Plant Foods Hum Nutr (2017) 72:205–210
DOI 10.1007/s11130-017-0611-8

ORIGINAL PAPER

Phenolic Compounds, Volatiles and Antioxidant Capacity of White Myrtle Berry Liqueurs

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Published online: 26 April 2017

Abstract The aim of this research was to evaluate the antioxidant capacity and physical-chemical characteristics of commercial white myrtle berry (*Myrtus communis* L. var. *leucocarpa* DC) liqueur (WMBL). The total phenolic (TP) content was measured spectrophotometrically, applying a modified Folin-Ciocalteu's method, and phenolic compounds were identified by high-performance liquid chromatography (HPLC) coupled with electrospray mass spectrometry, and quantified by HPLC coupled with ultraviolet/visible detection. The antioxidant capacities were evaluated by FRAP, CUPRAC, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$ assays. The volatiles were assessed by gas chromatography and mass spectrometry (GC-MS/FID) after headspace solid-phase microextraction (HS-SPME) and liquid-liquid extraction (LLE). WMBL showed lower TP levels (636.3 ± 39.2 mg GAE/L) than in purple myrtle berry liqueur (PMBL). Nevertheless, WMBL exhibited better antioxidant capacities, potentially due to high concentrations of gallic acid (294.2 ± 14.2 mg/L) and its derivatives (58.3 ± 2.1 mg/L). Other phenolic compounds detected by HPLC-DAD and LC-MS/MS were flavonols like myricetin and its derivatives (myricetin-3-*O*-galactoside and myricetin-3-*O*-rhamnoside) with concentrations similar to those found in PMBL. GC-MS/FID analysis revealed 44 compounds (terpenes, higher aliphatic compounds and shikimic acid pathway derivatives). 1,8-Cineole was the most abundant terpene in the liqueur (26.5% (HS-SPME) and 9.6% (LLE)).

Keywords Phenolic compounds · Volatiles · *Myrtus communis* L. var. *leucocarpa* DC liqueur · Antioxidant · Myricetin derivatives · 1,8-cineole

Electronic supplementary material The online version of this article (doi:10.1007/s11130-017-0611-8) contains supplementary material, which is available to authorized users.

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Abbreviations

ABTS$^{\bullet+}$	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate radical cation
CUPRAC	cupric ion reducing antioxidant capacity
DPPH$^{\bullet}$	1,1-diphenyl-2-picrylhydrazyl radical
FRAP	ferric ion reducing antioxidant power (ferric reducing ability of plasma)
GAE	gallic acid equivalent
HS-SPME	headspace solid-phase microextraction
LLE	liquid-liquid extraction
TEAC	Trolox equivalent antioxidant capacity
TPTZ	2,4,6-tris(2-pyridyl)-1,3,5-triazine
Trolox	(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

Introduction

Alcoholic beverages obtained by plant maceration are traditional foods, rich in natural compounds, with pleasant

