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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF SOME AROMATIC HERBS USED IN TRADITIONAL ROMANIAN CUISINE

Camelia PAPUC[1], Corina PREDESCU[2],
Gheorghe V. GORAN[3], Camelia PETRESCU[4]

Abstract. *Lovage (Levisticum officinale), parsley (Petroselinum crispum), tarragon (Artemisia dracunculus) and thyme (Satureja hortensis) extracts were obtained in 60% ethanol. Total phenolic content (TPC) was determined using the Folin-Ciocalteu phenol reagent method. Antioxidant activities of the extracts were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH▪) free radical-scavenging ability and ferric-reducing antioxidant power (FRAP) assay. The hydroalcoholic extract obtained from Satureja hortensis had the highest total phenolic content and the highest antioxidant activity. A significant and positive high Pearson's correlation between TPC and DPPH▪ assay and between TPC and FRAP assay respectively was observed for all plant extracts. The results indicated that phenolic compounds were the main contributor to antioxidant activity in the investigated aromatic herb extracts).*

Keywords: aromatic herbs, total phenolic content, antioxidant activity

1. Introduction

Aromatic herbs used in Romanian cuisine are herbaceous (leafy) plants that add flavour and colour to all types of meals. Aromatic herbs represent an important source of biologically active compounds, such as phytochemicals and phytoalexins, recognized for their beneficial health effects, and thus have also been used in folk medicine. Both phytochemicals and phytoalexins are made of simple phenolics and polyphenolics, which are known as bioactive compounds responsible for the antioxidant activity in plants, besides some vitamins (A, C and E) [2]. Phenolics are compounds that contain at least one hydroxyl group (-OH) attached to an aromatic ring. Phenolic compounds are ubiquitous components of plants and herbs that act as reactive oxygen species (ROS)/reactive nitrogen

[1]Prof., Ph.D., Senior Researcher, Faculty of Veterinary Medicine, Preclinical Sciences Department, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania, Associate Member of the Academy of the Romanian Scientists (e-mail: cami_papuc@yahoo.com).
[2]Lecturer, Ph.D., Researcher, Faculty of Veterinary Medicine, Preclinical Sciences Department, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania, (e-mail: durduncorina@yahoo.com).
[3]Lecturer, Ph.D., Researcher, Faculty of Veterinary Medicine, Preclinical Sciences Department, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania, (e-mail: durduncorina@yahoo.com).
[4]Researcher, Faculty of Titu Maiorescu, Bucharest, Romania, (e-mail: cameliapetrescu16@yahoo.com).

species (RNS) scavengers and also, some have antimicrobial, anti-inflammatory, antiallergic, antimutagenic, antiviral antithrombotic, and vasodilatory activities. More than 8,000 phenolic compounds as naturally occurring substances from plants have been reported [15]. Half of these phenolic compounds are flavonoids presenting as aglycone, glycosides and methylated derivatives [1].

The most used plants in traditional Romanian cuisine are thyme (*Satureja hortensis*), parsley (*Petroselinum crispum*), lovage (*Levisticum officinale*) and tarragon (*Artemisia dracunculus*). They are used in Romanian cuisine as seasoning due to their strong flavour. These plants have a distinctive taste and impart unique tastes and flavours of salads, cooked food, meat, and fish. The leaves are gathered before flowering and the flowering shoots can be used fresh or dried.

The objective of this study was the determination of total phenolic content (TPC) in a selected plant, as well as the evaluation of the antioxidant capacity of their hydroethanolic extract.

2. Materials and Methods

2.1. Plant Materials and Reagent

The plants were collected from the region of Ilfov County, in June. The fresh aromatic herbal was transferred in polyethylene bags to the laboratory on the same day. They were rinsed with de-ionized water, dried-plotted, weighed and dried to a constant weight in an air-forced oven maintained at 25 °C. Dried samples were grounded and stored until analysis in brown air-tight bottles.

All chemicals were from Fluka Chemicals, Sigma-Aldrich and Merck.

2.2. Extraction procedure

The extraction method used for dried samples has as follows: 40 mL of 60% aqueous ethanol was added to 0.5 g of dried sample. Then 10 mL of 6 M HCl was added. The extraction mixture was then sonicated for 15 min and refluxed in a water bath at 90 ◦C for 2 hrs. The mixture was then filtered and made up to 100 mL with ethanol.

