Pol. J. Environ. Stud. Vol. 31, No. 4 (2022), 3279-3285
DOI: *10.15244/pjoes/145617* ONLINE PUBLICATION DATE: 2022-04-13

Original Research

Evaluation of the Polyphenol Content and Antioxidant Activity of Wine Macerates (Medicinal Wines) With Sage (*Salvia Officinalis* L. *Lamiaceae*) and Sea Rush (*Juncus Martitimus* Lam. *Juncaceae*) Obtained Using Traditional Technology

Antoanela Popescu[1], Semaghiul Birghila[2]*, Marius Daniel Radu[3], Mihaela Mirela Bratu[1]

[1]Department of Pharmaceutical Sciences II, Ovidius University of Constanta, 6, Aleea Universitatii, Campus 1, 900470 Constanta, Romania
[2] Department of Chemistry and Chemical Engineering, Ovidius University of Constanta, 124, Mamaia Avenue, 900527 9 Constanta, Romania
[3]Department of Natural Sciences, Faculty of Natural and Agricultural Sciences, Ovidius University Constanta, 900470, Romania

Received: 8 October 2021
Accepted: 8 January 2022

Abstract

Two variants of medicinal wine were prepared, through the maceration of sage leaves (*Salvia officinalis L. Lamiaceae*) and sea rush rhizomes (*Juncus maritimus Lam. Juncaceae*) in natural red wine for 21 days. The ratio of dried plants added to wine was 0.1 % w/w for each in the first extract (medicinal wine 1) and 0.25% w/w for each in the second one (medicinal wine 2). The extraction conditions were similar in both products. Subsequently the two macerates were filtered and subjected to analysis (total polyphenols content Folin-Ciocalteu), monomeric anthocyanins content, polymerised compounds percentage, polyphenolic acids by HPLC and antioxidant activity (PHOTOCHEM). The two medicinal wines contain high amounts of polyphenols but also anthocyanins from the wine. Both medicinal wines contain higher polymerised compounds percentage given to the red wine most likely due to the polyphenol oxidases from dried plants. The increase of the antioxidant activities of the two macerates rendered to the raw wine corelates well with the increase of polyphenol amount

*e-mail: sbirghila@univ-ovidius.ro

during the extraction process in the two products. The wine behaves as a good selective extraction phase for some of the polyphenolic compounds.

Keywords: herbal wine, traditional technology, *Juncus maritimus*, *Salvia officinalis*

Introduction

The preparation and use of herbal wines (medicinal wines) have a long tradition in the empirical medicine starting from the ancient Egyptian culture and Ancient Oriental medicine [1, 2]. The use of alcoholic drinks with herbal extracts is also documented in European pharmacy since the 1st century CE [3]. In the late Victorian period, a marketing strategy of wine sellers re-launched the „special wines for the use of invalids" which contains coca, quinine or other plants with tonic effects [4]. During time, a wide range of herbs and tree resins were used to prepare medicinal wines and the recipes varied from country to country, from culture to culture and continued until nowadays [5, 6]. The medicinal plants proved to be efficient for health, due to the bioactive compounds extracted from the plants subjected to maceration [7, 8].

Sage (*Salvia officinalis* L.) is used as spice in foods and beverages as well as in herbal medicinal products. Depending on the purposes, the use of sage leaves is regulated by European law either as food or as medicine [9, 10]. These precautions were taken due to the recent toxicological review on thujone, which is a neurotoxic constituent of sage essential oil. Recent studies, however, see no risk associated with the occasional use of sage in food or medicine (especially in the traditional use as herbal tea) [11].

Other studies suggest the involvement of common and ubiquitous estrogenic flavonoids found in *Salvia officinalis* in the anti-hot flush effect [12].

