Studies Regarding the Determination of Antioxidant Properties of New Plant Extracts for Cosmetic Purposes

GABRIELA STANCIU[1]*, ELISABETA CHIRILA[1], SIMONA DOBRINAS[1], TICUTA NEGREANU-PIRJOL[2]

[1] "Ovidius" University, Chemistry Department, 124 Mamaia Blvd, 900527, Constanta, Romania

[2] "Ovidius" University, Pharmaceutical Sciences Department, 124 Mamaia Blvd, 900527, Constanta, Romania

This paper deals with recent original studies regarding the evolution in time of antioxidative properties for some alcoholic extracts of plant material from Constanta district, Romania: poplar leaf-buds, sea buckthorn male buds, snowdrop flowers and violet flowers, some of them reported for the first time as cosmetic raw materials to our knowledge. The total phenolic contents (TPC) and antioxidant capacity of lipid soluble substances (ACL) have been measured using modified Folin Ciocalteu, respectively photochemiluminescence methods in plant extracts after 1, 2, 3, 4, 5, 6, 7, 15 and 30 days of contact time of plant material with ethanol. In poplar leaf-buds TPC varied from 0.304 to 0.413 mg GAE/g dry weight, in sea buckthorn male buds from 0.087 to 0.333 mg GAE/g dry weight, in violet flowers from 0.226 to 0.269 mg GAE/g dry weight and in snowdrop flowers from 0.080 to 0.133 mg GAE/g dry weight. The results show that the ACL of the alcoholic extracts is high and increases continuously for poplar (from 183.85 to 391.48 nmol/mg dry weight) and sea buckthorn (from 30.71 to 246.70 nmol/mg dry weight). The other samples have a slightly increasing trend of ACL: from 119.19 to 125.08 nmol/mg dry weight (violet) and from 104.37 to 135.73 nmol/mg dry weight (snowdrop). The highest antioxidant capacity measured as TPC and ACL from the studied plant extracts belongs to poplar leaf buds, followed by sea buckthorn male buds, violet and snowdrop flowers. All the studied plant materials have high concentration of phenolic compounds that contribute to antioxidant properties explaining their use for cosmetic purposes.

Keywords: total phenolic compounds, antioxidant capacity, photochemiluminescence, plant extracts

There is a growing interest in the pharmaceutical and cosmetic industry to use plant extracts for various fields of applications [1-3]. At present, there are also some attempts to introduce new products, so called "nutraceuticals" and "cosmeceuticals" which can be defined as food and cosmetic products delivering specific health improvement [4].

According to their major active principles, plant extracts used in cosmetic products can be subdivided into essential oil plants, flavonoid drugs (extracts with anti-inflammatory activity and healing properties, extracts which stimulate the capillary blood pressure), tanniferous plant extracts or those containing silicic acid, saponin extracts, sedative or stimulating plant extracts, extracts used as additives for sun protection. Most of the drugs with dermatological use belong to the group owning anti-inflammatory activity [5].

Many species of fruits, vegetables, herbs, cereals, sprouts and seeds have been investigated for antioxidant activity during the past decade [6-9]. The majority of natural compounds with antioxidant properties contain phenolic groups. Phenolics are secondary plant metabolites that are involved in a wide range of specialized physiological functions. They appear to be very important for the normal growth, development and defence mechanisms of plants. It is evident that there is an increasing demand to evaluate the antioxidant properties of direct plant extract in order to assess their use in medical purposes.

Sea buckthorn (*Hippophae sp*.) is a deciduous, thorny and nitrogen-fixing shrub or small tree of 2-4 m height. Recent surveys indicate that this plant grows widely on riversides and slopes in dry temperate regions. The fruit of sea buckthorn is quite rich in vitamins and other bioactive substances, which have been utilized in Russia, China, Finland, Romania and some other countries for the industrial production of health protection food product, medicine and cosmetics. High contents of vitamin C, flavonoids, tannins, oils and oil soluble bioactive compounds as well as minerals are the characteristics of the berry [10-14].

Populus nigra L., commonly known as black poplar is a deciduous tree with broad green leaves and small yellowish-green flowers.

The leaf-buds of black poplar are used in traditional medicine as well as their alcoholic extracts. The constituents of alcoholic extracts and bee's propolis have been compared and shown to be similar in many respects. The bud exudates of P. nigra from different origin contain caffeic and isoferulic acids with their esters, chalcones, flavanones and flavones as the major components [15, 16].

