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The Seasonal Variation of Polyphenolic Compounds and Antioxidant activity of *Myrtus communis* L. in Iran

Raheleh Mirzaee[1] and Sharareh Najafian[2*]

1.MSc. student, Department of Agricultural Management, college of Agriculture, Shiraz Branch, Islamic Azad University, Shiraz, Iran
2.Department of Agriculture, Payame Noor University

Corresponding author: Sharareh Najafian

ABSTRACT: The seasonal variation of the antioxidant activity (DPPH assays) and the phenolic compounds (HPLC assay) of Myrtus communis L. (Myrtaceae), grown in Iran, as affected by four seasonals, namely summer, autumn, winter and spring were investigated. Considerable amounts of inhibitory effects were observed which range from 449.71 mg l-1 in summer to 99.296 mg l-1 during spring, at the same stage in spring, the extracts showed the highest antioxidant capacity, as well as the highest phenolic content (21.04 mg gallic acid/g and 4.9604mg coumarin/g plant). In contrast, the highest accumulation of catechin and trans-ferulic acid were observed during the cooler months (winter). In other phenolic compounds, certain trends were not observed in plants in different seasons. In the warmer months (summer), the levels of phenolic compounds, and antioxidant were in most cases, lower than those in the samples harvested in the cooler months (winter). All extracts were extremely rich in phenolic compounds, and provided good antioxidant properties, but the phenol phase in which the leaves were collected affected the phenolic composition of the myrtle extracts and consequently their biological activity. The spring (March-June) extract, the richest in main phenolic compounds (gallic acid and coumarin) showed the best antioxidant properties. Thus collection of the plants during spring seems the best choice for further use in pharmaceutical and food industry.

***Keywords*:** Seasonal variation, Essential oils, Antioxidant activity, Phenolics, *Myrtus communis* L

INTRODUCTION

Myrtle (*Myrtus communis* L.) a member of Myrtaceae family is widely distributed in the Mediterranean area and is used as a culinary spice, an antiseptic and anti-inflammatory agent in traditional Medicine (Appendino ., 2002). However, little research has been done on the pharmacological effects of the plant or its specific ingredients. Myrtle extracts have been reported to be effective as antibacterial (Al-Saimary ., 2002), antihyperglycemic (Onal ., 2005), and analgesic (Levesque and Lafont, 2000) treatment. In order to reduce risk of incidence of cardiovascular, other chronic diseases and certain types of cancer, a diet must be used rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E, which leads to the revival of interest in plants-based foods.(Choi ., 2007; Dorman and Hiltunen, 2004). In the modern age of pharmaceutics research teams of many countries have already been experimenting for their potential biological, therapeutic and pharmaceutical activities (Majhenic ., 2007; Sokovic and Van Griensven, 2006; Wannissorn ., 2005). At the moment, a large number of the food borne diseases are major dilemma in the whole world (Sokmen ., 2004). It has been reported the consumption of foods contaminated with some microorganisms represents a serious of health risk to humans, but also the subsistence and growth of microorganisms in foods may lead to spoilage, formation of toxins and quality deterioraion of food products (Celiktas

., 2007). Recently, the compound of essential oils and medicinal plant extracts have attracted a good deal of interest to scientists because of their potential as a source of natural antioxidants and biologically dynamic compounds (Bozin, 2006).

New research suggests that to explore the potential of some essential oils to the treatment of infectious diseases for substitute standard pharmaceutical remedies (Celiktas ., 2007). Polyphenols are a group of secondary metabolites involved in the H2O2 scavenging in plant cells. Interest in plant materials rich in polyphenolic compounds is on the increase due to their high antioxidant potency which may offer protection against cancer through the inhibition of oxidative damage, that is known to be a potential cause of mutation. Free radicals cause oxidative damage to lipids, proteins, and nucleic acids (Shui and Leong, 2004). The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity (Yang ., 2001). They possess ideal structural chemistry for radical scavenging activity and are more effective than tocopherol and ascorbate (Pandhair and Sekhon, 2006). The most commonly found polyphenolic compounds in plant extracts include Phenolic acids, flavonoids and tannins (Naik ., 2006). Therefore, the aim of this research was to investigate the effect of seasonal variation of polyphenolic compounds and antioxidant activity of Myrtle.

MATERIALS AND METHODS

Sample preparation

Samples of Myrtus communis were collected from “Abmordi”, Mian Jangal protected area in Fars province (South of Iran). The aerial parts of Myrtus communis were collected four times: during Autumn (Sep-Dec), Winter (Dec-March) in 2013 and Spring (March-June) and Summer (June-Sep) in 2014. The herb was air-dried at ambient temperature in the shade and stored in sacks in a dark, cool and dry depository. They were authorized by Ahmad Hatami (Department of biology Fars research center, Shiraz, Iran). A voucher specimen of the plants is deposited in the herbarium of the Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Extraction of Polyphenol and HPLC analysis

The procedure for extraction of polyphenols from plant material was used according to the modified method established by Justesen (Justesen ,. 1998). HPLC analysis was carried out on a Agilent 1200 series, equipped with a Zorbax Eclipse XDB-C18 column (10cm × 5 µm i.d.; × 150 mm film thickness, RP), and a photodiode array detector (PDA). Elution was monitored at 280 and 230 nm. Gradient elution was selected to achieve maximum separation and sensitivity. The elution was performed by varying the proportion of solvent A (formic acid 1% in deionized water) to solvent B (Methanol (v/v)) as follows: Methanol. Formic acid 1% (10:90), at 0 min; Methanol. Formic acid 1% (25:75), at 10 min; Methanol: formic acid 1% (60:40), at 20 min and finally, Methanol: formic acid 1% (70:30), at 30 min. The total running time was 30 min. The column temperature was 30 ºC. The injection volume was 20µL and it was done automatically using autosampler (Najafian and Rowshan, 2013).

