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ANTIMICROBIAL ACTIVITY AND SOME PHYTOCHEMICAL ANALYSIS OF TWO EXTRACTS *Vinca minor* L.

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ABSTRACT. This study investigated the antimicrobial activity as well as some phytochemical analysis of ethanol and diethyl ether extracts from plant species *Vinca minor* L. *In vitro* antimicrobial activity of extracts was studied on 20 strains of microorganisms (16 bacteria and four yeasts). Testing was performed by microdilution method and minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) were determined. The strongest antimicrobial activity was detected on G+ bacteria of the genus *Bacillus*. Tested G^- bacteria and yeasts were not sensitive to the action of the extracts or the sensitivity was insignificant.

Phytochemical analysis involved determining the amount of total phenolics, flavonoids and tannins, as well as the determination of antioxidant activity monitoring capability to neutralize free radicals (DPPH) and the reductive potential. Phytochemical examination indicates that the total phenolic compounds were more in the ethanolic extract and the content of flavonoids and tannins marginally higher in the diethyl ether extract. The antioxidant activity (DPPH) of the ethanolic extract of *V. minor* was significantly stronger as compared to the diethyl ether extract, and the reduction potential was approximately the same.

Keywords: *Vinca minor*, antimicrobial activity, antioxidant activity, phenols, flavonoids, tannins.

INTRODUCTION

The genus *Vinca (Apocynaceae)* comprises about seven species in the world. In Serbia, it is represented by *Vinca herbacea, Vinca minor* and *Vinca major*. *V. minor* or Lesser Periwinkle occurs throughout central Serbia (JOSIFOVIĆ *eds.*, 1973).

The leaves are oblong-ovate to elliptical, approximately 2-5 cm long and 1-2.5 cm wide, with petioles 1-3 mm long. The flower is perfect and without odor. It is showy, purple to blue and occasionally white, borne singly in an axillary position on a 1-1.5 cm pedice. The fruit is a non-fleshy follicle, approximately 2–2.5 cm long. It releases three to five seeds (DARCY *et al.*, 2002). *V. minor* flowers regularly from April to May and sporadically from May to September (FUNDERBUCK *et al.*, 1976). It is adapted to the mild climate. It grows best in partial shade and moist, well-drained soil. It is often found in woods and bluffs, and along cemeteries, roads, and other disturbed areas (KURT and CAROL, 2000). In folk medicine, it is used internally for

circulatory disorders, cerebral circulatory impairment and brain's metabolism support (FARAHANIKIAA *et al*, 2011).

Numerous literature data describe the compounds isolated from this plant. Phenols have been investigated for its antioxidant properties (NISHIBE *et al.,* 1996). Also flavonoids were tested for the anti-inflammatory (BAHADORI *et al.,* 2012) and antimicrobial effects (SZOSTAK and KOWALEWSKI, 1975). Extracts from *V. minor* species showed a pronounced antioxidant activity and the ability to reduce lipid peroxidation (NISHIBE *et al.,* 1996) as well as the ability in treatment of cardiovascular and neurodegenerative diseases (KHANAVI *et al.,* 2010; FERNANDES *et al.,* 1996).

There is plenty of information about antibacterial (YILDIRIM *et al.,* 2012; MEHRABIAN *et al.,* 1995; TURKER *et al.,* 2009) and antifungal (WILSON *et al.,* 1997; DOMENICO *et al.,* 2012) activities of *V. minor* ethanolic extract. In the available literature there are no data about the activity of *V. minor* diethyl ether extract. Considering that *V. minor* species is from the Balkans and has not been studied with these aspects, the aim of this study was to investigate the antibacterial and antifungal activities as well as phytochemical analysis with antioxidative activity of ethanolic and diethyl ether extracts of this plant.

