Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

IN VITRO EVALUATION OF ANTIOXIDATIVE PROPERTIES IN *CAPSICUM ANNUUM*, *VACCINIUM VITIS-IDAEA* AND *MELISSA OFFICINALIS* TINCTURES

Emanuel VAMANU, Adrian VAMANU, Elena DOPCEA

Department of Industrial Biotechnology, Faculty of Biotechnologies, University of Agronomical Sciences and Veterinary Medicine Bucharest, 59 Marasti Blvd., 011464, Bucharest, Romania

Corresponding author email: email@emanuelvamanu.ro

Abstract

Several commercial varieties of tinctures were analyzed for determining their antioxidant potential, since most knowledge relating to therapeutic properties of medicines are obtained from folk phytomedicine. Antioxidant potential was determined by the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radicals, reducing power, and chelating activity. The best results against scavenging of free radicals were obtained with tincture from Vaccinium vitis-idaea,followed by Capsicum annuum and Melissa officinalis. There were obvious significant differences between the free radicals' scavenging activities. Results were correlated with values obtained for reducing power and chelating ability. This finding was also confirmed by the low values of the EC_{50}. Also, the results were positively high when correlated with total phenolic and flavonoidic contents from the tinctures. The results given herein are the scientific proof that confirms the empirical medical knowledge on how to use the three tinctures in oxidative dysfunctions.

Keywords: *scavenging activity, phenolic content, tincture.*

INTRODUCTION

In recent decades, interest in the use of medicinal plants has significantly increased due to the isolation of biologically active substances that have a wide range of pharmacological effects. The phytotherapeutical action of herbal products is generated by the rich content of such substances, including phenolic compounds, vitamins, alkaloids, saponins, essential oils, and various minerals (Ivan, 2007). Their main and most evident effect is antioxidative, that acts upon the characteristics of reactive oxygen and nitrogen species. The antioxidative molecules existing in herbal products can react with free radicals and they are able to neutralize the latter by donating their own electrons. This process helps prevent the damage of cell tissues. Each cell protects itself from the influence of free radicals by its own prevention mechanisms, but in the event of excessive formation, oxidative stress occurs (Sen et al., 2010). Oxidative stress has been defined as the imbalance between the production of various reactive species and the ability of a living body's natural defense mechanisms (Oguntibeju et al., 2009). Under such circumstances, it is necessary to take antioxidative products which can restore the balance of a body's antioxidant status.

Polyphenols are the main secondary metabolites to be found in medicinal plants. Besides their antioxidant activity, such compounds are responsible for their antimicrobial actions (due to their content of flavonoids) and cytotoxic effects exerted against tumor cells Gharras, 2009). Their antimicrobial actions have been proven against potentially pathogenic strains, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida* strains, *Listeria* strains, or *Bacillus cereus* (Vamanu et al. 2011). On the other hand, their cytotoxic effects have also been associated with the content of alkaloids and triterpenes, which have been demonstrated in previous surveys as being directly related to the antioxidative response (Agarwal et al., 2012). This is the reason why the present research has undertaken to demonstrate the antioxidative properties of certain tinctures sold in Romania. Their properties have been linked to polyphenol and flavonoid contents. Highlighting the antioxidative effects has taken place by means

of the DPPH and ABTS radicals′ scavenging activity, reducing power, and chelating properties.

MATERIALS AND METHODS

Materials

The tinctures that have been used are: hot pepper tincture (*Capsicum annuum*), cranberry tincture (*Vaccinium vitis-idaea*), and lemon balm tincture (*Melissa officinalis*) manufactured by SC Dacia Plant SRL.

Figure 1. Marketing image of *Capsicum annuum, Vaccinium vitis-idaea* and *Melissa officinalis* tinctures

DPPH radical scavenging activity assay

The sample (100 µl) was mixed with 3 ml of ethanol solution of 0.004% DPPH and the absorbance was read at 517 nm 30 min later. Standard antioxidant, tert-Butylhydroquinone (TBHQ), was used for comparison as positive control. EC50 value (milligram extract/mL) is the effective concentration at which DPPH radicals were scavenged by 50%. The DPPH radical-scavenging activity of the samples was expressed as% = ((Acontrol − Asample) /Acontrol) × 100, where Acontrol is the absorbance of the blank control (DPPH solution without test sample) and Asample is the absorbance of the test sample (Vamanu et al. 2011).

