ANTIOXIDANT CAPACITY OF DIFFERENT WOODS TRADITIONALLY USED FOR COLORING HARD ALCOHOLIC BEVERAGES IN BULGARIA

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ABSTRACT

Introduction: According the folk traditions in Bulgaria for hard alcoholic beverages processing, *Quercus robur* L., *Morus nigra* L., *Robinia pseudoacacia* L., and *Cotinus coggygria* Scop. are the most popular species for manufacturing of wood barrels for storage and ageing, as well as for additionally coloring of drinks. The wood improves organoleptic characteristics by giving a specific taste, flavor and color to beverages. Popular believes suggest that colored drinks in small quantities could possess certain health beneficial effects (e.g. against gastric ulcer and diarrhea), probably due to the plant constituents extracted during ageing and coloration.

Methods: 40% aqueous-ethanol extracts were prepared based on traditional recipes for hard alcoholic drinks coloration. Antioxidant capacity and total polyphenol content of the extracts were measured and compared in a time course.

Results: The highest polyphenol content and antioxidant activity were estimated for *M. nigra* heartwood extract. High positive correlation between polyphenol content and antioxidant activity was calculated for all extracts studied.

Conclusions: The results suggest that polyphenol content significantly contributes to antioxidant activity of all studied extracts. The heartwood extract from *M. nigra*, identified to possess highest total polyphenol content and antioxidant activity, will be further investigated for cytotoxicity as well as for biological effects in different *in vivo* models.

***Key words**: Quercus, Morus, Robinia pseudoacacia, Cotinus coggygria, heartwood extracts.*

INTRODUCTION

One of the most important stages of hard alcoholic beverages production is the ageing process in wood barrels. The most popular woods for barrel manufacture are oak (*Quercus robur* L.), mulberry tree (*Morus nigra* L.), and black locust tree (*Robinia pseudoacacia* L.) [29]. Traditionally in Bulgaria hard alcoholic beverages (e. g. "rakia"), are colored additionally with stems and heartwood from these species and from smoke tree (*Cotinus coggygria* Scop.).

Each wood adds a specific taste, aroma and color to the beverages, probably due to the specific phytochemical content of the wood, to its physical properties, as well as to the geographical origin of the trees [3]. Many scientific studies reveal the rich composition of polyphenols and phenolic acids extracted from wood material during aging of beverages [1, 2, 8, 10, 26].

Along with improving the organoleptic characteristics of beverages, the extracted phytochemicals might contribute to some beneficial pharmacological properties of wood extracts. Scientific evidences exist that extracts from different parts of mulberry tree (mainly fruits, leaves and bark root) exhibit various beneficial effects such as antiviral, antihyperglycemic, antiatherogenic and hypotensive action [7, 18, 30]. Although phytochemical investigations confirm the high polyphenol content in heartwood ethanol extract [10], the biological properties of this part of mulberry tree are not yet clarified.

Flower infusion from black locust tree is recognized by folk medicine as a powerful remedy against neuralgia, gingivitis and gastrointestinal disorders [13]. Ethanol extract from young stems relieves gastro-intestinal inflammations and lowers the blood pressure [2]. Resorcinol – one of the most active polyphenol compounds extracted from black locust heartwood is used in

pharmaceutical industry for preparing ointments for treatment of dermatitis, acne and fungal infections [11, 12].

Smoke tree is a common medicinal plant used in Balkan folk medicine, predominantly for external administration as an anti-haemorrhagic and wound-healing remedy [19]. However, several authors report that aqueous infusions from leaves can be applied orally for treatment of throat and stomach inflammations, gastric ulcer, diarrhea and even diabetes mellitus. [9, 17, 23]. In recent *in vivo* studies, the beneficial effects of 1% leaves aqueous infusion and 20% heartwood aqueous-ethanol extract were estimated in respect of improving lipid profile and increasing serum antioxidant capacity [15, 24]. The diverse biological effects of *C. coggygria* are contributed to some extend to the high polyphenol content and antioxidant capacity of its aqueous and aqueous-ethanol extracts established in phytochemical studies [14, 16, 21].

Being the most popular source of material for barrels manufacturing *Q. robur* was used as a reference species in this study. According to Caldeira et al. [4] hundreds of volatile compounds derived from oak heartwood contribute to the aroma of brandies during ageing process. The antioxidant activity and the phenolic composition (total polyphenols, phenolic acids and hydrolysable tannins) of Portuguese and Spanish brandies stored in oak barrels were analyzed [5, 6, 27]. The compounds extracted from oak heartwood are recognized as potential antiulcer therapeutics in respect to their ability to inhibit *Helicobacter pylori* growth [20].