sensorial characteristics and physiological effects [1]. Specifically, berry liqueurs could be a source of bioactive compounds, because of the significant level of antioxidant phenolics in these fruits [2]. Myrtle liqueur is a traditional Sardinian beverage obtained by room temperature maceration of purple myrtle berries (*Myrtus communis* L.) in ethanol followed by dilution with water and sucrose [3, 4]. Commercially, two different myrtle liqueurs are produced: a purple-red liqueur, produced from purple myrtle berries (*M. communis* var. *melanocarpa* DC), and a white liqueur, produced by alcoholic maceration of young myrtle leaves. Less often, white liqueur is domestically produced from the yellowish berries of *M. communis* var. *leucocarpa* DC. Of these liqueurs, only the purple-red has been recognized with geographical designations, including the European Union "spirit drinks with geographical designations" [5] and the local Sardinian IGT [6]. At the moment, no liqueur from *M. communis* var. *leucocarpa* DC berries is commercially available, and several producers want to widen the choice of myrtle berry products through its promotion. A number of studies have been performed on the chemical composition of yellowish-white myrtle berries. The essential oil, fatty-acid composition, phenolic contents, and antioxidant activities were studied in samples from Tunisia [7]. Yellowish-white berries from Turkey were investigated for their total soluble solids, acidity, pH, tannic acid, ascorbic acid, phenolic and fatty acid composition [8]. Also, ash, crude protein, crude oil, water and alcohol soluble extracts, tartaric, malic and citric acids and minerals were studied in yellowish-white Turkish berries [9]. To the best of our knowledge, no studies on commercial liqueur from yellowish-white myrtle berries have been published so far. In the present study, the chemical composition of white myrtle berry liqueur was investigated, and results were compared with the macerate obtained from raw berries, before their final dilution with water and sucrose. The total phenolic (TP) content was measured with a modified Folin-Ciocalteu's method and phenolic compounds were identified and dosed by LC-MS/MS and HPLC-DAD. The volatiles were identified by GC-MS/FID after HS-SPME or LLE. In addition, the antioxidant capacity of both macerate and liqueur was evaluated by FRAP, CUPRAC, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$ assays.

Material and Methods

A more detailed description of the material and methods can be found as supplementary material.

Samples White myrtle berries (three batches of 90 kg) from wild growing plants were randomly harvested in December 2015 in Southern Sardinia (Monte Arcosu, Uta, CA, Italy) by professional pickers. The specimens were identified by Prof. Andrea Maxia (University of Cagliari, Italy) according to Pignatti [10] and Conti et al. [11] and different references for the right taxonomic status of taxa [12–14]. Voucher number DISVA.ALI.04.2015 was deposited at the Department of Life and Environmental Sciences of the University of Cagliari (Italy). After collection, the berries were cleaned and each batch was separately placed in an ethanol-water mixture and left for four months. The macerates were separated from the berries in April 2016, and the liqueurs were produced by adding sucrose and water to obtain a final percentage of 28% *v*/v (alcohol) and 32% *w*/*v* (sugar). Before bottling, the liqueur was filtered through IF350 cellulose acetate cardboard filter (Industrialfiltro srl, Cologno Monzese, MI, Italy).

Results and Discussion

Table 1 reports CIE chromaticity coordinates of the samples. The values describe a yellowish-amber product, which becomes darker from the macerate to final liqueur (lower L* and h°$_{ab}$, higher C* $_{ab}$). This darkening could be a

Table 1 CIE L*C*$_{ab}$h°$_{ab}$ chromaticity coordinates and *in vitro* antioxidant capacity of myrtle macerate and liqueur

Parameter	Myrtle white berries extract	
	macerate	liqueur
CIE chromaticity coordinates		
L* [a]	86.0 ± 0.4*	75.6 ± 0.0$^{\#}$
C*$_{ab}$ [b]	57.0 ± 0.3*	73.2 ± 0.2$^{\#}$
h°$_{ab}$ [c]	91.2 ± 0.1*	76.4 ± 0.1$^{\#}$
Antioxidant assays		
FRAP [d] (mmol Fe^{2+}/L)	33.51 ± 3.74*	30.21 ± 1.27*
CUPRAC [d] (mmol Fe^{2+}/L)	13.81 ± 0.87*	11.30 ± 1.48$^{\#}$
DPPH$^{\bullet}$ [e] (mmol TEAC/L)	4.98 ± 0.45*	3.72 ± 0.22$^{\#}$
ABTS$^{\bullet+}$ [e] (mmol TEAC/L)	14.89 ± 2.88*	11.66 ± 0.56*

Results are reported as the mean value ± standard deviation; $n = 3$

Means in the same line with different symbol are significantly different ($P \leq 0.05$)

[a] L*: Lightness (brightness), 0 = black and 100 = white;

[b] C*: Chroma (saturation), starting from 0, increases with higher brilliances;

