*https://doi.org/10.31925/farmacia.2022.4.12*

**ORIGINAL ARTICLE**

# INFLUENCE OF SOLVENT TYPE ON THE PHENOLIC CONTENT AND ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF *ECHIUM VULGARE* L. EXTRACTS

IVANA BOŠKOVIĆ [1]*, DRAGUTIN ĐUKIĆ [2], PAVLE MAŠKOVIĆ [2], LEKA MANDIĆ [2]

[1]*University of East Sarajevo, Faculty of Agriculture, 71 123 East Sarajevo, Bosnia and Herzegovina*
[2]*University of Kragujevac, Faculty of Agronomy, 32 000 Čačak, Serbia*

**corresponding author: ivana.boskovic@pof.ues.rs.ba*

*Manuscript received: January 2022*

**Abstract**

The aim of this study was to investigate influence of solvent type on the phenolic content and antimicrobial and antioxidant properties of *Echium vulgare* L. extracts. The following solvents were used in the study: ethanol, acetone, chloroform, petroleum and ethyl acetate. The content of phenols, flavonoids and tannins was determined by spectrophotometric methods, while the identification and quantification of polyphenolic compounds was performed by chromatographic method. Determination of antimicrobial activity was done by microdilution method, while several methods were used in the evaluation of antioxidant activity of plant extracts. The ethanolic extract had the highest content of phenols and flavonoids, while chloroform extract had the highest content of tannins. The ethanolic extract exhibited the best antioxidant potential compared to other extracts, and ethanolic and chloroform extracts manifested the best antimicrobial activity.

**Rezumat**

Scopul acestui studiu a fost de a investiga influența tipului de solvent asupra conținutului fenolic, a proprietăților antimicrobiene și antioxidante ale extractelor obținute de la specia *Echium vulgare* L. Au fost utilizați ca solvenți: etanol, acetonă, cloroform, petrol și acetat de etil, iar conținutul în fenoli, flavonoizi și taninuri a fost determinat prin metode spectrofotometrice. Identificarea și cuantificarea compușilor polifenolici s-a realizat prin metoda cromatografică. Determinarea activității antimicrobiene s-a realizat prin metoda microdiluției, în timp ce pentru evaluarea activității antioxidante a extractelor au fost utilizate mai multe metode. Extractul etanolic a avut cel mai mare conținut de fenoli și flavonoide, în timp ce extractul cloroformic a avut cel mai mare conținut de taninuri. Extractul etanolic a prezentat cel mai pronunțat potențial antioxidant, în timp ce extractele etanolice și cloroformice au manifestat cea mai bună activitate antimicrobiană.

**Keywords**: *Echium vulgare* L., solvent, antimicrobial activity, antioxidant activity

## Introduction

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind [28]. Numerous of biological properties such as antimicrobial, antiviral, antioxidant, anti-inflammatory, etc. are attributed to phenolic compounds that are present in the plant kingdom.

The selection of extraction solvents is critical, as it will determine the amount and type of phenolic compounds being extracted [11]. Numerous studies have shown that methanol has been more efficient in the extraction of lower molecular weight polyphenols, while the higher molecular weight flavanols are better extracted with aqueous acetone. A review of the literature indicated that aqueous mixtures organic solvents are better solvents for extraction of polyphenolic compounds from plant material [18, 29, 30, 32]. Humulescu *et al*. [22] point out that in addition to solvents, other factors such as dryness, dimension of plant material, temperature, pressure for processing and human factors affect yield extraction.

In plants, polyphenols perform a number of functions that have a great impact on the ecophysiology of plants: they act as antioxidants, antimicrobial agents, visual attractants of some insects important for flower pollination [19]. In recent years, medicinal plants have been extensively studied for their antioxidant activity and ability to neutralize free radicals. Due to the increasing resistance of bacteria to the action of a large number of antibiotics, the use of natural antimicrobial preparations of plant origin is becoming increasingly important. Therefore, intensive research is focused on finding new antimicrobial substances, originating from the plant kingdom.

The *Boraginaceae* family originates from Asia and includes a flowering family of about 2700 species and 147 genera [5]. The family comprises a group of plants that are important for pharmacology and cosmetology [14]. Some *Boraginaceae* species were used for burns

and wounds healing in folk medicine around the world [3].

