Original article

Improvement of polyphenolic content and antioxidant activity of Syrian myrtle berries (*Myrtus communis* L.) hydro-alcoholic extracts using flavoring additives

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Summary. Myrtle berries extracts were prepared using hydro-alcoholic solvent at different concentrations of alcohol ranging from 50 to 80% (v/v). Cloves and cinnamon were used as flavoring additives in different mixtures (myrtle berries; myrtle berries and cinnamon; myrtle berries and cloves; myrtle berries, cinnamon, and cloves) in order to enhance the phenolic content and the antioxidant activity of the liqueur. Total phenolic content was assessed by Folin-Ciocalteau assay, and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to evaluate the total antioxidant activity. The highest content of polyphenols reached 7.82 g/100 g DW after 60 days of myrtle berries and cloves mixture maceration in 50% hydro-alcoholic solution, and the antioxidant efficacy of this mixture was the highest with 80.02% of DPPH scavenging activity. In conclusion, adding cloves to the myrtle berries liqueur will provide the best yield of extracted bioactive compounds with the best antioxidant activity which will affect positively the vital properties for these liqueurs.

Key words: myrtle berries, cloves, cinnamon, liqueur, polyphenols

Introduction

Myrtus communis L, 'Common myrtle' is a Mediterranean aromatic evergreen shrub, from the Myrtacea family. It grows in the wild in hill regions and has important essential oils, tannins and anthocyanin. Different parts of *Myrtus communis* L. such as berries, leaves, flowers and roots has been used for medicinal, cosmetic purposes, spices and food industries (1-3). Several studies have confirmed the biological properties of myrtle extracts and essential oils, such as antioxidant (4-8), antimicrobial, and antifungal ones (9). The high level of phenolic compounds, including flavonoids, phenolic acids and tannins, and Pigment anthocyanins are responsible for their several activities as confirmed by previous researches (7, 10, 11).

Major current uses include production of leaf essential oil for aromatherapy or perfume industry, and production of special liqueurs by berries' maceration which is a very popular practice in the islands of Sardinia (Italy) and Corsica (France) (12, 13). The maceration process is considered an important action, which affects the bioactive material amount that will be extracted and the final product quality. Therefore it is important to control some variables that affect the extraction process such as solvents used and their polarity, temperature, and time of extraction (14-17). In addition, adding some aromatic plants as flavors such as cinnamon and cloves, which are rich in phenol compounds, to the myrtle liqueur may increase its content of biochemical materials particularly polyphenols.

Cloves are the dried, unopened inflorescence (buds) of the clove tree, *Syzygium aromaticum*, which belongs to the Myrtaceae family (18). The major volatile compounds identified from the n-hexane extract of the cloves buds using gas chromatography-mass spectroscopy (GC-MS) were eugenol and eugenol acetate, as well as some flavonoids (19, 20). Clove buds extracts and their essential oils have been known to support various biological activities such as antimicrobial and antioxidant properties (21, 22).

In addition, cinnamon is a well-known spice obtained from the inner bark of several trees of the genus *Cinnamomum*. It belongs to the Lauraceae family and is mainly used in the aroma and essence industries due to its fragrance, which can be incorporated into different varieties of foodstuffs, perfumes, and medicinal products (23). The most important constituents of cinnamon are cinnamaldehyde and trans-cinnamaldehyde (Cin), which are present in the essential oil, thus contributing to the fragrance and to the various biological activities observed with cinnamon (24). Cinnamon bark contains procyanidins and catechins (25). These procyanidins extracted from cinnamon possess antioxidant activities, potentiate insulin action, and may be beneficial in the control of glucose intolerance and diabetes (26, 27).

In this study, different extracts from Syrian wild myrtle berries and their mixtures with cloves and cinnamon were used for the production of liqueur. Hydroalcoholic mixtures were used in the range of 50 - 80% (v/v) to select the most efficient medium at room temperature in summer. The flavors, cinnamon and cloves, were added by small quantities in order to preserve the main taste of myrtle liqueur and at the same time increase the content of phenolic compounds, and therefore improve the vital properties of the liqueur. During the maceration period, the polyphenol content was measured every 10 days for five months. We mainly focused on the phenolic fractions, the major feature of this liqueur, since they strongly contribute to the color, taste, and texture of the product and because they are known to exhibit several health beneficial activities.