2.3. Determination of total phenolics content (TPC)

The total phenolic contents (TPC) were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [4]. Briefly, 20 μL extract was mixed with 1.16 mL of distilled water and 100 μL of Folin-Ciocalteu reagent, followed by the addition of 300 μL of Na_2CO_3 solution (20%). After 30 min of incubation at 40 °C, the absorbance of the reaction mixture was

measured at 760 nm. Results were expressed as mg of gallic acid/g dry weight sample (mg GAE/g DW).

2.4. Antioxidant activity

2.4.1. 1, 1-Diphenyl-2-Picrylhydrazyl radical (DPPH▪) scavenging activity

Experiments were carried out according to the method of Blois, modified by Proestos and Varzakas [13]. Briefly, a 1 mmol/L solution of DPPH radical solution in methanol was prepared and then, 1 mL of this solution was mixed with 3 mL of extract. After 30 min, the absorbance was read at 517 nm. Butylated hydroxytoluene (BHT) was used as positive controls. The percentage inhibition of the DPPH radical by the samples was calculated using the following equation:

$$\%\ DPPH\ radical - scavenging = \frac{Abs\ control - Abs\ sample}{Abs\ control}\ x\ 100 \qquad (1)$$

2.4.2. Ferric-reducing antioxidant power (FRAP) assay

The FRAP reagent was prepared by mixing 38 mM sodium acetate in distilled water pH 3.6, 20 mM $FeCl_3 \cdot 6H_2O$ in distilled water and 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl (10:1:1, v/v/v). The samples (50 µl) were incubated with FRAP reagent (4 mL) at 37 °C for 40 min in the dark. The absorbance of the resulting solution was measured at 593 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a reference antioxidant standard. FRAP values were expressed as g Trolox/100 g dray weight plant (g Trolox/100 g DW) [14].

2.5. Statistical Analysis

Excel (Microsoft Co, Redmond, WA, USA) and SPSS (version 19.0 statistical software, SPSS Inc., Chicago, USA) packages were used for statistical analysis. Results represented means ± s.d. of six determinations. Pearson's coefficient was used to evaluate the correlations between TPC and antioxidant activity.

3. Results and discussions

Aromatic herbs have long been reported as a prospective hub of natural antioxidant compounds, particularly plant secondary metabolites, such as, phenolic compounds which are generated by the plant to defend itself or to promote the growth under unfavourable conditions The phenolic compounds have attracted the attention of researchers due to their attractive biological properties,

such as their antioxidant activity. But, the content in polyphenols and their antioxidant activity differ from one plant to another and also depend on the climatic factors, but also the harvesting period.

The total phenolic contents of investigated aromatic plants are presented in Fig. 1. The highest level of total phenolics was found in leaves of thyme (*Satureja hortensis*), followed by leaves of tarragon (*Artemisia dracunculus*). Parsley (*Petroselinum crispum*) and Lovage (*Levisticum officinale*) had TPC low levels. Our results agree with the literature, which reports similar TPC.

