ORIGINAL PAPER

OBSERVATION OF THE EXTRACTION DYNAMICS OF POLYPHENOLIC COMPOUNDS FROM *ROSMARINUS OFFICINALIS* AND *SALVIA OFFICINALIS* IN NATURAL WINE

MIHAELA MIRELA BRATU[1], SEMAGHIUL BIRGHILA[2]*, MARIUS DANIEL RADU[3], ANTOANELA POPESCU[1]

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Abstract. *This study aims to highlight the extraction capacity of natural wine for polyphenolic acids from sage (Salvia officinalis L.) and rosemary (Rosmarinus officinalis L.). At the same time, the dynamics of the extraction process of polyphenolic acids, the evolution of the anthocyanin content and the antioxidant activity of the extract over 21 days were monitored. Observation of the extraction dynamics of polyphenolic acids from the selected plants showed that the wine has moderate extractive qualities for this class of compounds. Thus, some phenolic acids of major interest for antioxidant activity were less extracted (gallic acid, 3-methyl gallic acid, and chlorogenic acid) while other acids (p-coumaric acid, ferulic acid) showed better extraction. Also, during extraction, degradation of anthocyanin pigments in wine could be observed, which reduces the therapeutic value of extracts prepared by traditional methods. The chemical changes that occurred during the extraction process showed that a reduction of the extraction period to 8-11 days would be suitable for this type of preparations; thus, the development of degradation processes of polyphenolic compounds can be avoided.*

Keywords: *Rosmarinus officinalis L; Salvia officinalis; phenolic compounds; medicinal wine.*

1. INTRODUCTION

The practice of preparing herbal macerates in wine or other alcoholic solutions dates back to antiquity. For example, medical papyri from ancient Egypt mention various herbal macerates in date wine as therapeutic products [1]. The ancients observed that hydroalcoholic solutions have the property of extracting and concentrating different classes of phytocompounds, such as polyphenols, alkaloids, and essential oils. An example of the application of this observation is the preparation of hydroalcoholic extracts of alkaloids with euphoric and psychotropic properties from Egyptian blue lotus flowers, mentioned in ancient Egyptian papyri [2]. Also, amphorae containing wine residues in which plants such as: *Artemisia seibeni, Tanacetum annuum, Teucrium, Mentha, Salvia, Cassia, Rosmarinus officinialis* [3] were discovered in ancient Egyptian tombs. Later, in Ancient Greece,

[1] Ovidius University of Constanta, Department of Pharmaceutical Sciences II, 900470 Constanta, Romania. E-mails: antoniapopescu2002@yahoo.co.uk; antoniapopescu2002@yahoo.co.uk.
[2] Ovidius University of Constanta, Department of Chemistry and Chemical Engineering, 900527 Constanta, Romania.
**Corresponding author: sbirghila@univ-ovidius.ro.*
[3] Ovidius University of Constanta, Faculty of Natural and Agricultural Sciences, Department of Natural Sciences, 900470 Constanta, Romania. E-mail: drd_maryus@yahoo.com.

throughout the Roman Empire, but also in ancient China, wine was produced and used not only for recreational purposes, but also as part of medical therapies. For this, various medicinal herbs were macerated in wine, honey, incense, hashish or other products were added [4, 5].

Traditionally, various preparations resulting from the maceration of medicinal herbs in natural wine are still used, supposed to have tonic, digestive, invigorating or, if necessary, tranquilizing effects [6, 7]. Rosemary (*Rosmarinus officinalis L.*) and sage (*Salvia officinalis L.*) are spice plants commonly used in traditional cooking. In both plants, which belong to the same family (*Lamiaceae*), there are large amounts of polyphenolic compounds, such as: polyphenolic acids, phenolic diterpenes, flavonoids, having antitumor and antioxidant properties [8].

Grape wine also contains a wide range of polyphenolic compounds and organic acids with antioxidant properties, which bring many health benefits when consumed in small or moderate amounts [9-13]. The principle underlying the production of therapeutic preparations called medicinal wines is the ability to extract polyphenols from plants macerated by the hydroalcoholic phase (wine), enriching the preparation in compounds with antioxidant action. However, it has been observed that, as maceration takes place, the anthocyanin molecules in the wine undergo an oxidation process, transforming into tannins [14]. Therefore, step-by-step monitoring of the process is necessary to determine the optimal maceration time, so that the extract contains the maximum number of polyphenolic compounds, the least number of oxidized anthocyanins and the highest antioxidant activity.