Materials and methods

Chemicals

Folin-Ciocalteau (2N) reagent (Sigma-Aldrich, Switzerland), 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Sigma-Aldrich, USA), Sodium carbonate (Himedia, India), Gallic acid Sigma-Aldrich, China). Beverage grade ethanol with different concentrations (50-80%) was produced from grape wine via distillation.

Myrtle berry samples and hydro-alcoholic extract preparation

M. communis L. berries were collected from a mountainous region of Safita (in the Syrian coast) in December 2015. They were already mature and characterized by a blue dark color. After the harvest and removal of impurities, berries were stored at room temperature until the maceration process was carried out.

Cinnamon bark and clove buds samples were bought from local markets and then ground in a grinder.

The moisture content of myrtle berries, cinnamon, and cloves was determined and it was 13.27%, 12.40% and 21.79%; respectively.

Ethanolic Extracts (EEs) preparation

EEs were prepared following a procedure originally proposed by Tuberoso et al. (28).

Dried ground berries of myrtle were put in jars, a 1:10 (w/v) solid to solvent ratio was used. Cinnamon and cloves were added to samples in a ratio of 1:11 (w/w) cinnamon/cloves:myrtle berries and 1:1:10 (w/w) cinnamon:cloves:myrtle berries. Different extraction media 50, 60, 70, and 80% of beverage grade ethanol was used and evaluated for 5 months.

The jars containing the macerates were covered by foil and stored in the dark at room temperature.

Total phenolic content

The total phenolic yield in different extracts was measured by UV spectrophotometry, based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu reagent (29).

To 1 ml of 100 fold diluted extracts, 0.5 ml of Folin-Ciocalteu reagent (2N) was added, then diluted with 4.5 ml of distilled water. After a time interval of 5 min, 4ml of Na_2CO_3 (7.5%) were added. The samples were incubated for 30 min at 40°C. The absorbance of the resulting blue-colored solutions was measured at 734 nm. Analysis was conducted every 10 days during 5 months of maceration.

Measurements were performed, based on a standard calibration curve of gallic acid in ethanol. The mean (+SD) results of duplicate analyses were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry-material.

Antioxidant activity

A spectrophotometric test based on an electron-transfer reaction monitoring absorbance, DPPH, was used. DPPH assays were carried out according to Brand-Williams et al. (30). Extracts were diluted 100 fold with distilled water. 200 μL of diluted samples were added to 2 mL DPPH (100 μM/L methanol), the mixture was manually shacked. After 1h of incubation in the dark and at room temperature, the absorbance of the samples was measured at 520 nm.

The total antioxidant activity (TAA) was expressed in terms of percentage activity as calculated with the following equation:

$$TAA\% = [(Ac - As)/Ac]^{*}\ 100$$

Where **Ac** is the absorbance of the control and **As** is the absorbance of the tested sample.

Statistical analysis

All samples were assayed in duplicate to determine the mean and the standard error.

The results were analyzed statistically using the Statistica 7.0 software to determine the average value and standard error. For statistical evaluation, data were analyzed by two way analysis of variance (ANOVA) with a significance level of $p>0.001$.

Results and Discussion

Total phenolic content in alcoholic extracts of myrtle berries and its combinations with cinnamon, and cloves is shown in figure 1.

Many previous studies indicated that ethanol and water extracts of myrtle berries showed the highest amount of extracted compounds, while the highest antiradical and antioxidant activities were found in ethanol and ethyl acetate extracts (31). These extracts also had the highest content of phenolic compounds, because of their higher polarity. Ethanol, a traditional solvent for the preparation of the liqueur, was used in this study as a suitable solvent for polyphenol extraction and nutritionally safe for humans (32).

Many previous researches also had shown that the extraction yield of phenolic compounds is dependent on the plant material, type of solvent, method of extraction, solid-to-solvent ratio, and temperature (33-35). Our results were in accordance and they showed that the concentration of phenolic compounds in all extracts was dependent on the type of plant material used in the extracts, the concentrations of solvent, and period of maceration.

