural habitat. It is already known that genetic transformation could affect the production of centaury secondary metabolites. Transformed centaury roots obtained by inoculating using *A. rhizogenes* (strain A4M70GUS) accumulated only xanthones but no secoiridoids at all (Janković et al., 2002). There is also a literature which demonstrated that transformed centaury shoots inoculated by *A. rhizogenes* (strain LBA9402) produced two times higher amount of total secoiridoids compared to non-transformed shoots (Piatczak et al., 2006). In any case, it can be said that genetic transformation of centaury with *AtCKX* genes influenced the secondary metabolism of this plant species. Considering that control and transgenic centaury plants were grown *in vitro*, under the same temparature and light intensity conditions, it is possible that genetic transformation itself influenced on altered secondary metabolites content, especially on supression of gentiopicrin production.In the metabolic pathway of iridoids, secoiridoid gentiopicrin originates from the swertiamarin. In *AtCKX* transgenic centaury shoots increased accumulation of gentiopicrin and at the same time reduced swertiamarin content was detected. Accumulation of secoiridoid gentiopicrin is apparently result of increased activity of the enzyme that converts swertiamarin to gentiopicrin. It was also noted that in roots of centaury transgenic line *AtCKX*-29 the production of eustomin and demethyleustomin was stimulated. Although it is already known that *in vitro* culture could stimulate secondary metabolites production in response to abiotic stress, it can be also assumed that stress caused by genetic transformation itself additionally affected metabolic changes resulted in increased content of xanthones in *AtCKX1* transgenic centaury roots.

3.2. Antibacterial and antifungal activity of centaury plants grown in vitro

Methanol extracts of control and *AtCKX1*-29 transgenic centaury plants grown *in vitro* as well as pure secoiridoids (swertiamarin and gentiopicrin) and xanthones (eustomin and demethyleustomin) were assayed *in vitro* for their antibacterial activity against four Gram positive and four Gram negative bacteria. The results of antibacterial activity are presented in Table 2. Generally, all tested secondary metabolites (methanol extracts, pure secoiridoids and xanthones) showed MIC in the range of 0.004-0.030 mg/mL and MBC in the range of 0.005-0.040 mg/mL on all tested bacteria. Methanol extracts of all tested centaury shoots and roots showed antibacterial activity on all tested bacteria. MIC of centaury methanol extracts was in the range of 0.090-0.500 mg/mL, while MBC was in the range of 0.125-1.000 mg/mL. On the other hand, methanol extracts of control and *AtCKX1*-29 transgenic centaury roots showed low antibacterial activity (MIC/MBC were at 0.500/1.000 mg/mL) on *M. flavus*, *E. coli* and *E. cloacae*. Gentiopicrin also displayed low antibacterial activity and MIC/MBC were 0.030/0.040 mg/mL on *M. flavus*, *E. coli* and *E. cloacae*. These investigations showed that the most sensitive bacterial species was *S. aureus* (MIC – 0.004mg/mL and MBC – 0.005mg/mL). It was also interesting to note that extract of control centaury shoots was more effective than tested antibiotics, streptomycin and ampicillin, on *S. aureus*. Generally, streptomycin showed MIC in the range of 0.050-0.250 mg/mL and MBC in the range of 0.100-0.500 mg/mL while ampicillin showed inhibitory effect at 0.100-0.300 mg/mL and bactericidal at 0.100-0.500 mg/mL. Secondary metabolites characteristic for *C. erythraea* species (swertiamarin, gentiopicrin, eustomin and demethyleustomin) exhibited higher antibacterial activity on all tested bacteria than centaury methanol extracts and antibiotics.

Beside antibacterial, antifungal activity of methanol extracts of control and *AtCKX1*-29 transgenic centaury plants grown *in vitro* as well as pure secoiridoids and xanthones against eight species of microfungi was also tested. The results of antibacterial activity are presented in Table 3. All tested methanol extracts of centaury shoots and roots as well as pure secoiridoids and xanthones showed antifungal effect. MIC of methanol shoots and roots extracts was in the range of 0.008-1.000 mg/mL while MFC was in the range of 0.015-1.125 mg/mL. Methanol extract of control centaury roots showed high antifungal activity with MIC 0.008 mg/ml and MFC with 0.015 mg/ml on *P. funiculosum*. On the other hand, methanol extracts of *AtCKX1* transgenic centaury shoots and transgenic roots showed not so good antifungal activity with MIC 1.000 mg/mL and MFC 1.125 mg/mL against *A. fumigatus, A. ochraceus* and *P. ochrochloron*. Pure compounds such as eustomin, demethyleustomin, swertiamarin and gentiopicrin showed good antifungal effect and MIC value was 0.002-0.030 mg/mL and fungicidal activity was 0.004-0.060 mg/mL. The majority of these compounds showed similarly high activity againstall tested fungi. It was also noted that all analysed pure compounds were more effective than any methanol extracts against all fungi with the exception of control centaury root extract against *P. funiculosum*. The commercial antifungal agent, bifonazole, showed MIC at 0.100-0.200 mg/mL and MFC at 0.200-0.250mg/mL. Ketoconazole showed fungistatic activity at 0.150-2.500 mg/mL and fungicidal effect at 0.200-3.500 mg/mL. The methanol extracts of control centaury shoots and roots grown *in vitro* exhibited higher antifungal potential than mycotics against *A. Versicolor* and *P. funiculosum*. Contrarily, methanol extracts of *AtCKX1* transgenic centaury shoots and transgenic roots showed lower activity than both mycotics except for *P. funiculosum,* where oil possessed inhibitory activity higher than mycotics. Xanthone eustomin exhibited higher antifungal potential than both mycotics (even 100 times higher).

