Acta Poloniae Pharmaceutica – Drug Research, Vol. 70 No. 6 pp. 1021–1026, 2013 ISSN 0001-6837
Polish Pharmaceutical Society

PHENOLIC CONTENT, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *ERICA HERBACEA* L.

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Abstract: Antibacterial and antioxidant activity, total phenolic and flavonoid concentrations of aqueous, ethanol and ethyl acetate extracts from the leaves and flowers of *Erica herbacea* L. were studied. *In vitro* antibacterial activity of the extracts was determined by macrodilution method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been determined. Testing was performed on 30 clinical isolates, including different strains of *Escherichia coli*, *Enterococcus faecalis* and *Proteus vulgaris*. The values for MIC were in the range from 2.5 mg/mL to 40 mg/mL. The most sensitive bacterial strains were *Proteus vulgaris* strains. The aqueous extract from *E. herbacea* was found the most active. The total phenolic content was determined using Folin-Ciocalteu reagent and ranged between 14.98 and 119.88 mg GA/g. The concentration of flavonoids in extracts was determined using spectrophotometric method with aluminium chloride and obtained results varied from 16.19 to 26.90 mg RU/g. Antioxidant activity was monitored spectrophotometrically using DPPH reagent. The highest capacity to neutralize DPPH radicals was found in the aqueous extract from *E. herbacea*. The results of the total phenolic content determination of the examined extracts indicate that *E. herbacea* extracts are a rich source of phenolic compounds and also possess a significant antioxidant activity and moderate antibacterial activity.

Keywords: *Erica herbacea*, antibacterial activity, plant extracts, phenols, flavonoids, antioxidative

Winter heath, (*Erica herbacea* L., syn. *Erica carnea* L., fam. Ericaceae) is a prostrate, an evergreen shrub, densely branched, high 30-40 cm. The 4 needle-like leaves grow in whorls, on short stalks. The flowers are in clusters of peaks, on thin stems. The crown of the four leaves, bell-shaped, red. The fruit is a capsule. The flowers are produced in late winter to early spring, often starting to flower while the plant is still covered in snow. This plant inhabits variety of pine forests, spruce and oak forests (1).

Medicinal plants belonging to the genus *Erica* have been popularly used as antirheumatic, diuretic, laxative, astringent and for treatment of urinary infections (2, 3). It also has been shown that different extracts of leaves and flowers of *E. arborea* have considerable antioxidant effects (4, 5). *Erica* species contain many active compounds such as flavonoids, anthocyanins, coumarins and triterpenic compounds (6).

Erica herbacea is a plant that is used in traditional medicine for preventing infection of urinary tract. *E. herbacea* aerial parts are used as an ingredient in some uroantiseptic herbal mixture, because this specie contains a small amount of arbutin (1). This plant was the subject of the work that has analyzed the content of quercetin-3-rutinoside (rutin) in the selected plant species. It has been found to contain a small amount of rutin which is known for antioxidant activity (7, 8). Khadem and Marles reported that *Erica herbacea* contains a 2.3-dihydroksybenzoic acid which is a derivative of benzoic acid (9). Several studies demonstrated strong antioxidant activity of this compound (10).

High quantities of arbutin are found in some Ericaceae plant family. The pharmacologically active compound is hydroquinone which originates from arbutin (11). Several studies showed that

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arbutin exhibit antioxidant, antibacterial, antifungal, emmenagogue, diuretic and anti-inflammatory activities (12, 13).

Relevant literature review has shown very little data about the biological activity of extracts and isolated components from *E. herbacea*, which points to the fact that the plant has not been explored completely. To the best of our knowledge, there are no previous reports on the antibacterial activity of *E. herbacea* and there are limited data concerning the antioxidant activity of the plant.

The aim of this study was to investigate the antibacterial and antioxidant activity of aqueous, ethanol and ethyl acetate extract from leaves and flowers of this plant. The second aim was to determine a total phenol and flavonoid content in the extracts using spectrophotometric methods.

EXPERIMENTAL

Plant material

In summer of 2009, leaves and flowers of *E. herbacea* were collected from natural populations on Borja mountain in the region of Teslić city in south-east Republic of Srpska, Bosnia and Herzegovina. Plants identification was confirmed and voucher specimen was deposited at the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The collected plant material was air dried in darkness at ambient temperature (20°C). The dried plant material was cut up and stored in paper bags until needed.