As shown in Figure 1, the wild myrtle, and flavors used in this study are characterized with a high content of phenolic compounds. Snoussi *et al*. reported that all parts of the myrtle plant showed high content of total polyphenols, flavonoids, and proanthocyanidins contents, which was the highest in the berries (36). Barboni *et al*. investigated the polyphenol compounds that were extracted from myrtle berries, as reported they were characterized by two phenolic acids, four flavanols, three flavonols and five flavonol glycosides, and the major compounds were myricetin-3-*O*-arabinoside and myricetin-3-*O*-galactoside (37). In addition, cloves and cinnamon are also characterized by a high level of total phenolic compounds, and exhibited very strong radical scavenging activity (38). The major types of phenolic compounds found were phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins (38). Abo El-Maati *et al*. reported that clove had the highest extraction yield when extracted with ethanol 80%, and found that the best solvent for extracting phenolic compounds from clove was hexane followed by ethanol among other solvents used (39).

Maceration process was conducted at room temperature in summer, where the temperature ranged from 35 to 40C°. This increases the extraction yield of bioactive compounds, particularly phenolic compounds, by enhancing the solubility and the mass

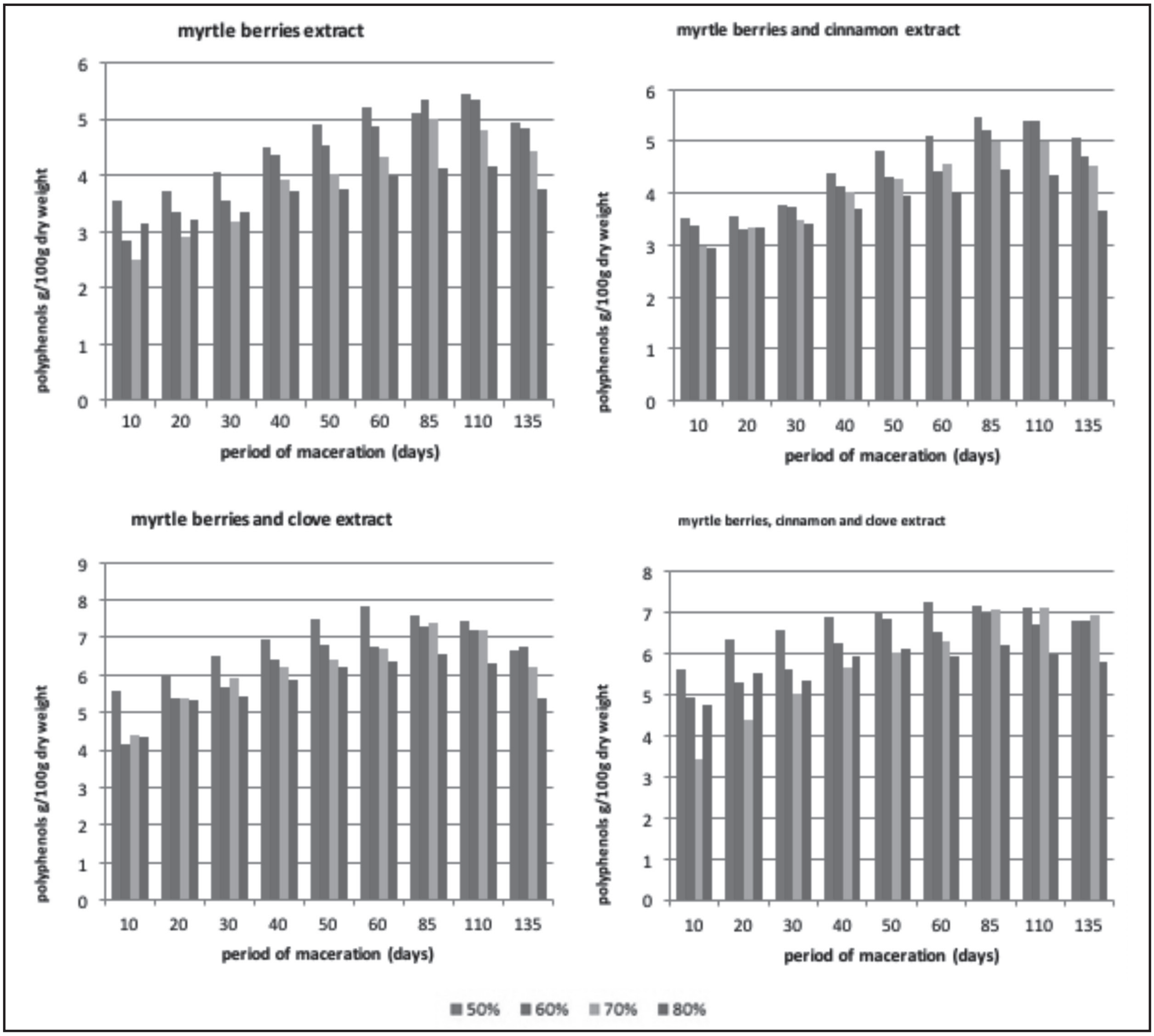

Figure 1. Total phenolic content (g/100g dry weight) in different alcoholic extracts of myrtle berries and its combinations with cinnamon, and cloves.

