International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 3, 716-719.

Determination of Total Phenols in Some Plants Used in Traditional Medicine in Bosnia and Herzegovina

Zilha Ašimović[1], Almina Čaušević[1], Davorka Završnik[2], Selma Špirtović-Halilović[2]*, Belma Imamović[3], Jasmin Mušanović[4]

[1]Department of Chemistry and Biochemistry, PAM Institute, Faculty of Agriculture and Food sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[3]Department of Control of Drugs, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[4]Department for Biology with Human Genetics, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Research Article

Please cite this paper as: Zilha Ašimović[1], Almina Čaušević[1], Davorka Završnik[2], Selma Špirtović-Halilović[2]*, Belma Imamović[3], Jasmin Mušanović[4]. Determination of Total Phenols in Some Plants Used in Traditional Medicine in Bosnia and Herzegovina. IJPTP, 2013, 4(3), 716-719.

Corresponding Author:

Selma Špirtović-Halilović,
selmaspirtovic@yahoo.com,
Faculty of Pharmacy, Zmaja od Bosne br. 8 (Kampus), 71000 Sarajevo,
Bosnia and Herzegovina,
Phone: 387 33 586180

Abstract

Objective: The medicinal plant species include those whose one or more parts containing biologically active substance can be used for therapeutic purposes or for the chemical synthesis of pharmaceuticals. Medicinal herbs contain natural antioxidants such as phenolic compounds, which attracted the attention of the public and scientists due to its positive effects on health.

The contents of total phenols in some medicinal herbs were determined in this paper. **Methods**: Determination was carried out on ten different types of medicinal herbs samples such as: *Matricaria chamomilla* L. (*Asteraceae*), *Thymus serpyllum* (*Lamiaceae*), *Melissa officinalis* (*Lamiaceae*), *Mentha piperita* L. (*Lamiaceae*), *Teucrium montanum* (*Lamiaceae*), *Arctostaphylos uva-ursi* (*Ericaceae*), *Salvia officinalis (Lamiaceae)*, *Lavandula officinalis* (*Lamiaceae*), *Achillea millefolium* L.(*Asteraceae*) and *Calluna vulgaris* (*Ericaceae*). Analyses were conducted to determine the differences between ethanol and water extracts of the dried herbs. The content of total phenols was determined spectrophotometrically using the Folin Ciocalteu.

Results: According to the results obtained, it was found that the samples, where solvent was distilled water, the highest content of total phenols had balm (64.98 mg GAE/100 g of DW) and the lowest content of total phenols had *Lavandula officinalis* (12.19mg GAE/100 g DW).

Ethanolic extracts of dried herbs *Thymus serpyllum* had the highest content (76.02 mg GAE/100 g DW) while the lowest total phenol content had *Achillea millefolium* (9.83 mg GAE/100 g DW). **Conclusion**: There is a difference in the content of phenolic compounds in medicinal plants. Extraction of phenolic compounds from plants depends on the solvent. As an effective solvent for the extraction of phenolic compounds from dried herbs 30% aqueous ethanol was showed. Dried herbs might be a potential resource of natural anti-oxidants.

Key words: herbs, total phenolic compounds, gallic acid, spectrophotometric method

INTRODUCTION

Vast natural resources of medicinal plants are being used for thousands of years for the cure of many diseases in all over the world [1,2].

Demand for medicinal plants is increasing in both developing and developed countries.

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions [3,4].

It has been reported that natural antioxidants in fruits and vegetables were inversely related with the risk of many chronic diseases, such as cardiovascular diseases and cancer [5, 6]. Because of potential health benefits of natural antioxidants [7,8], they are expected to be an alternative to synthetic ones. Therefore, there is an increasing interest for researchers in seeking for new resources of natural antioxidants. Several studies showed that phenolic compounds were the main antioxidant ingredients in several medicinal plants [9,10].

Flavonoids, phenolic acids, and phenolic diterpenes [11,12], lignans are the examples of phenolic components with antioxidant properties.

The purpose of this study was to evaluate ten plants which are often used in national medicine in area of Bosnia and Herzegovina as potential sources of natural phenolic compounds. Our study also demonstrates possible relationship between ethanolic and aqueous extract and contents of total phenols.