Sea rush (*Juncus maritimus* Lam.) is a plant commonly found in sand soils, salt marsh, on the sea coast or sand dunes. It contains high quantities of polyphenolic compounds [13, 14]. Our work revealed a high content of polyphenols mainly resveratrol in the rhizome [15]. Recent studies found antitumoral properties of *Juncus* extracts [16]. Due to the resveratrol content in the vegetable product, we used the *Juncus maritimus* rhizomes for the fabrication of medicinal wines.

In the present paper, two types of medicinal wines, containing sage leaves and *sea rush rhysoma,* were analysed in terms of polyphenolic compounds content and antioxidant activity; the two medicinal wines were prepared in two different mass ratios, according to traditional methods. The purpose of the article is to verify the ability of the wine to extract the polyphenolic compounds in sage and sea rush rhizomes, to observe the structural modifications of some polyphenolic compounds during the extraction process and to determine the antioxidant activity of medicinal wines that are obtained, according to the quantity of plants subjected to maceration.

Material and Methods

All chemicals and reagents used in this study were obtained from Merck and ChromaDex unless otherwise specified. All solvents and chemicals for this investigation were analytical and HPLC graded.

Plant Material

Salvia officinalis dried leaves were purchased from a pharmacy shop located in Constanta, Romania. *Juncus maritimus* rhizome were collected from Vadu village, Constanta County, in September 2018. The rhizomes were washed and dried in warm air. The botanical identification was performed according to the protocols [17] (Fig. 1). For the chemical analysis of the vegetal products, the ethanolic extracts were prepared using 10 g grounded dried plant with 100 mL 50% ethanol under reflux for 6 hours (Table 1). The extracts were then filtered and kept at room temperature in the dark until analyse.

Herbal Wine (Medicinal Wine) Preparation

As extraction phase, natural red wine (Vitis × labruscana) with 10% alcohol content with no added sulphites was used. Wine without the addition of plants was considered a control wine. The dried and ground sage leaves and sea rush rhizomes were suspended in wine in ratios of 0.1% w/w each (medicinal wine 1) and 0.25% w/w each, respectively (medicinal wine 2). Both extracts were stored in darkness at 20ºC and occasionally stirred for 21 days. The extraction process was repeated three times.

Hydro Alcoholic Plant Extracts

10 g grounded dried sage leaf was extracted with 50% ethanol (w/v) under reflux for 6 hours. The extract was then filtered and kept at room temperature in the dark until analyse. 10 g grounded dried sea rush rhizome was extracted with 50% ethanol (w/v) under reflux for 6 hours. The extract was then filtered and kept at room temperature in the dark until analysis.

Determination of Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu reagent according to the method reported by Pereira da Silva et al. [18]. For this experiment, an aliquot (1 mL) of sample was added

a) b)

