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Antimicrobial activity, total phenolic content and flavonoid concentrations of *Teucrium* species

Research Article

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Abstract: *In vitro* antimicrobial activity of 21 crude extracts obtained from seven taxa of the genus *Teucrium* (T. *chamaedrys, T. montanum, T. arduini, T. polium, T. scordium* subsp. *scordium, T. scordium subsp. scordioides* and *T. botrys*) was tested against bacterial and fungal species. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using a microdilution analysis method. Total phenolic content and flavonoid concentrations were measured spectrophotometrically. Total phenols were determined using Folin-Ciocalteu reagent and their amounts ranged from 28.49 up to 159.84 mg CA/g of extract (chlorogenic acid equivalent). The amounts of flavonoids ranged from 38.17 up to 190.45 mg RU/g of extract (rutin equivalent).The plant extracts showed greater potential of antibacterial than antifungal activity. A relationship was found between total phenolics and biological activity. The highest level of total phenols was measured in the methanol extracts, which demonstrated higher antimicrobial activity than acetone and ethyl acetate extracts. *Staphylococcus aureus* ATCC 25923 appeared to be the most sensitive organism. Our results indicate that *Teucrium* spp extracts are rich sources of phenolic compounds and are promising candidates for further development as natural antimicrobial agents.

Keywords: *Teucrium • Minimum inhibitory concentration • Minimum bactericidal concentration • Quantification of phenolics*

1. Introduction

The products of secondary metabolism are not of essential importance for plants, but are most often the result of adaptation to biotic and abiotic environmental factors. Main secondary metabolites in plants are terpenes, phenolics and nitrogen-containing compounds. Secondary metabolites have important biological and pharmacological activities, such as antibiotic, antioxidative, anti-allergic, hypoglycemic, and anticarcinogenic properties [1,2].

Plants are an abundant natural source of effective antibiotic compounds. Plant antibiotics are chemicals that kill pathogen cells or inhibit their division and are classified into antibacterial, antifungal, antiviral and antineoplastic types according to their target. They affect cell membrane permeability, regulate or disable enzymatic processes, and inhibit protein translation [3,4].

Due to their natural origin, the antibiotics obtained from plants are of greater benefit in comparison to synthetic ones. The use of natural antibiotics from plants does not induce side effects such as abdominal pain, diarrhea, cramping, headache, nausea and vomiting. Plant products may be used as antibiotic alternatives and do not cause resistance in bacteria [5].

The genus *Teucrium* (germander) belongs to the family Lamiaceae, within the subfamily Ajugoideae. In the flora of Europe the genus *Teucrium* has been divided into six sections with 49 species. In the flora of Serbia it is represented by 6 species. The section Stachyobotrys is represented by the species *T. arduini* L., the section Scorodonia by *T. scordium* L. (*T. scordium* subsp. *scordium*, *T. scordium* subsp. *scordioides*), the section

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Chamaedrys by *T. chamaedrys* L. and the section Polium by *T. polium* L. and *T. montanum* L. These are mostly perennial herbs, shrubs or subshrubs, while *T. botrys* is an herbaceous annual plant. The species of this genus are widespread in all continents of the world but a very large number of species are present in the Mediterranean [6,7].

A large number of known medicinal species belonging to the genus *Teucrium* are used in folk medicine and pharmacy, in food industry as spices and for bitter beverages. *T. chamaedrys*, *T. montanum* and *T. polium* are the most popular species of this genus in the flora of Europe and Asia, and they are used in treatment of digestive and respiratory disorders, abscesses, gout, and conjunctivitis. They also enhance fat and cellulite decomposition and possess anti-inflammatory, antioxidative, antimicrobial, antidiabetic and antihelmintic effect [8,9].

Qualitative phytochemical analysis of *Teucrium* species revealed the presence of different secondary metabolites such as phenolic acids [10-12] and flavonoids with very strong biological activity [13,14]. However, little is known about the antimicrobial properties of *Teucrium* spp extracts. Only some species of this genus have previously been evaluated for antimicrobial activities. Gursoy and Tepe [15] tested polar and non-polar sub-fractions of methanol extracts of *Teucrium chamaedrys* from Turkey. The polar sub-fraction exhibited weaker antimicrobial activity than the non-polar samples. Different extracts of *Teucrium montanum* were investigated for their antimicrobial activity [16]. The ethyl acetate, n-butanol and chloroform extracts of *Teucrium montanum* were the most active while the extracts of *Teucrium arduini* and *Teucrium polium* showed low antibacterial and antifungal activity [17-19].