Chemicals

Organic solvents and sodium hydrogen carbonate were purchased from „Zorka pharma" Šabac, Serbia. Gallic acid, rutin hydrate and aluminium chloride hexahydrate ($AlCl_3$) were purchased from Acros Organics, New Jersey, USA. Chlorogenic acid, Folin-Ciocalteu phenol reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemicals Co., St. Louis, MO, USA. Sodium carbonate (Na_2CO_3) was obtained from MP-Hemija, Belgrade, Serbia. Nutrient liquid medium, a Mueller-Hinton broth was purchased from Liofilchem, Italy. An antibiotic, amoxicillin, was purchased from Panfarma, Belgrade, Serbia.

Preparation of plant extracts

Prepared plant material (10 g) was transferred to dark-colored flasks with 200 mL of solvent (water, ethanol, ethyl acetate) and stored at room temperature. After 24 h, infusions were filtered through Whatman No. 1 filter paper and residue was reextracted 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. Aqueous extract of herb was prepared at 80°C. The obtained extracts were kept in sterile sample tubes and stored at –20°C.

Determination of total phenolic contents in the plant extracts

The *E. herbacea* extracts were analyzed for total phenolics spectrophotometrically by the Folin-Ciocalteu procedure (14). 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 reagent dissolved in water. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 mL of 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 ($y = 0.008x + 0.0077$, $R_{\epsilon} = 0.998$). Then, the total phenolic content was expressed as gallic acid equivalents in milligrams per gram of extract (mg GA/g of extract).

Determination of flavonoid concentrations in the plant extracts

The concentrations of flavonoids was determined using spectrophotometric method with aluminium chloride (15). The sample contained 1 mL of methanolic solution of the extract in the concentration of 1 mg/mL and 1 mL of 2% $AlCl_3$ solution dissolved in methanol. The 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 ($y = 0.021x + 0.040$, $R_{\epsilon} = 0.999$). Then, the concentrations of flavonoids were expressed as the milligram of rutin equivalent per gram of extract (mg of RU/g of extract).

Evaluation of DPPH scavenging activity

The ability of the plant extract to scavenge DPPH free radicals was assessed using the method

described by Takao et al. (16). 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 (2 mL each) were mixed with 2 mL of DPPH methanolic solution (80 µg/mL). After 30 min in darkness at room temperature, the absorbance was recorded in a spectrophotometer at 517 nm. The control samples contained 2 mL of methanol added to 2 mL of DPPH solution. Chlorogenic 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.

Bacterial strains

Antibacterial activity of aqueous, ethanol and ethyl acetate extract from leaves and flowers of *E. herbacea* was tested against 30 strains of bacteria including ten different *Escherichia coli* strains (MFBL-Ec1, MFBL-Ec2, MFBL-Ec3, MFBL-Ec4, MFBL-Ec5, MFBL-Ec6, MFBL-Ec7, MFBL-Ec8, MFBL-Ec9, MFBL-Ec10), ten *Enterococcus faecalis* strains (MFBL-Ef1, MFBL-Ef2, MFBL-Ef3, MFBL-Ef4, MFBL-Ef5, MFBL-Ef6, MFBL-Ef7, MFBL-Ef8, MFBL-Ef9, MFBL-Ef10) and ten different strains of *Proteus vulgaris* (MFBL-Pv1, MFBL-Pv2, MFBL-Pv3, MFBL-Pv4, MFBL-Pv5, MFBL-Pv6, MFBL-Pv7, MFBL-Pv8, MFBL-Pv9, MFBL-Pv10).

The *Escherichia coli* strains and *Proteus vulgaris* strains represented Gram-negative bacteria. Bacterial strains of *Enterococcus faecalis* were Gram-positive. All clinical isolates were a generous gift from the Institute of Public Health, Banja Luka.

Suspension preparation

The original density of the bacterial suspension was 0.5 Mc Farland after which the additional dilution in saline at the proportion of 1 : 10 was made. The final concentration of the bacteria in the test tubes was 10^6 colony forming units (CFU)/mL.