*Echium vulgare* L. is a biennial herb occurring in weed communities in Europe [27]. Numerous studies have reported antibacterial, antiviral, anti-inflammatory, antiproliferative, antioxidative and analgesic properties of extracts of the *Echium* genus [1, 4, 16, 25].

The aim of this study was to investigate influence of solvent type on the polyphenolic content and anti-microbial and antioxidant properties of *Echium vulgare* L. extracts.

## Materials and Methods

*Plant material and extraction*

The plant *E. vulgare* L. was collected in the flowering stage on Brđanska gorge, Serbia. The aboveground part of the plant, previously degreased with petroleum ether (40°C) is extracted with a series of solvents in Soxhlet's apparatus (ethanol, acetone, chloroform, petroleum and ethyl acetate). The solutions were evaporated on a rotary evaporator at a temperature of 40°C.

*Determination of the total phenolic compounds, flavonoids and tannins content*

The amount of total phenols in plant was determined by the Folin-Ciocâlteu method [33]. The total phenol content is expressed in milligram equivalents of gallic acid *per* gram of extract (mg GA/g of extract).

The total quantity of flavonoid is carried out by the method of Brighente *et al*. [9]. Total flavonoids content is expressed in milligram equivalents of routine *per* gram of extract (mg RU/g extract).

In order to determine the tannins content, by the method of Verrmeris and Nicholson was used [36]. The concentration of tannin is expressed in milligram equivalents of gallic acid *per* gram of extract (mg GA/g of extract).

*Determination of the phenolic compounds in extracts by HPLC analysis*

Determination of polyphenol components in the tested extracts have been performed on the appliance HPLC Agilent 1200 Series UV-Vis DAD for a multi detection wavelengths (Table II). The 5 mL of sample separation is performed using a column Agilent Eclipse XDB-C18 column (4.6 × 50 mm, 1.8 microns). The column was thermostated at 25°C. Two solvents were used to elute gradient A ($H_2O$ + 2% HCOOH) and B (80% ACN + 2% HCOOH + $H_2O$). Separation of the components was performed using the following linear gradient: from 0 to 10 min, 0% B; from 10 to 28 minutes, gradually increasing 0 - 25%; B from 28 to 30 min, 25% B; 30 - 50% B gradually increases over 30 to 35 minutes; 35 - 40 min gradually increases 50 - 80% B; and finally in the last 5 minutes it gradually decreases by 80 - 0% B. All identifications of each component were based on the retention time of the original standards. The polyphenolic components present in the samples were identified by comparing their retention times and spectra with the retention time and standard spectrum for each component [5].

*Determination of antioxidant activity of extracts*

The total antioxidative capacity was determined by the phosphor-molybdenum method [31]. The results of the total antioxidant potential are expressed in micrograms of ascorbic acid *per* gram of extract (μg AA/g). Possibility of neutralization of DPPH radicals with plant extracts was studied by the method of Takao *et al*. [35]. Determination of inhibition of lipid peroxidation was carried out by using the ammonium thiocyanate method, based on the initiation of lipid autoxidation at elevated temperature [23]. The ability of extracts, as substrates suitable for the neutralization of hydroxyl radicals formed in the Fenton reaction, was determined by the method of Hinneburg *et al*. [24]. The results of the antioxidant activity are presented as means ± standard deviations (mean ± SD) of three analytical determinations. The results of inhibition lipid peroxidation, hydroxyl radical scavenging activity and DPPH scavenging activity are presented as a number of $IC_{50}$ values, the concentration of the test compound that reduces concentration of free radical species by 50%. As is known, the lower is the $IC_{50}$ value, the more pronounced is the antioxidant activity.

*Determining the antimicrobial activity of plant extracts of microdilution method*

The determination of the antimicrobial potential of the tested extracts was carried out by a microdilution method [10] on the bacteria: *Listeria ivanovii* ATCC 19119, *Listeria inocuu* ATCC 33090, *Listeria mono-cytogenes* ATCC 19112, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 6057, *Bacillus spieizenii* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophiticus* ATCC 15035, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 13315, *Proteus mirabilis* ATCC 14153, *Salmonella enteritidis* ATCC 13076, *Salmonella typhimurium* ATCC 14028, *Enterobacter aerogenes* ATCC 13048, *Citrobacter freundi* ATCC 43864, *Pseudomonas aeruginosa* ATCC 27853 and yeast: *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Minimum inhibitory concentration (MIC) and MBC (minimum bactericidal concentration) were determined. The microplates were incubated for 24 h at 37°C for bacteria and 48 h for the yeasts, and after this period a minimum inhibitory concentration (MIC) was determined. The contents of all wells, in which no microbial growth was visible, were transferred to new Petri plates with appropriate solid medium (MH agar) and after incubation at 37°C for 24 h, the counting colonies grown was performed (MBC).