Table 2. Antibacterial activity of the methanol extracts of centaury control shoots and roots cultured *in vitro*, transgenic *AtCKX1*-29 shoots and roots cultured *in vitro*, xanthones (eustomin and demethyleustomin) and secoiridoids (swertiamarin and gentiopicrin). Mean values of MIC (minimum inhibitory concentration) and MBC (minimal bactericidal concentration) are presented in mg/mL± SD. Different letters in each row indicate significant differences between the extracts ($p \leq 0.05$)

Bacteria			*Staphylococcus aureus*	*Bacillus cereus*	*Listeria monocytogenes*	*Micrococcus flavus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhimurium*	*Enterobacter cloacae*
shoots	control shoots *in vitro*	MIC	0.090±0.003^{b}	0.090±0.005^{c}	0.250±0.003^{cd}	0.250±0.010^{b}	0.375±0.008^{f}	0.375±0.008^{d}	0.187±0.004^{d}	0.250±0.001^{d}
		MBC	0.125±0.008^{c}	0.125±0.002^{bc}	0.500±0.02^{c}	0.500±0.020^{d}	0.500±0.007^{ef}	0.500±0.030^{b}	0.250±0.020^{d}	1.000±0.070^{b}
	transgenic shoots *in vitro*	MIC	0.187±0.003^{d}	0.125±0.000^{d}	0.250±0.020^{c}	0.375±0.120^{c}	0.187±0.004^{e}	0.375±0.008^{d}	0.125±0.008^{c}	0.250±0.020^{d}
		MBC	0.250±0.007^{e}	0.250±0.010^{d}	0.500±0.030^{c}	0.500±0.010^{d}	0.250±0.000^{d}	0.500±0.020^{b}	0.250±0.020^{cd}	1.000±0.030^{b}
roots	control roots *in vitro*	MIC	0.250±0.001^{e}	0.250±0.020^{e}	0.250±0.010^{d}	0.500±0.007^{c}	0.375±0.008^{f}	0.500±0.010^{e}	0.375±0.008^{e}	0.500±0.010^{e}
		MBC	0.500±0.007^{g}	0.500±0.020^{e}	1.000±0.030^{d}	1.000±0.070^{e}	0.500±0.010^{e}	1.000±0.100^{c}	0.500±0.070^{d}	1.000±0.200^{b}
	transgenic roots *in vitro*	MIC	0.090±0.003^{b}	0.125±0.006^{d}	0.375±0.008^{e}	0.500±0.010^{c}	0.375±0.008^{f}	0.500±0.001^{e}	0.187±0.010^{d}	0.500±0.020^{e}
		MBC	0.125±0.002^{c}	0.250±0.010^{d}	0.500±0.001^{c}	1.000±0.050^{e}	0.500±0.010^{f}	1.000±0.200^{c}	0.250±0.010^{cd}	1.000±0.10^{b}
xanthones	eustomin	MIC	0.008±0.002^{a}	0.008±0.001^{a}	0.025±0.002^{a}	0.025±0.001^{a}	0.010±0.001^{a}	0.025±0.002^{a}	0.010±0.002^{a}	0.025±0.002^{ab}
		MBC	0.010±0.001^{ab}	0.010±0.002^{a}	0.030±0.007^{a}	0.030±0.002^{a}	0.020±0.003^{a}	0.030±0.003^{a}	0.020±0.001^{a}	0.030±0.001^{a}
	demethyl-eustomin	MIC	0.008±0.001^{a}	0.008±0.001^{a}	0.010±0.001^{a}	0.025±0.001^{a}	0.025±0.002^{ab}	0.025±0.001^{a}	0.010±0.003^{a}	0.010±0.002^{a}
		MBC	0.010±0.001^{ab}	0.010±0.002^{a}	0.030±0.002^{a}	0.030±0.001^{a}	0.030±0.002^{a}	0.030±0.001^{a}	0.020±0.002^{a}	0.030±0.002^{a}
secoiridoids	swertiamarin	MIC	0.010±0.001^{a}	0.005±0.001^{a}	0.010±0.001^{a}	0.030±0.001^{a}	0.030±0.001^{b}	0.025±0.002^{a}	0.025±0.002^{a}	0.010±0.001^{a}
		MBC	0.020±0.001^{b}	0.020±0.002^{a}	0.030±0.003^{a}	0.040±0.001^{a}	0.040±0.002^{a}	0.030±0.002^{a}	0.030±0.001^{a}	0.030±0.002^{a}
	gentiopicrin	MIC	0.004±0.001^{a}	0.020±0.001^{a}	0.020±0.002^{a}	0.030±0.001^{a}	0.020±0.001^{ab}	0.030±0.002^{a}	0.015±0.002^{a}	0.030±0.001^{ab}
		MBC	0.005±0.003^{a}	0.030±0.002^{a}	0.030±0.007^{a}	0.040±0.002^{a}	0.030±0.001^{a}	0.040±0.002^{a}	0.020±0.001^{a}	0.040±0.002^{a}
positive controls	strepto-mycin	MIC	0.250±0.010^{e}	0.050±0.010^{b}	0.150±0.010^{b}	0.130±0.007^{a}	0.050±0.003^{c}	0.050±0.010^{b}	0.050±0.001^{b}	0.050±0.002^{b}
		MBC	0.500±0.010^{f}	0.100±0.007^{b}	0.300±0.020^{b}	0.250±0.010^{c}	0.100±0.001^{b}	0.100±0.020^{a}	0.100±0.001^{b}	0.100±0.010^{a}
	ampicillin	MIC	0.100±0.007^{c}	0.100±0.010^{d}	0.150±0.007^{b}	0.100±0.007^{a}	0.100±0.010^{c}	0.300±0.010^{c}	0.150±0.020^{c}	0.150±0.010^{c}
		MBC	0.150±0.070^{d}	0.150±0.010^{c}	0.300±0.007^{b}	0.150±0.010^{b}	0.200±0.010^{a}	0.500±0.030^{b}	0.200±0.002^{c}	0.200±0.020^{a}