transfer, which is compatible with what has been reported by Al-Farsi et al. (40). The heat also might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions (33), thus more phenolic compounds would distribute to the solvent. The amount of polyphenols increased positively during maceration period in all extracts and was dependent on the concentrations of alcohol. The highest amount was observed in alcoholic extract of myrtle berries and cloves, at 50% alcohol, and after 60 days of maceration (7.82 g/100 g dry weight).

The changes in the yield of polyphenols with different concentrations of alcohol used for extraction during maceration period are shown in figure 2.

EE 50% (v/v) showed the highest amount in total polyphenols during the maceration period for all types of extracts, with the best yield for myrtle berries:cloves, and myrtle berries:cinnamon:cloves mixtures. There was no significant difference between EE 60% (v/v) and EE 70% (v/v) ($P<0.01$) regarding the phenolic content, while EE 80 % (v/v) exhibited the lowest amount in total polyphenols.

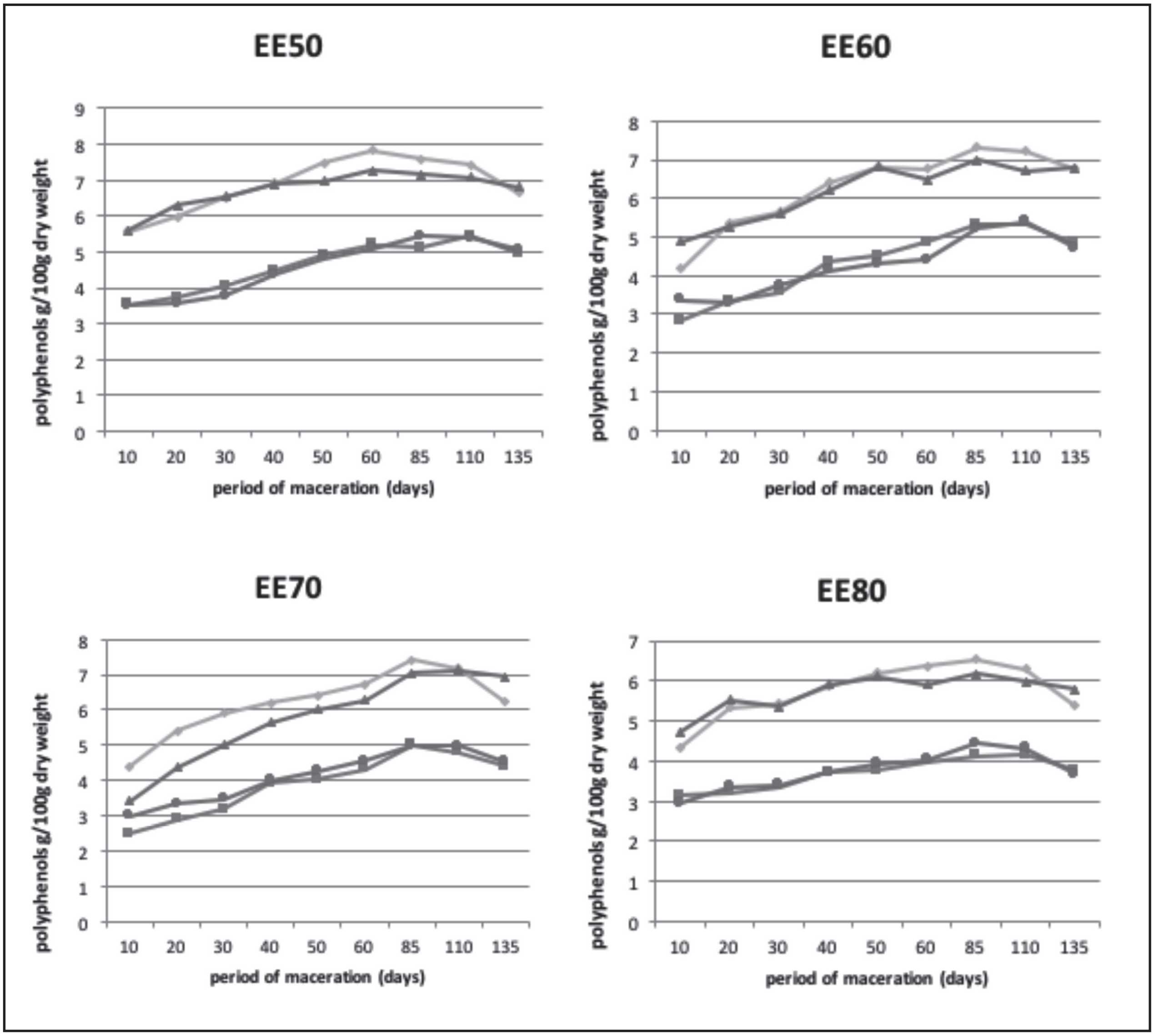

Figure 2. Changes in the yield of polyphenols with different concentrations of hydro-alcoholic mixtures (50-80% (v/v)) during the maceration period.

Our results indicated that higher content of polyphenols was obtained by increasing the polarity of the solvent used. Same results were reported and 50% ethanol was found to be more efficient in the extraction of polyphenols than 80%, or 100% ethanol in *Mentha* species, soybeans and black tea (41-43). This could be explained by the denaturation of protein by high concentrations of ethanol which will prevent the dissolution of polyphenols and so affect the extraction yield (44). On the other hand, low concentrations of ethanol could access into cells and dissociate the complex phenolic compounds bound to proteins and polysaccharides into the cell walls of plant cell (45).