Our work aims to highlight the extraction capacity of natural wine with 10 degrees alcohol for polyphenolic acids from sage (*Salvia officinalis L.*) and rosemary (*Rosmarinus officinalis L.*). The dynamics of the extraction process of polyphenolic acids, the evolution of the anthocyanin content and the antioxidant activity of the extract over 21 days were also monitored.

The final aim was to observe the chemical changes that occur during the extraction process, in order to determine the optimal time for the maceration process. The aim was also to determine the final composition of the macerate to certify the possible therapeutic effects supported by traditional practice.

2. MATERIALS AND METHODS

2.1. MATERIALS

All phenolic standards and solvents used in the present work were HPLC-grade (purity >99%). Methanol, ethanol, acetonitrile were purchased from Sigma Aldrich (Steinheim, Germany). 2,4,6-Tripyridyl-s-triazine (TPTZ) and Folin–Ciocalteu reagent were obtained from Merck (Darmstadt, Germany). *Salvia officinalis* and *Rosmarinus officinalis* dried leaves were purchased from a pharmacy shop located in Constanta, Romania.

2.2. METHODS

Preparation of extracts in wine

Red wine (Vitis × labruscana) with 10% alcohol content, prepared without added sulphites, was used as the extractive phase. In 100 mL of wine 0.25 g of powdered dried rosemary leaves and 0.25 g of powdered dried sage leaves were suspended. The prepared suspensions were kept at 4°C in the dark with daily shaking. Three suspensions were similarly prepared, which were kept macerated and chemically analysed daily for 21 days. Finally, the polyphenolic acid content of the three macerates was analysed by HPLC.

Determination of Total Phenolic Content

The total phenolic content was determined spectrophotometrically (Jasco V-630, Japan) according to the method reported by Pereira da Silva et al. [15]. Absorbance was read at 725 nm and TPC content, expressed in gallic acid equivalent (GAE/L) were calculated from calibration curve (range 1^{-10} mg·L^{-1}; R^2 = 0.9990).

Determination of anthocyanins

The determination of total monomeric anthocyanins content and polymerised compounds percentage in the control wine and the two wine macerates was performed according to the pH differential method [16].

Ferric Reducing Antioxidant Power (FRAP) Assay

The reducing capability of ethanolic extracts was measured as ferric reducing antioxidant power (FRAP) as described by Benzie and Strain [17]. The fresh working solution was prepared by mixing 300 mM acetate buffer (pH=3.6) with 10 mM TPTZ solution (dissolved in 49 mM HCl) and 20 mM $FeCl_3$ solution (10:1:1, v/v/v). The plant extract to be analysed was first adequately diluted to fit within the linearity range (0.8–16.6 mM/L). The absorbance value was measured at 593 nm (JascoV-630 UV-VIS spectrophotometer) after 30 minutes incubation at 37°C. The results were calculated using regression equations (y=297.03x; R^2=0.9953) and expressed as mM Trolox equivalents/L extracts. All determinations were performed in triplicate.

Determination of phenolic compounds by HPLC

Chromatographic analyses of phenolic compounds were performed on an Agilent 1200 HPLC system, equipped with a diode array detector (DAD), quaternary pump, and an autosampler (Agilent Technologies, Santa Clara, CA, USA), using a reference method (USP 30-NF25, 2007), [18]. Phenolic acids were separated on a Zorbax XDB C18 analytical column (250 mm x 4 mm, i.d. 5 µm sizes) maintained at 35^0C. The mobile phase used in the analysis consisted of 0.1 % phosphoric acid in water (solvent A) and acetonitrile (solvent B). The solvent gradient conditions for phenolic compounds in volume ratio were the following: 0-13 min 10% B, 13-14 min 22 % B, 14-17 min 40 % B, 17-17.5 min 10% B and 17.5-22 min 10% B. The injection volume was 20 µL and the flow rate was 1.5 mL/min and the chromatogram was recorded at 310 nm. Calibration curves were built for each of the compounds by injecting the standards at six different concentrations (0.22-0.50 mg/mL). The performance of the chromatographic method was verified by evaluating some parameters:

linearity (R^2), limit of detection (LOD), limit of quantification (LOQ) and precision (LOQ) (Table 1).

Table 1. Performance parameters of the chromatographic method.