There are numerous reports describing antiviral, antibacterial, antifungal and anti-inflammatory properties of plants (Fierascu et al., 2017; Kapoor et al., 2015; Namita and Mukesh, 2012; Pushpa et al., 2013; Saranraj and Sivasakthi, 2014; Silva and Fernandes, 2010; Tanase et al., 2018). Some of these observations helped in identifying active compounds responsible for specific activities and certainly helped in developing novel drugs for therapeutic use in humans. In the present study secondary metabolites characteristic for *C. erythraea* species (swertiamarin, gentiopicrin, eustomin and demethyleustomin) exhibited higher antibacterial activity on all tested bacteria than centaury methanol extracts and antibiotics. All the tested methanol extracts of centaury shoots and roots exhibited antibacterial activity. Pure swertiamarin, gentiopicrin, eustomin and demethyleustomin exhibited higher antibacterial activity on all tested bacteria than centaury methanol extracts. These compounds showed higher antibacterial activity even better than commercial antibiotics (streptomycin and ampicillin) used as positive control. It can be concluded that high antimicrobial activity could be ascribed to bitter secoiridoid glycosides as well as xanthones. These results are in accordance with previous findings considering the antibacterial activity of swertiamatin and gentiopicrin (Šiler et al., 2010, 2014). Beside antibacterial all tested methanol extracts of centaury shoots and roots as well as pure secoiridoids and xanthones showed antifungal effect. The majority of these compounds showed high activity against all tested fungi. It was also noted that all analysed pure secoiridoids and xanthones were more effective than any centaury methanol extracts against all fungi. Interestingly, xanthone eustomin exhibited higher antifungal potential, even 100 times higher, than both applied mycotics (ketoconazole and bifonazole).