To our knowledge this is the first report concerning the comparative antimicrobial activity analysis for investigated *Teucrium* species. The aim of this study was to screen the *in vitro* antimicrobial activity as well as total phenolic content and flavonoid concentrations of *Teucrium* species from Serbia and Montenegro as potential sources of natural antimicrobial agents.

2. Experimental Procedures

2.1 Chemicals

Acetone, methanol, ethyl acetate and sodium hydrogen carbonate were purchased from "Zorka pharma" Šabac, Serbia. Standards of phenolic acids (chlorogenic acid) and flavonoids (rutin hydrate) were obtained from Sigma Chemicals Co., St Louis, MO, USA. The Folin-Ciocalteu's phenol reagent and aluminium chloride ($AlCl_3$) were from Fluka Chemie AG, Buchs, Switzerland. All solvents and chemicals were of analytical grade. Nutrient media, Mueller–Hinton broth was from Liofilchem, Italy, while Sabouraud dextrose broth was from Torlak, Belgrade. An antibiotic, amoxicillin was purchased from Galenika A.D., Belgrade and antimycotic, fluconazole was from Pfizer Inc., USA. Resazurin was purchased from Alfa Aesar GmbH & Co. KG, Germany.

2.2 Plant material

Teucrium species were collected from July to September 2009, from the region of Serbia and Montenegro. The voucher specimens were confirmed and deposited in Herbarium at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The collected plant material was air-dried in darkness at room temperature. Dried plant parts were cut up and stored in tight-seal dark containers until needed.

2.3 Preparation of plant extracts

Prepared plant material (10 g) was transferred to dark-coloured flasks and mixed with 200 ml of solvents with different polarities (methanol, acetone, or ethyl-acetate) 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. 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.

2.4 Test microorganism

Five bacterial species, Gram-positive (*Staphylococcus aureus* ATCC 25923 and clinical isolate of *Staphylococcus aureus* from ulcerous boil), Gram-negative (*Escherichia coli* ATCC 25922, clinical isolate of *Escherichia coli* and *Pseudomonas aeruginosa* from urine), and three fungal species (*Candida albicans* ATCC 10231, clinical isolate of *Candida albicans* from oral cavity and environmental isolate of *Aspergillus niger*) were used for antimicrobial tests. All clinical isolates were a generous gift from the Institute of Public Health, Kragujevac. The other microorganisms were provided by the Microbiology Laboratory, Faculty of Science, University of Kragujevac. The microorganisms used in this study are medically significant. *S. aureus*, *E. coli*, *P. aeruginosa and C. albicans* are opportunistic pathogens while *A. niger* is a saprophytic fungus. *E. coli* and *P. aeruginosa* are frequently associated with infection of the urinary tract. Also, *P. aeruginosa* is commonly found in hospital environment and can easily infect immunosuppressed patients. *S. aureus* can infect skin and wounds causing acne, boils, pimples. *C. albicans* is responsible for a

range of systematic infections, many of which can start as superficial infections (oral, skin, vaginal infection). *A. niger* is a saprophytic fungus widely distributed in nature but, because it produces potent allergens, often causes asthma and other hypersensitivity reactions.

Bacterial and yeast suspensions were prepared by direct colony method. The turbidity of initial suspension was adjusted by comparing with 0.5 McFarland's standard (approximately 10^8 colony forming units (CFU)/ml for bacteria, 10^6 CFU/ml for yeast) and 1:100 diluted in sterile 0.85% saline. The suspension of fungal spores was prepared by gentle stripping of spores from nutrient agar slants with growing aspergilli. The resulting suspension was 1:1000 diluted in sterile 0.85% saline.

