

EVALUATION OF TOTAL POLYPHENOL CONTENT OF THE EXTRACTS OBTAINED FROM SELECTED ROMANIAN PLANT SPECIES

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ABSTRACT. The study focused on the use of native plants as a source of raw materials to obtain hydroalcoholic extracts by two extraction methods, percolation and respectively Soxhlet. The plants used in the study were: ivy (*Hedera helix*), cocklebur (*Xanthium strumarium*), wormwood (*Artemisia spp.*), sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*). The extracts were chemically characterized to identify bioactive compounds. It was determined their polyphenol acid content (expressed as gallic acid) and flavonoid compounds content expressed in rutin. These are compounds with proven antifungal activity. Observing these values can be seen that both the content in flavonoids and polyphenol acids is higher in species of rosemary, wormwood and cocklebur, which explains their use in the Romanian traditional medicine. This indicates that these extracts have major antifungal properties.

Keywords: hydroalcoholic vegetal extracts, polyphenolic compounds, flavonoids

INTRODUCTION

In recent years could be observed a remarkable interest of the scientific community for herbs because they are an inexhaustible source of raw material for preparing some important phytochemicals. (Ateba et al., 2003).

Importance of current agronomical technology lies in increasing the share of vegetal products in the works and treatments applied in agriculture to about 40-50%. (Amorim et al., 2008; Wong et al., 2006).

Thus, we aimed to study the extracts obtained from five species used in traditional medicine treatments (Aqil et al., 2006), for the isolation of bioactive compounds of plant or constituents that could be exploited in the development of a natural antifungal treatment for the horticultural crops (Dulger et al., 2004; Popa et al., 2011). One of the main advantages of the products that are based on plant extracts is the ability to effectively control the emergence of plant diseases (Sher, 2009). They also have a positive impact on the environment and are not detrimental to plants that undergo treatment. Extraction products have an antifungal effect, in addition to their phytosanitary protective effect (Shan et al., 2005).

Plant based products have a much lower impact on plants and environment than synthetic products. These natural products are particularly active, even when using low concentrations (Nascimento et al., 2000).

The plants studied in this work were as follows cocklebur (*Xanthium strumarium*), ivy (*Hedera helix*), wormwood (*Artemisia spp.*), rosemary (*Rosmarinus officinalis*) and sage (*Salvia officinalis*). These are native plants, cultivated or from wild flora, widespread in our country. They began to be investigated in detail in recent decades due to their complex action (analgesics, antibiotics, antimicrobials) which is a result of their constituent bioactive substances: azulenes and procamazulenes, flavonoids, phytosterols, sesquiterpenes, organic acids, vitamins and minerals. Many of these secondary metabolites have proven antifungal effect. (Katalinić et al., 2004; Farooq, 2005; Li, 2008).

Selection of extracts to be used was suggested by previous research results from the specialty literature, obtained on a much larger number of species and genera of plants (Mahesh et al., 2008).

MATERIALS AND METHODS

Vegetal material

We used five plant species namely: cocklebur (*X. strumarium*), ivy (*H. helix*), wormwood (*Artemisia spp.*), rosemary (*R. officinalis*) and sage (*S. officinalis*) purchased from Plafar Bucharest. These plants were carefully selected based on observations and results of previous research and bibliographic data (Liang et al., 2004; Erdemoglu et al., 2006).

Table 1. Characteristics of the vegetal material used

Nr.Crt.	Common name	Scientific name	Family	Part of plant used
1	Cocklebur	<i>Xanthium strumarium</i>	<i>Asteraceae/Compositae</i>	Herba
2	Ivy	<i>Hedera helix</i>	<i>Araliaceae</i>	Folium
3	wormwood	<i>Artemisia spp.</i>	<i>Asteraceae/ Compositae</i>	Herba

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4	Rosemary	<i>Rosmarinus officinalis</i>	<i>Lamiaceae</i>	Herba
5	Sage	<i>Salvia officinalis</i>	<i>Lamiaceae</i>	Folium

Reagents and equipment

All chemicals and reagents used were of analytical grade or of the purest quality purchased from certified companies. Reagents for standard curves were from ROTH (rutin) and Aldrich (gallic acid). For the Folin reagent was used sodium tungstate, and phosphoric acid (Merck). We have used sodium carbonate, sodium acetate and aluminum chloride (Sigma) and double distilled water.

Absorption spectra were recorded using UV-Vis T60 PG Instruments Ltd spectrophotometer. (Leicestershire, UK).

Plant extracts

In this study, we used two methods of obtaining the plant extracts: percolation and Soxhlet extraction, in order to obtain optimal extraction efficiency of total content of phenolic compounds and flavonoid compounds (Nackz et al., 2004).

The solvent used was ethanol at a concentration of 50 % and a mass of plant material: volume of solvent was 1:10. For each method of extraction were carried out three repetitions. At the end of each extraction process, the extract obtained was filtered under vacuum Buchner funnel using Whatman no.1 filter paper.

