ANALYSIS OF THE POLYPHENOLS FROM THE ANGIOSPERMATOPHYTA AND SPERMATOPHYTA PLANT SPECIES

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Abstract

The aim of this paperwork is to quantitatively determine the polyphenolic compounds from the Angiospermatophyta and Spermatophyta plant species.

The quantitative analyses of the polyphenols were carried out using the Folin-Ciocalteu analysis method.

The researched vegetal species were: Alchemilla vulgaris – Lady's Mantle (aerial part), Allium ursinum – Wild Garlic (leafs), Acorus calamus – Sweet Flag (roots), Solidago virga-aurea – Golden Rod (aerial part), Agrimonia eupatoria - sticklewort (aerial part), Viscum album – European mistletoe (leafs and twigs) and Veronica officinalis – Heath Speedwell (aerial parts).

Keywords: polyphenols, Folin-Ciocalteu method, antioxidant, vegetal extracts

INTRODUCTION

The polyphenols found in vegetal extracts are more and more looked into lately due to their antioxidant capacities.

In order for a phenol to have significant antioxidant capacities it is necessary that the hydroxyl group to be hindered by at least one large volume substituent such as t-butyl, cyclohexyl, α-metylbenzyl (Harborne, 1986).

The hindered phenols form stable aroxyls by reacting with the peroxy radicals, shown via RPE spectra or isolated in inert atmosphere.

For the phenols containing O, N, S, heteroatoms in the substituents from the 2 or 4 position it is noted that the oxygen atoms have a negative impact on the antioxidant activity when they are found in the substituent from the 4 position, while their presence in the substituent from the 2 position has a favorable effect.

In the case of nitrogen atoms the situation is reversed, while sulfur atoms have a positive effect in both cases (Giurginca and Meghea, 1986).

Pyrocatechole and its alchyled derivates are efficient antioxidants while the derivates of the 2-alcoxypehols have a reduced antioxidant activity.

Numerous studies regarding the antioxidant activity of phenols have shown that the most powerful antioxidant effect is noted for the 2,6-di-tertiary-butyl-hydroquinone.

The reduce of the antioxidant activity due to the implication of the hydroxyl group in a hydrogen bond can be explained if one takes into consideration the fact that the antioxidant activity also depends on the reactivity of the phenols towards the peroxy radicals.

Polyphenols are a representative class of substances due to their multiple actions, such as: anti-allergic, anti-cancer, anti-cataract, anti-colitis, anti-diabetic, anti-inflammatory, anti-mutating, anti-HIV, anti-ulcer, bactericidal and vasodilator (Eleanor and Şharon, 1996; Hoffer, 1999; Mindell, 1996;Null, 2000; Packer and Colman, 2000; Reuben, 1995).

The increased antioxidant efficiency is also due to their synergy, the summing of their combined action, each functioning via different mechanisms and at various levels of the evolution chain of the free radicals in the living body (Burati, 2001).

The specialized literature does not provide a great variety of information regarding the quantitative composition in polyphenols of vegetal extracts from the Spermatophyta and Angiospermatophyta plant species, although their therapeutical properties are well documented (Ciulei et. al.,1993).

For these reasons a more detailed study on the composition of hydro-alcoholic extracts that are rich in phenols is necessary.

MATERIAL AND METHODS

The determination of the total polyphenols content from the vegetal sources was carried out by measuring the optical density of a primary extract which mixed with the Folin-Ciocalteu reagent absorbs in the VIS domain at the wavelength $\lambda = 750$ nm.

The reagents used are as follows:

- Ethanol, analytical purity (96% ;40 %)(Merck)
- Folin-Ciocalteu reagent (0,1 N)
- Sodium carbonate (sol. 7,5 %)(Merck)
- Distilled water
- Gallic acid - standard (99% purity)(Roth)

The vegetal extracts were obtained by the following method: vegetal material, previously dried up and finely minced, was subjected to static extraction (maceration) with alcohol (ethanol 96%) for 10 days at room temperature and kept in the dark, using a molar report of 5 : 50 of vegetal material : alcohol (Farmacopeea, 1993).

The obtained extractive solutions were filtered through 4 layers of cloth, the residue was washed with solvent (ethanol 96%) and then brought to 50 mL.

Limpid, specific colored hydro-alcoholic were obtained. The tinctures were stored at different temperatures and in the dark (in order to avoid the degradation of light sensitive substances).

The following materials and instruments were also used:

- pippetes (1, 5, 20 mL);
- flasks (25, 100 mL);
- Kern analytical scale, measuring domain: 0,0001g - 120 g;
- Ultrasound bath TPC-25;
- Biotek multiple detection spectrophotometer, measuring domain: UV-VIS 190-800 nm.

A standard etalon curve was drawn. The total content in polyphenols is expressed using a gallic acid etalon curve.

For standard preparation 25 mg gallic acid are measured using the analytic scale and then the entire quantity is transferred to a 25 mL flask. 15 mL of ethanol 40% is then added and after this the solution is placed in the ultrasound bath resulting a 1mg/mL solution.

The obtained solution is left at room temperature to rest and then is brought to 25 mL using solvent. The final solution is the stock standard out of which 5 dilutions are prepared: 1mg/100 mL; 0,5 mg/100 mL; 0,25 mg/100 mL 0,125 mg/mL and 0,0625 mg/mL.