2.5 Microdilution method

In vitro antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) using microdilution method with resazurin [20]. The plant extracts were first dissolved in 100% dimethylsulfoxide (DMSO) (10% of total volume) and then in nutrient liquid medium (up to 100% of total volume). The stock concentrations of plant extracts were 40 mg/ml. Serial twofold dilutions of plant extracts were made in a concentration range from 20 mg/ml to 0.078 mg/ml in sterile 96-well plates containing Mueller–Hinton broth for bacteria and Sabouraud dextrose broth for fungi and yeasts. 10 µl of diluted bacterial, yeast, and spore suspensions were added to each well to give a final concentration of $5x10^5$ CFU/ml for bacteria and $5 x 10^3$ CFU/ml for fungi and yeast. Finally, 10 µl of resazurin solution, as an indicator of microbial growth, was added to each well inoculated with bacteria and yeast. Resazurin is a blue non-fluorescent dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. The inoculated plates were incubated at 37°C for 24 h for bacteria, 28°C for 48 h for yeast and 28°C for 72 h for fungi. MIC was defined as the lowest concentration of tested substance that prevented resazurin color change from blue to pink. For fungi, MIC values of the tested substance were determined as the lowest concentration that visibly inhibited mycelia growth.

Amoxicillin and fluconazole, dissolved in nutrient liquid medium, were used as the antimicrobial positive controls. A solvent negative control test was performed to determine the effect of 10% DMSO on the growth of microorganism. It was observed that 10% DMSO did not inhibit the growth of the microorganisms. Also, in the experiment, the concentration of DMSO was subsequently decreased because of the twofold serial dilution assay, resulting in a working DMSO concentration of 5% or lower. Each test included a positive growth control and a negative sterility control. All tests were performed in duplicate and MICs were constant.

Minimum microbiocidal 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 the minimum microbicidal concentration.

2.6 Determination of total phenolic contents in the plant extracts

The concentration of phenolics in plant extracts was determined using a spectrophotometric method [21]. A methanolic solution of 1mg/ml plant extract was used in the analysis. The reaction mixture was prepared by combining 0.5 ml of methanolic extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% $NaHCO_3$. The spectrophotometic blank used consisted of 0.5 ml methanol 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% $NaHCO_3$. The samples were incubated at 45°C for 45 min. The absorbance was measured at λ_{max}=765 nm. The samples were prepared in triplicate for each analysis and the mean absorbance was obtained. The same procedure was repeated for the standard solution of chlorogenic acid to produce a calibration curve. The concentration of phenolics in extracts (mg/ml) was determined from the calibration line and expressed in terms of chlorogenic acid equivalent (mg of CA/g of extract).

2.7 Determination of flavonoid concentrations in the plant extracts

The flavonoid content in the examined plant extracts was determined using a spectrophotometric method [22]. The sample contained 1 ml of 1 mg/ml plant methanol extract and 1 ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was measured at λ_{max}=415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was determined. The concentration of flavonoids was read (mg/ml) on the calibration line and the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

3. Results

3.1 *In vitro* antimicrobial activity

The antimicrobial activity and MIC/MMC values exhibited by methanol, acetone and ethyl acetate extracts of *Teucrium* species against tested bacterial and fungal species are shown in Table 1. The solvent (10% DMSO)

Plant species	Extract	*E. coli* ATCC 25922		*S. aureus* ATCC 25923		*E. coli*		*S. aureus*		*P. aeruginosa*		*C. albicans* ATCC 10231		*C. albicans*		*A. niger*	
		MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
T. chamaedrys	M	5	5	0.078	0.078	5	5	5	5	1.25	2.5	>20	>20	>20	>20	>20	>20
	Ac	20	20	5	5	>20	>20	20	>20	20	>20	>20	>20	>20	>20	10	10
	Et	10	>10	0.6	0.6	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	10	10
T. montanum	M	5	5	0.15	0.3	5	5	5	5	2.5	5	>20	>20	>20	>20	>20	>20
	Ac	10	10	0.15	0.15	20	20	10	10	5	>20	>20	>20	>20	>20	20	20
	Et	>10	>10	0.3	0.3	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10
T. polium	M	10	10	0.3	0.3	20	>20	5	5	>20	>20	>20	>20	>20	>20	>20	>20
	Ac	10	10	0.3	0.3	20	20	10	10	5	5	>20	>20	>20	>20	>20	>20
	Et	>10	>10	0.3	0.3	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10
T. arduini	M	10	10	0.15	0.15	10	10	5	5	5	10	>20	>20	>20	>20	>20	>20
	Ac	20	>20	0.6	0.6	>20	>20	20	20	10	20	>20	>20	>20	>20	>20	>20
	Et	20	20	0.6	0.6	20	>20	20	>20	20	20	>20	>20	>20	>20	>20	>20
T. scordium subsp. *scordioides*	M	10	20	0.3	0.3	10	20	5	10	10	20	>10	>10	>10	>10	>10	>10
	Ac	>10	>10	0.6	0.6	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
	Et	>10	>10	0.3	0.3	>10	>10	>10	>10	10	>10	>10	>10	>10	>10	>10	>10
T. scordium subsp. *scordium*	M	5	10	0.3	0.3	20	20	10	10	5	10	>20	>20	>20	>20	>20	>20
	Ac	>20	>20	0.6	0.6	>20	>20	20	20	10	>20	>20	>20	>20	>20	>20	>20
	Et	>10	>10	0.6	0.6	>10	>10	10	>10	10	10	10	10	10	10	5	5
T. botrys	M	>20	>20	1.25	1.25	>20	>20	>20	>20	20	>20	>20	>20	>20	>20	>20	>20
	Ac	>10	>10	2.5	5	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	10	10
	Et	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	5	>10	5	>10	10	10
Amoxicillin		3.91	250	0.24	0.24	1.95	250	>500	>500	>500	>500	/	/	/	/	/	/
Fluconazole		/	/	/	/	/	/	/	/	/	/	15	>1000	15	>1000	1000	1000