ABTS Radical Scavenging Assay

ABTS radical was obtained by adding 7mM of the ABTS stock solution to 2.45mM potassium persulfate. The mixture was left to stand in the dark, at room temperature, for 12−16 h before use. The ABTS radical solution was then diluted with 5mM phosphate-buffered saline (pH 7.4) to an absorbance at 730nm of 0.70 ±0.02. After adding 10 µL of the sample to 4mL of the diluted ABTS radical solution, the absorbance was measured at 30 min. The ABTS radical-scavenging activity of the samples was expressed as% = ((Acontrol − Asample) /Acontrol) × 100, where Acontrol is the absorbance of the blank control (ABTS solution without test sample) and Asample is the absorbance of the test sample (Vamanu, 2012a). Standard antioxidant (TBHQ) was used for comparison as positive control. EC50 value (milligram extract/mL) is the effective concentration at which ABTS radicals were scavenged by 50%

Reducing Power

Each sample (2.5 mL) was mixed with 200 mM sodium phosphate buffer (2.5 mL, pH 6.6) and 1% potassium ferricyanide (2.5 mL), and the mixture was incubated at 50 °C for 20 min. Next, 10% trichloroacetic acid (2.5 mL) was added, and the mixture was centrifuged at 3,000 g for 10 min. The upper layer (2.5 mL) was mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL). Finally, the absorbance was measured at 700 nm and compared to a blank. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm plotted against the extract concentration. TBHQ was used as positive control (Vamanu, 2012b).

Ferrous Ion Chelating Assay

1mL of the sample was mixed with 3.7mL of ultrapure water, following which the mixture was reacted with ferrous chloride (2 mmol/L, 0.1mL) and ferrozine (5 mmol/L, 0.2mL) for 20 min. The absorbance at 562nm was determined spectrophotometrically. TBHQ was used as a positive control. The chelating activity on the ferrous ion was calculated using the equation following: chelating activity (%) = [(*Ab* −*As*) */Ab*] ×100, where *Ab* is the absorbance of the blank without the extract or TBHQ and As is the absorbance in the presence of the extract or TBHQ (Vamanu and Nita, 2013).

Determination of total phenolic and flavonoid content

The total phenolic and flavonoid content of ethanolic extract, and several organic fractions, were determined using Folin-Ciocalteu reagent and aluminium chloride colorimetric method,

respectively (Vamanu, 2012c; Premanath et al., 2011).

Statistical analysis

All the essays were assessed in triplicate, and the results were expressed as mean ±SD values of three observations. The mean values and standard deviation were calculated with the EXCEL program from Microsoft Office 2010 package.

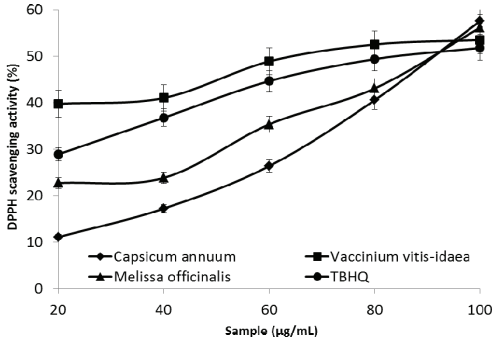

Figure 2. DPPH scavenging activity of *Capsicum annuum, Vaccinium vitis-idaea* and *Melissa officinalis* tinctures

RESULTS AND DISCUSSIONS

Evaluating antioxidative potential by the DPPH and ABTS determination of radicals' scavenging activity requires a modern method to be applied due to the stability of these free radicals, and to the precise results that ensue thereof. The discoloration level of the reaction mixture (turning from mauve into beige for the DPPH scavenging assay; or turning from turquoise into colorless for the ABTS scavenging assay) is allocated in direct proportion to the ability of donating a hydrogen atom derived from the antioxidative compounds in the examined assay. The process leads to the reduction of existing free radicals, and also generating the cessation of the radical chain reaction which has determined the propagation of the oxidation mechanism (Kumaraswamy and Satish 2008). DPPH and ABTS free radicals accept hydrogen radical and become stable molecules (Nikhat et al., 2009). In this research, the standard (TBHQ) has proven a moderate scavenging activity against DPPH radicals, and a high scavenging activity against ABTS radicals. At a concentration of 100 µg/mL, the descending order of the scavenging activity against DPPH radicals has been: *C. annuum >M. officinalis >V. vitis-idaea* (Figure 2). Thus, in terms of the hot pepper tincture, the scavenging activity's difference from the standard has been on average 10% higher. With respect to ABTS radicals, the descending order has been: *V. vitis-idaea >M. officinalis >C. annuum* (Figure 3). In this context, the set value of the TBHQ reaching 100 µg/mL has been on average 2% higher. The differences noticed in relation with the three tinctures for scavenging properties against the two free radicals have been generated by the existing molecules that possess antioxidative effects. The type and presence of certain molecules have caused specificity differences as compared with the DPPH or ABTS radicals which have been best monitored in the *C. annuum* tincture.