Snowdrop (*Galanthus sp.*) are native to many parts of Europe including Bulgaria, the eastern parts of Turkey and the Caucasus mountain range. Overall little is known about the use of this genus in Europe but there are new researches about the use of some active principles in medicine [17-19].

Violet (*Viola sp.*) is native to Europe and Asia, but has also been introduced to North America and Australasia. In India it is commonly used as remedy to cure sore throat and tonsilitis [20].

There are a variety of spectrometric and chemiluminometric analytical methods available, applied for antioxidant properties evaluation [21-25].

The paper presents original results of recently performed studies concerning the antioxidant properties characterization as total phenolic contents (TPC) and antioxidant capacity of lipid soluble substances (ACL) evolution in time of sea buckthorn, poplar, snowdrop and

* *email: gstanciu@univ-ovidius.ro*

violet ethanolic extracts used for cosmetic purposes in Constanta district, Romania, some of them reported for the firs time as cosmetic raw materials.

Experimental part

Sampling

Different part of plants: poplar (*Populus nigra L.)* leaf-buds, sea buckthorn (*Hippopae rhamnoides L.*) male buds, snowdrop (*Galanthus nivalis L.*) flowers and violet (*Viola odorata L.*) flowers have been collected in the early spring of 2008 from forest area in Dobrogea County, Romania. Poplar leaf-buds and sea buckthorn male buds were dried in air, on wood grid, during 10 days, snowdrop and violet flowers were processed immediately after collection. 100g of poplar, sea buckthorn and violet plant materials was leave in contact with 300 mL of ethanol 95%, and 100g of snowdrop flowers with 500mL of ethanol 95%, in brown recipients at dark in order to obtain the extracts. The mixture was strongly shaken three times every day.

After 1, 2, 3, 4, 5, 6, 7, 15 and 30 days of contact time 5 mL alcoholic extract, previously filtered, were collected in glass recipients and stored at 4^0C. For determinations each extract was diluted with ethanol in ratios from 1:625 to 1:1250.

Reagent and apparatus

All used reagents were of analytical reagent grade. Gallic acid was purchased from Fluka (Buchs, Switzerland) and Folin – Ciocalteau reagent from Merck (Darmstadt, Germany). Gallic acid (standard phenolic compound) 1×10^{-2} mol$\times$L^{-1} was prepared by dissolving 0.1881g of gallic acid in 100mL of methanol 50%. Folin – Ciocalteau reagent was diluted with distilled water 1:2 (V:V).

Spectrometric measurements were carried out using a Jasco 550 UV VIS scanning spectrophotometer. The antioxidant capacity of lipid soluble substances determination has been performed with Photochem instrument with ACL kit (Analytic Jena AG, Germany).

Measurements

The evolution of antioxidant properties of studied plant extract on time: (i) the total phenolic content (TPC) determination using a modified Folin Ciocalteu method and (ii) the antioxidant capacity of lipid soluble substances (ACL) using Analytik Jena AG Germany method based on photochemiluminescence, was performed [25]. In parallel, UV-Vis spectra of each alcoholic extract were registered and overlaid. For the final data report the humidity of initial processed plant material at 100^0C was determined.

The used method for TPC determination is based on the reduction of a phosphowolframate – phosphomolibdate complex to blue products by soluble phenolic compounds, in sodium carbonate media and the measurement of the absorption of the formed complex at the wavelength of 680nm. The absorbance relative to a gallic acid standard curve was measured and results are expressed as gallic acid equivalents (mg GAE/ g dry weight).

To plot the calibration curve in the range of 0.022 – 0.15 mg GAE/L, 1 mL Folin Ciocalteu reagent 1:2 was added in 50mL calibrated flasks to different volumes of standard gallic acid solution, then 1 mL ethanol, 1 mL sodium carbonate solution 20%, then the mixture was mixed and let standing 10 min at room temperature and fill up to the mark with distilled water. At the end the mixture was homogenized and let under room temperature 30 minutes for the color stabilization and after that the absorbance was read at 680 nm.

For ACL determination, in principle a photo sensitive compound provides free radicals by optical excitation, the antioxidants from the sample eliminate partially the free radicals and the residual radicals react with luminol and produce luminescence [14]. The calibration curve was plotted using Trolox as standard. 20 µL of diluted plant extracts were used for ACL determinations. Three individual measurements were performed and the mean value is reported.

Results and discussions

Superimposed spectra of alcoholic extracts after 1, 2, 3, 4, 5, 6, 7, 15 and 30 days have been plotted to establish some correlations with the obtained values of antioxidant properties.