The aim of the present study was to determine and compare total polyphenol content (TPC) as well as antioxidant activity (AOA) of heartwood aqueous-ethanol extracts from four arboreal species, traditionally used in Bulgaria for coloring of hard alcoholic beverages.

MATERIALS AND METHODS

Heartwood samples processing

Heartwood samples from four species (*Q. robur, M. nigra, R. pseudoacacia* and *C. coggygria*) were subjected to fumigation following the popular technology: the wooden chips were boiled for 10 minutes and then saturated with cold water for 24 hours. Finally the material was dried for 15 minutes at 150-190°C.

Aqueous-ethanol extracts preparation

The extracts were prepared following the traditional Bulgarian recipe for coloring of hard alcoholic drinks. Briefly, the wood materials (2g) were put in dark glass bottles with 40% ethanol (1L per sample) for 60 days. In order to follow the changes of polyphenol content and antioxidant activity at different time points, samples (1ml) were taken periodically from the four extracts.

Biochemical methods

Total polyphenol content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent [28] and calculated as mM quercetin equivalents (mM QE).

Antioxidant activity (AOA) of extracts was measured applying the spectrophotometric method [25] based on the ability of the antioxidants to reduce preformed ABTS cation radical [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)]. Values were expressed as mM uric acid equivalents (mM UAE).

Statistical analysis

Statistical analyses were performed by analysis of variance (ANOVA). Data were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

The results from measurements of TPC and AOA of the four studied extracts are presented graphically in figures 1 and 2.

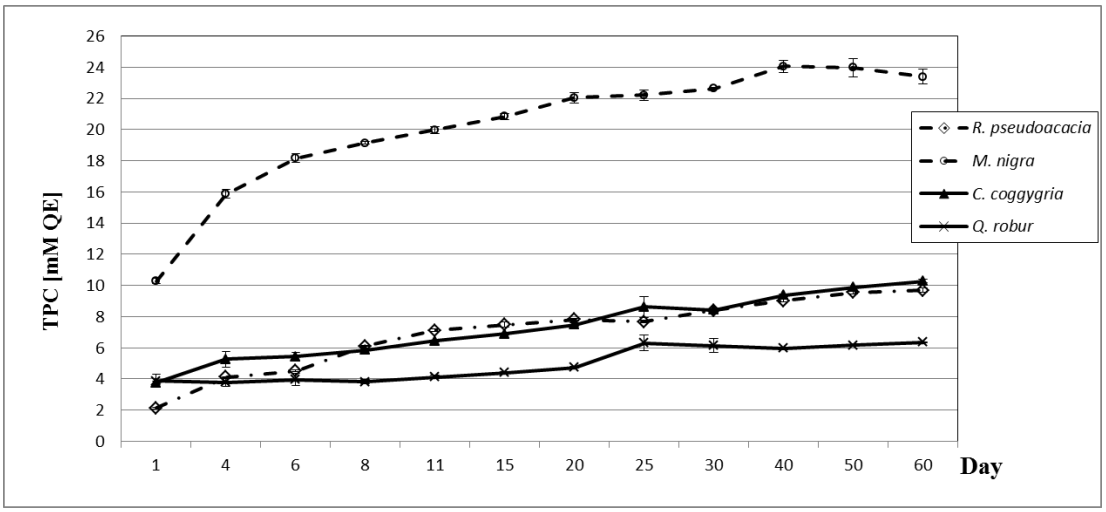

Figure 1. Changes in total polyphenol content (TPC) of 40% aqueous-ethanol wood extracts at different time points, ranging from day 1 to day 60 of extraction. *Data are presented as mean±SD*