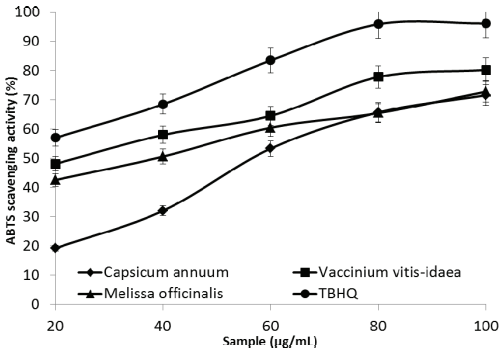

Figure 3. ABTS scavenging activity of *Capsicum annuum, Vaccinium vitis-idaea* and *Melissa officinalis* tinctures

For reducing power assays, the antioxidative molecules to be found in the three tinctures will determin the reduction of Fe^{3+} into Fe^{2+} (Moein et al., 2008). The molecules' reducing power in the three plant tinctures serves as a direct indicator of the extent of the antioxidative potential. According to the research in the field of natural supplements, reducing power is related to the existence of reductones (Li and Lin, 2010). Ascorbic acid is the best known reductone, and high reduction ability is generally associated with a significant amount of this compound which is frequently found in hydroalcoholic extracts. The absorbance increase has been set at 700 nm, as compared with the standard value (Figure 4). The value

set at the concentration of 100 µg/mL has ranged between 0.404 and 1.643. Compared with the standard, the reducing power value in the *V. vitis-idaea* tincture is 3.8 times higher which has indirectly confirmed the presence of molecules with obvious reduction properties. The same behavior has been noticed in the *M. officinalis* tincture as well. The difference between the two tinctures has been 28.4%, in favor of the cranberry tincture.

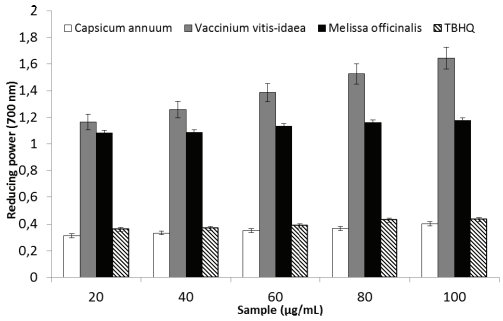

Figure 4. Reducing power of *Capsicum annuum, Vaccinium vitis-idaea* and *Melissa officinalis* tinctures

As far as the determination of ferrous chelating capacity is concerned, ferrozine forms Fe^{2+} compounds, whereas in the presence of chelating agents from the three tinctures, compound formation is disturbed. According to the sample concentration, the reaction mixture is colored in several shades of red and pink in an inverse proportion. The decrease in absorbance has been measured spectrophotometrically (Grace-Lynn et al., 2012). Ferrum is essential as it is very important for oxygen conduction and acts as an activating agent for various enzymes. The ferrous ion (or copper ion) intervenes in a range of mechanisms that may contribute in the emergence of oxidative processes. Its role is very well known in the lipid peroxidation of the cell membrane, and its action upon the protein content is also recognized (Karthika et al., 2012). In the present research, the capacity of the examined tinctures to link to Fe^{2+} in the presence of ferrozine as compared with the (TBHQ) standard has been shown in Figure 5. The maximum value of the ferrous ion chelating activity is 80.17%, at 100 µg/mL, with respect to *C. annuum* tincture. It has been 1.07% higher than the *V. vitis-idaea* tincture, and 5.61% higher than the tincture obtained from the *M. officinalis*. The standard has shown a low ferrous chelating activity at a significantly lower value than the three assays, up to a maximum of 46.61% in the highest concentration of the assay.