*Statistical analysis*

Statistical analysis of the results was performed using analysis of variance mono-factorial trial. The probability value of 0.05 was considered significant.

## Results and Discussion

*The total phenolic compounds, flavonoids and tannins content*

Due to their beneficial properties and effectiveness, medicinal herbs have been used for centuries in the prophylaxis and treatment of many diseases. The medicinal properties of plants are attributed to a number of secondary metabolites produced by plants. Successful separation and determination of biologically active compounds from plant material, it mainly depends on the type of solvent used in the extraction procedure [15]. Therefore, the aim of this study was to investigate the influence of solvent type on the polyphenolic content and antimicrobial and antioxidant properties of *Echium vulgare* L. extracts.

The content of phenols, flavonoids and tannins of the tested extracts of *Echium vulgare* obtained in our study are summarized in Table I. The total phenolic content is expressed as gallic acid equivalents, total flavonoids content are expressed in terms of rutin equivalents and concentration of tannins are expressed as gallic acid equivalents. The phenolic content in the extracts ranged from 89.22 ± 0.07 to 100.21 ± 0.57 mg GA/g, the flavonoid content ranged from 31.22 ± 0.25 to 35.16 ± 0.17 mg RU/g, and the tannin content ranged from 65.39 ± 0.38 to 70.35 ± 0.42 mg GA/g.

**Table I**

Total phenolics, flavonoids and tannin contents in extracts of plant *Echium vulgare* L. (mgGA/g, mgRU/g)

| Extracts | Total phenolics (mg GA/g) | Flavonoids (mg RU/g) | Tannins (mg GA/g) |
|---|---|---|---|
| **Chloroform** | $97.86 \pm 0.13^{b}$ | $31.22 \pm 0.25^{c}$ | $70.35 \pm 0.42^{a}$ |
| **Ethyl acetate** | $89.22 \pm 0.07^{d}$ | $34.40 \pm 0.18^{ab}$ | $69.86 \pm 0.64^{a}$ |
| **Ethanol** | $100.21 \pm 0.57^{a}$ | $35.16 \pm 0.17^{a}$ | $65.39 \pm 0.38^{b}$ |
| **Acetone** | $95.38 \pm 0.32^{c}$ | $34.21 \pm 0.21^{b}$ | $70.33 \pm 0.47^{a}$ |
| **Petroleum** | $85.20 \pm 0.19^{e}$ | $34.10 \pm 0.46^{b}$ | $66.11 \pm 0.13^{b}$ |

*Statistically significant difference between the levels of 0.05% is significant if it is next obtained concentration values is a different letter. If they are the same letters that difference is not significant. If there are two letters, the significance is seen again after that is there at least one letter that is the same or different. The results are presented as average ±SD of three analytical determinations

The ethanolic 100.21 ± 0.57 mg GA/g, chloroform 97.86 ± 0.13 mg GA/g and acetone extracts 95.38 ± 0.32 mg GA/g had the highest concentration of phenols, and ethanol extract had the highest concentration of flavonoids 35.16 ± 0.17 mg RU/g. The highest concentrations of tannin had the chloroform 70.35 ± 0.42 mg GA/g and acetone extracts 70.33 ± 0.47 mg GA/g.

Research has shown that all five solvents had a statistically significant effect on phenol content. Statistically significant difference is found between the content the flavonoids from the ethanolic extract in comparison to the other tested extracts. Based on the obtained results, we can conclude that the extraction yield was higher in polar solvents compared to less polar solvent, which is in accordance with the results with the results obtained by other researchers [15, 34].

*HPLC analysis* of *phenolic compounds*

The results of the HPLC analysis of the tested extracts of the *Echium vulgare* are indicated in Table II. In our study the acetone (24.82 mg/g) and the ethanolic extract (19.06 mg/g) had the highest values of the identified compounds. The lowest values of the identified compounds were recorded in ethyl acetate extract (4.91 mg/g).