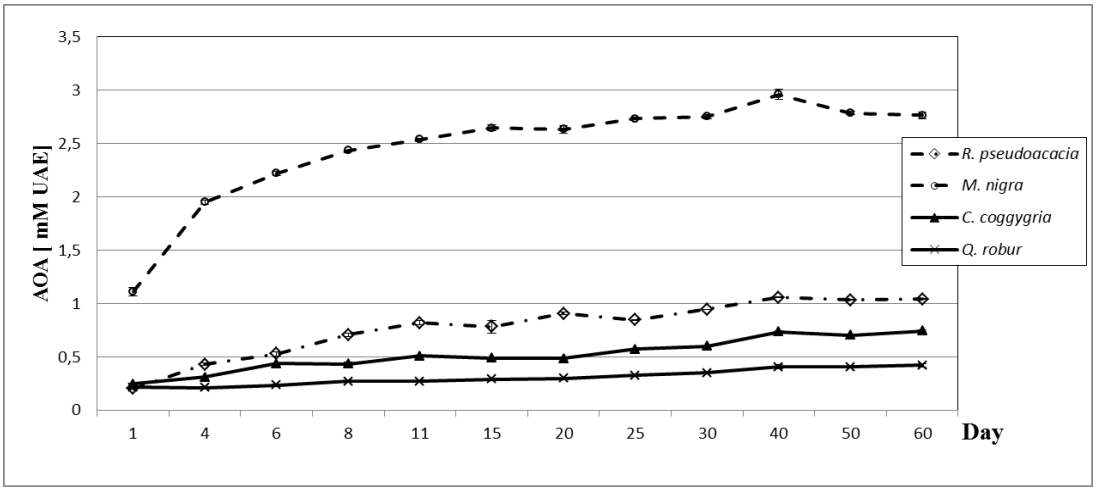

Figure 2. Changes in antioxidant activity (AOA) of 40% aqueous-ethanol wood extracts at different time points, ranging from day 1 to day 60 of extraction. *Data are presented as mean±SD*

The aqueous-ethanol extract of mulberry exhibited the highest AOA and TPC. Similar results were reported by a comparative study of 50% aqueous-ethanol wood extracts from different species where the highest TPC was estimated in mulberry extract followed by acacia and oak ones [8].

The correlation between TPC and AOA of all studied extracts was calculated. High positive correlation was estimated for all studied extracts: r=0,99 (black locust and mulberry), r=0,97 (smoke tree) and r=0,91 (oak). These data are in accordance with results reported by other authors [1, 10], suggesting that polyphenols and phenolic acids are the compounds contributing to the antioxidant capacity of wood extracts.

Although that TPC of black locust and smoke tree extracts were very similar, the AOA of the former was significantly higher (Fig. 2). Differences in antioxidant capacity of the studied extracts are probably due to the different phytochemical composition of the woods. According to Alañón et al., [1] the presence of phenolic acids as gallic, ellagic and *p*-coumaric greately contribute to antioxidant capacity of heartwood extracts. Scientific literature provides quite few data about the

phytochemical composition of mulberry wood, perhaps because of its limited usage for the manufacturing of barrels for aging of wine and spirits.

Time points analyses indicate that changes in AOA and TPC become negligible after 40 days of extraction and this fact could be considered in further investigations.

Interestingly, *M. nigra* is recognized as a medicinal plant mainly for therapeutic potential of its fruits, leaves and root bark. The established high AOA and TPC of heartwood of the tree by our study provides a basis for further investigations on the biological effects of the extract in experimental models of pathologies involving oxidative stress.

CONCLUSION

The presented results suggest that polyphenol content significantly contributes to antioxidant activity of heartwood extracts. The *M. nigra* extract was identified to possess highest total polyphenol content and antioxidant activity among all studied extracts and will be further investigated for cytotoxicity as well as for biological effects in different *in vivo* models.

REFERENCES

1. Alañón, M.E., Castro-Vázquez, L., Díaz-Maroto, M. C., Hermosín-Gutiérrez, I., Gordon, M. H., Pérez-Coello, M. S., 2011. Antioxidant capacity and phenolic composition of different woods used in cooperage, Food Chemistry 129, 1584–1590.

2. Antal, D., Schwaiger, S., Ellmerer-Muller, E., Stuppner, H., 2010. Cotinus coggygria Wood: Novel flavanone dimer and development of an HPLC/UV/MS method for the simultaneous determination of fourteen phenolic constituents. Planta Med 76, 1-8.

3. Cabrita, M. J. B., Garcia, R., Martins, N., Gomes da Silva, M. D. R., Costa Freitas, A. M., 2012. Gas chromatography in the analysis of compounds released from wood into wine, analytical chemistry. In ”Advanced gas chromatography - progress in agricultural, biomedical and industrial applications”, edited by Mustafa Ali Mohd, ISBN 978-953-51-0298-4.

4. Caldeira, I., Belchior, A., Clímaco, M., de Sousa, B., 2002. Aroma profile of portuguese brandies aged in chestnut and oak woods. Analytica Chimica Acta, 458: 55–62.

5. Caldeira, I., Clímaco, M., de Sousa, B., Belchior, A. 2006. Volatile composition of oak and chestnut woods used in brandy ageing: Modification induced by heat treatment. J Food Eng 76, 202-211.

6. Canas, S., Casanova, V., Belchior, A., 2008. Antioxidant activity and phenolic content of portuguese wine aged brandies. J Food Compos Anal, 21, 626–633.

7. Chan, K.C., Yang, M.Y., Lin, M.C., Lee Y.J., Chang, W.C., Wang, C.J., 2013. Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in cultured aortic vascular smooth muscle cells. J Agric Food Chem 61 (11), 2780–2788.