Table 3. Antifungal activity of the methanol extracts of centaury control shoots and roots cultured *in vitro*, transgenic *AtCKX1*-29 shoots and roots cultured *in vitro*, xanthones (eustomin and demethyleustomin) and secoiridoids (swertiamarin and gentiopicrin). Mean values of MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) are presented in mg/mL± SD. Different letters in each row indicate significant differences between the extracts ($p \leq 0.05$)

Fungi			***Aspergillus fumigatus***	***Aspergillus versicolor***	***Aspergillus ochraceus***	***Aspergillus niger***	***Trichoderma viride***	***Penicillium funiculosum***	***Penicillium ochrochloron***	***Penicillium verrucosum* var. *cyclopium***
shoots	control shoots *in vitro*	MIC	0.500±0.020^{c}	0.060±0.001^{ab}	0.125±0.008^{a}	0.500±0.020^{f}	0.125±0.008^{d}	0.125±0.008^{ab}	0.250±0.020^{d}	0.125±0.025^{bc}
		MFC	1.000±0.070^{d}	0.125±0.008^{ab}	0.250±0.020^{c}	1.125±0.040^{d}	0.250±0.020^{cd}	0.250±0.020^{b}	0.500±0.020^{c}	0.250±0.020^{b}
	transgenic shoots *in vitro*	MIC	1.000±0.070^{e}	0.500±0.030^{d}	1.000±0.001^{b}	0.250±0.007^{d}	0.125±0.080^{cd}	0.500±0.020^{c}	1.000±0.070^{e}	0.500±0.020^{d}
		MFC	1.250±0.080^{d}	1.000±0.100^{de}	1.250±0.08^{d}	0.500±0.007^{c}	0.250±0.010^{cd}	1.000±0.070^{c}	1.250±0.080^{de}	1.250±0.080^{d}
roots	control roots *in vitro*	MIC	1.000±0.050^{d}	0.500±0.001^{de}	1.000±0.070^{b}	0.500±0.020^{f}	0.250±0.010^{f}	0.008±0.001^{a}	1.000±0.001^{e}	0.250±0.020^{c}
		MFC	1.250±0.080^{d}	1.000±0.200^{e}	1.250±0.050^{d}	1.125±0.120^{e}	0.500±0.020^{e}	0.015±0.002^{a}	1.500±0.070^{e}	1.000±0.100^{c}
	transgenic roots *in vitro*	MIC	1.000±0.001^{de}	0.500±0.050^{e}	1.000±0.200^{b}	0.500±0.020^{e}	0.125±0.001^{cd}	0.125±0.008^{ab}	1.000±0.001^{e}	1.000±0.070^{e}
		MFC	1.125±0.040^{d}	1.000±0.050^{d}	1.125±0.020^{d}	1.000±0.070^{d}	0.250±0.007^{cd}	0.250±0.010^{b}	1.125±0.020^{d}	1.125±0.040^{c}
xanthones	eustomin	MIC	0.015±0.002^{a}	0.015±0.002^{a}	0.015±0.060^{a}	0.004±0.001^{a}	0.003±0.007^{a}	0.004±0.001^{a}	0.015±0.002^{ab}	0.008±0.007^{a}
		MFC	0.060±0.003^{a}	0.060±0.003^{ab}	0.060±0.003^{ab}	0.015±0.002^{a}	0.004±0.003^{a}	0.007±0.001^{a}	0.060±0.007^{a}	0.015±0.005^{a}
	demethyl-eustomin	MIC	0.015±0.002^{a}	0.015±0.002^{a}	0.015±0.001^{a}	0.030±0.002^{a}	0.030±0.003^{b}	0.030±0.003^{ab}	0.030±0.002^{ab}	0.015±0.002^{a}
		MFC	0.060±0.007^{a}	0.060±0.007^{ab}	0.060±0.003^{ab}	0.060±0.003^{a}	0.060±0.001^{b}	0.060±0.001^{a}	0.060±0.003^{a}	0.030±0.007^{a}
secoiridoids	swertiamarin	MIC	0.015±0.001^{a}	0.015±0.001^{a}	0.015±0.002^{a}	0.015±0.002^{a}	0.015±0.002^{ab}	0.030±0.003^{ab}	0.030±0.080^{bc}	0.015±0.001^{a}
		MFC	0.060±0.003^{a}	0.060±0.001^{ab}	0.060±0.001^{a}	0.030±0.007^{a}	0.030±0.003^{a}	0.060±0.003^{a}	0.060±0.005^{a}	0.030±0.007^{a}
	gentiopicrin	MIC	0.008±0.001^{a}	0.002±0.001^{a}	0.004±0.001^{a}	0.002±0.001^{a}	0.002±0.001^{a}	0.004±0.001^{a}	0.004±0.001^{a}	0.004±0.001^{a}
		MFC	0.015±0.002^{a}	0.004±0.001^{a}	0.008±0.001^{a}	0.008±0.001^{a}	0.008±0.001^{a}	0.008±0.001^{a}	0.008±0.001^{a}	0.008±0.001^{a}
positive controls	keto-conazole	MIC	0.200±0.070^{b}	0.200±0.010^{b}	0.150±0.020^{a}	0.200±0.007^{c}	0.200±0.010^{e}	2.500±0.200^{d}	0.200±0.020^{cd}	1.000±0.100^{e}
		MFC	0.500±0.020^{c}	0.500±0.050^{b}	0.200±0.020^{bc}	0.500±0.001^{c}	0.300±0.020^{d}	3.500±0.100^{d}	0.500±0.030^{c}	1.000±0.001^{c}
	bifonazole	MIC	0.150±0.0100^{b}	0.100±0.020^{c}	0.150±0.001^{a}	0.150±0.007^{b}	0.100±0.007^{c}	0.200±0.020^{b}	0.200±0.030^{d}	0.150±0.010^{b}
		MFC	0.200±0.001^{b}	0.200±0.007^{c}	0.200±0.001^{bc}	0.200±0.007^{b}	0.200±0.010^{c}	0.250±0.020^{b}	0.250±0.020^{b}	0.200±0.020^{b}