In the first case, extraction by percolation, the flow rate must be so arranged that in the 24 hours to percolate an amount of liquid equal to 1.5 x the amount of plant material. In our case, the grounded plant material came in contact with the solvent in counterflow for 24 hours, the rate of leaching is a drop to 5 seconds. To keep their therapeutic properties, the hydro-alcoholic extracts were obtained at room temperature.

Soxhlet extraction method is based on a large difference between the boiling point of the solvent and the extracted bioactive compounds. Based on this property the mixture is brought to the boiling point of the solvent, which will condense a refrigerant and return back into the cartridge containing the vegetal sample to be extracted.

Spectrophotometric analysis

We identified specific absorption areas for wavelengths (Fan et al., 2006; Gong et al., 2009) and were measured the intensity of absorption in accordance with the specifications in the scientific literature (Sakakibara et al., 2003; Wojdylo et al., 2007).

- Range 390-420 nm is attributed to flavonoid compounds and quinones derived by oxidation of polyphenols - flavones

- Range 600-660 nm is attributed to polyphenolic carboxylic acid (Stratil et al., 2006)

1. Determination of total polyphenolic compounds

Quantitative determination of polyphenol acids was achieved by adapting the classical Folin- Ciocalteu method (Singleton et al., 1965; Singleton et al., 1977;

Singleton et al., 1999) because it is simple, specific and rapid. The method is based on oxidation reaction with Folin phenol groups in a highly alkaline environment (Vermerris et al., 2006).

Were used the following reagents: Folin reagent and 20% Na₂CO₃ solution. The product obtained is colored blue and the absorbance is measured at $\lambda = 660$ nm. Concentration of polyphenolic compounds from the extracts was calculated using a standard curve made for gallic acid. Standard curve was established in parallel and under the same conditions as the sample, for 0,1 - 1mg GA / ml.

For each test sample were prepared dillution of 1:50 and were performed 3 repetitions.

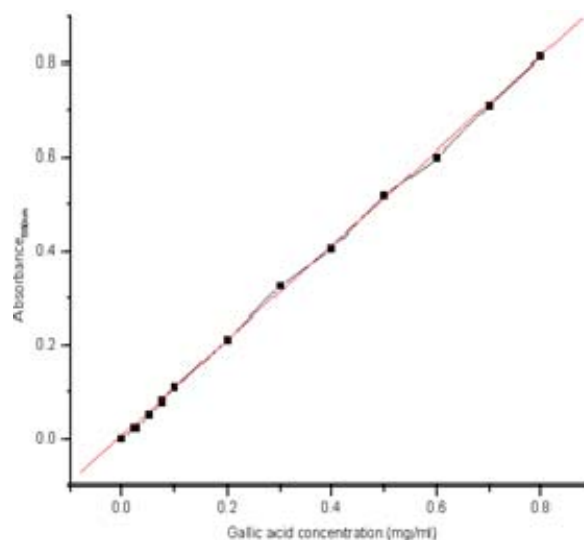


Figure 1. Standard curve for gallic acid

The standard curve of gallic acid:

Linear regression was $Y = 0.00888 + 1.01063 * X$

where Y is the absorbance and X is the concentration of polyphenol acids (mg GAE/100ml) .

The calibration curve has good linearity with a regression coefficient $R = 0.99744$, Standard deviation $SD = 0.02146$ and difference statistically not significant $P < 0.0001$.

2. Determination of the total concentration of flavonoids

The total content of flavonoids was determined using a colorimetric method based on their reaction with aluminum chloride (Chu et al., 2000; Kim et al., 2003; Martens et al., 2005; Dai et al., 2010)

The method involves incubation in the dark at room temperature of 3 parallel samples, previously diluted 1:50. After incubation in the dark, at room temperature for 45 minutes, the absorbance of the reaction sample was measured spectrophotometrically at $\lambda = 420$ nm against a blank: 2,5 ml CH₃COONa 10% with 2 ml

AlCl₃ 2,5% and 5,5 ml distilled water (Harbona et al., 2000; Miliuskas et al., 2004).

The content of flavonoids in plant extracts was calculated using a standard curve, established in parallel under the same conditions with the sample solution, for 0,1 - 1 mg rutin/ml.

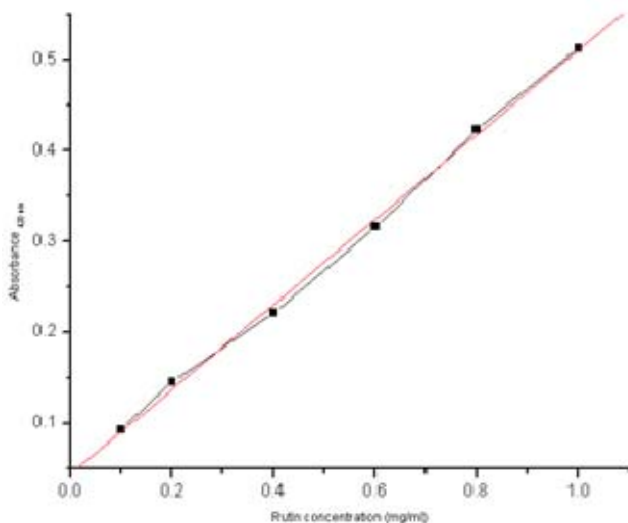


Figure 2. Standard curve for rutin

Standard curve for rutin:

Linear regression equation was $Y = 0.42551 + 0.46764 * X$,

where Y is the absorbance and X concentration of flavonoid compounds (mg RE/100ml).