[c] h°: hue, the value performs a circumference anticlockwise starting from the red tone and forming an angle of 0° with the positive semi-axis of a *, to yellow at 90°, 180° through the green and blue at 270 °, and then returning to red tone for one complete rotation

[d] FRAP (ferric ion reducing antioxidant power) and CUPRAC (cupric ion reducing antioxidant capacity) values are expressed as Fe^{2+} millimolar concentration, obtained from a $FeSO_4$ solution having an antioxidant capacity equivalent to that of the dilution of the myrtle extract

[e] DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl radical) and ABTS$^{\bullet+}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate radical cation) values are expressed as TEAC millimolar concentration, obtained from a Trolox solution having an antiradical capacity equivalent to that of the dilution of the myrtle extract

consequence of polyphenol oxidation, by polyphenol oxidase and other enzymes that create melanins and benzoquinones from natural phenols, resulting in a brown colour [15], or the consequence of non-enzymatic browning related to pigments degradation (e.g., chlorophylls) [16].

Total phenolic (TP) content dosed by Folin-Ciocalteu's assay and phenolic compounds investigated by HPLC-MS and dosed by HPLC-DAD are reported in Table 2. TP content was ca. 2–3 folds lower than the average in purple berry liqueur [4]. This confirms previous observations regarding lower amounts of phenolic compounds in yellowish-white myrtle berries compared to the purple-red ones [7]. The TP content of this liqueur is comparable to that of strawberry, raspberry, and blackcurrant liqueurs [17] and to that of bitter herbal liqueurs [18], whereas it was more concentrated than in cherry and cranberry liqueurs [19]. HPLC-DAD was applied to investigate the phenolic fraction and LC-MS/MS analysis was used to confirm peaks' attribution (Table 1). It can be observed that all detected polyphenols were also found in the purple berries as has been previously reported [3, 4]. Large amounts of hydroxybenzoic acids were found (408.2 ± 19.9 mg/L), mainly gallic and ellagic acids (294.2 ± 14.2 and 55.8 ± 2.6 mg/L, respectively). Myrtle liqueur also contained large amounts of flavonols, mainly myricetin-3-*O*-galactoside and myricetin-3-*O*-ramnoside. Recently, these compounds have been recognized as effective in limiting postprandial hyperglycemia, which is typical of the type 2 diabetes mellitus [20]. Myrtle macerate contained traces of malvidin-3-*O*-glucoside that were not detectable in the liqueur, confirming the observations of other authors regarding the small amounts of anthocyanins found in white-yellow berries [7]. This finding could be visually verified by observing small dark stains present on the skin of ripened white berries.

All results of antioxidant activity assessed with FRAP, CUPRAC, DPPH$^{\bullet}$, and ABTS$^{\bullet+}$ assays are reported in Table 1. The antioxidant capacities (total and antiradical) were comparable to those of purple myrtle berry liqueur, despite the lower amount of total phenols [4]. This can be explained by high concentrations of gallic and ellagic acids, as it is well-known that these acids possess the highest antioxidant activity among phenolic compounds [21–23]. In particular, gallic acid has been strongly correlated with the antioxidant activity of blackcurrant liqueurs (r = 1.00), unlike the TP content, which correlation was weak (r = 0.40) [24]. Besides, myricetin and its glycosides (myricetin-3-*O*-galactoside and myricetin-3-*O*-rhamnoside) and gallic acid derivates strongly inhibit free

Table 2 Phenolic composition of myrtle white berries macerate and liqueur (mg/L)