Although centaury secondary metabolites and their bioactivity have previously been investigated in details (Kumarasamy et al., 2003a, 2003b; Van der Sluis, 1985a) there are no literature data considering pure xanthones eustomin and demethyleustomin as potential antimicrobials. Thus, the results obtained in this work present the first report considering centaury xanthones as compounds with high antifungal activity.

3.3. DPPH radical scavenging activity and total phenolic content

The antioxidant activity of plant extracts is one of the most important factor in protection against oxidative damage. The potential antioxidant activity of centaury extracts was assessed on the basis of the scavenging activity for the stable DPPH radicals. The results showed in Table 4 indicated that the highest radical scavenging activity was observed in *AtCKX1* transgenic centaury roots cultured *in vitro* where the concentration of methanol extracts lower than 1 mg/mL (IC_{50} = 0.65 mg/mL) already inhibited 50% of the DPPH reference signal. Similarly, high antioxidant activity was also detected in control centaury roots (IC_{50} = 1.10 mg/mL) and *AtCKX1* transgenic centaury shoots (IC_{50} = 1.81 mg/mL). On the other hand, control centaury shoots *in vitro* indicated the lowest antioxidant activity where IC_{50} value was 3.12 mg/mL. It was also noted that centaury root extracts were more effective than shoot extracts, with activities two to five times higher for root extracts.

Based on the results presented in Table 4 total phenolic content was higher in the centaury roots compared to shoots. The highest level of total phenolic content was detected in *AtCKX1* transgenic centaury roots grown *in vitro* (163.28 mg GA/g DW). Similar total phenolic level was determined in control centaury roots. In aerial parts of centaury control and transgenic shoots significantly lower total phenolic content revealed.

Table 4. DPPH radical scavenging activities and total phenolic content of methanol extracts of centaury control shoots and roots cultured *in vitro*, transgenic *AtCKX1*-29 shoots and roots cultured *in vitro*. Results are presented as mean ± SE. Different letters in each row indicate significant differences between the extracts ($p \leq 0.05$)

Centaury methanol extracts	*IC_{50} ± SE (mg/mL)*	*Total phenolic content (mg GA/g DW)*
Control shoots *in vitro*	3.12 ± 0.22^d	113.27 ± 1.62^a
Transgenic *AtCKX1*-29 shoots	1.81 ± 0.03^c	128.24 ± 1.29^b
Control roots *in vitro*	1.10 ± 0.02^b	162.04 ± 1.75^c
Transgenic *AtCKX1*-29 roots	0.65 ± 0.03^a	163.28 ± 3.32^c

Generally, the antioxidant activities of phenolic compounds are mainly due to their ability to act as hydrogen donors, reducing agents and radical scavengers (Mai et al., 2009). It is known that there is a positive relationship between antioxidant activity potential and amount of phenolic compounds of the crude extracts of *Camellia sinensis* (Mello and Quadros, 2014). Significantly higher phenolics content and antioxidant activity were also detected in *Capsella bursa-pastoris* and *Marrubium vulgare* (Neagu et al., 2019) As phenolic compounds, xanthones have already been described for their antioxidant properties (Pinto et al., 2005). Antioxidant properties of *C. erythraea* have been already previously characterised (Valentão et al., 2001). In this study *AtCKX* transgenic centaury methanol extracts were analysed for the first time for their antioxidant activities.

The results showed that methanol extract of transgenic roots, line *AtCKX1*-29, with highest phenolic content exhibited the highest capacity for the scavenging of the DPPH radicals. Considering that in roots of centaury transgenic line *AtCKX1*-29 detected stimulated production of eustomin and demethyleustomin, antioxidant properties of analysed methanol extracts could be assigned to elevated xanthone compounds.

4. Conclusions

The results of present investigation clearly indicate that the antibacterial and antifungal activity depend on plant material. All tested methanol extracts of control and transgenic *AtCKX1* centaury shoots and roots showed better antibacterial activity, while pure secoiridoids (gentiopicrin and swertiamarin) and xanthones (eustomin and demethyleustomin) were more active against fungi.