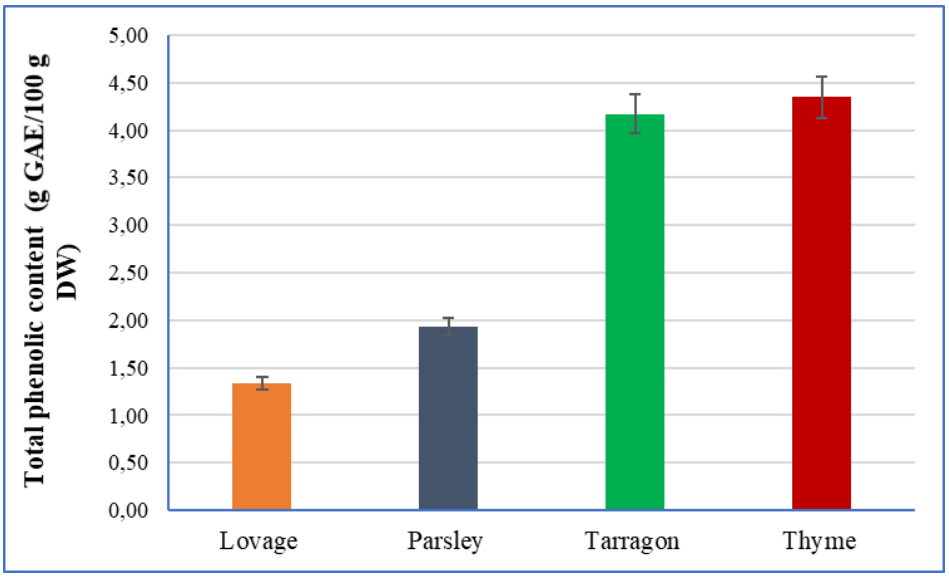

Fig. 1. Total phenolic content in the aromatic herbs

Thus, for *Thymus vulgaris*, the values reported for TPC were between 4.67 and 5.90 g GAE/100 g DW, according to the harvesting period [17]. In the leaves of *Artemisia dracunculus*, dried at room temperature and darkness [7], reported TPC of 58 mg GAE/g DW. TPC levels in parsley and lovage leaves, frozen at −20 °C until analysis, were 360.89 and 577.04 mg GAE/g FW [10]. The total phenolic content level depends on the drying methods. TPC levels in *Thymus vulgaris* were between 1,400 and 3,920 mg/100g DW, the highest level being reported for oven drying at 50 °C with microwave pre-treatment.

Phenolic compounds act as antioxidants by various mechanisms such as donation of H atoms, donation of electrons or chelation of transitional metal ions. This study investigated the antioxidant activity of four aromatic herbs by two methods, DPPH▪ scavenging activity, and FRAP antioxidant power. However, these

methods have different reaction mechanisms and do not necessarily measure the same activity [12]. DPPH▪ assay is based on hydrogen transfer, whereas the FRAP assay is based on electron transfer reactions.

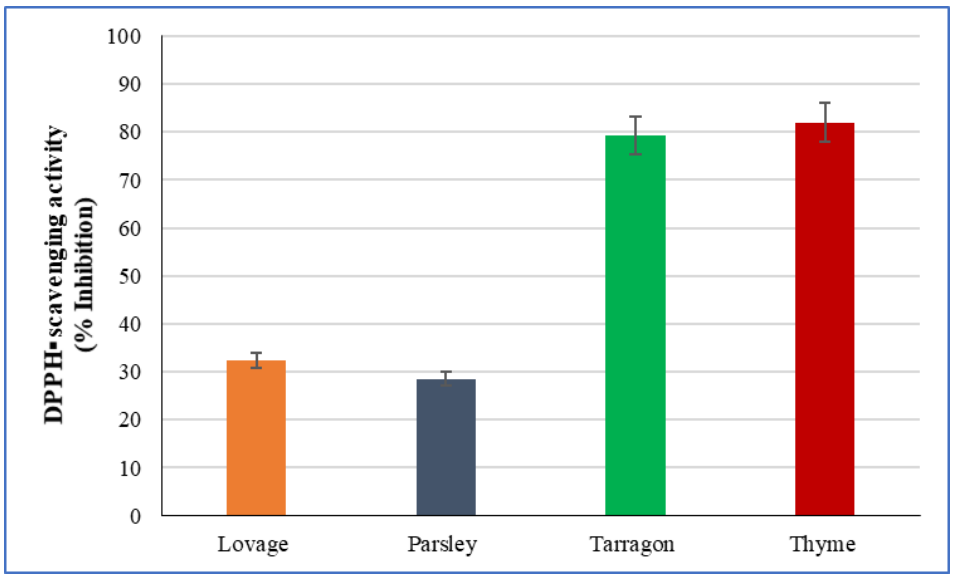

Fig. 2. DPPH radical scavenging activities of aromatic herbs extracts

The DPPH radical scavenging ability of aromatic herbs extracts is given in Fig. 2. The capacity of extracts to act as H atom donors ranged between 82.02% and 28.50%. The highest DPPH▪ scavenging activity was recorded for thyme extract, followed by tarragon, lovage, and parsley, following the same ranking order as for total phenolics content. In this experiment, there was a positive correlation between total phenolic content and the percentage of DPPH radical scavenging.