**Table II**

HPLC analysis of the obtainedextracts of plant *Echium vulgare* L. (mg/g)

| Compounds | Chloroform | Ethyl acetate | Ethanol | Acetone | Petroleum |
|---|---|---|---|---|---|
| **p-Hydroxibenzoic acid** | 1.83 | n.d. | n.d. | n.d. | 3.36 |
| **Chlorogenic acid** | n.d. | n.d. | 0.79 | n.d. | 1.54 |
| **p-Coumaric acid** | n.d. | n.d. | n.d. | n.d. | 0.29 |
| **Ferulic acid** | n.d. | n.d. | 0.26 | n.d. | 0.21 |
| **Synapic acid** | n.d. | n.d. | 1.32 | n.d. | n.d. |
| **Rutin** | 3.49 | 4.04 | 7.77 | 16.73 | 2.22 |
| **Luteolin glycoside** | n.d. | n.d. | n.d. | 0.73 | n.d. |
| **Rosmarinic acid** | n.d. | n.d. | 8.00 | 6.34 | 0.84 |
| **Quercetin** | n.d. | 0.41 | 0.44 | n.d. | n.d. |
| **Kaempferol** | n.d. | 0.45 | 0.47 | 1.02 | 0.62 |
| **Apigenin** | n.d. | n.d. | n.d. | n.d. | n.d. |
| **Σ** | 5.33 | 4.91 | 19.06 | 24.82 | 9.07 |

(n.d.) – not determined.

The most dominant components in the tested extract were rutin and rosmarinic acid, which is in accordance with the research of phenolic compounds of plants from the family *Boraginaceae* [5-7]. Rutin exerts many pharmacological activities, including anti-inflammatory, antimicrobial, anticarcinogenic, neuroprotective, anti-thrombotic and antiviral activities [2, 17]. Rosmarinic acid exhibits a wide range of biological activities, and

various studies have shown antioxidant, antimicrobial, antiviral, anticancer, and antiallergic properties of rosmaric acid [20, 21].

*Antioxidant activity of extracts*

Plants intensively produce secondary metabolites, which play a major role in biological systems, because they neutralize the action of free radicals. The importance of antioxidants is that they have the ability to stabilize free radicals before damaging a cell. Evaluation of the antioxidant activity of the extracts of *Echium vulgare* L. included the determination of the total antioxidant potential, inhibition of lipid peroxidation, hydroxyl radical (OH•) scavenging activity and DPPH free radical scavenging activity (Table III).

**Table III**

The total antioxidant capacity (μgAA/g), inhibition of lipid peroxidation (μg/mL), hydroxyl radical (OH•) scavenging activity (μg/mL) and DPPH radical scavenging activity (μg/mL)

| Extract | Total antioxidant capacity | Inhibition of lipid peroxidation $IC_{50}$ | Hydroxil radical scavenging activity $IC_{50}$ | DPPH radical scavenging activity $IC_{50}$ |
|---|---|---|---|---|
| **Chloroform** | 101.24 ± 0.88 | 51.34 ± 1.06 | 69.03 ± 0.18 | 70.01 ± 1.06 |
| **Ethyl acetate** | 102.28 ± 0.35 | 55.22 ± 1.27 | 67.30 ± 0.27 | 74.07 ± 0.88 |
| **Ethanol** | 105.22 ± 0.32 | 49.48 ± 1.33 | 68.58 ± 0.11 | 71.60 ± 0.40 |
| **Acetone** | 101.23 ± 0.29 | 50.50 ± 1.10 | 70.18 ± 0.77 | 72.51 ± 0.86 |
| **Petroleum** | 91.01 ± 1.42 | 56.38 ± 1.02 | 75.19 ± 0.17 | 78.76 ± 0.79 |

Average antioxidans activity (± SD) of three analytical determinations

The ethanolic extract of *Echium vulgare* L. had the highest total antioxidant potential (105.22 ± 0.32 μg AA/g) and the best ability to inhibit lipid peroxidation (49.48 ± 1.33 μg/g). The ethyl acetate extract exerted the highest ability to capture the hydroxyl radicals (67.30 ± 0.27 μg/g), and the chloroform extract had the strongest DPPH radical scavenging activity (70.01 ± 1.06 μg/mL).

Ethanol extract exhibited the strongest antioxidant potential compared to other solvents which is in accordance with the research by Do *et al*. [13] who pointed out that 100% ethanol extract gave the highest antioxidant capacity in all *in vitro* assays studied. Numerous bioactive components of *E. vulgare* extracts have certainly contributed to their antioxidant activity [5].