Generally, transgenic *AtCKX1* centaury shoots and roots showed higher antioxidant activity compared to the control shoots and roots. The highest antioxidant activity (IC_{50} = 0.65 mg/mL) was determined in transgenic *AtCKX1*-29 roots which is in correlation with the highest total phenolic content. It can be concluded that all tested methanol extracts as well as pure secoiridoid and xanthone compounds represent potential bacterial and mold inhibitors confirming the possibility of using them in agronomy, veterinary, medicine and food industry. Thus, centaury plants with increased content of secondary plant metabolites, especially xanthones, could be of significant interest in the development of novel drugs.

Acknowledgements
This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. ON173015 and ON173032).

Abbreviations
HPLC – High pressure liquid chromatography
MS – Murashige and Skoog medium
MS spectrometry – Mass spectrometry
NMR – Nuclear magnetic resonance spectroscopy
UV – Ultraviolet–visible spectroscopy

References

Berkan T., Ustünes L., Lermioglu F., (1991), Antiinflamatory, analgesic and antipyretic effects of an aqueous extract of *Erythraeacentaurium*, *Planta Medica*, **57**, 34-37.

Blumenthal M., Busse W.R., Goldberg A., Gruenwald J., Hall T., Riggins C.W., Rister R.S., (1998), *The Complete German Commission E Monographs, Therapeutic Guide to Herbal Medicines*, American Botanical Council, Austin, Texas, Integrative Medicine Communications, Boston, Massachusetts.

Božunović J., Živković S., Gašić U., Glamočlija J., Ćirić A., Matekalo D., Šiler B., Soković M., Tešić Ž., Mišić D., (2018), *In vitro* and *in vivo* transformations of *Centaurium erythraea* secoiridoid glucosides alternate their antioxidant and antimicrobial capacity, *Industrial Crops and Products*, **111**, 705-721.

Brand-Williams W., Cuvelier M.E., Berset C., (1995), Use a free radical method to evaluate antioxidative activity, *LWT Food Science and Technology*, **28**, 25-30.

Bravo L., (1998), Polyphenols: chemistry, dietary sources, metabolism and nutritional significance, *Nutrition Reviews*, **56**, 317-333.

Capasso F., Mascolo N.,Morrica P.,Ramundo E., (1983), Phytotherapeutic profile of some plants used in folk medicine, *Bollettino-Societa Italiana Biologia Sperimentale*, **59**, 1398-1404.

Chevallier A., (2000), *Encyclopedia of Herbal Medicine*, Dorling Kindersley, London.

CLSI, (2009), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Approved Standard*, 8th Edition, CLSI publication M07-A8, Clinical and Laboratory Standards Institute (CLSI), Wayne, PA.

Cui K., Luo X., Murthy M.R.V., (2004), Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants, *Progress in Neuropsychopharmacology and Biological Psychiatry*, **28**, 771-799.

Dhama K., Latheef S.K., Mani S., Samad H.A., Karthik K., Tiwari R., Khan R.U., Alagawany M., Farag M.R., Alam G.M., Laudadio V., Tufarelli V., (2015), Multiple beneficial applications and modes of action of herbs in poultry health and production – a review, *International Journal of Pharmacology*, **11**, 152-176.

Espinel-Ingroff A., (2001), Comparation of the E-test with the NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi, *Journal of Clinical Microbiology*, **39**, 1360-1367.

Fierascu R.C., Ion R.M., Fierascu I, (2017), Antifungal effect of natural extracts on environmental biodeteriogens affecting the artifacts, *Environmental Engineering and Management Journal*, **16**, 2435-2442.

Haloui M., Louedec L., Michel J.B., Lyoussi B., (2000), Experimental diuretic effects of *Rosmarinus officinalis* and *Centaurium erythraea*, *Journal of Ethnopharmacology*, **71**, 465-472.

Hanel H., Raether W., (1988), A more sophisticated method of determining the fungicidal effect of water-insoluble preparations with a cell harvester, using miconazole as an example, *Mycoses*, **31**, 148-154.

Hänsel R., Keller K., Rimpler H., Schneider G., (1992), *Centauriumerythraea. Hagers Handbuch der Farmazeutischen Praxis, Drogen: A-D*, 5th Edition, Springer Verlag, Berlin-Heidelberg, 756-763.

Janković T., Krstić D., Šavikin-Fodulović K., Menković N., Grubišić D., (1997), Comparative investigation of secoiridoids compounds of *Centaurium erythreae* growth in nature and cultured *in vitro*, *Pharmaceutical and Pharmacological Letters*, **7**, 30-32.