1 mL standard stock solution is collected using a pipette and is transferred into a 100 mL flask, 60-70 mL distilled water is added, then the solution is agitated; 5 mL Folin-Ciocalteu reagent is added to the solution and homogenized. After 1 minute, but no later than 8 minutes, 15 mL solution sodium carbonate 7.5% is added. This moment is noted as the “0” moment and then the obtained solution is homogenized once more.

The above solution is brought to 100 mL using distilled water. The obtained solution is the 1 mg/100 mL standard dilution. After 2 hours the absorbance at $\lambda = 750$ nm is measured against the blank sample. The blank sample is prepared in the same method as above by replacing 1 mL standard stock solution with 1 mL ethanol 40%.

The same procedure is used for the remaining 4 dilutions, the standard stock solution used being 0.5 mL, 0.25 mL, 0.125 mL and 0.0625 mL respectively.

For the standard curve the measured absorbance in correlation with the gallic acid concentration are represented graphically. The obtained calibration curve for gallic acid is shown in figure 1.

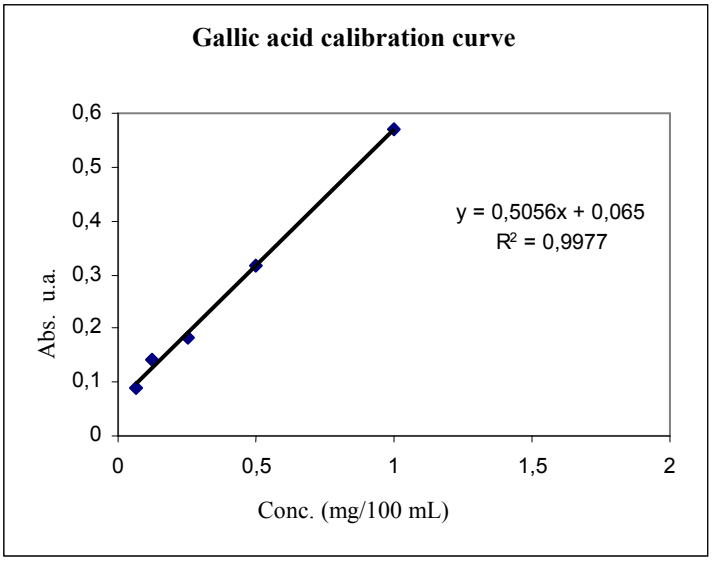

Figura1.

Standard calibration curve for gallic acid

After this the determinations of the total polyphenols from the vegetal samples were carried out.

The alcoholic extracts were filtered through qualitative paper-filter. 1 mL of the filtrate is taken and introduced into a 100 mL flask, 60-70 mL distilled water is added, then the solution is agitated. 5 mL Folin-Ciocalteu reagent is added to the solution and homogenized.

After 1 minute, but no later than 8 minutes, 15 mL solution sodium carbonate 7.5% is added. This moment is noted as the "0" moment and then the obtained solution is homogenized once more. The solution is then brought to 100 mL using distilled water.

After 2 hours the absorbance at λ = 750 nm is measured against the blank sample. The total concentration of polyphenols is calculated using the equation of the standard calibration curve and is expressed as mg/mL vegetal extract.

RESULTS AND DISCUSSIONS

The results obtained for the 7 researched vegetal extracts are shown in table 1.

The results of the quantitative analysis show that a high content in polyphenols is present in the Alchemilla vulgaris (Lady's Mantle), Agrimonia eupatoria (sticklewort) and Solidago virga-aurea (Golden Rod), while the extract prepared from the Acorus calamus (Sweet Flag) roots has the lowest content in polyphenols.

Table 1.

Total content in polyphenols of the researched vegetal extracts

Vegetal extract	**Alchemilla vulgaris (*Lady's Mantle*)**	**Allium ursinum (*Wild Garlic*)**	**Acorus calamus (*Sweet Flag*)**	**Solidago virga-aurea (*Golden Rod*)**	**Agrimonia eupatoria (*sticklewort*)**	**Veronica officinalis (*Heath Speedwell*)**	**Vâscum album (*European mistletoe*)**
Extract absorbance	0,573	0,331	0,240	0,536	0,541	0,300	0,365
extract absorbance – blank absorbance	0,523	0,281	0,190	0,486	0,491	0,250	0,315
Total polyphenols [mg/mL]	0,905	0,427	0,247	0,833	0,842	0,365	0,493

Based on the obtained results one can observe that the content in polyphenols is influenced by the type of vegetal part that was used.

The extracts obtained from the aerial parts of the studied plants (Lady's Mantle, sticklewort and Golden Rod) have a higher content in polyphenols. The high content in phenolic compounds of the Alchemilla vulgaris (Lady's Mantle) and Solidago virga-aurea (Golden Rod) extracts along with the other results obtained and previously published (Condrat et. al.,2009; Condrat et. al.,2007) prove once more that the studied plants are representative in comparison with the other studied plants from the point of view of contained active principles.

CONCLUSIONS

The obtained results could be used as a starting point for the evaluation of the antioxidant potential for the researched extracts against the effects of the free radicals and can contribute to the studies regarding the applicability of these extracts after purification in food, pharmaceutical and cosmetic industry.

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