Figures 1-4 show the superimposed spectra of alcoholic plant extracts and it can be observed a continuous increase of the absorbance in time, showing the enhancement of intensity due to the extractible organic compounds increase.

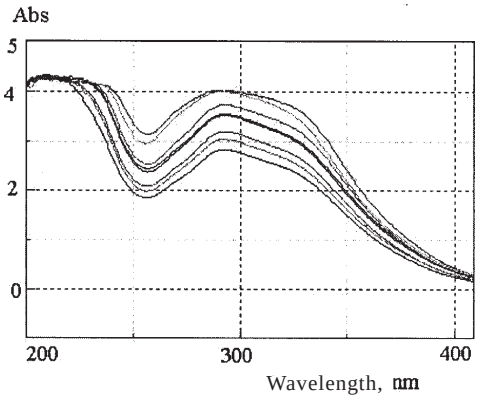

Fig. 1. Superimposed spectra of poplar leaf buds ethanol extracts in time

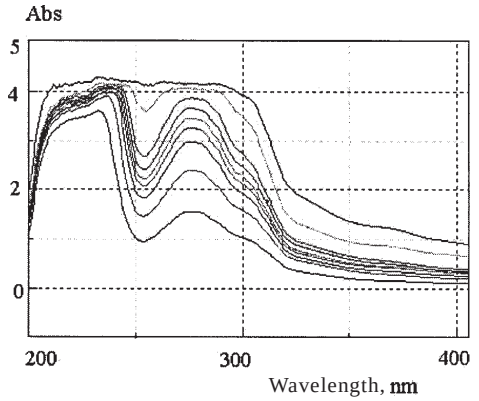

Fig. 2. Superimposed spectra of sea buckthorn male buds ethanol extracts in time

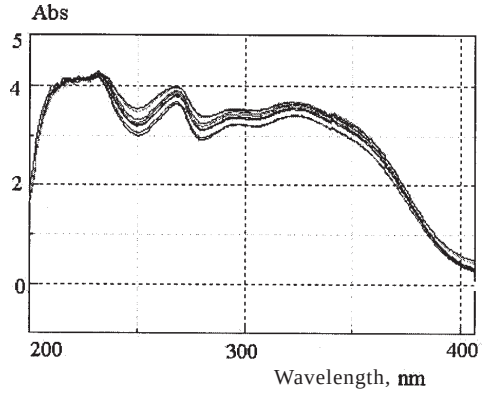

Fig. 3. Superimposed spectra of violet flowers ethanol extracts in time

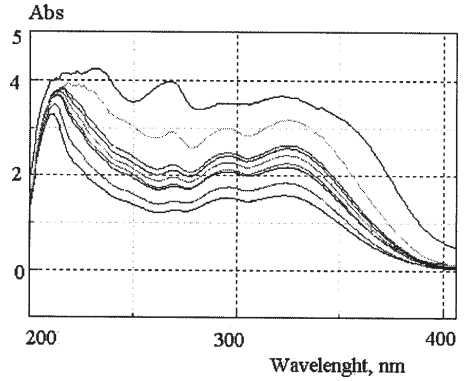

Fig. 4. Superimposed spectra of snowdrop flowers ethanol extracts in time

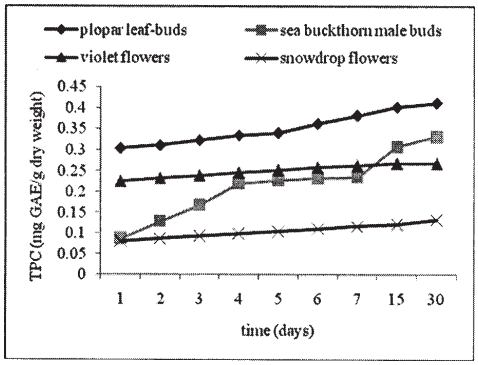

Fig. 5

Fig. 5. In time evolution of total phenolic content (mg GAE/g dry weight) for plants extracts in ethanol

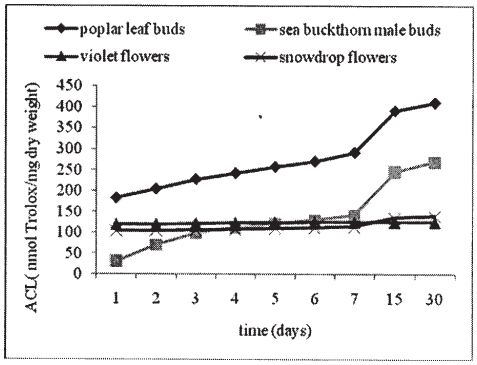

Fig. 6. In time evolution of antioxidant capacity (nmol trolox / mg dry weight) for plants extracts in ethanol