*Antimicrobial activity of plant extracts*

The phenolic compounds present in the medicinal plants are responsible for a range of biological activities, including antimicrobial action [26]. Therefore, in the last decade there has been increased interest in the antimicrobial activity of various extracts obtained from traditional medicinal plants.

The tested extracts of *Echium vulgare* L. exhibited a certain degree of antimicrobial activity, while chloroform and ethanol extracts exhibited the best antimicrobial activity compared to the other tested extracts and standard antibiotics – tetracycline and ketoconazole – Table IV.

**Table IV**

Antimicrobial activity of *Echium vulgare* L. plant extracts MIC and MBC (μg/mL)

| Bacteria/Extract MIC/MBC | Chloroform | Ethyl acetate | Ethanol | Acetone | Petroleum | Tetracycline | Ketoconazole |
|---|---|---|---|---|---|---|---|
| ***P. mirabilis*** | 3.91/7.82 | 7.81/15.625 | 15.625/31.25 | 31.25/62.5 | 125.00/250.00 | 0.49 | 0 |
| ***P. vulgaris*** | 7.81/15.625 | 3.91/7.82 | 7.81/15.625 | 31.25/62.5 | 15.625/31.25 | 0.49 | 0 |
| ***K. pneumoniae*** | 15.625/31.25 | 62.5/125.00 | 3.91/7.82 | 62.5/125.00 | 250.00/500.00 | 0.49 | 0 |
| ***E. coli*** | 7.81/15.625 | 62.5/125.00 | 250.00/500.00 | 3.91/7.82 | 15.625/31.25 | 0.97 | 0 |
| ***C. freundii*** | 62.5/125.00 | 7.81/15.625 | 3.91/7.82 | 250.00/500.00 | 125.00/250.00 | 0.49 | 0 |
| ***E. aerogenes*** | 125.00/250.00 | 250.00/500.00 | 15.625/31.25 | 62.5/125.00 | 500.00/1000 | 0.97 | 0 |
| ***S. enteritidis*** | 125.00/250.00 | 7.81/15.625 | 3.91/7.82 | 31.25/62.5 | 62.5/125.00 | 0.49 | 0 |
| ***S. typhimur.*** | 3.91/7.82 | 500.00/1000 | 7.81/15.625 | 31.25/62.5 | 62.5/125.00 | 0.49 | 0 |
| ***L. monocytog.*** | 15.625/31.25 | 7.81/15.625 | 31.25/62.5 | 250.00/500.00 | 62.5/125.00 | 0.97 | 0 |
| ***L. ivanovii*** | 3.91/7.82 | 62.5/125.00 | 250.00/500.00 | 31.25/62.5 | 125.00/250.00 | 0.97 | 0 |
| ***L. inocuu*** | 62.5/125.00 | 15.625/31.25 | 7.81/15.625 | 3.91/7.82 | 250.00/500.00 | 0.49 | 0 |
| ***B. spieizeni*** | 250.00/500.00 | 3.91/7.82 | 125.00/250.00 | 31.25/62.5 | 125.00/250.00 | 0.97 | 0 |
| ***P. aeruginosa*** | 125.00/250.00 | 31.25/62.5 | 250.00/500.00 | 500.00/1000 | 7.81/15.625 | 0.97 | 0 |
| ***E. faecalis*** | 7.81/15.625 | 31.25/62.5 | 500.00/1000 | 62.5/125.00 | 15.625/31.25 | 0.97 | 0 |
| ***E. faecium*** | 7.81/15.625 | 125.00/250.00 | 62.5/125.00 | 31.25/62.5 | 250.00/500.00 | 0.49 | 0 |
| ***S. aureus*** | 3.91/7.82 | 250.00/500.00 | 31.25/62.5 | 7.81/15.625 | 31.25/62.5 | 0.97 | 0 |
| ***S. saprophiticus*** | 7.81/15.625 | 250.00/500.00 | 125.00/250.00 | 62.5/125.00 | 3.91/7.82 | 0.97 | 0 |
| ***A. niger*** | 125.00/250.00 | 7.81/15.625 | 500.00/1000 | 7.81/15.625 | 31.25/62.5 | 0 | 0.97 |
| ***C. albicans*** | 250.00/500.00 | 125.00/250.00 | 62.5/125.00 | 15.625/31.25 | 7.81/15.625 | 0 | 1.95 |