Janković T., Krstić D., Šavikin-Fodulović K., Menković N., Grubišić D., (2002), Xanthones and secoiridoids from hairy root cultures of *Centaurium erythraea* and *C. pulchellum*, *Planta Medica*, **68**, 944-946.

Jensen S.R., Schripsema J., (2002), *Chemotaxonomy and Pharmacology of Gentianaceae*, In: *Gentianaceae-Systematics and Natural History*, Struve L., Albert V. (Eds.), Cambridge University Press, UK, 573-631.

Kapoor A., Kaur G., Kaur R., (2015), Antimicrobial activity of different herbal plants extracts: a review, *World Journal of Pharmacy and Pharmaceutical Sciences*, **4**, 422-459.

Kumarasamy Y., Nahar L., Cox P.J., Jaspars M., Sarker S.D., (2003a), Bioactivity of secoiridoid glycosides from *Centauriumerythraea*, *Phytomedicine*, **10**, 344-347.

Kumarasamy Y., Nahar L., Sarker S.D., (2003b), Bioactivity of gentiopicroside from the aerial parts of *Centauriumerythraea*, *Fitoterapia*, **74**, 151-154.

Ksouri R., Falleh H., Megdiche W., Trabelsi N., Mhamdi B., Chaieb K., Bakrouf A., Magné C., Abdelly C., (2009), Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarixgallica* L. and related polyphenolic constituents, *Food and Chemical Toxicology*, **47**, 2083-2091.

Mai T.T., Fumie N., Chuyen N.V., (2009), Antioxidant activities and hypolipidemic effects of an aqueous extract from flower buds of *Cleistocalyxoperculatus* (Roxb.) merr. and perry, *Journal of Food Biochemistry*, **33**, 790-807.

Mello L.D., Quadros G.P., (2014), Correlation between antioxidant activity and total phenolic content with physicochemical parameters of blended extracts of *Camellia sinensis*, *Acta Scientarium*, **36**, 97-103.

Mok D.W.S., Mok M.C., (2001), Cytokinin metabolism and action, *Plant Physiology and Plant Molecular Biology*, **52**, 89-118.

Mroueh M., Saab Y., Rizkallah R., (2004), Hepatoprotective activity of *Centauriumerythraea* on acetaminophen-induced hepatotoxicity in rats, *Phytotherapy Research*, **18**, 431-433.

Namita P., Mukesh R., (2012), Medicinal plants used as antimicrobial agents: a review, *International Research Journal of Pharmacy*, **3**, 39-40.

Neagu E., Paun G., Ungureanu O., Radu G.L., (2019), Antioxidant activity and phenolics content of *Capsella bursa-pastoris* and *Marrubium vulgare* depending on environmental factors, *Environmental Engineering and Management Journal*, **18**, 1553-1560.

Newall C.A., Anderson L.A., Phillipson J., (1996), *Herbal Medicines. A Guide for Health-Care Professionals*, London: The Pharmaceutical Press.

Piatczak E., Wielanek M., Wysokinska H., (2005), Liquid culture system for shoot multiplication and secoiridoid production in micropropagated plants, *Centaurium erythraea* Rafn, *Plant Science*, **168**, 431-437.

Piatczak E., Krolicka A., Wysokinska H., (2006), Genetic transformation of *Centaurium erythraea* Rafn. by *Agrobacterium rhizogenes* and production of secoiridoids, *Plant Cell Reports*, **25**, 1308-1315.

Prior R.L., Cao G., (2000), Antioxidant phytochemicals in fruits and vegetables: diet and health implications, *Hort Science*, **35**, 588-592.

Pushpa R., Nishant R., Navin K., Pankaj G., (2013), Antiviral potential of medicinal plants: an overview, *International Research Journal of Pharmacy*, **4**, 8-16.

Saranraj P., Sivasakthi S., (2014), Medicinal plants and its antimicrobial properties: a review, *Global Journal of Pharmacology*, **8**, 316-327.

Schmülling T., Werner T., Riefler M., Krupková E., Bartrina y Manns I., (2003), Structure and function of cytokinin oxidase/dehydrogenase genes of maize, rice, *Arabidopsis* and other species, *Journal of Plant Research*, **116**, 241-252.

Silva N.C.C., Fernandes J.A., (2010),Biological properties of medicinal plants: a review of their antimicrobial activity, *The Journal of Venomous Animals and Toxins including Tropical Diseases*, **16**, 402-413.

Singelton V.R., Orthifer R., Lamuela-Raventos R.M., (1999), Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent, *Methods in Enzymology*, **299**, 152-178.