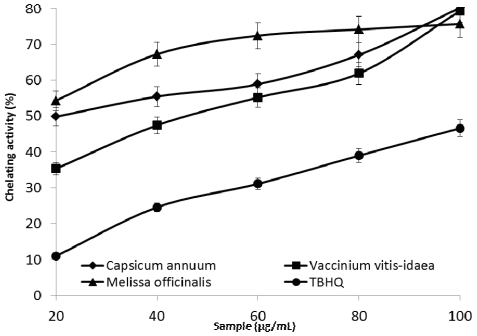

Figure 5. Ferrous ion chelating activity of *Capsicum annuum, Vaccinium vitis-idaea* and *Melissa officinalis* tinctures

The results of the above tests have been relatively different based on each individual biochemical test performed. This finding proves that certain molecules can exert their antioxidative properties in their own individual ways. This confirmation has ensued from the inversely proportional values of the EC_{50} regarding its chelating capacity. From the perspective of this parameter, the maximum value has been reached for *M. officinalis* tincture (< 10 µg/mL). The EC_{50} values of the other two tinctures have been ≈30 µg/mL in the *C. annuum*, and ≈65 µg/mL in *V. vitis-idaea*. The latter results confirm the high biological values of these tinctures, especially that of the cranberry one, because Ferrum immobilization within compounds stops its accumulation which has toxic effects at cellular level (Grace-Lynn et al., 2012). The same behavior has been assessed in the DPPH scavenging activity where the lemon balm tincture has shown its EC_{50} value as being inferior to 90 µg/mL. On the contrary, the EC_{50} values of the other two tinctures have been superior to the former, namely ≈95 µg/mL.

The scavenging activity against free radicals is due to the amount of phenol compounds. Subsequent to all the measurements performed, the *V. vitis-idaea* tincture has contained an amount of 106 ± 8.15 mg gallic acid/g extract,

M. officinalis tincture 96.4 ± 4.74 mg gallic acid/g extract, and 76.8 ± 3.98 mg gallic acid/g extract in the *C. annuum* tincture. The flavonoid content has proven a similar trend, with a maximum level of 4.43 ± 0.5 mg quercetin/g extract in the *V. vitis-idaea* tincture. This value has been 35.44% higher than the lemon balm tincture, and 64.78% higher than the hot pepper tincture. The calculated values of the main antioxidative compounds have corresponded with the increase in the EC_{50} index value in terms of the scavenging activity, reducing power and ferrous ion chelating activity (Zhu et al., 2006).

Interpreting the results of the antioxidative properties′ analysis relating to the three tinctures has also taken place by calculating the index value of the correlation among various examination methods. In the hot pepper tincture, the R^2 value has been 0.8811, while the same value among the remaining methods has ranged between 0.9711 and 0.9891 ($p<$ 0.0005). The correlation coefficient has ranged from 0.828 to 0.9305, with a minimum value calculated for the ABTS ratio between radicals′ scavenging activity and ferrous ion chelating activity. The value ensuing thereof has confirmed the behavior of this tincture when expressing its antioxidative feedback. As to the lemon balm, there has been a low R^2 value in the relationship between the ferrous ion chelating activity and the reducing power, a value of 0.6311. In the relation with the ABTS assay, a 0.7129 value of the correlation index has been measured, whereas, the correlation level has been high in the other outcomes, ranging between 0.8621 and 0.9446.

Owing to the generally neutral pH contained in tinctures, the DPPH assay has been a less appropriate method where the sample′s correlation degree influences the final result. The differences when calculating the R^2 value related to the ABTS assay more precisely indicate the distinctions among the three tinctures, as the method is valid irrespective of the pH value, and the results do not directly depend on the coloring level of the sample (Bhoyar et al., 2011; Zhu et al., 2006).

CONCLUSIONS

To conclude, the total phenolic content has been determined as significantly higher in the lemon balm tincture as compared with the other two. This ratio has been correlated in the case of flavonoid contents as well. According to the EC_{50} values, the *V. vitis-idaea* tincture has had a more obvious antioxidative activity in compliance with its correlation to the scavenging activity against free radicals and the chelating activity, than in the hot pepper (*C. annuum*) tincture. These measurements comply with similar research, thus confirming that a tincture′s expression of its antioxidative value is not automatically and directly connected with its phenolic content. The antioxidative feedback is very complex due also to the existence of additional compounds that exert specific effects.

ACKNOWLEDGEMENTS

This work was partially supported by the project PNCDI II CNCSIS—Human Resources, Theme 9/2010 (http://www.emanuelvamanu.ro).