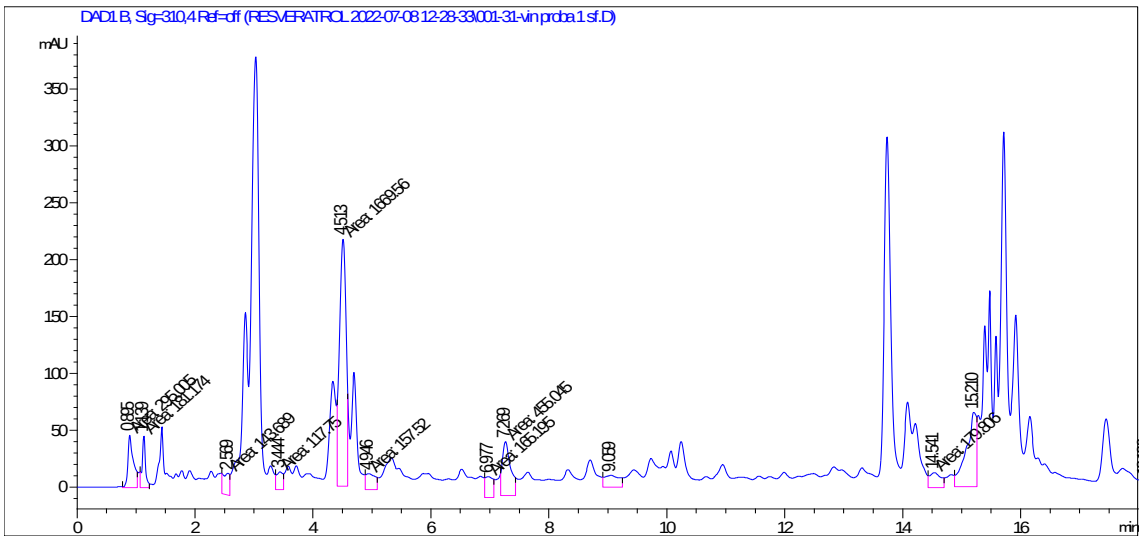
No.	Compound	Retention tine [min]	Linearity [R^2]	LOD [mg/L]	LOQ [mg/L]
1	Gallic acid	0.990 ± 0.025	0.99965	0.13	0.39
2	3-Methylgallic acid	2.606± 0.008	0.99729	0.11	0.34
3	Chlorogenic acid	3.501 ± 0.015	0.99999	0.12	0.37
4	Caffeic acid	4.598 ± 0.036	0.99619	0.12	0.36
5	Syringic acid	5.054± 0.021	0.99999	0.11	0,34
6	p-Coumaric acid	7.187± 0.019	0.99691	0.17	0.51
7	Ferulic acid	8.565± 0.058	0.99537	0.16	0.48
8	*E* - resveratrol	14.467 ± 0.017	0.99863	0.12	0.37
9	Ellagic acid	15.303± 0,027	0.99885	0.14	0.43
10	Cinnamic acid	15.867 ± 0,007	0.99563	0.19	0.58

The accuracy of the method was evaluated by the recovery test; most compounds showed values between 85 and 95%, acceptable within the limits specified by ANVISA guidelines [19].