Parameter	Identification[a]	LOD	LOQ	Myrtle white berries extract	
				macerate	liqueur
Total polyphenols[b] (mg GAE/L)				786.6 ± 20.7*	636.3 ± 39.2#
Polyphenols by HPLC (HPLC-DAD, mg/L)					
Total				564.6 ± 22.3*	466.4 ± 23.4#
Hydroxybenzoic acids				289.6 ± 15.4*	408.2 ± 19.9#
Gallic acid	rt, UV–Vis, MS	0.4	1.1	79.1 ± 3.5*	294.2 ± 14.2#
Ellagic acid	rt, UV–Vis, MS	0.3	1.0	106.0 ± 4.8*	55.8 ± 2.6#
Gallic acid derivatives[c]	UV–Vis, MS			104.4 ± 3.7*	58.3 ± 2.1#
Flavonols				275.0 ± 11.2*	58.1 ± 1.7#
Myricetin-3-*O*-galactoside	rt, UV–Vis, MS	0.6	1.7	46.9 ± 2.2*	2.1 ± 0.1#
Myricetin-3-*O*-rhamnoside	rt, UV–Vis, MS	0.5	1.6	168.7 ± 7.3*	23.0 ± 1.2#
Myricetin	rt, UV–Vis, MS	0.5	1.4	28.9 ± 1.5*	25.6 ± 1.0#
Other flavonols[d]	UV–Vis, MS			30.4 ± 0.9*	7.4 ± 0.2#
Anthocyanins				tr	nd
Malvidin-3-*O*-glucoside	rt, UV–Vis, MS	0.4	1.3	tr	nd
Other anthocyanins[e]	UV–Vis, MS			nd	nd

Results are reported as the mean value ± standard deviation; n = 3

Means in the same line with different symbols are significantly different ($P \leq 0.05$)

nd not detected (below the limit of detection, LOD), *tr* traces (below the limit of quantification, LOQ)

[a] rt: comparison with retention time of pure standard. UV–Vis: comparison with typical UV–Vis spectra of pure compound or similar pure standards. MS: comparison with MS spectra of pure compound or literature data

[b] GAE: gallic acid equivalent

[c] dosed as gallic acid

[d] dosed as myricetin

[e] dosed as malvidin-3-*O*-glucoside

radical and lipid peroxidation [25, 26]. The macerate's antioxidant activity was similar to that of the liqueur, although it exhibited different composition of phenolics: the macerate composition was richer in ellagic and in gallic acid derivatives, while the liqueur contained larger amounts of gallic acid. It can therefore be assumed that degradation of both ellagic and gallic acid derivatives occurred during the processing of the macerate to obtain the liqueur, leading to the increased gallic acid concentration. The antioxidant capacity of the white myrtle liqueur proved to be higher than that of walnut [27], cherry, and raspberry liqueurs [19], reaching levels similar to those of strawberry and blackcurrant liqueur [17].

Table 3 reports the volatiles composition of the myrtle macerate and liqueur determined by GC-MS/FID analyses, assessed after two different extractions (HS-SPME and LLE). The analyses allowed us to highlight several differences between the

Table 3 The volatiles composition of the myrtle macerate and liqueur determined by GC-MS/FID analysis after HS-SPME (headspace solid-phase microextraction) and LLE (liquid-liquid extraction)