Macrodilution method

The minimum inhibitory concentration of the extracts had been determined by the tube dilution method through the series of dilutions (17). In the test tubes filled with the Mueller Hinton broth, the solution of the extracts is added and the series of double dilutions have been made. In each of the test tubes, the 100 µL of the suspension of the tested bacteria was added. The 24 h incubation at the temperature of 37°C was conducted. MIC was the lowest concentration of the extract which inhibits the growth of the bacteria. The same method was used to identify the value of the MIC for the amoxicillin. The minimum bactericidal concentration (MBC) is the lowest concentration of the tested substance which has the bactericidal effect. These values have been collected by inoculation of the Mueller Hinton agar with the test tube content. It was the content from the test tubes in which the MIC has been found and all the test tubes more than MIC were found. Amoxicillin was used as a positive control. Whereas the extracts were dissolved in 10% DMSO, solvent control test was performed to study the effects of 10% DMSO on the growth of bacterial strains. It was observed that 10% DMSO did not inhibit the growth of bacteria. All tests were performed in duplicate and MICs were constant.

Statistical analysis

Data are presented as the means ± standard deviations where appropriate. All statistical analyses were performed using SPSS package.

RESULTS AND DISCUSSION

Total phenolic content and flavonoid concentrations

Total phenolic content in the plant extracts was measured by the Folin-Ciocalteu method. The results are presented in Table 1. The aqueous extract

Table 1. Total phenolic contents and concentrations of flavonoids in leaves and flowers of *E. herbacea* extracts

Type of extract	Total phenolic content[1] (mg GA/g of extract)	Flavonoid concentration[1] (mg RU/g of extract)
Water	119.88 ± 0.50	26.90 ± 0.31
Ethanol	39.26 ± 0.94	24.46 ± 0.44
Ethyl acetate	14.98 ± 0.28	16.19 ± 0.12

[1]Values represent the mean ± standard deviation

had the highest phenolic content with 119.88 mg of GA/g of extract while the lowest content was measured for the ethyl acetate extract with 14.98 mg of GA/g of extract. The results of analysis of different extracts indicate that water and ethanol are the most effective solvents for extraction of phenolic compounds from leaves and flowers of *E. herbacea*, respectively.

The concentration of flavonoids in extracts of *E. herbacea* was determined using spectrophotometric method with aluminium chloride. The content of flavonoids was expressed in terms of rutin equivalents. The summary of quantities of flavonoids identified in the tested extracts is shown in Table 1. Total flavonoid content in plant extracts was ranged between 16.19 and 26.90 mg RU/g of extract.

Antioxidant activity

The antioxidant activity of different plant extracts of *E. herbacea* was determined using DPPH reagent. The scavenging activity was measured as the decrease in absorbance of the samples *versus* DPPH standard solution. The antioxidant activity of three different extracts from *E. herbacea* is presented in Figure 1.

The highest scavenging activity has been shown by *E. herbacea* aqueous extract, which contained the highest amount of flavonoids (26.90 mg RU/g) and phenols (119.88 mg GA/g).

Phenolic compounds exist in most plant tissues as secondary metabolites and may play roles in interactions between the plant and its biological environment. Phenolics are also important components of the human diet due to their potential antioxidant activity (18), and their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases (19).

Based on these results, each extract of *E. herbacea* showed a phenol concentration-dependent scavenging effect. Numerous investigations of the antioxidant activity of plant extracts have confirmed significant linear correlation between total phenolic content and antioxidant activity.

In previous study (20), the antioxidant activity of extract from *E. herbacea* was investigated and it was determined that this plant possesses significant antioxidant effect.

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

Antioxidative properites of the investigated extracts were tested by means of DPPH free radical scavenging activity.

Antibacterial activity

The results of *in vitro* antibacterial activities of aqueous, ethanol and ethyl acetate extracts from leaves and flowers of *E. herbacea* against 30 strains of Gram-positive and Gram-negative pathogenic bacteria are presented in Table 2. Antibacterial activity of tested extracts was evaluated by determining MICs and MBCs values. For comparison,