MATERIAL AND METHODS

Chemicals

Organic solvents (ethanol and diethyl ether) and concentrated hydrochloric acid (HCl) were purchased from Zorka Pharma (Šabac, Serbia). 2.2-diphenyl-1-picrylhydrazyl (DPPH), chlorogenic acid, ascorbic acid (vitamin C), cyanidin chloride and Folin-Ciocalteu phenol reagent were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Gallic acid, rutin hydrate, aluminium chloride hexahydrate ($AlCl_3 \times 6H_2O$), sodium dihydrogen phosphate (NaH_2PO_4) and trichloroacetic acid (TCA) were purchased from Acros Organics (New Jersey, USA). Sodium carbonate (Na_2CO_3) was obtained from MP-Hemija (Belgrade, Serbia). Dimethyl sulfoxide (DMSO), n-Butanol, ferric ammonium sulfate ($NH_4Fe(SO_4)_2 \times 12H_2O$), potassium ferricyanide ($K_3[Fe(CN)_6]$ x H_2O) and Iron(III)chloride ($FeCl_3$) were purchased from Centrohem (Stara Pazova, Serbia). Disodium hydrogen phosphate (Na_2HPO_4) was obtained from Merck (Alkaloid, Skoplje, FYR Macedonia). Resazurin was obtained from Alfa Aesar GmbH & Co. (KG, Karlsruhe, Germany). Nutrient liquid medium, a Mueller–Hinton broth was purchased from Liofilchem (Italy), while a Sabouraud dextrose broth was from Torlak (Belgrade, Serbia). An antibiotic, doxycycline, was purchased from Galenika A.D. (Belgrade, Serbia) and antimycotic, fluconazole, was from Pfizer Inc. (USA).

Plant material

In April 2012 leaves of species *V. minor* L. were collected at the time of flowering. The leaves were harvested at noon on the west side of the mountain Goč. Identification and classification of the plant material was performed at the Faculty of Science, University of Kragujevac. The voucher sample is deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac (No. MB01/12). The collected plant material was air-dried in darkness at ambient temperature. The dried plant material was cut up and stored in paper bags until needed.

Preparation of plant extracts

Dried ground plant material was extracted by maceration with ethanol and diethyl ether. 30 g of plant material was soaked with 150 ml of solvent for 24 h at room temperature. The resulting extract was then filtered through filter paper (Whatman No.1). The residue from the filtration was extracted again twice using the same procedure. The filtrates obtained were combined and then evaporated to dryness using a rotary evaporator at 40 °C. The obtained

amounts of crude extracts of *V. minor* were 4.52 g for ethanol extract and 1.28 g for ethyl ether extract. The extracts were kept in sterile sample tubes and stored at -20 °C.

Phytochemical analysis

Determination of total phenolic content

The total phenolic content was determined by using Folin-Ciocalteu's method (WOOTTON-BEARD *et al.*, 2011). The reaction mixture was prepared by mixing 0.2 ml of methanolic solution of extract (1 mg/ml) and 1.5 ml of 1:10 Folin-Ciocalteu's reagent dissolved in water. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml 6 % Na_2CO_3 solution. After incubation for 90 min at room temperature in darkness, the absorbance of the mixture was read at 725 nm against a blank using spectrophotometer. The blank was prepared with methanol instead of extract solution. The samples were prepared in triplicate and the mean value of absorbance was obtained. The same procedure was repeated for gallic acid which was used for calibration of standard curve. Total phenol content is reported as gallic acid equivalents by reference to linear equation of the standard curve (y = 0.008x + 0.0077, R2 = 0.998). Then the total phenolic content was expressed as a milligram of gallic acid equivalent per gram of extract (mg GAE/g of extract).