Šiler B., Živković S., Banjanac T., Cvetković J., Nestorović-Živković J., Ćirić A., Soković M., Mišić D., (2014) Centauries as underestimated food additives: Antioxidant and antimicrobial potential, *Food Chemistry*, **147**, 367-376.

Šiler B., Mišić D., Nestorović J., Banjanac T., Glamočlija J., Soković M., Ćirić A., (2010), Antibacterial and antifungal screening of *Centauriumpulchellum* crude extracts and main secoiridoid compounds, *Natural Products Communications*, **5**, 1525-1530.

Pinto M.M.M., Sousa M.E., Nascimento M.S.J., (2005), Xanthone derivatives: new insights in biological activities, *Current Medicinal Chemistry*, **12**, 2517-2538.

Tanase C., Cosarca S., Toma F., Mare A., Man A., Miklos A., Imre S., Boz I., (2018), Antibacterial activities of beech bark (*Fagus sylvatica* L.) polyphenolic extract, *Environmental Engineering and Management Journal*, **17**, 877-884.

Tosun M., Celik F., Ercisli S.O., Yilmaz S., (2011), *Bioactive Contents of Commercial Cultivars and Local Genotypes of Walnut* (*Juglansregia* L.), Proc. of the International Conference on Environmental and Agriculture Engineering (IPCBEE), Singapore, vol. 15, 110-114.

Trifunović M., Cingel A., Simonović A., Jevremović S., Petrić M., Dragićević I.Č., Motyka V., Dobrev P.I., Zahajská L., Subotić A., (2013), Overexpression of Arabidopsis cytokinin oxidase/dehydrogenase genes AtCKX1 and AtCKX2 in transgenic C*entaurium erythraea* Rafn., *Plant Cell Tissue and Organ Culture*, **115**, 139-150.

Trifunović M., Motyka V., Cingel A., Subotić A., Jevremović S., Petrić M., Holík J., Malbeck J., Dobrev P.I., Dragićević I.Č., (2015), Changes in cytokinin content and altered cytokinin homeostasis in *AtCKX1* and *AtCKX2*-overexpressing centaury (*Centaurium erythraea* Rafn.) plants grown *in vitro*, *Plant Cell Tissue and Organ Culture*, **120**, 767-777.

Tsukatani T., Suenaga H., Shiga M., Noguchi K., Ishiyama M., Ezoe T., Matsumoto K., (2012), Comparison of WST-8 colorimetric method and CLSI broth microdilution method for susceptibility testing against resistant bacteria, *Journal of Microbiological Methods*, **90**, 160-166.

Valentão P., Andrade P.B., Silva E., Vincente A., Santos H., Bastos M.L., Seabra R., (2002), Methoxylatedxanthones in the quality control of small centaury (*Centauriumerythraea*) flowering tops, *Journal of Agricultural and Food Chemistry*, **50**, 460-463.

Valentão P., Fernandes E., Carvalho F., Andrade P.B., Seabra R.M., Bastos M.L., (2001), Antioxidant activity of *Centauriumerythraea* infusion evidenced by its superoxide radical scavenging and xanthine oxidase inhibitory activity, *Journal of Agricultural and Food Chemistry*, **49**, 3476-3479.

Valentão P., Fernandes E., Carvalho F., Andrade P.B., Seabra R.M., Bastos M.L., (2003), Hydroxyl radical and hypochlorous acid scavenging activity of small Centaury *(Centauriumerythraea)* infusion. A comparative study with green tea *(Camellia sinensis)*, *Phytomedicine*, **10**, 517-522.

Valko M., Leibfritz D., Moncol J., Cronin M.T.D., Mazur M., Telser J., (2007),Free radicals and antioxidants in normal physiological functions and human disease, *The International Journal of Biochemistry and Cell Biology*, **39**, 44-84.

Van de Sande-Bruinsma N., Grundmann H., Verloo D., Tiemersma E., Monen J., Goossens H., Ferech M., (2008),Antimicrobial drug use and resistance in Europe, *Emerging Infectious Diseases*, **14**, 1722-1730.

Van der Sluis W.G., (1985a), *Secoiridoids and Xanthones in the Genus Centaurium Hill* (*Gentianaceae*) – *A Pharmacognostical Study*, Rijksuniversiteitte Utrecht.

Van der Sluis W.G., (1985b), Chemotaxonomical investigations of the Genera *Blackstonia* and *Centaurium* (*Gentianaceae*), *Plant Systematics and Evolution*, **149**, 253-286.

Van der Sluis W.G., Van der Nat J.M., Spek Á.L., Ikeshiro Y., Labadie R.P., (1983), Secoiridoids and xanthones in the genus *Centaurium*. Part VI: Gentiogenal, a conversion product of gentiopikrin (gentiopicroside), *Planta Medica*, **49**, 211-215.