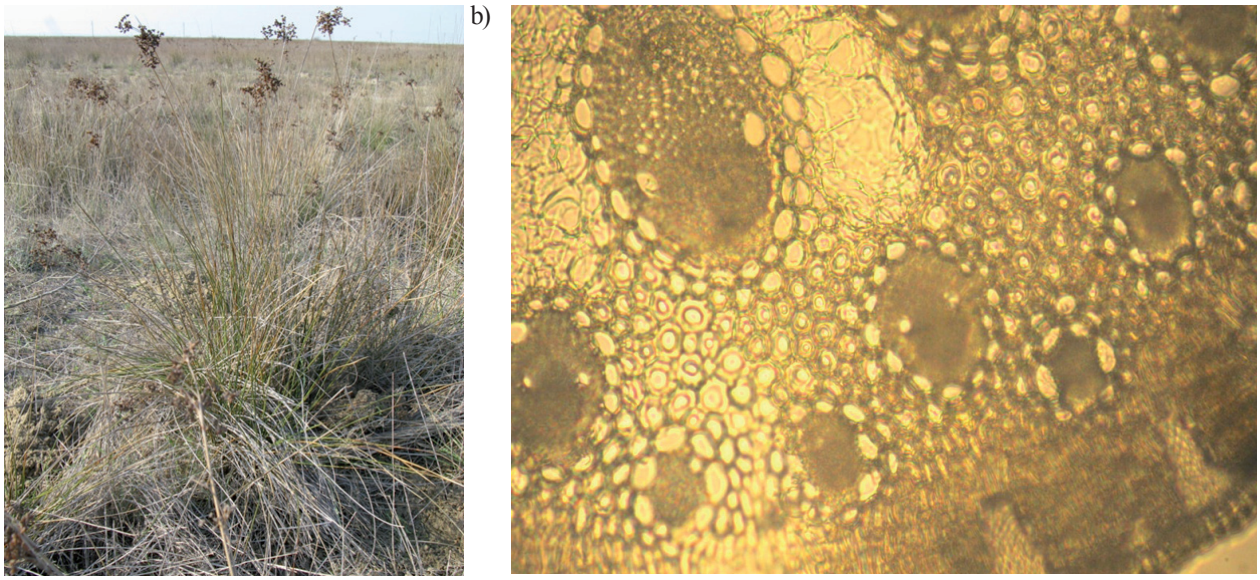

Fig. 1. a). Sea rush (*Juncus maritimus* Lam.) in the field, b) Transversal section trough the *Juncus maritimus* Lam. stem (2 x 20).

to 1 mL of Folin-Ciocalteu reagent (diluted 1:1 with distilled water), and the mixture was added to 25 mL distilled water (solution B). Then, 1 mL of solution B was mixed with a 20% sodium carbonate solution to a total of 5 mL. After 40 min of storage in the dark at room temperature, the absorbance was measured at 725 nm, using a UV-VIS spectrophotometer (Jasco V-630, Japan). Phenolic content of the wine sample was expressed as mg·L^{-1} GAE, using a calibration curve in the concentration 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 [19, 20].

HPLC Analysis of Phenolic Compounds

Chromatographic analyses of common phenolic compounds were performed on an Agilent 1200 HPLC system equipped with a diode array detector (DAD), and auto-sampler (Agilent Technologies, Santa Clara, CA, USA) using a reference method [21]. Phenolic acids were separated on a Zorbax XDB C18 analytical column (250mm × 4 mm, i.d. 5 µm) maintained at 35ºC. The mobile phase used in the analysis consisted of 0.1% phosphoric acid in water (solvent A) and acetonitrile (solvent B). Gradient elution was programmed according to the following scheme: 0-13 min, 10% B; 13-14 min, 22% B; 14-17 min, 40% B; 17-22 min, 10% B. The injection volume was 20 µL, the flow rate 121 was 1.5 mL min-1, and the chromatograms were recorded at 310 nm.

In order to evaluate the accuracy of the method, the following parameters were evaluated: linearity (R^2), limit of detection (LOD), limit of quantification (LOQ), and recovery coefficients (Table 2).

Calibration curves were created for each compound by injecting standards (concentration of stock solution ranged between 0.22 and 0.50 mg mL^{-1}) at six different concentrations.

The recovery efficiency of the method was evaluated by the analysis of filters spiked with a known concentration of standard phenolic compounds. Most of the compounds provided high recoveries with mean values ranging between 80 to 95%, acceptable within the limits specified by the guidelines of ANVISA (2003b) [22].

Table 1. Chemical analysis of vegetal products used for the preparation of medicinal wines.

Vegetal plant	Total phenol content (TPC) (mg·L^{-1})	Antioxidant Activity AA (mmol of Trolox eqv· L^{-1})	Polyphenolic compounds (mg·L^{-1})				
			E-resveratrol	Chlorogenic acid	Caffeic acid	Vanillin	Gallic acid
Juncus maritimus	5600±210^{d}	84.91±5.12^{c}	8.25±1.40^{c}	25.74±2.10^{d}	8.29±1.20^{b}	16.20±2.50^{c}	335.70±16.47^{d}
Salvia officinalis	6450±340^{d}	14.69±1.40^{b}	-	52.25±3.17^{c}	58.73±4.14^{d}	-	755.56±24.19^{d}

Data are expresed as mean±standard deviations. Different letters indicate statistical significance at the $p<0.05$ level for each concentration

Table 2. Retention time and linearity of curves.