The calibration curve has good linearity with a regression coefficient $R = 0.99884$, standard deviation $SD = 0.08803$, the difference statistically not significant $P < 0.0001$.

Statistical analysis

Each experiment was performed in triplicate. Mean values and standard deviations were calculated using Excel (Microsoft USA) and the data obtained were processed using programs UVWin5 Software v5.2.0 and Origin v 6.0 (MicroCal USA)

RESULTS AND DISCUSSIONS

In the following are presented the results obtained from the quantitative determination of total polyphenols and total flavonoids of the hydroalcoholic plant extracts studied, and the dry weight of the extracts.

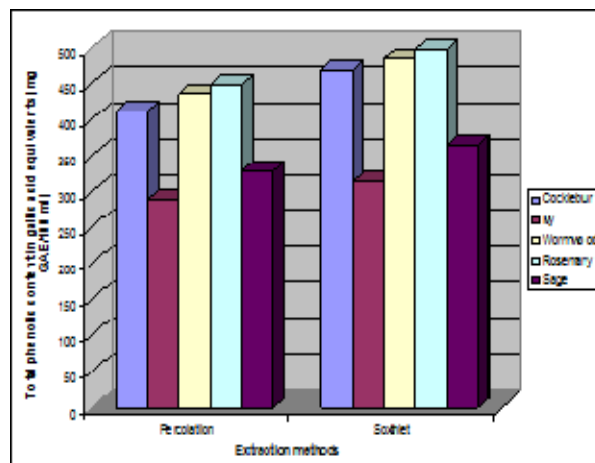


Figure 3. Total content of the polyphenolic acids from the plant extracts obtained by the two extraction methods

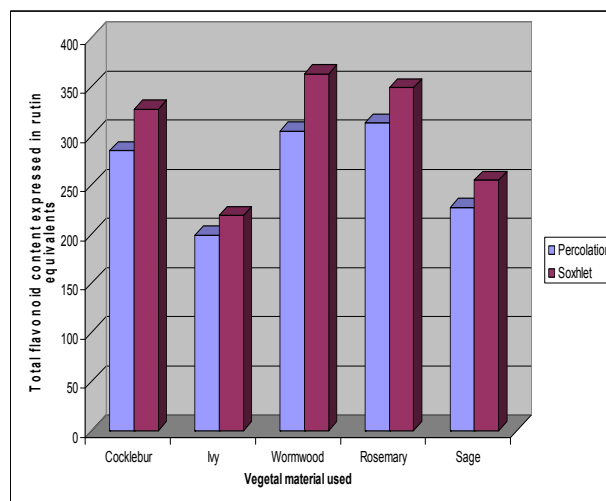


Figure 4. Total content of flavones

Table 2. Total content of dry weight

Nr.crt.	Plant used	d.w. (%)	
		Percolation	Soxhlet
1	cocklebur	4.84	4.32
2	ivy	3.98	3.67
3	wormwood	4.67	4.29
4	rosemary	3.87	3.24
5	sage	3.82	3.21

Generally, the results showed that the total bioaccumulation of polyphenolic compounds expressed in gallic acid are almost equal between the two extraction methods, with a slight increase in Soxhlet extraction.

Regarding the biosynthesis of flavonoid compounds expressed in rutin equivalent the results were lower than the values of polyphenolic acids, but almost equal between the two extraction methods.

Explanation would be that the flavonoids in plants vary from year to year, even if the location is the same, depending on the duration and intensity of solar light because they are screen substances from UV. To the extent that the plant does not require a high level of flavonoids (in years with rainy summers and cloudy), the amount of polyphenol acids increases, both groups share the same active ingredients having biogenetic precursors (Schmidt, 2008). Observing these values can be seen that both the content in flavonoids and polyphenol acids is higher in the species of rosemary, wormwood and cocklebur, which explains their use in Romanian traditional medicine.

CONCLUSIONS

Observing the results we were able to conclude the following:

- extracts that presented the highest phenolic content (300-400 mgGAE/100ml) showed also high content in flavonoids (300-400 mg RE/100 ml) and a correlation with antifungal activity (Rice-Evans et al., 2003; Williams et al., 2004);
- the high content of bioactive compounds indicate that they are major contributors to the antifungal properties of these plants.

Our results suggest the high value of these species studied for their use in phytoprotection. Based on this information, it can be concluded that plants are natural sources of antifungal substances of great importance.

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