The FRAP assay is based on the ability of phenolic compounds to reduce Fe^{3+} to Fe^{2+}. When the reduction of Fe^{3+} to Fe^{2+} occurs in the presence of 2,4,6-trypyridyl-s-triazine, the reaction is accompanied by the formation of a coloured complex with Fe^{2+} (absorption at 593 nm). The reducing power appears to be related to the degree of hydroxylation and the extent of conjugation in phenolics. FRAP values in the aromatic herbs examined ranging from 1.22 ± 0.66 g Trolox/100g DW in parsley to 8.52 ± 0.97 g Trolox/100g DW in thyme (Fig. 3). For tarragon FRAP values were close to those obtained for thyme (6.81 ± 0.92 g Trolox/100g DW), whereas, those obtained for lovage were close to those obtained for parsley (1.98 ± 0.59 g Trolox/100g DW).

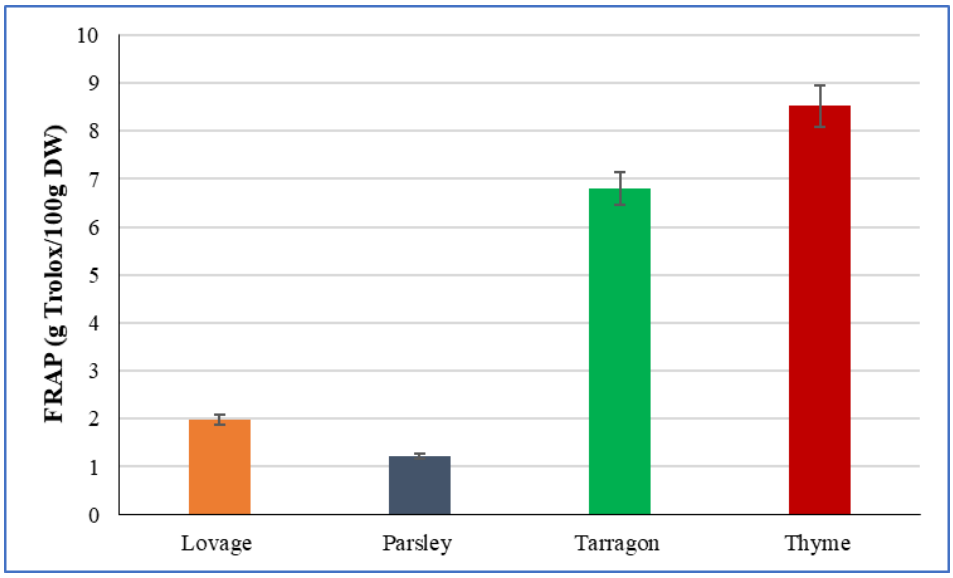

Fig. 3. Antioxidant activity of aromatic herbs extracts expressed as ferric-reducing antioxidant power (FRAP)

It is established a significant correlation between DPPH▪ scavenging and TPC content $R^2 = 0.9546$, $p<0.05$, whereas the correlation between FRAP and TPC is $R^2 = 0.9196$. A significant correlation is between DPPH▪ scavenging and FRAP ($R^2 = 0.9743$, $p<0.05$).

The correlation between total phenolic contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [6, 8, 18]. Antioxidant activity of fruits and vegetables significantly increases with the presence of a high concentration of total phenolic content [5, 16]. For this reason, many researchers reported a positive correlation between phenolic content and the antioxidant activity [3, 9, 11].

Conclusions

(1). Lovage (*Levisticum officinale*), parsley (*Petroselinum crispum*), tarragon (*Artemisia dracunculus*) and thyme (*Satureja hortensis*) leaves contains important levels of phenolic compounds.

(2). Thyme and tarragon leaves have the highest content of phenolic compounds.

(3). The phenolic compounds from the investigated aromatic herbs used in Romanian cuisine have antioxidant properties acting as an electron and hydrogen donors.

(4). Thyme and tarragon leave hydroethanolic extracts have the highest antioxidant activity.

(5). Between the antioxidant activity and the total phenolic content, there is a significant positive correlation.