Nr. Crt.	Compound	Retention time (min)	Linearity (R^2)	LOD $mg \cdot mL^{-1}$	LOQ $mg \cdot mL^{-1}$
1.	*E* - resveratrol	14.46	0.9996	0.12	0.37
2.	*Z* – resveratrol	15.75	0.9972	0.07	0.22
3.	Caffeic acid	4.59	0.9961	0.12	0.36
4.	Chlorogenic acid	3.50	0.9999	0.12	0.37
5.	Cinnamic acid	15.86	0.9984	0.19	0.58
6.	Vanilin	6.91	0.9969	0.14	0.42
7.	Gallic acid	0.99	0.9953	0.13	0.39
8.	Ferulic acid	8.56	0.9986	0.16	0.48
9.	Ellagic acid	15.30	0.9988	0.14	0.43
10.	p-Coumaric acid	7.18	0.9979	0.17	0.51
11.	3-Methylgallic acid	2.60	0.9956	0.11	0.34

Determination of Antioxidant Activity Using Chemiluminometry

The antioxidant activity of the product was measured using a photochemiluminometer PHOTOCHEM, Analytik Jena AG, Germany, according to the recommended protocols [23]. The antioxidant activity of the samples were quantified using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as calibration standard in a range of values 1-3 $nmol \cdot mL^{-1}$ ($y = 3.1062x$; $R^2 = 0.9986$). Each sample was analysed in triplicate and the results were expressed as mm Trolox $equiv \cdot L^{-1}$.

All results were expressed as mean±standard deviation (SD) of triplicate determinations. The correlation values were assessed using Pearson correlation. Differences at $p<0.05$ were considered significant.

Results and Discussion

Total Phenolic Content and Antioxidant Activity

Total phenolic content (TPC) is an important parameter widely used for the evaluation of wines and other foods. The concentration and composition of the phenolics present in wines largely depend on the source of fruit, environmental factors in vineyard, and the method of wine-making [24]. Most of the phenolic compounds are concentrated in the grape skin, and therefore, greater concentrations of phenolics can be expected in red wines. The values of total phenolic content and antioxidant activity for the two medicinal wine samples are presented in Table 3. It was observed that a higher amount of TPC was found in the medicinal wines given to the wine used as extraction phase.

The phenolic compounds are considered a major contributor to the antioxidant activity of wines [25]. The antioxidant activity of the medicinal wine 2 is higher than the sum of the antioxidant activities of its compounds (wine, sage leaf and sea rush rhizome); (Table 3). This fact proves that the wine is a better extraction phase for polyphenols than the 50% ethanol.

Anthocyanins Content

Anthocyanins present in wines are water-soluble flavonoid pigments that are present in almost all tissues of higher plants including roots, stems, leaves, flowers, and fruits. In grapes, they are located mainly in the skin and in the flesh. Monomeric anthocyanins in young red wines contribute the majority of wine colour. During wine maturation and aging, these monomeric anthocyanin compounds form complex and stable anthocyanin-derived pigments resulting in a colour variation [26].

Table 3 presents the total anthocyanin content (TAC) in analysed vines. It can be noticed that during maceration the two products lost a large amount of wine anthocyanins which turned into polymerised compounds. Anthocyanin pigments are labile compounds that can undergo several degradative reactions, due to the presence of polyphenoloxidase, or other peroxidases in the vegetal products and/or light exposure.

The presence of active polyphenol oxidase and peroxidases in the vegetal products subjected to extraction could be the main reason for the wine anthocyanins degradation during the maceration process. The effect of plants polyphenol oxidases upon the anthocyanins is well known and different treatments are applied on food products in order to avoid this process [27, 28]. The anthocyanins degradation through oxidation could be due partially to the temperature

Table 3. Total phenolic content (TPC), antioxidant activity (AA).