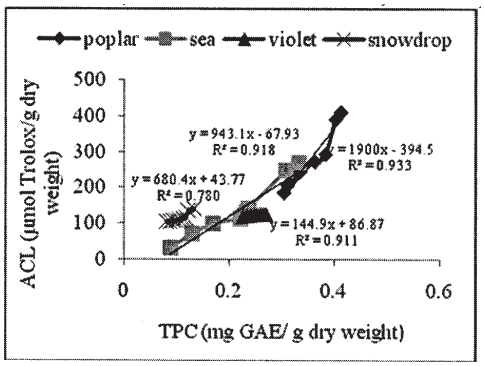

Fig. 7. ACL versus TPC variation in time for plants extracts in ethanol

Polyphenols possess ideal structural chemistry for free radical scavenging activity. Antioxidant properties of polyphenols arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electrons, and from their ability to chelate transition metal ions [26].

For antioxidant properties characterization of the studied plants alcoholic extracts, there were determined TPC using a modified Folin–Ciocalteu method and ACL using photochemiluminescence.

Figure 5 presents the evolution of total phenolic content in time (mg GAE/g dry weight) for alcoholic plants extracts. Total phenolic compounds were found to be for poplar leaf buds from 0.304 to 0.413 mg GAE/g dry weight, for sea buckthorn male buds from 0.087 to 0.333 mg GAE/g dry weight, for violet flowers from 0.226 to 0.269 mg GAE/g dry weight and for snowdrop flowers from 0.080 to 0.133 mg GAE/g on dry weight. From the obtained data it can be observed that the extraction yield has increasing shape. Poplar leaf buds show the highest TPC content, followed by sea buckthorn male buds, violet and snowdrop extracts.

Figure 6 presents the evolution in time of the antioxidant capacity (expressed in nmol Trolox/mg dry weight), for the studied alcoholic plants extracts.

For all studied plant materials after different contact time with ethanol were observed different increasing trends of ACL from 1 to 30 days. The results show that the ACL of the alcoholic extracts is high and increases continuously for poplar (from 183.85 to 391.48 nmol/mg dry weight) and sea buckthorn (from 30.71 to 246.70 nmol/mg dry weight). The other samples have a slightly increasing trend of ACL: from 119.19 to 125.08 nmol/mg dry weight (violet) and from 104.37 to 135.73 nmol/mg dry weight (snowdrop).

The differences between obtained values of TPC and ACL in different plant extracts could be explained by their content in organic compounds which are responsible for antioxidant capacity.

Poplar leaf buds extracts have the highest TPC value from the first day of extraction and this value increases in time, reaches the maximum value in the 30th day having higher content from all other analyzed samples.

Sea buckthorn male buds extract presents the most important increase of TPC (fig. 5), having at the beginning low value and in the final reaching values in the vicinity of poplar extract TPC.

The TPC variation in time for violet and snowdrop flowers extracts is lower than the others despite the fact that in the first day they have TPC values higher or equal with those of sea buckthorn male buds.

ACL evolution for studied plant extracts is more or less in the same shape with TPC (see figure 6). As a conclusion, the highest antioxidant capacity measured as TPC and ACL from the studied plant extracts belongs to poplar leaf buds, followed by sea buckthorn male buds, violet and snowdrop flowers.

This assumption is correlated with the ACL versus TPC variation in the studied time period for all extracts, showed in the figure 7. The trend lines have more or less linear increase with different slopes. The maximum slope is of poplar, and decreases in the following order: poplar leaf buds, sea buckthorn male buds, snowdrop and violet flowers.

Alcoholic extracts of buds show higher antioxidant properties than those of the flowers. Leaf buds of poplar and male buds of sea buckthorn are promising plant materials for more detailed investigation of their antioxidant properties and application possibilities.

Conclusions

Recent original studies regarding the evolution in time of antioxidative properties for some alcoholic extracts of plant material: poplar leaf-buds, sea buckthorn male buds, snowdrop flowers and violet flowers are reported for the first time as cosmetic raw materials.

The ACL determination using photochemiluminescence which combines the very fast photochemical radical generation with the highly sensitive luminometric detection represents a suitable technique to evaluate the antioxidant properties for any mixture.

The highest antioxidant capacity measured as TPC and ACL from the studied plant extracts belongs to poplar leaf buds, followed by sea buckthorn male buds, violet and snowdrop flowers.