Generally, there was no significant difference in total polyphenol content between myrtle berries extract and myrtle berries:cinnamon extract ($p<0.001$), and between myrtle berries:cloves extract and myrtle berries:cloves:cinnamon extract ($p<0.001$). This indicates that the addition of cloves to myrtle liqueur increases the content of total polyphenols, whereas the addition of cinnamon exhibited no effect on the total amount of phenolics. This could be explained by the chemical composition of cinnamon which impeded the extraction of phenolic compounds in these mixtures. Cinnamon contains large amounts of fiber and carbohydrates that can cause problems during extraction under these conditions. Dvorackova et al. reported that extraction of phenolic compounds from cinnamon is dependent on solvent, time, temperature used in extraction and technique employed, in which it was observed that maceration produced large amounts of secondary products and slime, therefore, was an ineffective method for obtaining good yield of cinnamon's phenolics (46).

Antioxidant activity

Results of the evaluation of the antioxidant activity measured by DPPH of the tested hydro-alcoholic extracts and their variability during the maceration time and with variable solvent concentrations were shown in Figure 3. Many previous studies had shown that plant extracts and essential oils have an important antioxidant activity (47-51). Several researches exhibited a positive and significant linear relationship between antioxidant activity and total phenolic content (52, 53), with phenolic acids, flavonoids, isoflavones, flavones,