Sample	Total phenol content (TPC) ($mg \cdot L^{-1}$)	Antioxidant Activity (mmol Trolox eqv· L^{-1})	Anthocyanins (cyanidin3-glucoside $mg \cdot L^{-1}$)	Polymerised compounds %
Control wine	6931.0±109^{c}	0.9960±0.18^{b}	353.0 ± 5.0^{c}	10.82%
Medicinal wine 1	8928.6±410^{b}	6.0540±0.96^{c}	191.03± 7.6^{b}	67.64%
Medicinal wine 2	10416.7±620^{d}	16.2420±1.47^{b}	171.33± 4.1^{c}	76%

Data are expresed as mean±standard deviations. Different letters indicate statistical significance at the $p<0.05$ level for each concentration

under which the macerates have been kept (25°C), as estimated by Sinela et al. [29], as well.

For the therapeutic value of medicinal wines, the anthocyanins presence brings important benefits, since it was demonstrated that anthocyanins have remarkable antioxidant properties, contributing to the increase of visual acuity, control of type II diabetes mellitus, reduction of coronary diseases and prevention of malignant diseases [30, 31]. It should be also mentioned that the degradation of these compounds does not bring therapeutic benefits to medicinal wines. The phenolic analyses are frequently used effective tools in characterising different wines. Wines with higher TPC are considered to be high-quality [32]. The results of the study show that untreated wine used as a control, indicates values of total polyphenols and anthocyanins within the limits presented for red wines [33, 34].

HPLC Analysis of Polyphenolic Acids

Phenolic compounds including hydroxybenzoic acids (gallic, 3-methyl gallic), some hydroxycinnamic acids (chlorogenic, cinnamic, caffeic, p-coumaric, ellagic) and vanillin were identified by comparison with the retention times of standards under identical conditions (Table 2). The quantitative data were calculated from their respective calibration curves and amounts of identified phenolic compounds by chromatographic method are summed up in Table 4.

The analysis of polyphenolic acids extracted in the wine during maceration lead to the conclusion that some compounds with high interest for the antioxidant activity were poorly extracted (e.g. resveratrol) while others (such as gallic acid, 3 methylgallic acid or ellagic acid) were better extracted. Some of them, which were under detection limits in the control wine, were concentrated in the macerates (3 methylgallic acid, ellagic acid and ferulic acid). The wine behaves as a selective extraction phase but contrary to our expectations, it did not extract resveratrol from the sea rush rhizome, a compound with important therapeutic effects.

The decrease of chlorogenic acid in the wine macerate 2 could be explained as an oxidation of this compound during maceration. The macerate 2 contains a higher number of dried plants (sage or sea rush),

Table 4. Phenolic compounds ($mg \cdot L^{-1}$).

Nr. Crt.	Compound	Control wine	Medicinal wine 1	Medicinal wine 2
1.	*E* – resveratrol	1.802±0.12^{b}	1.058±0.24^{c}	1.944±0.17^{c}
2.	*Z* – resveratrol	-	-	3.777±0.73^{b}
3.	Caffeic acid	-	9.441±1.01^{c}	4.088±1.09
4.	Chlorogenic acid	4.297±0.78^{b}	5.374±0.51	3.796±1.02
5.	Cinnamic acid	-	12.077±1.04	-
6.	Vanilin	-	-	-
7.	Gallic acid	171.179±6.34^{d}	187.286±5.12^{c}	257.263±10.51^{d}
8.	Ferulic acid	-	1.981±0.18^{b}	-
9.	Ellagic acid	-	-	80.785±4.32^{c}
10.	p-Coumaric acid	-	-	-
11.	3-Methylgallic acid	-	-	38.062±3.12^{c}

Data are expresed as mean±standard deviations. Different letters indicate statistical significance at the $p<0.05$ level for each concentration.