Determination of total flavonoid content

The concentrations of flavonoids were determined by using aluminium chloride method (QUETTIER-DELEU *et al.*, 2000). The mixture contained 2 ml of methanolic solution of extract (1 mg/ml) and 2 ml of 2 % methanolic $AlCl_3$×6H2O solution. The mixture was vigorously shaken, and after 10 min of incubation at room temperature, the absorbance versus a prepared blank was read at 430 nm using spectrophotometer. The samples were prepared in triplicate and the mean value of absorbance was obtained. Rutin was used as a standard for calibration of standard curve. The concentrations of flavonoids were calculated from the linear equation of standard curve (y = 0.021x + 0.040, R2 = 0.999). Then the concentrations of flavonoids were expressed as milligram of rutin equivalent per gram of extract (mg RU/g of extract).

Determination of condensed tannins

Condensed tannins were determined by using the butanol-HCl method as described by PORTER *et al.*, (1986). The mixture contained 0.5 ml of liquid extract was transferred to glass test tube, adding 3 ml of the butanol-HCl reagent (butanol-HCl 95:5 v/v) and 0.1 ml of the ferric reagent (2 % ferric ammonium sulfate in 2N HCl). Covered tubes were heated in a water bath at 97 to 100 °C for 60 min. After cooling, the absorbance was determined at 550 nm. A blank was measured as the absorbance of the unheated mixture. The samples were prepared in triplicate and the mean value of absorbance was obtained. Cyanidin chloride was used as a standard for calibration of standard curve. The concentrations of proanthocyanidins were calculated from the linear equation of standard curve (y j 0.0094x ю 0.006, R2 j 0.999). Then the concentrations of proanthocyanidins were expressed as milligram of cyanidin chloride equivalent per gram of extract (mg of CChE/g of extract).

Determination of antioxidant activity

DPPH radicals scavenging capacity assay

The ability of the plant extract to scavenge DPPH free radicals was assessed by using the method described by TAKAO *et al.*, (1994). The stock solution of the plant extract was prepared in methanol to achieve the concentration of 2000 μg/ml. Further, two-fold dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5 μg/ml. Diluted solutions of extract (1 ml each) were mixed with 1 ml of DPPH methanolic solution (80 μg/ml). After 30 min in darkness at room temperature, the absorbance was read in a spectrophotometer at 517 nm. The control

samples consisted of 1 ml of methanol added to 1 ml of DPPH solution. Ascorbic acid was used as a positive control. The experiment was performed in triplicate. Scavenging activity is expressed as the inhibition percentage calculated using the following equation:

$$\text{Scavenging activity (\%)} = 100 \times [(A_{control} - A_{sample}) / A_{control})]$$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the extract.

The IC_{50} value is the effective concentration at which 50 % of DPPH radicals were scavenged. It was obtained from the graph of scavenging activity (%) versus concentration of samples. Low IC_{50} value indicates strong ability of the extract to act as DPPH scavenger.

Reductive power of plant extract

As well as in previous assay the tested concentrations of the extracts ranged from 62.5 μg/ml to 1000 μg/ml. The mixture contained 0, 5 ml of methanolic solution of extract (1 mg/ml), 1.25 ml of 1 % $K_3Fe(CN)_6$ and 1.25 ml of phosphate buffer (pH =6.6; 200 mM).

After 20 min of incubation at temperature 50 °C, 1.25 ml of a 10% solution of trichloroacetic acid was added. In 1.25 ml of prepared mixture 1.25 ml of distilled water and 0.25 ml of 0.1% solution of iron chloride. After incubation (30 min), the absorbance versus a prepared blank was read at 700 nm using spectrophotometer. Increase in absorbance of the reaction mixture indicates greater reducing capability. Ascorbic acid was used as a standard. The samples were prepared in triplicate and the mean value of absorbance was obtained, through which the activity was expressed (LIM *et al.*, 2009).