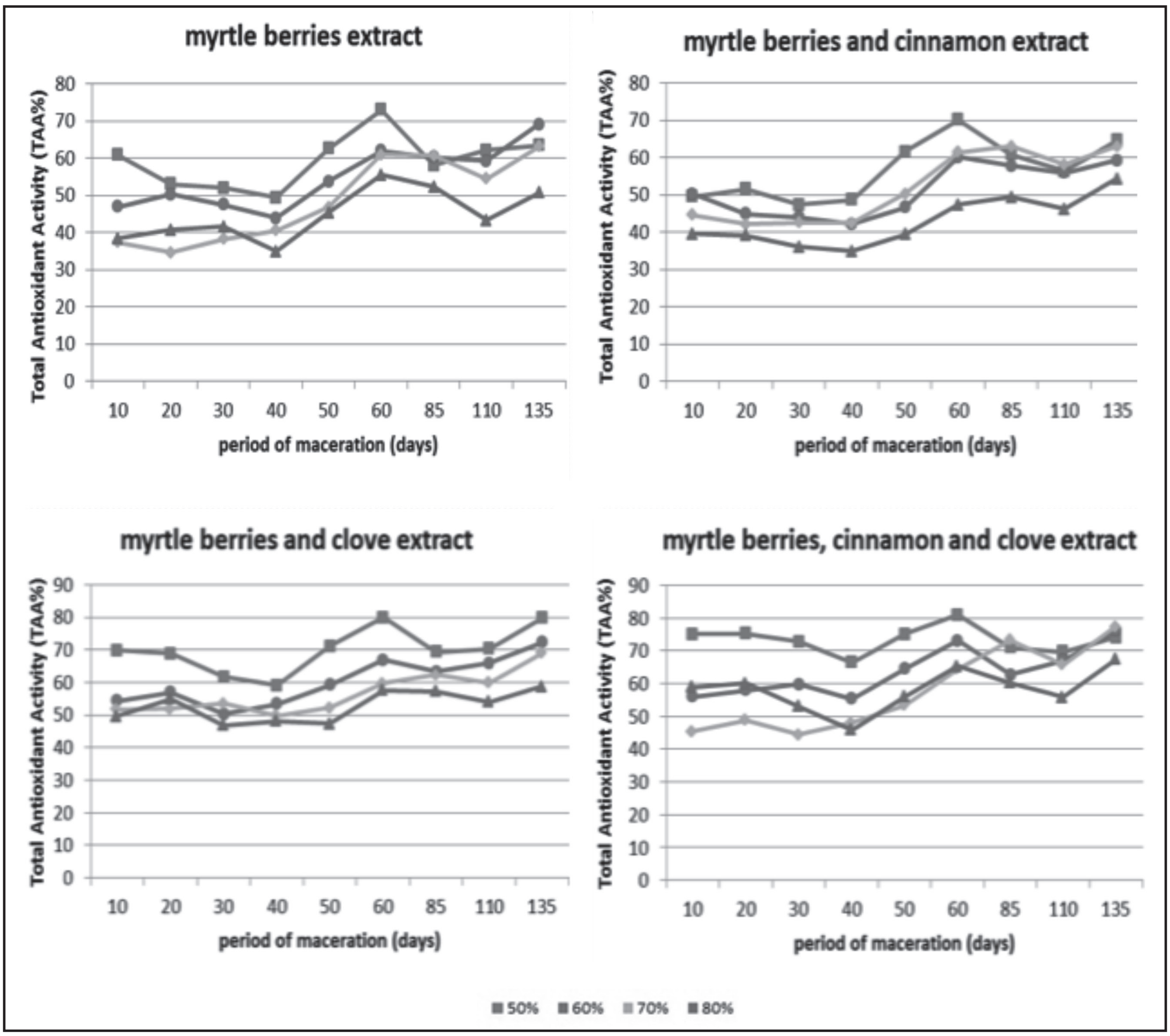

Figure 3. Changes in total antioxidant activity, measured by DPPH assay, of tested alcoholic extracts during the maceration period and with variable solvent concentrations.

anthocyanins, coumarins, lignans, catechins, and isocatechins as the main phenolic compounds known to possess antioxidant properties (54, 55). Montoro et al. also showed that anthocyanins, the main pigments in dark blue berries of myrtle, may contribute to raise radical scavenging activity of this berries (7). This is consistent with our study, where, as shown in Figure 3, all extracts showed variable antioxidant activity according to the type of extract, concentration of alcohol and time of maceration. EE50 showed the highest scavenging efficiency of free radicals after 60 days of maceration in myrtle berries:cinnamon:clove and myrtle berries:clove mixtures (80.95% and 80.02%, respectively), while the lowest efficiency was in myrtle berries and myrtle berries:cinnamon extracts (72.91% and 70.06%, respectively). This corresponds with the results we have obtained regarding the content of total polyphenols, which indicate a positive correlation between the total content of polyphenols and the antioxidant activity of the mixture, so it can be speculated that this activity is attributed to the combined effect of phenolic compounds from wild myrtle berries cloves together, whereas the effect of cinnamon was not significant in different mixtures and under different extraction conditions.

Conclusion

To the best of our knowledge, this is the first study of the influence of cloves and cinnamon addition to the traditional myrtle liqueur on its phenolic composition. Our results showed that the addition of cloves enhances the phenolic content of myrtle liqueur, whereas the effect of cinnamon was not significant. This addition also raised the total antioxidant efficacy of the mixture after 60 days of maceration. In conclusion 50% hydro-alcoholic extract of myrtle berries with cloves provide the better characteristics for liqueur preparation, in addition to the potential use of this extract as a commercial source of polyphenol.

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