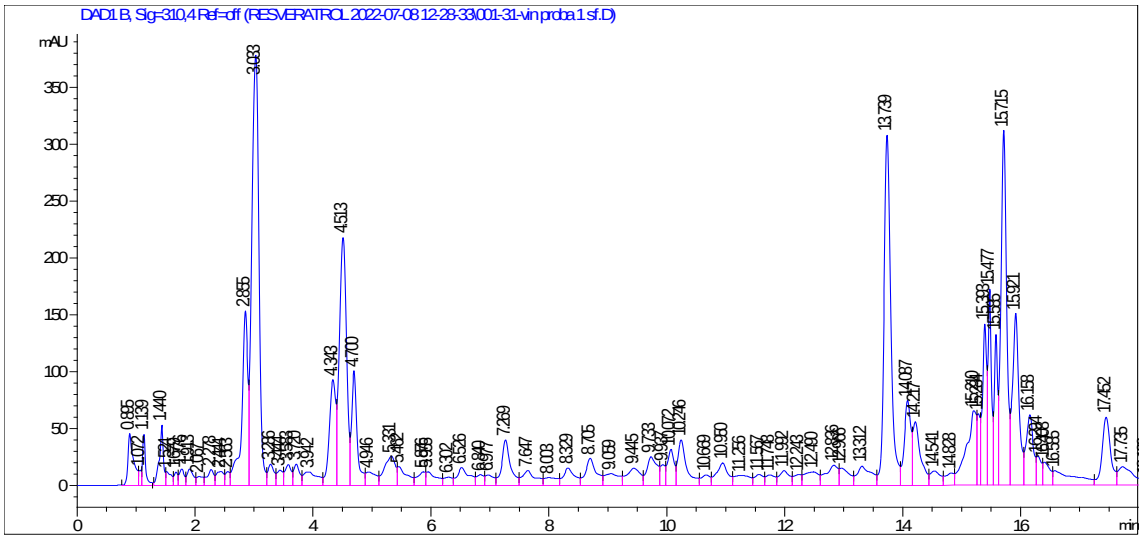
3. RESULTS AND DISCUSSION

3.1. RESULTS

Phenolic acids including hydroxybenzoic acids (i.e., gallic and 3-metil gallic) and hydroxycinnamic acids (i.e., chlorogenic, cinnamic, caffeic, p-coumaric, ellagic, syringic, *E* – resveratrol and ferulic) were identified by comparison with the retention times of standards under identical conditions (Table 1). Typical HPLC-DAD chromatograms for *Salvia officinalis* L., *Rosmarinus officinalis* L., control wine and medicinal wine are presented in Fig. 1. The quantitative data were calculated from their respective calibration curves and amounts of identified phenolic compounds by chromatographic method are summed up in Table 2. Fig. 2 shows the dynamics of the polyphenolic acid extraction process, the evolution of the anthocyanin content and the antioxidant activity of the extract over 21 days.



a)



b)

Figure 1. Typical HPLC-DAD chromatograms of phenolic compounds in control wine (a) and medicinal wine (b).

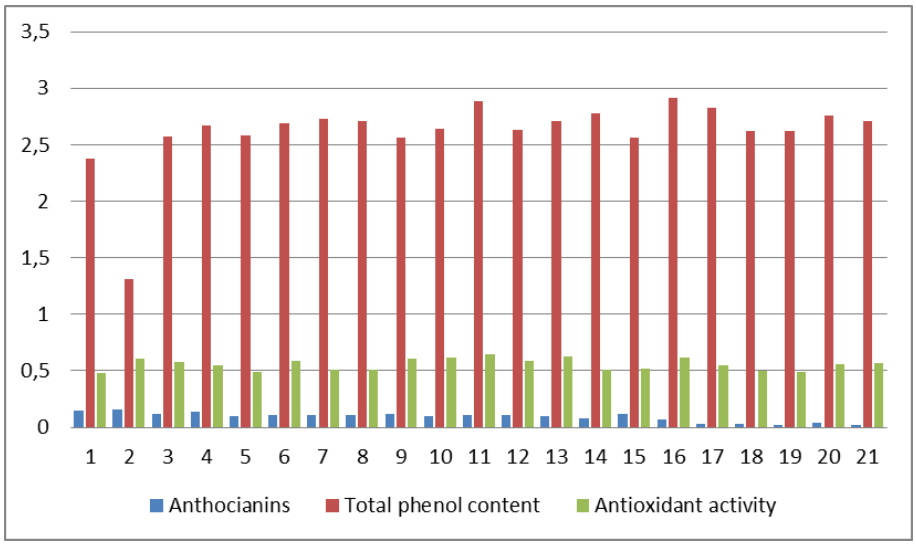


Figure 2. Dynamics of the extraction process of phenolic compounds

Table 2. Phenolic acids (mg/L, means ±SD, n=3)

No.	Compound	*Salvia officinalis* L.	*Rosmarinus officinalis* L.	Control wine	Medicinal wine
1	Gallic acid	143.744±12.37	362.355±18.45	38.598±5.12	98.159±8.74
2	3-Methylgallic acid	-	26.542±3.21	15.483±2.78	15.913±3.02
3	Chlorogenic acid	-	51.440±6.18	1.657±0.28	4.970±1.09
4	Caffeic acid	5.556±1.26	-	11.269±2.01	43.335±4.67
5	Syringic acid	-	-	5.376±1.32	5.307±1.05
6	p-Coumaric acid	2.918±0.98	4.086±1.45	-	11.547±2.19
7	Ferulic acid	2.218±0.78	-	-	5.897±1.65
8	*E* - resveratrol	-	-	8.661±2.43	9.007±2.79
9	Ellagic acid	-	-	-	1001.654±59.87
10	Cinnamic acid	-	-	-	170.182±13.27

3.2. DISCUSSION

There was a slight increase in antioxidant activity measured by the FRAP method, which peaked at mid-interval (starting on day 9). After day 14, the values decreased slightly but remained high. Compared to the antioxidant activity of pure wine, sage and rosemary extracts in wine showed higher activities until the end of the extraction. This may be due to compounds extracted from the macerated plants, of which polyphenolic acids make an important contribution to the increased antioxidant activity.