MATERIAL & METHODS

Plant materials

Whole parts of the plants *Matricaria chamomilla* L. (*Asteraceae*), *Thymus serpyllum* (*Lamiaceae*), *Melissa officinalis* (*Lamiaceae*), *Mentha piperita* L. (*Lamiaceae*), *Teucrium montanum* (*Lamiaceae*), *Arctostaphylos uva-ursi* (*Ericaceae*), *Salvia officinalis (*Lamiaceae*)*, *Lavandula officinalis* (*Lamiaceae*), *Achillea millefolium* L. (*Asteraceae*), *Calluna vulgaris* (*Ericaceae*) were collected from the local market.

Sample preparation

The plant sample was ground to fine powder with a special grinder for herbal medicine. A precisely weighed amount (1.00 g) of the powder was extracted with 40 mL of distilled water (30% ethanole solution in second case) at 90-95°C for 15 min using reflux. The resulting extract was filtered through filter paper into a volumetric flask of 50 mL and solvent was added up to the mark.

Determination of total phenolic compounds

The amounts of phenolics in the selected medicinal plant extracts were determined with Folin-Ciocalteu's reagent.

250 µL of extract, pre-diluted with solvent, 15 mL distilled water**,** and 1.25 mL dilution of Folin-Ciocalteu reagent (diluted with distilled water in ratio 1:4) was added in volumetric flask (25 mL). After 5 minutes 3.75 mL of Na_2CO_3 (saturated solution) were added and added solvent to the mark. The resulting mixture was incubated at 50ºC for 20 minutes. In the same way blank was prepared, but with distilled water.

The absorbance of all samples was measured at 760 nm using a spectrophotometer (Model UV-VIS 2200 Shimadzu) The standard curve was prepared using 25, 33,3, 50 and 100 mg/L Results were expressed as milligrams of gallic acid equivalents per 100 grammes dry mass (mg GAE/100 g DW), which is a common reference compound. All samples were analysed in triplicate.

Statistical analysis

Microsoft office Excel 2003 was used for statistical analysis.

The difference between the arithmetic mean of two samples was determined using t test, while the given data was processed by statistical methods.

Chemical reagents

- The chemical reagents such as Na_2CO_3, gallic acid [3,4,5-Trihydroxybenzoic acid] and Folin-Ciocalteu`s phenol reagent were purchased from Semikem, Sarajevo, Bosnia and Herzegovina.
- Saturate solution of Na_2CO_3

200 g Na_2CO_3 was dissolved in 800 mL hot distilled water, and then cooled to room temperature. A few crystals of sodium carbonate are added and a 1000 mL volumetric flask filled with distilled water and than filtered after 24 hours.

- Solution of gallic acid

Gallic acid (0.03 g gallic acid was dissolved in methanol (into a volumetric flask of 100 mL and solvent was added up to the mark).

RESULTS

For the tested plants statistically analyzed data were shown (mean, standard deviation, coefficient of variation, standard error, min and max values) (Table 1 and Table 2).

Table 1. Total phenolic content of each plant aquatic extract expressed as mg GAE/100 g DW

Plants	Total phenolic content mg GAE/mL	min	max	Standard error	Coefficient of variation (%)
Matricaria chamomilla	23.87±0.20	23.75	24.10	0.12	0.835
Arctostaphylos uva-ursi	24.56±0.02	24.55	24.58	0.01	0.0622
Lavandula officinalis	12.19±0.10	12.09	12.29	0.06	0.822
Achillea millefolium	16.533±0.06	16.48	16.66	0.04	0.370
Melissa officinalis	64.98±0.02	64.97	65.00	0.01	0.027
Calluna vulgaris	18.12±0.03	18.10	18.16	0.02	0.191
Teucrium montanum	16.093±0.04	16.05	16.12	0.02	0.235
Mentha piperita	32.603±0.04	32.56	32.64	0.02	0.124
Thymus serpyllum	45.71±0.41	45.35	46.16	0.24	0.902
Salvia officinalis	22.7867±0.06	22.75	22.85	0.03	0.242

Table 2. Total phenolic content of each ethanolic extract expressed as mg GAE/ 100 g DW

Plants	Total phenolic content mg GAE/mL	min	max	Standard error	Coefficient of variation (%)
Matricaria chamomilla	66.67±0.05	66.63	66.73	0.03	0.077
Arctostaphylos uva-ursi	16.21±0.05	16.17	16.26	0.03	0.283
Lavandula officinalis	1510±0.01	15.09	15.11	0.01	0.076
Achillea millefolium	9.83±0.02	9.81	9.85	0.01	0.203
Melissa officinalis	56.79±0.07	56.72	56.85	0.04	0.115
Calluna vulgaris	30.38±0.02	30.36	30.39	0.01	0.057
Teucrium montanum	32.21±0.03	32.17	32.23	0.02	0.100
Mentha piperita	39.47±0.05	39.42	39.52	0.03	0.128
Thymus serpyllum	76.02±0.05	75.98	76.07	0.03	0.062
Salvia officinalis	54.59±0.02	54.57	54.61	0.01	0.037