8. de Rosso, M.D., Cancian, D., Panighel, A., Vedova A.D., Flamini, R., 2009. Chemical compounds released from five different woods used to make barrels for aging wines and spirits: volatile compounds and polyphenols. Wood Sci Tech 43, 375-385.

9. Dulger, B., Hacioglu, N., Bilen, S., 2009. Antimicrobial Activity of *Cotinus coggyria* from Turkey. Asian J Chem 21, (5), 4139-4140.

10. Flamini, R., Vedova, A. D., Cancian, D., Panighel, A., de Rosso M., 2007. GC/MS-positive ion chemical ionization and MS/MS study of volatile benzene compounds in five different woods used in barrel making, J. Mass Spectrom 42, 641–646.

11. Hahn, S., Kielhorn, J., Koppenhöfer, J., Wibbertmann, A., Mangelsdorf, I., 2006. Concise International Chemical Assessment Document 71: Resorcinol. WHO Report.

12. Hosseinihashemi, S.K., Kanani, S., 2012. Heartwood extractives of *Robinia pseudoacacia* wood. J Adv Lab Res Biol 3 (3), 156-160.

13. Ikonomov, P., Boichinov, A., Nikolov, P., 1947. Medicinal plants. “Progres”, Sofia (in Bulgarian).

14. Ivanova, D, Gerova, D, Chervenkov, T, Yankova, T., 2005. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacol 96,145–150.

15. Ivanova, D., Pavlov, D., Eftimov, M., Kalchev, K, Nashar, M., Tzaneva, M., Valcheva-Kuzmanova, S., 2013. Subchronic toxicity study of ethanol infusion from *Cotinus coggygria* wood in rats. Bulg J Agric Sci, 19 (2) (*in press*)

16. Katalinic, V., Milos, M., Kulisic, T., Jukic M., 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 94, 550–557.

17. Kultur S., 2007. Medicinal plants used in Kırklareli Province (Turkey). J Ethnopharmacol 111, 341–364.

18. Kumar A, Chauhan, P. K., Bhardwaj, V.S., Kumar, R., Tyagi, A., 2011. In vitro antioxidant and phytochemical investigations of ethanolic extracts of *Viola serpens* and *Morus nigra*. J.Chem Pharm Res 3(4), 166-171.

19. Landzhev, I., 2010. In: Encyclopedia of Medicinal Plants in Bulgaria, Sofia, Trud Publ, 372–374 (in Bulgarian).

20. Lin, Y., Vattem, D., Labbe, R., Shetty, K., 2005. Enhancement of antioxidant activity and inhibition of *Helicobacter pylori* by phenolic phytochemical enriched alcoholic beverages. Process Biochem 40 (6), 2059–2065.

21. Nićiforović N., Mihailović V., Mašković P., Solujić S., Stojković A., Pavlović D., Muratspahić, 2010. Antioxidant activity of selected plant species; potential new sources of natural antioxidants. Food Chem Toxicol 48, 3125-3130.

22. Nazina, Y., Mogileva I., Shilov V., 2005. Treatment of stomach and intestine diseases: Call for help the nature. Rostov na Don: Phenix Publ, p. 320 (in Russian).

23. Novakovic, M., Vuckovic, I., Janackovic, P., Sokovic, M., Tesevic, V., Milosavljevic, S., 2007. Chemical composition, antibacterial and antifungal activity of the essential oils of *Cotinus coggygria* from Serbia. J Serb Chem Soc 72, 1045–1051.

24. Pavlov, D., Nashar, M., Eftimov, M., Kalchev, K., Valcheva-Kuzmanova, S., Tzaneva, M., Ivanova D., 2013. Subchronic toxicity study of aqueous infusion from *Cotinus coggygria* leaves in Wistar rats. Compt rend Acad bulg Sci 66 (5), 749-756.

25. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice- Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med 26, 1231-1237.

26. Sanz, M., Fernández de Simón, B., Esteruelas, E., Muñoz, A. M., Cadahía, E., Hernández, M.T., Estrella, I., Martinez, J., 2012. Polyphenols in red wine aged in acacia (*Robinia pseudoacacia*) and oak (*Quercus petraea*) wood barrels. Anal Chim Acta 732, 83-90.

27. Schwarz, M., Rodríguez, M., Martínez, C., Bosquet, V., Guillén, D., Barroso, C., 2009. Antioxidant activity of Brandy de Jerez and other aged distillates, and correlation with their polyphenolic content. Food Chem 116, 29–33.

28. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 299, 152-178.

29. Spirov, N. 1995. The home made wine. Slavena, Varna (in Bulgarian).

30. Venkatesh, K. R. and Chauhan, S., 2008. Mulberry: Life enhancer. J Med Plants Res 2(10), 271-278.