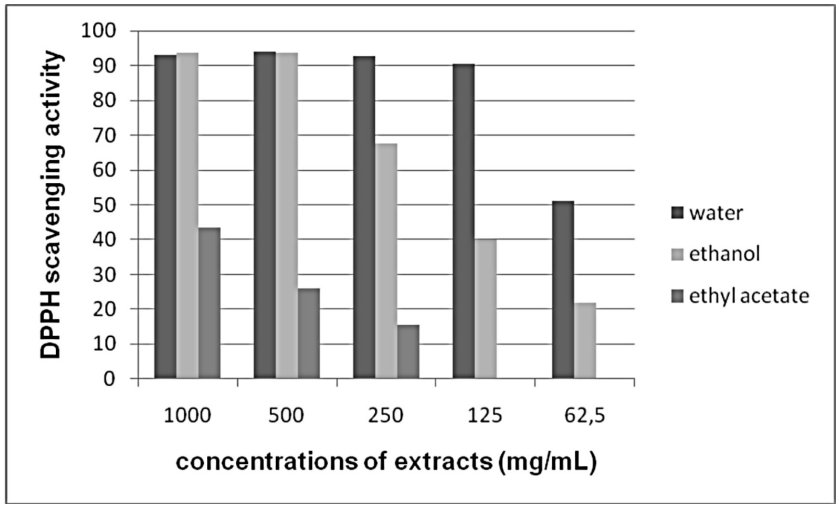

Figure 1. Antioxidant (DPPH scavenging) activity of aqueous, ethanol and ethyl acetate extracts of *E. herbacea*

Table 2. Antibacterial activities of aqueous, ethanol and ethyl acetate extracts from leaves and flowers of *E. herbacea* against tested strains of bacteria based on macrodilution method.

Species	Aqueous extract		Ethanol extract		Ethyl acetate extract		Amoxicillin	
	MIC[1]	MBC[2]	MIC	MBC	MIC	MBC	MIC	MBC
E. coli MFBL-Ec1	20	40	40	>40	20	40	1000	2000
E. coli MFBL-Ec2	20	40	40	>40	20	40	4000	>4000
E. coli MFBL-Ec3	20	40	40	>40	20	40	4	8
E. coli MFBL-Ec4	20	40	40	>40	20	40	2	4
E. coli MFBL-Ec5	20	40	40	>40	20	40	2000	4000
E. coli MFBL-Ec6	10	20	40	>40	20	40	2000	4000
E. coli MFBL-Ec7	10	20	40	>40	20	40	4	4
E. coli MFBL-Ec8	10	20	40	>40	20	40	1000	2000
E. coli MFBL-Ec9	10	20	40	>40	20	40	4000	>4000
E. coli MFBL-Ec10	10	20	40	>40	20	40	1000	2000
E. faecalis MFBL-Ef1	10	40	40	>40	40	>40	0.977	125
E. faecalis MFBL-Ef2	10	20	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef3	10	40	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef4	10	40	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef5	10	40	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef6	10	40	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef7	10	40	40	>40	40	>40	0.977	>125
E. faecalis MFBL-Ef8	10	20	40	>40	40	>40	0.488	125
E. faecalis MFBL-Ef9	10	20	40	>40	40	>40	0.488	>125
E. faecalis MFBL-Ef10	10	20	40	>40	40	>40	0.488	>125
P. vulgaris MFBL-Pv1	2.5	5	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv2	2.5	5	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv3	2.5	5	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv4	2.5	5	20	>40	40	>40	0.977	7.812
P. vulgaris MFBL-Pv5	5	10	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv6	5	10	20	>40	40	>40	0.977	7.812
P. vulgaris MFBL-Pv7	5	10	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv8	5	10	20	>40	40	>40	2000	>4000
P. vulgaris MFBL-Pv9	5	10	20	>40	40	>40	>4000	>4000
P. vulgaris MFBL-Pv10	5	10	20	>40	40	>40	>4000	>4000

[1] Minimum inhibitory concentration (MIC) and [2]minimum bactericidal concentration (MBC) values are given as mg/mL for plant extracts and µg/mL for antibiotic

MIC and MBC values for amoxicillin are also listed in Table 2. In general, the tested extracts demonstrated selective antibacterial activity and the activity depended both on the species of bacteria and on the type and concentration of extract.

The aqueous extract showed the strongest antibacterial activity against all tested bacterial strains. The ethanol extract showed the highest activity against strains of *Proteus vulgaris* and ethyl acetate extract against strains of *Escherichia coli*. The most sensitive strains of tested bacteria towards water extract of *E. herbacea* were strains of *Proteus vulgaris*. The best antibacterial activity has been shown by *E. herbacea* aqueous extract (MIC 2.5 mg/mL).

Comparison of the antibacterial effects of different extracts from flowers and leaves of *E.*

herbacea showed that phenolic compounds and flavonoids were responsible for the growth inhibition of bacterial strains and most of the antibacterial effects probably originated from compound such as arbutin.