No	Compound	RI[a]	Area (% ± SD)			
			HS-SPME		LLE	
			macerate	liqueur	macerate	liqueur
1.	Diethyl acetal	< 900	-	0.5 ± 0.0*	-	-
2.	2,4-dimethylpentan-3-one[b]	< 900	-	0.5 ± 0.1*	-	-
3.	Ethyl 2-methylbutyrate[b]	< 900	-	1.0 ± 0.1*	-	-
4.	α-Pinene	939	38.5 ± 2.0*	1.0 ± 0.1#	0.9 ± 0.1#	0.1 ± 0.0§
5.	Benzaldehyde	966	-	1.7 ± 0.1*	-	-
6.	*p*-Cymene	1030	1.2 ± 0.1*	0.8 ± 0.1#	-	-
7.	Limonene	1034	21.3 ± 1.2*	3.9 ± 0.3#	0.5 ± 0.1§	0.1 ± 0.0^
8.	1,8-Cineole	1036	16.7 ± 0.9*	26.5 ± 1.1#	7.1 ± 0.4§	9.6 ± 1.3^
9.	Benzyl alcohol	1040	-	-	-	1.6 ± 0.2*
10.	Linalool	1102	1.0 ± 0.1*	23.3 ± 2.5#	0.4 ± 0.1§	4.0 ± 0.2^
11.	2-Phenylethanol	1119	-	-	-	2.0 ± 0.2*
12.	*trans*-Pinocarveol	1144	-	-	0.1 ± 0.0*	-
13.	Ethyl benzoate	1175	-	9.2 ± 0.8*	-	-
14.	Diethyl succinate	1181	-	-	0.5 ± 0.1*	2.8 ± 0.5#
15.	Terpinen-4-ol	1182	-	7.4 ± 0.5*	-	-
16.	α-Terpineol	1194	0.2 ± 0.1*	7.7 ± 0.6#	0.8 ± 0.1§	4.4 ± 0.5^
17.	4-Vinylphenol	1226	-	-	-	1.2 ± 0.1*
18.	Geraniol	1261	-	-	1.4 ± 0.4*	2.4 ± 0.4#
19.	*trans*-Anethole	1288	1.2 ± 0.1*	6.0 ± 0.4#	0.7 ± 0.1§	0.1 ± 0.0^
20.	2-Methoxy-4-vinylphenol	1316	-	-	-	1.6 ± 0.3*
21.	α-Terpenyl acetate	1353	1.4 ± 0.1*	4.0 ± 0.1#	1.4 ± 0.4*	0.3 ± 0.0§
22.	β-Elemene	1393	0.6 ± 0.1*	-	-	-
23.	Methyleugenol	1406	0.1 ± 0.0*	1.1 ± 0.1#	0.4 ± 0.1§	2.4 ± 0.3^
24.	*trans*-Caryophyllene	1421	9.7 ± 0.4*	1.7 ± 0.1#	1.2 ± 0.3§	0.2 ± 0.0^
25.	4-Hydroxybenzyl alcohol	1432	-	-	-	10.8 ± 0.9*
26.	α-Humulene	1456	2.6 ± 0.2*	0.9 ± 0.1#	0.4 ± 0.1§	0.1 ± 0.0^
27.	Ethyl 4-hydroxybenzoate[b]	1535	-	-	-	8.4 ± 0.6*
28.	4-Hydroxybenzoic acid	1537	-	-	-	2.0 ± 0.2*
29.	Vanillic acid	1572	-	-	-	2.4 ± 0.5*
30.	*trans*-Caryophyllene oxide	1584	-	-	0.9 ± 0.1*	-
31.	Ethyl vanillate	1589	-	-	-	1.2 ± 0.1*
32.	Homovanillic acid	1653	-	-	-	0.8 ± 0.1*
33.	Tetradecanoic acid	1766	-	-	0.3 ± 0.0*	3.2 ± 0.4#
34.	Ferulic acid	1873	-	-	-	3.6 ± 0.3*
35.	Hexadecan-1-ol	1883	-	-	-	3.2 ± 0.3*
36.	Hexadecanoic acid	1975	-	-	9.2 ± 0.7*	-
37.	Ethyl palmitate	1996	0.7 ± 0.1*	-	21.7 ± 2.3#	-
38.	(*Z*)-Octadec-9-en-1-ol	2058	-	-	-	12.0 ± 0.4*
39.	Octadecan-1-ol	2083	-	-	-	4.4 ± 0.3*
40.	Oleic acid	2137	-	-	-	3.6 ± 0.2*
41.	Linoleic acid	2141	-	-	12.3 ± 0.6*	-
42.	Ethyl linoleate	2160	-	-	32.5 ± 1.8*	-
43.	Octadecanoic acid	2165	-	-	-	2.4 ± 0.3*
44.	Ethyl stearate	2195	-	-	1.3 ± 0.3*	-

Results are reported as the mean value ± standard deviation (SD); $n = 3$

Means in the same line with different symbol are significantly different ($P \leq 0.05$)

[a] RI: retention indices on HP-5MS column relative to *n*-alkanes (C_9-C_{25})