The chloroform extract of *E. vulgare* L. exhibited the strongest activity against the bacteria *P. mirabilis, S. Typhimurium, L. ivanovii* and *S. aureus* (MIC = 3.91 μg/mL). The ethanolic extract exhibited the greatest antimicrobial activity against *K. pneumoniae, S. enteritidis* and *C. freundii* (3.91 μg/mL). The lowest

value of minimum bactericidal concentration (7.82 μg/mL) was obtained for the chloroform extract against the bacteria *P. mirabilis, S. Typhimurium, L. ivanovii* and *S. aureus,* and the ethanolic extract was effective against the bacteria *K. pneumoniae, S. enteritidis* and *C. freundii*. The antimicrobial effect of ethanol and chloroform extract is due to the high antioxidant capacity and the presence of large amounts of flavonoids and tannins, which is in accordance with the results of research by Cushnie and Lamb [12]. Also, good antimicrobial potential of ethanol extract can be associated with the stronger extraction capacity of alcohol could have produced greater number of active constituents responsible for antibacterial activity [8].

## Conclusions

The results of these studies showed that the applied solvents significantly affected the content of phenolic compounds in and their antimicrobial and antioxidant properties. Ethanol extract had the highest content of phenols and flavonoids, while chloroform extract had the highest content of tannins. Ethanol extract exhibited the best antioxidant potential compared to other extracts, and ethanolic and chloroform extracts manifested the best antimicrobial activity. The tested extracts are abundant in pharmacologically active substances, so further research on phenolic compounds of plants from the *Boraginaceae* family would be of great importance in the scientific field.

## Conflict of interest

The authors declare no conflict of interest.

## References


1. Abolhassani M, Antibacterial effect of borage (*Echium amoenum*) on *Staphyloccocus aureus*. *Braz J Infect Dis.*, 2004; 8(5): 382-385.
2. Agrawal PK, Agrawal C, Blunden G, Rutin: A potential Antiviral for Repurposing as a SARS-CoV-2 Main Protease ($M^{pro}$) Inhibitor. *Nat Prod Commun.*, 2021; 16(4): 1-12.
3. Aliasl J, Barikbin B, Khoshzaban F, Naseri M, Sedaghat R, Kamalinejad M, Talei D, Emadi F, Akbari Z, Aliasl F, Jalaly NY, Mohseni-Moghaddam P, Effect of *Arnebia euchroma* ointment on post-laser wound healing in rats. *J Cosmet Laser Ther.*, 2015; 17(1): 41-45.
4. Bentabet N, Boucherit K, Boucherit-Otmani Z, Rahmoun N, Phytochemical Screening and Evaluation of Antibacterial Activity of Extracts from Leaves and Roots of *Echium Vulgare* of the Region of Tlemcen. *Der Pharma Chem.*, 2021; 13(1): 33-39.
5. Bošković I, Antimicrobial and antioxidant activity of plant extracts from family *Boraginaceae*. PhD thesis, University of Montenegro, Podgorica, 2017: 1-57.
6. Bošković I, Đukić D, Mašković P, Mandić L, Phytochemical composition and biological activity of *Echium italicum* L. plant extracts. *Bulg Chem Commun.*, 2017; 49(4): 836-845.
7. Bošković I, Đukić D, Mašković P, Mandić L, Perović S, Phytochemical composition and antimicrobial, antioxidant and cytotoxic activities of *Anchusa officinalis* L. extracts. *Biologia*, 2018; 73(4): 1035-1041.
8. Bhattacharjee I, Chatterjee SK, Chatterjee S, Chandra G, Antibacterial potentiality of *Argemone mexicana* solvent extracts against some pathogenic bacteria. *Mem Inst Oswaldo Cruz*, 2006; 101(6): 645-648.
9. Brighente IMC, Dias M, Verdi LG, Pizzolatti MG, Antioxidant Activity and Total Phenolic Content of Some Brazilian Species. *Pharm Biol.*, 2007; 45(2): 156-161.
10. CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition, 2012; M07/A9, 32(2): 1-88.
11. Chan SW, Lee CY, Yap CF, Wan Aida WM, Ho CW, Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels. *Int Food Res J.*, 2009; 16(2): 203-213.
12. Cushnie TPT, Lamb AJ, Antimicrobial activity of flavonoids. *Int J Antimicrob Agents*, 2005; 26(5): 343-356.
13. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH, Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Limnophila aromatica*. *J Food Drug Anal.*, 2013; 22(3): 296-302.
14. Dresler S, Szymczak G, Wojcik M, Comparison of some secondary metabolite content in the seventeen species of the *Boraginaceae* family. *Pharm Biol.*, 2017; 55(1): 691-695.
15. Ezez D, Tefera M, Effects of Solvents on Total Phenolic Content and Antioxidant Activity of Ginger Extracts. *Hindawi J Chem.*, 2021; 2021: Art. ID 6635199: 1-5.
16. Farahani M, Antiviral effect assay of aqueous extract of *Echium Amoenum*-L against HSV-1. *Zahedan J Res Med Sci.*, 2013; 15(8): 46-48.
17. Ganeshpurkar A, Saluja AK, The Pharmacological Potential of Rutin. *Saudi Pharm J.*, 2017; 25(2): 149-164.
18. Guyot S, Marnet N, Drilleau J, Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. *J Agric Food Chem.*, 2001; 49(1), 14-20.
19. Heim KE, Tagliaferro AR, Bobilya DJ, Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem.*, 2002; 13(10): 572-584.
20. Hooker CW, Lott WB, Harrich D, Inhibitors of human immunodeficiency virus type 1 reverse transcriptase target distinct phases of early reverse transcription. *J Virol.*, 2001; 75(7): 3095-3104.
21. Huang SS, Zheng RL, Rosmarinic acid inhibits angiogenesis and its mechanism of action *in vitro*. *Cancer Lett.*, 2006; 239(2): 271-280.
22. Humulescu I, Flutur MM, Cioanca O, Mircea C, Robu S, Marin-Batir D, Spac A, Corciova A, Hancianu M, Comparative chemical and biological activity of selective herbal extracts. *Farmacia*, 2021; 69(5): 861-866.