As in a previous experiment [14], we observed that during the maceration period the concentration of total monomeric anthocyanins in the wine decreases sharply, reaching almost zero on day 21. The explanation could be the action of polyphenol oxidases from the plant products subjected to maceration, capable of transforming anthocyanin molecules into polymeric compounds without therapeutic value. The fact that the extraction process was carried out in the dark rules out the hypothesis that the degradation of anthocyanin pigments in wine was due to exposure to light. Another cause of the accelerated degradation of anthocyanins during extraction could be the stirring process to which the extracts were periodically subjected, which increased the concentration of oxygen in the environment, thus favouring the oxidation process. The general mechanism of oxidation involves a first step in which the oxygen molecule accepts electrons from Fe(II) and Cu(I) ions, changing into a superoxide ion (hydroperoxyl radical OH-O´ at wine pH). Phenolic compounds with catechol group are oxidised to quinones, the hydroperoxyl radical changing to a peroxide radical [20, 21].

The total polyphenolic content recorded higher values than the pure wine used as extractive phase. The exception was day 2 of extraction, when a lower value was recorded. As in the case of antioxidant activity, the values of total polyphenolic content tended to increase slightly until the end of extraction. The dynamics of the variation of total polyphenolic content values could not be correlated with that of antioxidant activities. Changes in the structures of polyphenolic compounds in the prepared extracts compared to polyphenolic compounds identified in pure wine may be due to the accelerated oxidation process triggered by periodic stirring of the extracts, which led to a higher exposure to oxygen [22].

As a conclusion, the observation of the extraction dynamics of polyphenolic acids from sage and rosemary using natural wine with 10% alcoholic content strength as the extraction phase demonstrates that wine has moderate extractive qualities for this category of compounds. Also, during extraction, degradation of anthocyanin pigments in wine could be observed, which decreases the therapeutic value of extracts prepared by traditional methods.

The natural wine used in the experiment, without the addition of antioxidants, degraded slightly during the process, generating anthocyanin degradation products without therapeutic value. Probably a shortening of the extraction period to 8-11 days would be appropriate for this type of preparations, in order to avoid to some extent the development of degradation processes of polyphenolic compounds. Considerable variation was observed in the phenolic profiles of the selected spices, with gallic acid being the most prevalent phenolic compound.

The phenolic constituents in *Salvia officinalis L.*, were identified as gallic acid, caffeic acid, p-coumaric acid and ferulic acid. In *Rosmarinus officinalis L.,* chlorogenic acid is a hydroxycinnamic acid identified in a higher amount. It has been reported that chlorogenic acid shows moderate antioxidant activity and therefore low free radical scavenging power. [23]. The *Lamiaceae* (rosemary, sage) are known plants containing a number of phenolic acids such as gallic acid, caffeic acid or its derivatives (rosmarinic or chlorogenic) p-coumaric, ferulic [24]. It was reported that rosmarinic acid (RA) is the main phenolic

component in *Rosmarinus officinalis* and *Salvia officinialis* in concentrations varying between 0.05 and 26 g/kg dry weight [25]. Other studies have indicated that gallic acid is present in *Rosmarinus officinalis*, caffeic acid is found in *Salvia officinalis L*., and p-coumaric acid is found in *Origanum vulgare L*. [26]

The analysis of polyphenolic acids extracted in wine during maceration leads to the conclusion that some compounds of major interest for antioxidant activity were less extracted (gallic acid, 3-methyl gallic acid, and chlorogenic acid) while other compounds (p-coumaric acid, ferulic acid) showed better extraction. Also, some polyphenolic acids, which were below detection limits in the control wine, were concentrated in the macerated samples (ellagic, ferulic and cinnamic).

It can be stated that wine behaves as a selective extraction phase for phenolic compounds. Although gallic acid was present in significant amounts in the spices that have been used, its contribution to the final product is very small; probably the maceration period led to the formation of other undetected compounds.

4. CONCLUSIONS

Extraction dynamics of polyphenolic acids from sage and rosemary using natural wine as the extraction phase showed that wine has moderate extractive qualities for this category of compounds. Degradation of anthocyanin pigments in wine was observed, which decreases the therapeutic value of extracts prepared by traditional methods. Oxidation processes during extraction led to changes in the structure of polyphenolic compounds in medicinal wine. By establishing an optimal extraction period for this type of preparations, the development of degradation processes of polyphenolic compounds could be reduced.

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