Table 1. Comparative antibacterial and antifungal activity of extracts of Teucrium species.

MIC and MMC values are expressed in mg/ml
M-methanol extract, Ac-acetone extract, Et-ethyl acetate extract
Amoxicillin and fluconazole are expressed in μg/ml

did not inhibit the growth of tested microorganisms. The extracts showed different degree of antimicrobial activity. The intensity of antimicrobial action varied depending on the species of microorganism, plant species and the type of extract.

In general, plants showed similar antimicrobial activity, except *T. botrys* which was almost inactive against the tested microorganisms. MIC and MMC values ranged from 0.078 mg/ml up to >20 mg/ml. The antibacterial inhibitory effect of at least one type of extract against all bacterial strains were recorded for all plants, except for *T. botrys*. On the other hand, only *T. botrys* and *T. scordium* subsp. *scordium* showed antifungal activity against all the tested strains of fungi.

All three extract types of *T. arduini* inhibited the growth of four bacteria, *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *S. aureus* and *P. aeruginosa*. The extracts of *T. chamaedrys* and *T. scordium* subsp. *scordium* showed a similar inhibition spectrum. *T. chamaedrys* inhibited *E. coli* ATCC 25922, *S. aureus* ATCC 25923 and *P. aeruginosa*, while *T. scordium* subsp. *scordium* inhibited *Staph. aureus* ATCC 25923, *S. aureus* and *P. aeruginosa*. *T. montanum* and *T. polium* extracts were effective against two strains of bacteria, *S. aureus* ATCC 25923 and *P. aeruginosa*. All extracts of *T. scordium* subsp. *scordioides* affected only *S. aureus* ATCC 25923, while the extracts of *T. botrys* appeared to have no bacterial inhibitory effects.

Among the solvents tested, the methanol extracts showed the greatest inhibitory effects, followed by acetone and ethyl acetate extracts respectively. Detectable MIC and MMC values for methanol extracts were 0.078–20 mg/ml, for acetone extracts 0.15–20 mg/ml, and for ethyl acetate extracts 0.6–20 mg/ml. The methanol plant extracts inhibited all bacterial strains, with the exception of *T. polium* and *T. botrys* extracts. The methanol extracts did not demonstrate antifungal activity. The acetone extract of *T. montanum* and *T. polium* inhibited the growth of all bacterial strains, while acetone extracts from other tested plants each showed resistance against at least one bacterial strain. The MICs/MMCs of acetone extracts were higher than those of the methanol extracts. The acetone extract of *T. chamaedrys*, *T. montanum* and *T. botrys* showed anti-*Aspergillus niger* activity. The ethyl acetate extract of *T. arduini* inhibited all tested bacterial strains while the ethyl acetate extract of *T. botrys* and *T. scordium* subsp. *scordium* demonstrated antifungal activity. Two or more tested microorganisms showed resistance to the ethyl acetate extracts of all other tested plants.