DISCUSSION & CONCLUSION

This study examined the influence of the two solvent on medicinal herbs phenol content. Total phenol content in dried medicinal herbs was specified using distilled water as a solvent extraction, and then the same was done for samples using 30% of ethanol aqueous solution. The total amount of phenol was specified using spectrophotometric method based on colour reaction of phenol with Folin-Ciocalteu reagent. Absorbance of samples
were measured at 760 nm and the amount of total phenolics in mg GAE/100 g DW
extract were then analyzed and interpreted.
By manipulating the regression equations of gallic acid calibration curve (y = 0.0500x, R^2 = 0.9990, for distilled water, and y = 1.2936x, R^2 = 0.9913, for 30% of ethanol aqueous solution), the total phenolic content of each extract was calculated and expressed as gallic acid equivalent (GAE).
The influence of those two solvents (distilled water and 30% ethanol aqueous solution) was examined and each sample results were recorded. A wide range of total phenolics (TP) content was found in studied. The total phenol content within dried medicinal herbs samples varied from 9.83-76.02 mg GAE/100 g DW. These values can be compared to data found in related literature, where general medicinal herbs phenol content varies between 27.94-48.86 mg GAE/100g DW [13]. Our results shows that the highest total phenol content was found in *Melissa officinalis* when using distilled water as a solvent (64.98±0.02 mg GAE/100 g DW), while the lowest TP content was found in *Lavandula officinalis* (12.19±0.10mg GAE/100 g DW). The highest phenol content in medicinal herbs ethanol extracts was found in *Thymus serpyllum* (76.02±0.05 mg GAE/100 g DW), while the lowest content was found in *Achillea millefolium* (9.83±0.02 mg GAE/100 g DW). Obtained data can be related to other references, which claims that balm has highest general phenol content (48.86 mg GAE/100 g DW) [13].

Due to the effect of different environmental factors there could be some variations in relations to phenol content in similar or same medical herbs. The period of picking, handling with the herbs, the ways of preserving them, as well as the techniques of sample preparation during the analytical method, can highly influence phenol compounds concentration differences in the obtained results.

According to the results obtained after laboratory analysis can be concluded that there is a difference in the content of phenolic compounds in medicinal plants, and extraction of phenolic compounds from plants depends on the solvent. As an effective solvent for the extraction of phenolic compounds from dried herbs 30% aqueous ethanol was showed.
The results obtained by the study show that dried herbs might be a potential resource of natural anti-oxidants.

References

1. Dibyajyoti S, Swati P. Antifungal Activity of Ethanol Extract of *Pouzolzia zeylanica (L.) Benn* IJPTP 2012, 3(2): 272-274.
2. Gupta S, Kohli S, Sumeet D. In-Vitro anti-inflammatory activity of Sarcostemma acidum Wight. & Arn. Indian herb by Human red blood cell membrane stabilization method. IJPTP 2011, 2 (4): 184-188.
3. Velioglu YS, Mazza, G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem 1998; 46: 4113–4117.
4. Emmons CL, Peterson DM, Paul GL. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocopherol antioxidants. J Agric Food Chem 1999; 47: 4894-4898.
5. Duthie GG., Duthie SJ, Kyle JAM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 2000; 13: 79-106.
6. Leifert WR, Abeywardena MY. Cardioprotective actions of grape polyphenols. Nutr Res 2008; 28: 729-737.
7. Eberhardt M.V, Lee CY, Liu RH. Antioxidant activity of fresh apples. Nature 2000; 405: 903-904.
8. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. New Engl J Med, 2003; 348: 2599-2608.
9. Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci, 2004; 74: 2157-2184.
10. Liu HY, Qiu NX, Ding HH, Yao RQ. Polyphenols content and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res. Intern, 2008; 41: 363-370.
11. Pietta PG. Flavonoids in medicinal plants. In C. A. Rice- Evans, & L. Packer (Eds.), Flavonoids in health and disease (pp. 61–110). New York: Dekker. 1998.
12. Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. Critical Reviews of Food Science & Nutrition, 1992; 32(1): 67–103.
13. Atanassova M, Georgieva S, Ivancheva K. Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. Journal of University of Chemical Technology and Metallurgy, 2011; 46(1): 81-88.

AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.