Table 5. Pearson's correlation coefficients between the phenolic compounds and antioxidant activities.

Total contents of phenolic compounds	Antioxidant activity		
	Control wine	Medicinal wine 1	Medicinal wine 2
TPC	0.927**	0.824**	0.912**
TA	0.263	0.182	0.160

**Highly significant correlation, $p<0.05$

which means a larger amount of polyphenol oxidases in the extracts.

As an explanation for the presence of Z resveratrol in the wine macerate 2, it is assumed that an amount of E resveratrol turned into Z resveratrol during extraction process (Table 4).

Correlation Coefficients (R^2) of Total Anthocyanins, Total Phenolic and Antioxidant Activity

A correlation of total phenolic content and anthocyanin content with antioxidant activity is shown in Table 5. The phenolic content presented higher correlation with antioxidant activity in analysed wines, ($p<0.05$). The differences observed between samples are probably associated to composition of antioxidant compounds, since each compound has its antioxidant activity [35].

The resulting p-values at the significance level of 0.05 indicate that the TPC is one of the main factors responsible for the antioxidant activity. Similar results have also reported a linear relationship between antioxidant activity and total phenols [36]. On the other hand, no statistically significant correlations were found between the antioxidant activity and the TA and phenolic acid concentrations. In these cases, the correlation coefficients were generally low, (below 0.55). Values below 0.55 of the Pearson's correlation coefficients calculated between the antioxidant activities and individual phenolic compound contents suggest that the constituents that occur separately in the wines could not be responsible for the antioxidant properties. These correlations are similar to literature reports [33].

Conclusions

The two wine macerates contain high amount of polyphenols extracted from the dried plants but also anthocyanins from the red wine. Both wine macerates have a higher content in polyphenols than the wine itself. Some compounds were better extracted from the plant material than others (e. g. resveratrol).

The two macerates contain a higher polymerised compounds percentage, probably due to the sage and sea rush polyphenol oxidases and peroxidases. The antioxidant activities increased in both products, mainly in the medicinal wine 2.

This study concludes that the wine behaves as a good selective extraction phase for the polyphenolic compounds and the obtained medicinal wines have enhanced the antioxidant activities.

Acknowledgments

This work was supported by the Department of Pharmaceutical Sciences, University of Constantza.

Conflict of Interest

The authors declare no conflict of interest.

References

1. MCGOVERNA P.E., MIRZOIANB A., HAL G.R. Ancient Egyptian herbal wines, PNAS. **106**, 7361, **2009**.
2. ZHAN B.Y., CHE X.F., WEI B.C. Progress of Chinese medicine wine products. Inform Tradit Chin Med. **27**, 120, **2010**.
3. EGEA T., SIGNORINI M.A., BRUSCHI P. RIVERA D., OBÓN C., ALCARAZ F. PALAZÓN J.A. Spirits and liqueurs in European traditional medicine: Their history and ethnobotany in Tuscany and Bologna (Italy). J. Ethnopharmacol. **175**, 241, **2015**.
4. HANDS T. Drinking for Health: Proprietary Tonic Wines. In: Drinking in Victorian and Edwardian Britain. Palgrave Macmillan, Cham. **2018**.
5. MCGOVERN P.E., VOIGT M.M., GLUSKER D.L., EXNER L.J. Neolithic resinated wine. Nature. **381**, 480, **1996**.
6. NUNN J.F. Ancient Egyptian Medicine, Norman: University of Oklahoma Press, (Appendix B). 24, **1996**.
7. KHAN A.S., ARIF K., MUNIR B., KIRAN S., JALAL F., QURESHI N., HASSAN S.M., SOOMRO G.A., NAZIR A., GHAFFAR A., TAHIR M.A., IQBAL M. Estimating Total Phenolics in *Taraxacum officinale* (L.) Extracts**.** Pol. J. Environ. Stud. **28** (1), 497, **2019**.
8. ANJUM F., SHAHID M., JILANI M.I., ORANAB S., FAROOQ S., NAZIR A., NAZ S., IQBAl M. Evaluation of Antioxidant Potential and Cytotoxic Behavior of Different Varieties of *Allium sativum*. Pol. J. Environ. Stud. **29** (6), 4447, **2020**.
9. ZHAN J.Y.X., ZHENG K.Y.Z., ZHU K.Y., ZHANG W.L., BI C.W.C., CHEN J.P. DU C.Y.Q., DONG T.X., LAU D.T.W., TSIM K.W.K. Importance of Wine-Treated Angelica Sinensis Radix in Si Wu Tang, a Traditional Herbal Formula for Treating Women's Ailments. Planta Med. **79**, 533, **2013**.
10. EMA: Public statement on the use of herbal medicinal products containing thujone. EMA/HMPC/732886/2010 London, UK. European Medicines Agency, **2011**.
11. GHORBANI A., ESMAEILIZADEH M. Pharmacological properties of Salvia officinalis and its components. J. Tradit. Complement. Med. 7, 433, **2017**.
12. RAHTE S., EVANS R., EUGSTER P.J., MARCOURT L., WOLFENDER J-L., KORTENKAMP A., TASDEMIR D. *Salvia officinalis* for Hot Flushes: Towards Determination