23. Hsu CK, Chiang BH, Chen YS, Yang JH, Liu CL, Improving the antioxidant activity of buckwheat (*Fagopyrum tataricm Gaertn*) sprout with trace element water. *Food Chem.*, 2008; 108(2): 633-641.
24. Hinneburg I, Dorman HJD, Hiltunen R, Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem.*, 2006; 97(1): 122-129.
25. Jin J, Boersch M, Nagarajan A, Davey AK, Zunk M, Antioxidant Properties and Reported Ethnomedicinal Use of the Genus *Echium* (*Boraginaceae*). *Antioxidants*, 2020; 9(8): 722-731.
26. Jesus RS, Piana M, Freitas RB, Thiele FB, Alves CFS, Belke BV, Jank Mossmann NJ, Cruz RC, Santos RCV, Dalmolin TV, Bianchini BV, Campos MMA, Freitas Bauermann L, *In vitro* antimicrobial and antimycobacterial activity and HPLC–DAD screening of phenolics from *Chenopodium ambrosioides* L. *Braz J Microbiol.*, 2018; 49(2): 296-302.
27. Kiraly G, New Hungarian Herbal book. The Vascular Plants of Hungary. Identification key. Aggteleki Nemzeti Park Igazgatóság, Jósvafo, 2009: 6-93, (available in Hungarian).
28. Krishnaiah D, Sarbatly R, Nithyanandam R, A review of the antioxidant potential of medicinal plant species. *Food Bioprod Process.*, 2011; 89(3): 217-233.
29. Labarbe B, Cheynier V, Brossaud F, Souquet JM, Moutounet M, Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J Agric Food Chem.*, 1999; 47(7): 2719-2723.
30. Metivier RP, Francis FJ, Clydesdale FM, Solvent extraction of anthocyanins from wine pomace. *J Food Sci.*, 1980; 45(4): 1099-1100.
31. Prieto P, Pineda M, Aguilar M, Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal Biochem.*, 1999; 269(2): 337-341.
32. Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF, Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J Agric Food Chem.*, 2001; 49(3): 1270-1276.
33. Singleton VL, Orthofer R, Lamuela-Raventos RM, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth Enzymol.*, 1999; 299: 152-178.
34. Sultana B, Anwar F, Ashraf M, Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, 2009; 14(6): 2167-2180.
35. Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K, A Simple Screening Method for Antioxidants and Isolation of Several Antioxidants Produced by Marine Bacteria from Fish and Shellfish. *Biosci Biotechnol Biochem.*, 1994; 58(10): 1780-1783.
36. Vermerris W, Nicholson R, Phenolic compound biochemistry, Springer; 2006: 1-151.