REFERENCES

Agarwal N., Majee C., Chakraborthy G.S., 2012. Natural Herbs as Anticancer Drugs. International Journal of PharmTech Research, Vol. 4 (3), p. 1142-1153.

Bhoyar M.S., Mishra G.P., Naik P.K., Srivastava R.B., 2011. Estimation of antioxidant activity and total phenolics among natural populations of Caper (*Capparis spinosa)* leaves collected from cold arid desert of trans-Himalayas. Australian Journal of Crop Sciences, Vol. 5 (7), p. 912-919.

Gharras H. E., 2009. Polyphenols: food sources, properties and applications − a review. International Journal of Food Science and Technology, Vol. 44 (12), p. 2512−2518.

Grace-Lynn C., Darah I., Chen Y., Latha L.Y., Jothy S.L., Sasidharan S., 2012. *In Vitro* Antioxidant Activity Potential of Lantadene A, a Pentacyclic Triterpenoid of *Lantana* Plants. Molecules, Vol. 17 (9), p. 11185-11198.

Ivan S., Medicina naturistă pentru toți, 2007. CNI Coresi Publishing House, p. 108.

Karthika K., Paulsamy S., Jamuna S., 2012. Evaluation of in vitro antioxidant potential of methanolic leaf and stem extracts of Solena amplexicaulis (Lam.) Gandhi. Journal of Chemical and Pharmaceutical Research, Vol. 4 (6), p. 3254-3258.

Kumaraswamy M.V., Satish S., 2008. Free radical scavenging activity and lipoxygenase inhibition of Woodfordia fructicosa Kurz and Betula utilis Wall.

African Journal of Biotechnology, Vol. 7 (12), p. 2013-2016.
Li C.C., Lin E.S., 2010. Antiradical capacity and reducing power of different extraction method of Areca catechu seed. African Journal of Biotechnology, Vol. 9 (46), p. 7831-7836.
Moein M.R., Moein S., Ahmadizadeh S., 2008. Radical Scavenging and Reducing Power of *Salvia mirzayanii* Subfractions. Molecules Vol. 13 (11), p. 2804-2813.
Nikhat F., Satynarayana D., Subhramanyam E.V.S., 2009. Isolation, Charectrisation and Screening of Antioxidant Activity of the Roots of Syzygiumcuminii (L) Skeel. Asian Journal of Research in Chemistry, Vol. 2 (2), p. 218-221.
Oguntibeju O. O., Esterhuyse A.J., Truter E.J., 2009. Cardiovascular disease and the potential protective role of antioxidants. African Journal of Biotechnology, Vol. 8 (14), p. 3107-3117.
Premanath R., Sudisha J., Devi N.L., AradhyaS.M., 2011. Antibacterial and anti-oxidant activities of fenugreek (Trigonella foenum graecum L.) leaves. Research Journal of Medicinal Plant, Vol. 5 (6), p. 695-705.
Vamanu E., 2012a. Biological Activities of the Polysaccharides Produced in Submerged Culture of Two Edible *Pleurotus ostreatus* Mushrooms. Journal of Biomedicine and Biotechnology, Article ID 565974.
Vamanu E., 2012b. *In Vitro* Antimicrobial and Antioxidant Activities of Ethanolic Extract of Lyophilized Mycelium of *Pleurotus ostreatus* PQMZ91109. Molecules, Vol. 17 (4), p. 3653-3671.
Vamanu E., 2012c. Determination of antioxidant and antimicrobial properties of *Agaricus bisporus* from Romanian markets. Ovidius University Annals of Chemistry, Vol. 23 (1), p. 47-52.
Vamanu E., Nita S., 2013. Antioxidant Capacity and the Correlation with Major Phenolic Compounds, Anthocyanin, and Tocopherol Content in Various Extracts from theWild Edible Boletus edulis Mushroom. Journal of Biomedicine and Biotechnology, Article ID 313905.
Vamanu E., Vamanu A., Nita S., Colceriu S., 2011. Antioxidant and Antimicrobial Activities of Ethanol Extracts of *Cynara Scolymus* (Cynarae folium, Asteraceae Family). Tropical Journal of Pharmaceutical Research, Vol. 10 (6), p. 777-783.
Zhu K., Zhou H., Qian H., 2006. Antioxidant and free radical-scavenging activities of wheat germ protein hydrolysates (wgph) prepared with alcalase. Process biochemistry, vol. 41 (6), p. 1296-1302.