Determination of antimicrobial activity

Test microorganisms

Antimicrobial activity of diethyl ether and ethanol extracts were tested against 20 microorganisms including sixteen 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, *B. pumilus* NCTC 8241, *Proteus mirabilis* ATCC 12453; and clinical isolates: *Escherichia coli, Staphylococcus aureus, S. aureus* PMFKG-B12, *Enterococcus faecalis, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella enterica, S. typhimurium* and *Bacillus subtilis*) and four species of yeast: *Candida albicans* ATCC 10231, *C. 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 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 suspensions contain about 10^8 colony forming units (CFU)/ml and yeast suspensions contain 10^6 CFU/ml. 1:100 dilutions of initial suspension were additionally prepared into sterile 0.85 % saline.

Microdilution method

Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) by using microdilution method with resazurin (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 yeast, into each well. A 100 μl from the stock solution of tested extracts (concentration of 5000 μg/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 2500 to 19.53 μg/ml. A 10 μl

of diluted bacterial and yeast suspension was added to each well to give a final concentration of 5×10^5 CFU/ml for bacteria and 5×10^3 CFU/ml for yeast. Finally, 10 µl resazurin solution (6.75 mg/ml) was added to each well inoculated with bacteria and yeast. Resazurin is an oxidation–reduction indicator used for the evaluation of microbial growth. It 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 the yeast. MIC was defined as the lowest concentration of tested substance that prevented resazurin color change from blue to pink.

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, dissolved in nutrient liquid medium, were used as positive controls. Stock solutions of crude extracts were obtained by dissolving in DMSO and then diluted into broth to achieve a concentration of 10 % DMSO. 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 two-fold serial dilution assay (the working con-centration was 5 % and lower). Each test included growth control and sterility control. All tests were performed in duplicate and MICs were constant.

Data analysis

All data were presented as means ± standard deviations (mean ± SD) where appropriate. All calculations were performed using Microsoft Excel software.

RESULTS AND DISCUSSION

Total phenol content, flavonoid and tannins concentrations

Since there are various possibilities of the extraction of biological active plant compounds, we used different solvents (ethanol and diethyl ether) in this study. Concentration of total phenols, flavonoids and tannins in extracts of *V. minor* are shown in Table 1.

Using the method with Folin-Ciocalteu reagent, the concentrations of total phenols were examined. The highest concentration was found in ethanol extract of *V. minor* (36.142 mg GA/g).

Using the method with aluminium chloride we obtained the concentrations of flavonoids. The highest concentration of flavonoids had diethyl ether extract (39.079 mg RU/g).

Table 1. Concentration of total phenols, flavonoids and tannins in extracts of *V. minor*.

Type of extracts	Total phenolic content* (mg GA/g of extract)	Flavonoid concentration* (mg RU/g of extract)	Tannin concentration* (mg CCh/g of extract)
Ethanol	36.14 ± 0.08	36.82 ± 0.08	1.9 ± 0.03
Diethyl ether	20.24 ± 0.10	39.08 ± 0.06	2.92 ± 0.06

*Each value shown is the mean value ± standard deviation

In determining the amount of tannins, a method with butanol-HCl reagent was used. The obtained results show that diethyl ether extract has higher concentration of tannin than the ethanolic extract (Table 1).

Antioxidant activity

The antioxidant activity of different plant extracts of *V. minor* is determined by the use of two methods: DPPH radicals scavenging capacity assay and reductive power of plant extract.

The capability of ethanol and diethyl ether extracts of *V. minor* to neutralize free radicals (DPPH) is expressed in the form of IC_{50} values (μg/ml). The results are shown in Table 2. In addition, activity was determined for the control substance as a standard parameter in the experiment, also expressed in the form of IC_{50} values. Antioxidative efficiency of ethanolic extract was significantly higher compared to diethyl ether extract.

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

Type of extract	IC_{50} value* (μg/ml)
Ethanol	496.14±0.91
Diethyl ether	1101.85±8.97
Ascorbic acid	5.25

*Each value shown is the mean value ± standard deviation.

Reduction potential of tested extracts was lower than control (ascorbic acid) and extracts did not differ for its reduction power (Table 3).

Table 3. The reductive potential of investigated plant extracts and standard substance.