Staphylococcus aureus ATCC 25923 was the most sensitive strain tested. It was inhibited by different extracts of all tested plants, except by the ethyl acetate extract of *T. botrys*. The inhibitory and bactericidal concentrations, in most cases, were lower than 1 mg/ml. Susceptibility of *S. aureus* and *P. aeruginosa* was moderate with MIC values between 5–20 mg/ml and 1.25-20 mg/ml, respectively. *E. coli*, clinical isolate and standard strain, exhibited minimal susceptibility to all extracts. Also, the tested fungi demonstrated low sensitivity to the extracts.

3.2 Total phenol content and flavonoid concentration

Total phenolic contents of different plant extracts are shown in Table 2. The methanol extracts had a higher total phenolic content (53.91–159.84 mg CA/g), followed by acetone extracts (50.12–157.89 mg CA/g) and ethyl acetate extracts (32.01–82.15 mg CA/g). The highest concentrations of total phenols were measured in *T. scordium* subsp. *scordium*, *T. chamaedrys*, *T. montanum* and *T. polium*. The lowest levels of total phenols were measured in *T. botrys* extracts.

The concentrations of flavonoids are presented in Table 3. The methanol and acetone extracts contained higher flavonoid concentrations than ethyl acetate

Plant species	methanol extract	acetone extract	ethyl acetate extract
T. chamaedrys	159.84±1.02	132.73±0.84	28.49±0.32
T. montanum	149.16±0.94	70.34±0.12	48.01±0.28
T. polium	115.48±0.88	97.25±0.23	40.38±0.81
T. arduini	86.39±0.52	76.30±0.78	41.00±0.65
T. scordium ssp. *scordioides*	155.94±0.71	157.89±1.06	82.15±1.00
T. scordium ssp. *scordium*	163.79±1.11	108.34±0.96	50.32±0.55
T. botrys	53.91±0.45	50.12±0.66	32.01±0.31

Table 2. Total phenolic contents[1] of *Teucrium* extracts expressed in terms of chlorogenic acid equivalent, (mg of CA/g of extract).

[1]*mean value ± standard deviation.*

Plant species	methanol extract	acetone extract	ethyl acetate extract
T. chamaedrys	61.80±0.18	78.03±0.94	87.17±0.21
T. montanum	46.75±0.14	48.55±1.12	38.17±0.59
T. polium	78.82±1.14	61.88±0.98	47.76±0.84
T. arduini	79.89±0.89	110.01±1.23	63.94±1.29
T. scordium ssp. *scordioides*	111.93±1.22	109.17±1.31	92.86±0.96
T. scordium ssp. *scordium*	152.58±0.91	177.07±1.17	190.45±1.33
T. botrys	85.23±1.11	150.55±1.51	74.20±1.01

Table 3. Flavonoid concentrations[1] of *Teucrium* extracts expressed in terms of rutin equivalent (mg of RU/g of extract).

[1]*mean value ± standard deviation.*

extracts. The concentration of flavonoids ranged from 46.75 up to 152.58 mg RU/g in the methanol extracts, 48.55-177.07 mg RU/g in the acetone extracts, and 38.17-190.45 mg RU/g in the ethyl acetate extracts. Among the tested plants, *T. scordium* subsp. *scordium* contained the highest flavonoid levels.

4. Discussion

The inhibitory effects of 21 plant extracts obtained from 7 different taxa were investigated. Determination of MIC and MMC showed that all the plant extracts exhibited antimicrobial effects against some of the eight tested microorganisms. In general, the plant extracts exhibited higher antibacterial activity than antifungal activity. Compared to standard strains of *E. coli*, *S. aureus*, *C. albicans* and clinical isolates of *E. coli* and *C. albicans*, the activity of extracts was less effective than positive controls (amoxicillin and fluconazole). However, the positive control compounds were not effective against every pathogen tested. The clinical isolates of *S. aureus* and *P. aeruginosa* showed resistance to amoxicillin. Resistance of *S. aureus* is likely due to production of β-lactamase, an enzyme which inactivates penicillins by opening their β-lactam rings. In the case of *P. aeruginosa*, amoxicillin is not effective. *P. aeruginosa* possesses natural mechanisms of resistance to many of the widely used antibiotics. Also, *A. niger* showed resistance to fluconazole. Fluconazole is one of the newer azole antifungals which has proved highly effective in the treatment of infection caused by *C. albicans* but showes limited activity against *Aspergillus*. This could be the reason why *A. niger* displayed resistance in our experiments [23]. Tested extracts of *Teucrium* species with antimicrobial activity could have significant medicinal contributions particularly by inhibiting amoxicillin-resistant *S. aureus, P. aeruginosa* and fluconazole-resistant *A. niger*. The recorded MMC values, which were not more than fourfold of their corresponding MICs, indicated the bactericidal effect of tested extracts [24]. The plants differ significantly in their activities against the tested microorganisms. Most of the extracts showed better activity against *S. aureus* ATCC 25923, *S. aureus* and *P. aeruginosa* than against *E. coli* ATCC 25922 and *E. coli. C. albicans* strains were inhibited only by the extracts from *T. scordium* subsp. *scordium* and *T. botrys,* while *A. niger* was inhibited by *T. chamaedrys* and *T. montanum*.