of Mechanism of Activity and Active Principles. Planta Med. **79**, 753, **2013**.
13. EL-SHAMY A.I., ABDEL-RAZEK, A.F., NASSAR M.I. Phytochemical review of *Juncus L.* genus (Fam. Juncaceae). Arab. J. Chem. **8**, 614, **2015**.
14. RODRIGUES M.J., GANGADHAR K.N., ZENGIN G., MOLLICA A., VARELA J., BARREIRA L., CUSTÓDIO L. Juncaceae species as sources of innovative bioactive compounds for the food industry: In vitro antioxidant activity, neuroprotective properties and in silico studies. Food Chem. Toxicol. **107**, 590, **2017**.
15. POPESCU A., NEGREANU-PÎRJOL T., ARCUŞ M., BUCUR L., ISTUDOR V. HPLC analysis of polyphenols and antioxidant activity determination of the *Juncus maritimus* Lam. rhizoma as impurity of the product *Holoschoeni rhizoma*. Rev. Med. Chir. Soc. Med. Nat. **113**, 332, **2009**.
16. STEFKÓ D., KÚSZ N., BARTA A., KELE Z., BAKACSY L., SZEPESI Á., FAZAKAS C., WILHELM I., KRIZBAI I.A., HOHMANN J. Gerardiins A–L and structurally related phenanthrenes from the halophyte plant *Juncus gerardii* and their cytotoxicity againsttriple-negative breast cancer cells. J. Nat. Prod. **83**, 3058, **2020**.
17. USDA NRCS National Plant Data Team, New York Metropolitan Flora database. New York Metropolitan Flora Project, Brooklyn Botanic Garden, New York. Glenn, S.D, **2013**.
18. PEREIRA DA SILVA C.H.T., PEREIRA DA SILVA T.P.S., NOBRE DE ALMEIDA C.V.T., LIMA D.C.A.; CAVALCANTI DE AMORIM E.L. Antioxidant activity and phenolic content of Caesalpinia pyramidalis Tul and *Sapium glandulosum* (L.), Morong from North-eastern Brazil. Molecules. **16**, 4728, **2011**.
19. LEE J., DURST R.W., WROLSTAD R.E. Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method. J AOAC International. **88**, 1269, **2005**.
20. GIUSTI M.M., WROLSTAD R.E. Unit F1.2, Characterization and measurement of anthocyanins by UV-visible spectroscopy. R. E. Wrolstad (Ed.), Handbook of analytical food chemistry New York, John Wiley & Sons. 19, **2005**.
21. NF 25, United States Pharmacopeia and the National Formulary (USP 30/NF 25). Rockville (MD): The United States Pharmacopeial Convention, Inc. 914, **2007**.
22. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2003b. Resolution RE, 899, 29 May 2003. Guide for validation of analytical and bioanalytical methods. Retrieved November 16, **2017**.
23. POPOV I., LEWIN G. Antioxidative homeostasis: Characterization by means of chemiluminescent technique, Methods Enzymol. **300**, 437, **1999**.