[b] tentatively identified

two samples, which were probably caused by the manufacturing techniques. The major headspace compounds of the macerate were the monoterpenes α-pinene (38.5%), limonene (21.3%), 1,8-cineole (16.7%) and *trans*-caryophyllene (9.7%). Beyond them, the importance of the presence of limonene in other ethanolic extracts has been highlighted because of its functional properties as an antioxidant [28]. On the other hand, higher aliphatic compounds (such as ethyl palmitate (21.7%), linoleic acid (12.3%) and ethyl linoleate (32.5%)) dominated in the macerate extract. Monoterpenes were less abundant in the liquid-liquid extract in comparison with the macerate headspace, and the major ones were 1,8-cineole (7.1%), α-pinene (0.9%), *trans*-caryophyllene (1.2%) and limonene (0.5%). The headspace of the liqueur contained 1,8-cineole (26.5%) and linalool (23.3%) as the major compounds. Terpinen-4-ol, α-terpineol and *trans*-anethole appeared only in the liqueur headspace. Monoterpenes were present among the minor constituents of the liqueur extract; the major ones were linalool (4.0%), 1,8-cineole (9.6%) and α-terpineol (4.4%), in very different percentages from purple-red myrtle [29]. (*Z*)-Octadec-9-en-1-ol (12.0%) was the most abundant among the higher aliphatic compounds. However, shikimic acid pathway derivatives were found in the liqueur extracts, such as: 4-hydroxybenzyl alcohol (10.8%), ethyl 4-hydroxybenzoate (8.4%), 4-hydroxybenzoic acid (2.0%), vanillic acid (2.4%) and ethyl vanillate (1.2%). Variability in the volatile composition from macerate to liqueur can be explained by long storage in the hydro-alcoholic solution. During four months of extraction, these compounds could have been extracted from harder parts as the seeds and different reactions could have occurred. Moreover, dilution of the macerate, and the addition of sucrose, could have modified the native chemical composition. The similarities between the macerate and liqueur headspace composition and the essential oil (EO) of *M. communis* var. *leucocarpa* DC plant was expected, as was an abundance of 1,8-cineole, α-pinene and limonene [30]. It should be noticed that in the referenced study, large amounts of myrtenyl acetate was found in the EOs from areal parts of *M. communis* var. *leucocarpa* DC, but this compound was not detected in the macerate and liqueur from white berries. Such differences could be related to the different parts of myrtle used (leaves or berries), or to the existence of different chemotypes of *M. communis* var. *leucocarpa* DC. This last observation also raises the problem that the actual botanical classification of the variety of *M. communis* with white berries is based solely on the colour of berries [14]. A more appropriate morphological and genetic investigation of *M. communis* var. *leucocarpa* DC is needed.

Conclusions

This research represents the first investigation on liqueur from white myrtle berries, a beverage that remains almost unexplored and unexploited. The preliminary characterization of this product revealed an interesting content of phenolic compounds and good antioxidant activity, higher than that of purple myrtle berry liqueur. The volatile fraction is similar to purple myrtle berry liqueur, and is rich in oxygenated compounds that contribute to the pleasant flavour of this liqueur. This research could well help producers to both protect and improve this typical product, obtaining a legal recognition that, at present, is still limited to the purple berry liqueur.

Acknowledgements The authors thank Distillerie Mario Pacini s.r.l (Elmas, CA, Italy) for supplying samples, Prof. Andrea Maxia (University of Cagliari, Italy) for white berry characterization and Cesare Omissi MA (University of Oxford, UK) for helpful discussion. This study was partially funded by the Croatian Science Foundation as part of the project (IP-11-2013-8547) "Research of Natural Products and Flavours: Chemical Fingerprinting and Unlocking the Potential".

Compliance with Ethical Standards

Human or Animal Studies This article does not contain any studies with human or animal subjects.

Conflict of Interest The authors declare that they have no conflict of interest.

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