Antimicrobial activity of plant extracts is attributed to the presence of numerous bioactive secondary metabolites. Phenolic compounds, terpenoids and alkaloids are very important components in antimicrobial activity [25]. For this reason, total phenolic content as well as flavonoid concentrations were quantified. The highest concentrations of total phenolics were measured in the methanol extracts. These findings confirm the relationship between the amount of phenolics and biological activities. Namely, the methanol extracts were most active in comparison with acetone and ethyl acetate extracts. The greater effectiveness of the methanol extracts resulted from the fact that methanol is a very useful solvent capable of extracting a wide range of compounds [26]. Weak antimicrobial activity was observed in the extracts of *T. botrys*, which showed a low total phenolic content.

The concentration of flavonoids and the antimicrobial activity of plant extracts are not heavily correlated. This suggests that *Teucrium* extracts are rich in active compounds that are not solely flavonoids, but also a variety of different active phenolic compounds.

The results in this study are in accordance with previous biological data for the genus *Teucrium*. The antimicrobial activity has been demonstrated for a number of *Teucrium* species such as *T. chamaedrys*, *T. montanum*, *T. arduini*, *T. polium*. Gursoy and Tepe [15] tested polar and non-polar sub-fractions of methanol extracts from *T. chamaedrys* in 2009.

While polar-subfractions were inactive, non-polar subfractions inhibited the growth of *Acinetobacter lwoffi*, *C. albicans*, *C. krusei*, *Clostridium perfringens*, *E. coli* and *Streptococcus pneumonia*. *Bacillus cereus* and *Klebsiella pneumoniae* were resistant. Detectable MIC values were equal or higher than MIC values in this study. Different extracts from *T. montanum* were investigated for their antimicrobial activity [16]. The ethyl acetate, n-butanol and chloroform extracts expressed inhibitory activity against tested Gram-positive and Gram-negative bacteria, while petroleum ether and water extracts were inactive. The tested yeast strains and *E. coli* strain were resistant to *T. montanum* extracts, which our results also indicate. *Staphylococcus aureus* ATCC 6538 was most sensitive to leaf and flower infusions of *T. arduini* [17]. None of the tested infusions exhibited antimicrobial activity against Gram-negative bacteria (*E. coli* ATCC 10535, *P. aerugimosa* ATCC 27853), or the tested fungi (*C. albicans* ATCC 10231, *A. niger* ATCC 16404). Previous studies observed low antibacterial and antifungal activity of *T. polium* extracts [18,19]. In our study, *T. polium* showed moderate antibacterial and no antifungal activity.

5. Conclusions

The antimicrobial activity of *T. scordium* subsp. *scordium*, *T. scordium* subsp. *scordioides* and *T. botrys* was represented in this study for the first time. Our results indicate the species of genus *Teucrium* can be considered as a rich natural source of polyphenolic compounds, possessing prominent antimicrobial activity.

The phenolic content of extracts depends on tissue type and solvents used for extraction. The concentration of phenolic contents as well as the properties of these compounds contribute to the activites of different extracts. For the extraction of active components it is most effective to use solvents of high polarity. In this study, *Teucrium* species extracts were found to contain significant levels of total phenols.

Our results describe broad-spectrum antimicrobial activity of *Teucrium* species extracts. These properties could indicate the use of crude extracts from *Teucrium* species in medical and pharmaceutical applications as potential antimicrobial agents and as effective preservatives. Since in this study antimicrobial activity of plant extracts against human pathogenic bacteria and fungi was tested, and there were no isolates from food, authors decided not to discuss potential application of plant extracts as antimicrobial preservatives.

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