24. FIGUEIREDO-GONZÁLEZ M., CANCHO-GRANDE B., SIMAL-GÁNDARA J., TEIXEIRA N., MATEUS DE FREITAS V. The phenolic chemistry and spectrochemistry of red sweet wine-making and oak-aging**.** Food Chem. **152**, 1522, **2014**.
25. RUIZ-RODRIGUEZ B.M., DE ANCOS B., SANCHEZ-MORENO C., FERNANDEZ-RUIZ V., SANCHEZ-MATA M.C., CAMARA M., TARDIO J. Wild blackthorn (*Prunus spinosa* L.) and hawthorn (*Crategus monogyna Jacq.)* fruits as valuable sources of antioxidants. Fruits. **69**, 61, **2014** .
26. TANG K., LIU T., HAN Y., XU Y., LI J.M. The Importance of Monomeric Anthocyanins in the Definition of Wine Colour Properties. S. Afr. J. Enol. Vitic. **38**, 1, **2017**.
27. DE AGUIAR CIPRIANO P., EKICI L., BARNES R.C, GOMES C., TALCOTT S.T. Pre-heating and polyphenol oxidase inhibition impact on extraction of purple sweet potato anthocyanins Food Chem. **180** (1), 227, **2015**.
28. MARTYNENKO A., CHEN Y. Degradation kinetics of total anthocyanins and formation of polymeric color in blueberry hydrothermodynamic (HTD) processing. J Food Eng. **171**, 44, **2016**.
29. SINELA A., RAWAT N., MERTZ C., ACHIR N., FULCRAND H., DORNIER M. Anthocyanins degradation during storage of Hibiscus sabdariffa extract and evolution of its degradation products. Food Chem. **214**, 234, **2017**.
30. PELEG M., KIM A., NORMAND M.D. Predicting anthocyanins' isothermal and non-isothermal degradation with the endpoints method. Food Chem. **187**, 537, **2015**.
31. THILAKARATHNA S.H., RUPASINGHE H. Flavonoid bioavailability and attempts for bioavailability enhancement. Nutrients. **5**, 3367, **2013**.
32. TZACHRISTAS A., PASVANKA K., CALOKERINOS A., PROESTOS C. Polyphenols: Natural Antioxidants to Be Used as a Quality Tool in Wine Authenticity. Appl. Sci. **10**, 5908, **2020**.
33. PANDEYA A., RAYAMAJHI S., POKHREL P., GIRI B. Evaluation of secondary metabolites, antioxidant activity and color parameters of Nepali wines, Food Sci Nutr. **6**, 2252, **2018**.
34. CASSINO C., GIANOTTI V., BONELLO F., TSOLAKIS C., CRAVERO M.C., OSELLA D. Antioxidant composition of a selection of Italian red wines and their corresponding free-radical scavenging ability. J. Chem. **1**, 8, **2016**.
35. GÓMEZ-PLAZA E., OLMOS O., BAUTISTA-ORTÍN A.B. Tannin profile of different Monastrell wines and its relation to projected market prices. Food Chem. **204**, 506, **2016**.
36. DE CAMARGO A.C., REGITANO-D'ARCE M.A.B., BIASOTO A.C.T., SHAHIDI F. Low molecular weight phenolics of grape juice and winemaking byproducts: Antioxidant activities and inhibition of oxidation of human low-density lipoprotein cholesterol and DNA strand breakage. J. Agric. Food Chem. **62**, 12159, **2014**.