Type of extract	1000 (μg/ml)*	500 (μg/ml)	250 (μg/ml)	125 (μg/ml)	62.5 (μg/ml)
Ethanol	0.343±0.0008	0.153±0.001	0.097±0.0004	0.053±0.0006	0.034±0.0005
Diethyl ether	0.353±0.001	0.163±0.0001	0.116±0.0006	0.053±0.0006	0.045±0.0003
Ascorbic acid	2.943±0.17	2.943±0.17	2.843±0.00	2.667±0.00	1.190±0.02

*Each value shown is the mean value of absorbance ± standard deviation

The main phenolics in *V. minor* were found. Kaempferol-3-O-(6-O-rhamnosyl-glucoside)-7-O-glucoside, 2,3-dihydroxybenzoic acid, vincoside and chlorogenic acid were isolated from the leaves of *V. minor*. 2,3-Dihydroxybenzoic acid showed a potent radical-scavenging activity (NISHIBE *et al.,* 1996).

Other authors consider that antioxidant activity is derived from alkaloids present in large quantities in this plant (SCHEINDLIN and RUBIN, 2006). It has been found that *V. minor* aquatic alkaloid extract exhibited significant DPPH free radical scavenging activity, while their aquatic non-alkaloid extract showed only weak activity (BAHADORI *et al.,* 2012).

Antimicrobial activity

The results of *in vitro* antibacterial and antifungal activities of ethanol and diethyl ether leaves extracts of *V. minor* are shown in Table 4 and 5. For comparison, the tables also give the results of the activities of doxycycline and fluconazole. The solvent (10 % DMSO) had no effect on the growth of tested microorganisms. Antimicrobial activities of tested extracts were assessed by determining the MIC and MMC values for 20 species of microorganisms.

The values of minimum inhibitory concentrations and minimum microbicidal concentrations obtained in this experiment ranged from 19.53 to >2500 μg/ml. Efficacy of antimicrobial activity depended on the species of microorganism.

The strongest antimicrobial activity was showed on G+ bacteria, especially from genus *Bacillus*, while the activity on other bacteria was moderate. Ethanol and diethyl ether extracts

were active in concentration from 19.53 µg/ml to >2500 µg/ml. The most sensitive bacteria were *B. pumilus* NCTC 8241 (MIC 19.53 µg/ml) and *B. subtilis* (MIC 78.13 µg/ml) for the ethanol extract and *B. pumilus* NCTC 8241 (MIC 19.53 µg/ml) and *B. subtilis* (MIC 156.25 µg/ml) for diethyl ether extract. G- bacteria did not show sensitivity within the tested extract concentrations. The exception is *P. aeruginosa* ATCC 27853 where MIC was 2500 µg/ml.

When analyzing the effectiveness of extracts in preventing the growth of yeasts, that activity was generally weak. All species of yeasts have shown high resistance. MMC values are often coincided with MICs. Ethanol extract stood out with a stronger effect on all the tested yeasts compared to other extract with the noticeable difference.

Table 4. Antibacterial activities of ethanol and diethyl ether extracts of *V. minor* leaves against tested bacteria.