The arbutin-containing plant-drugs have been used for the treatment of urinary infections. Arbutin is a phenolic glycoside, which splits into hydroquinone and glucose. The total amount of hydroquinone in urine is crucial for the therapeutic activity of the herbal preparation (21, 22).

This study indicates that extracts of *E. herbacea*, showed a certain level of antimicrobial activity and may have an additional biologic potential in industrial use, but that additional research is needed, especially on the aqueous extract in order to identify the active component(s).

A possible application of these findings in the pharmaceutical industry for the production of compounds supporting antibiotics for treating urinary infection seems to be worth exploring.

CONCLUSIONS

The results of this research suggest that aqueous, ethanol and ethyl acetate extracts from the leaves and flowers of *E. herbacea* inhibit the growth of human pathogens and aqueous extract can have the significant effect on the prevention of the urinary tract infection. Antibacterial compounds from *E. herbacea* may have important applications as natural antibacterial agents. Therefore, the leaves and flowers of this plant are natural sources of antioxidant substances of high importance.

Acknowledgments

This investigation was supported by the Ministry of Education and Science of the Republic of Serbia, grants No. OI173032 and No. 41010.

REFERENCES

1. Sarić M.: Medicinal plants of SR Serbia, p. 243-244, Serbian Academy of Sciences and Arts, Belgrade 1989.
2. Akkol E.K., Yeşilada E., Güvenç A.: J. Ethnopharmacol. 116, 251 (2008).
3. Tuzlaci E., Eryasar Aymaz FP.: Fitoterapia 72, 323 (2001).
4. Mehmet A., Bahadori F., Öztürk M., Kolak U., Topçu G.: Fitoterapia 78, 571 (2007).
5. Nazemiyeh H., Bahadori F., Delazar A., Ay M., Topcu G., Nahar L., Majinda R.R., Sarker S.D.: Nat. Prod. Res. 16, 1385 (2008).
6. Güvenç A., Kendir G., Eken A., Aydin A.: FABAD J. Pharm. Sci. 32, 121 (2007).
7. Sofić E., Čopra-Janićijević A., Salihović M., Tahirović I., Kroyer G.: Medicinal Plants 2, 97 (2010).
8. Choi J.S., Kang S.W., Li J., Kim J.L., Bae J.Y., Kim D.S., Shin S.Y.et al.: J. Agric. Food Chem. 57, 2079 (2009).
9. Khadem S., Marles R.J.: Molecules 15, 7985 (2010).
10. Sroka Z., Cisowski W.: Food Chem. Toxicol. 41, 753 (2003).
11. Pop C., Vlase L., Tamas M.: Not. Bot. Hort. Agrobot. Cluj 37, 129 (2009).
12. Azadbakht M., Ramezani M., Marston A., Hostettmann K., Jahromi M.M.: J. Med. Plants 3, 9 (2004).
13. Lee H.J., Kim K.W.: Inflamm. Res. 61, 817 (2012).
14. Wootton-Beard P.C., Moran A., Ryan L.: Food Res. Int. 44, 217 (2011).
15. Quettier-Deleu C., Gressier B., Vasseur J., Dine T., Brunet C., Luyckx M., Cazin M., Cazin J.C., Bailleul F., Trotin F.: J. Ethnopharmacol. 72, 35 (2000).
16. Takao T., Kitatani F., Watanabe N., Yagi A., Sakata K.: Biosci. Biotechnol. Biochem. 58, 1780 (1994).
17. NCCLS (National Committee for Clinical Laboratory Standards): Approved Standard M7-A4: Wayne PA (1997).
18. Martin K.R., Appel C.L.: Nutr. Dietary Suppl. 2, 1 (2010).
19. Bravo L.: Nutr. Rev. 56, 317 (1998).
20. Pavlović R.D., Lakušić B., Došlov-Kokoruš Z., Kovačević N.: Pharmazie 64, 656 (2009).
21. Schindler G., Patzak U., Brinkhaus B., von Niecieck A., Wittig J., Krahmer N., Glockl I., Veit M.: J. Clin. Pharmacol. 42, 920 (2002).
22. Quintus J., Kovar K.A., Link P., Hamacher H.: Planta Med. 71, 147 (2005).

Received: 17. 01. 2013