All the studied plant materials have high concentration of phenolic compounds that contribute to antioxidant properties explaining their use for cosmetic purposes.

References

1. LORETZ, L., API, A. M.,. BARRAJ, L., BURDICK, J., DE DAVIS, A., DRESSLER, W., GILBERTI, E., JARRETT, G., MANN ,S., PAN ,Y.H.L., RE T., K. RENSKERS, SCRAFFORD, C., VATER, S., Food Chem. Toxicol. **44**, 2006, p.2008
2. YANISHLIEVA, N. V., MARINOVA, E., POKORN, Y, J., European J. of Lipid Sci. and Tech., **108**, 2006,p. 776
3. POKORNÝ, J., European J. of Lipid Sci. and Tech., **109,** 2007, p. 629
4. SORG ,O., ANTILLE, C., KAYA, G., SAURAT, J. H., Dermatol. Therapy, **19**, 2006, 289
5. STOBIECKI, M., KACHLICKI, P., Isolation and Identification of Flavonoids in Grotewold E. (Ed) The Science of Flavonoids: Springer New York: 2006, p. 47
6. ELMASTAS, M., GULCIN, I., OZTURK, L., GOKCE, I., Asian J. Chem. **17,** 2005, p. 137
7. ELMASTAS, M., DEMIRTAS ,I., ISILDAK, O., ABOUL-ENEIN, H. Y., J. Liq. Chromatogr. Rel. Techol. **29**, 2006, p. 1465
8. ELMASTAS, M., I. GULCIN, S. BEYDEMIR, O.I. KUFREVIOGLU, H. Y. ABOUL-ENEIN, Anal. Letters, **39,** 2006, p. 47
9. ISILDAK O., TURKEKUL I., ELMASTAS M., TUZEN M., Food Chem. **86**, 2004, p. 547
10. SAVA C., CHIRILA E., BUCUR L., Proceedings "12thRomanian Conference on Chemistry and Chemical Engineering, Bucarest, Romania, 2001, p 89
11. NEGI, P. S., CHAUHAN A. S., SADIA G. A., ROHINISHREE Y. S., RAMTEKE, R. S., Food Chem. **92**, 2005, p. 119
12. GEETHA, S., SAI RAM M., SINGH, V., ILAVAZHAGAN, G., SAWHNEY, R.C., J. Ethnopharmacol. **79**, 2002,p. 373
13. ARIMBOOR, R., SARIN KUMAR, K., ARUMUGHAN, C., J. Pharm. Biomed. Anal. **47**, 2008, p. 31
14. YANG B., Food Chem. **112**, 2009, p. 89
15. POPOVA, M., SILICI, S., KAFTANOGLU, O., BANKOVA, V., Phytomedicine, **12**, 2005, p. 221
16. JERKOVIC, I., MASTELIC, J., Phytochemistry, **63**, 2003, p. 109
17. MALAKOVA, J., NOBILIS, M., SVOBODA, Z., LISA ,M., HOLCAPEK, M., KVETI J., KLIMS J., PALICKA V., J. Chromatogr. B, **853**, 2007, p. 265
18. ASCHAN G., PFANZ H., Flora, **201**, 2006, p. 623
19. HEINRICH M., TEOH H.L., J. Ethnopharmacol. **92**, 2004, p. 147
20. HERRMANN A., BURMAN R., MYLNE J.S., KARLSSON G., GULLBO J., CRAIK D.J., CLARK R.J., GORANSSON U., Phytochemistry, **69**, 2008, 939.
21. GÜLÇINI., ELMASTAS M., ABOUL-ENEIN H. Y., Phytotherapy Res. **21,** 2007, p. 354
22. BILIA A. R., GIOMI M., INNOCENTI M., GALLORI S., VINCIERI F. F., J. Pharm. Biomed. Anal. **46**, 2008, p. 463
23. HOSAKA S., OBUKI M., NAKAJIMA J., SUZUKI M., Luminescence, **20**, 2005, p. 419
24. PASCUAL C., ROMAY C., J. Biolumin. Chemilumin. **7**, 2005, p. 123
25. POPOV I. N., LEWIN G., Photosintesitized chemiluminiscence. Its medical and industrial applications for antioxidizability tests. In: A. M.Garcia-Campana, WRG Baeyens (Eds) Chemiluminiscence in Analytical Chemistry, Marcel Decker Inc., New York, Bassel. 2001
26. OSZOY N., CAN A., YANARDAG R., AKEV N, Food Chemistry **110**, 2008, p. 571

Manuscript received: 4.02.2009