	Ethanol extract		**Diethyl ether extract**		**Doxycycline**	
Species	**MIC***	**MMC***	**MIC**	**MMC**	**MIC**	**MMC**
Bacillus subtilis	78.13	625	156.25	1250	0.112	1.953
Bacillus subtilis ATCC 6633	>2500	>2500	>2500	>2500	1.953	31.25
Bacillus pumilus NCTC 8241	19.53	1250	19.53	1250	0.112	7.81
Staphylococcus aureus	1250	1250	1250	1250	0.448	7.81
S. aureus PMFKGB12	>2500	>2500	>2500	>2500	-	-
S. aureus ATCC 25923	625	625	625	625	0.224	3.75
Enterococcus faecalis	>2500	>2500	>2500	>2500	7.81	62.5
E. faecalis ATCC 29212	625	>2500	1250	>2500	7.81	62.5
Proteus mirabilis	>2500	>2500	>2500	>2500	250	> 250
Proteus mirabilis ATCC 12453	>2500	>2500	>2500	>2500	15.625	62.5
Pseudomonas aeruginosa	>2500	>2500	>2500	>2500	250	> 250
P. aeruginosa ATCC 27853	2500	>2500	2500	>2500	62.5	125
Escherichia coli	>2500	>2500	>2500	>2500	7.81	15.63
Escherichia coli ATCC 25922	>2500	>2500	>2500	>2500	15.625	31.25
Salmonella typhimurium	>2500	>2500	>2500	>2500	15.625	125
Salmonella enterica	>2500	>2500	>2500	>2500	15.625	31.25

*Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) values are given as µg/ml for plant extract and antibiotic (doxycycline); "-" means not determined.

Table 5. Antifungal activities of ethanol and diethyl ether extracts of *V. minor* leaves against tested yeast

	Ethanol extract		**Diethyl ether extract**		**Fluconazole**	
Species	**MIC***	**MMC***	**MIC**	**MMC**	**MIC**	**MMC**
Rhodotorula sp. PMFKG-F27	625	2500	2500	2500	62.5	1000
Candida albicans	1250	>2500	>2500	>2500	62.5	1000
Candida albicans ATTC 10231	1250	>2500	>2500	>2500	31.25	1000
Saccharomyces boulardii PMFKG-P34	>2500	>2500	>2500	>2500	31.25	1000

*Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) values are given as µg/ml for plant extract and antimycotic (fluconazole).

Although there are numerous phytochemical and biochemical studies of this plant, the diethyl ether extract from *V. minor* leaves has not been investigated enough.

The effect of ethanolic extract of *V. minor* on pathogenic bacteria isolated from aquatic organisms was studied by TURKER *et al.* (2009). They came to conclusion that the ethanolic extract of *V. minor* exhibited a broad-spectrum activity against both G+ (*Streptococcus*

agalactiae, *Lactococcus garvieae* and *E. faecalis*) and G- bacteria (*Aeromonas hydrophila* and *Yersinia ruckeri*). As well as in our study, the effect of the ethanolic extract was significantly lower than positive controls and effect was better in G+ bacteria with the exception of *E. faecalis*. *In vitro* antibacterial activities of *V. minor* plant extracts, growing in Turkey, showed the best antibacterial activity, just as in our study, with ethanolic extract. Cosidering results obtained against *S. typhimurium* there was no match between this study and our study. Ethanolic extract of *V. minor* leaves exhibited moderate inhibitory activity against *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *S. aureus* (YILDIRIM *et al.*, 2012). MEHRABIAN *et al.*, (1995) reported the antimicrobial effect of *V. minor* on some pathogen bacteria (*S. aureus* and *P. aeruginosa*) where similar effect of extracts was observed on both mentioned bacteria. In our study the results varied between *S. aureus* and *P. aeruginosa*. Ethanolic extract hadn't expressed activity or it was a soft spot for G- bacteria.

With these results, tested plant has some scientific justification as a medicinal plant. In the future, identification of active components can be studied for plant extracts having strong bioactivity. This study indicates that extracts of *V. minor* showed a certain level of antimicrobial activity and may be an additional biologic potential in pharmaceutical use, but that additional research is needed, especially in order to identify the active components.

CONCLUSION

The results of antimicrobial activity indicate that tested extracts showed different degree of antimicrobial activity in relation to the tested species. Extracts of *V. minor* demonstrated more potent inhibitory effects on the growth of G+ bacteria than to the other tested microorganisms. Therefore, the leaves of this plant can be a potential source of antibacterial substances.

The results of our study suggest the great value of the *V. minor* species, suggesting it for use in pharmaceutical, phytotherapy and food industry.

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