R E F E R E N C E S

[1] Ahmed, S.I., Hayat, M.Q., Tahir, M., Mansoor, Q., Ismail, M., Keck, K., Bates, R.B., Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of *Cassia angustifolia* Vahl, BMC Complementary Medicine and Therapies **16,** 460 (2016). DOI: 10.1186/s12906-016-1443-z

[2] Al-Laith, A.A., Alkhuzai, J., Freije, A., Assessment of antioxidant activities of three wild medicinal plants from Bahrain, Arab. J. Chem, **12**, 2365-2371 (2015). doi: http://dx.doi.org/10.1016/j.arabjc. 2015.03.004

[3] Andreu, L., Nuncio-Jáuregui, N., Carbonell-Barrachina, A.A., Legua, P., Hernández, F., Antioxidant properties and chemical characterization of Spanish *Opuntia ficus-indica* Mill. cladodes and fruits. J Sci Food Agric. **98.** 1566-1573 (2018). https://doi.org/10.1002/jsfa.8628

[4] Arabshahi-Delouee, S., Urooj, A., Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves, Food Chemistry. **102,** 1233–1240 (2007). https://doi.org/10.1016/j.foodchem.2006.07.013

[5] Fidrianny, I., Suhendi, H., Insanu, M. Correlation of phytochemical content with antioxidant potential of various sweet potato (*Ipomoea batatas*) in West Java, Indonesia**.** Asian Pac. J. Trop. Biomed. **8**, (1), 25-30 (2018). DOI: 10.4103/2221-1691.221131

[6] Hossain, M.A., Mizanur Rahman, S.M. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple, Food Res. Inter. **44**, (3), 672-676 (2011). doi: 10.1016/j. foodres.2010.11.036

[7] Khezrilu, B.J., Heidari, R., The Evaluation of Antioxidant Activities and Phenolic Compounds in Leaves and Inflorescence of Artemisia dracunculus L. by HPLC, J. Med. Plant Research, (**13)**, 41-50 (2014).

[8] Kumar, S., Sandhir, R., Ojha, S., Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves, BMC Res Notes **7**, 560 (2014). https://doi.org/10.1186/1756-0500-7-560

[9] Mishra, A., Sharma, A.K., Kumar, S., Saxena, A.K., Pandey, A.K., *Bauhinia variegata*, Leaf Extracts Exhibit Considerable Antibacterial, Antioxidant, and Anticancer Activities, Biomed Res Int. **2013,** 915436 (2013). https://doi.org/10.1155/2013/915436.

[10] Nour, V., Trandafir I., Cosmulescu S., Bioactive Compounds, Antioxidant Activity and Nutritional Quality of Different Culinary Aromatic Herbs, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **41**(1), 136-142, (2017). DOI: https://doi.org/10.15835/nbha4119026

[11] Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N., Suda, I., Involvement of Anthocyanins and other Phenolic Compounds in Radical-Scavenging Activity of Purple-Fleshed Sweet Potato Cultivars. J. Food Sci. **67**, (5) 1752–1756 (2002). doi: 10.1111/j.1365-2621. 2002. tb08718.x

[12] Prior, R. L., Wu, X., Schaich, K., Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements, J. Agric. and Food Chem.; **53**, 4290–4302 (2005). http://dx.doi.org/10.1021/jf0502698

[13] Proestos, C., Varzakas, T., Aromatic Plants: Antioxidant Capacity and Polyphenol Characterisation, Foods **6**, (4) 28 (2017). doi: 10.3390/foods6040028.

[14] Stratil, P., Klejdus, B., Kuban, V., Determination of Total Content of Phenolic Compounds and Their Antioxidant Activity in Vegetables Evaluation of Spectrophotometric Methods, J Agric Food Chem. **54**, 607-616 (2006). DOI: 10.1021/jf052334j

[15] Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A., Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview, Medicines (Basel). **5, (3)** 93 (2018). doi:10.3390/medicines5030093

[16] Ulewicz-Magulska, B., Wesolowski, M. Total Phenolic Contents and Antioxidant Potential of Herbs Used for Medical and Culinary Purposes. Plant Foods Hum. Nutr. 74, 61–67 (2019). https://doi.org/10.1007/s11130-018-0699-5

[17] Vábková, J., Neugebauerová, J., Determination of total phenolic content, toatl flavonoid content and frap in culinary herbs in relation to harvest time, Acta Univ. Agric. Silvic. Mendel. Brun. Vol. LX, 29(1), 167-172 (2012).

[18] Vamanu, E., Nita, S. Antioxidant Capacity and the Correlation with Major Phenolic Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from the Wild Edible Boletus edulis Mushroom. Bio Med Research International. **2013**, Article ID313905, http://dx.doi.org/10.1155/2013/313905