Preparation of plant extraction for antioxidant

20 g of plant dry weight was soaked in 200 ml methanol/water (90/10) for 2 days. The solvent was changed after one day. The extract was filtered and then concentrated in a rotary evaporator in a period of <10 min time. The yield was determined by weighing the powders. The powders were maintained at temperature of at -20°C before using. Just before each measurement, the powder was dissolved in methanol at the desired concentration and its antioxidant activity and its total phenol content were determined (Bejeli ., 2012)

Using DPPH for determination of antioxidant

The antioxidant activity of plant extract and the standard antioxidants were determined based on the radical scavenging effect of the stable DPPH free radical. The standard solution was prepared by Gallic acid. In a modified assay (Bruits ., 2001) 200 µl of a 100 mM DPPH radical solution in methanol was mixed with 20 µl of 12.5-3200 µg ml-1 extracts, gallic acid, respectively. The solutions were kept at room temperature for about 30 min. The inhibition of DPPH radical was measured using a micro-plate reader model Biotek ELx 808 at 515nm. The IC50 of each sample (concentration in µg ml-1 required to inhibit DPPH radical formation by 50%) was calculated by MATLAB software packages. The methanol solution extract without DPPH was considered as a blank and was subtracted from all of the measurements. The antioxidant activity was determined by using the following equation:

$$\text{antioxidant activity} = 100 - \frac{(\mathrm{A_{sample}} - \mathrm{A_{blank}}) \times 100}{\mathrm{A_{control}}}$$

Where "A" is the absorbance of the samples in wells. DPPH (without plant extract) and methanol were used as control and blank, respectively.

The IC50 value for each sample was defined as concentration of the test sample resulting in 50% reduction of the concentration of initial DPPH.The IC50 values were calculated using the non-linear regression between the Log concentrations of the test extract (µg ml-1) against the mean percentage of the radical scavenging activity (Bruits ., 2001).

Statistical analysis

Descriptive of data are expressed as mean ± SD. The means of Polyphenols were compared by using the two-way analysis of variance (ANOVA). The differences of individual means were deemed to be significant at $p < 0.05$. All calculation were done by SPSS (V16) software and checked with software, Minitab (v16).

RESULTS AND DISCUSSION

Antioxidant activity

The biological activities, the antioxidant properties of the isolated essential oils were recorded. Free radical capacities of the oils were measured by the DPPH assay and the results are given in fig 1. *Myrtus communis* essential oils obtained from spring and winter crops showed greater radical-activity than those collected during autumn and summer, exhibiting IC50 values: 99.3, 158.9 and 184.9, and 449.7 mg/L, respectively. Based on these results, the order of antioxidant activity of *Myrtus communis* essential oils was as follows: spring > winter > autumn > summer. No data are available in the literature regarding the antioxidant activity of *Myrtus communis* essential oil with respect to seasonal variations so that the results of the present analysis can be compared with Abdullah Ijaz ., who investigated that the essential oils of *Ocimum basilicum* of winter sample exhibited better antioxidant activity than autumn and antioxidant activity of *O. basilicum* essential oils was as follows: winter > spring > autumn > summer. In this regard, our findings are in agreement with Abdullah Ijaz . (2008) who recorded the lowest antioxidant activity in August (summer).

Polyphenols

The polyphenols as Gallic acid, Catechin, Chloregenic acid, Rutin, Quercetin, p-Coumaric acid, Coumarin, Carvacerol, Trans-ferulic acid and Ellagic acid were detected of *Myrtus communis* at different seasons (Table 1). Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Naik ., 2006). The results showed that the maximum gallic acid were (21.04 mg g−1) found in *Myrtus communis* during spring, while the minimum gallic acid of essential oil were (10.84 mg g−1) during the summer. Gallic acid is found both free and as part of hydrolyzable tannins. Salts and esters of gallic acid are termed 'gallates'. Despite its name, it does not contain gallium. gallic acid is commonly used in the pharmaceutical industry (Fiuza, 2004). Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal hemorrhaged. It is also used to treatalbuminuria and diabetes. Some ointments to treat psoriasis and external haemorrhoids contain gallic acid (phytochemicals.info). fig2.

All the tested *Myrtus communis* demonstrated higher *Catechin* in winter (when the plants reached the end of their growing cycle) than in summer (when the plants were in full bloom). Catechins are photochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Based on their structure, catechins are classified as flavanols (Williamson and Manach, 2005). Chlorogenic acid was detected (0.10 mg g−1) during autumn whereas *Rutin* was found (20.98 mg g−1) in winter and *Ellagic acid* was seen during spring (2.9 mg g−1). *Chlorogenic acid* (CGA) is a natural chemical compound which is the ester of caffeic acid and quinic acid. It is an important biosynthetic intermediate (Boerjan ., 2003) Chlorogenic acid is an important intermediate in lignin biosynthesis. This compound, known as an antioxidant, may also slow the release of glucose into the bloodstream after a meal (Johnston., 2003). Rutin is one of the phenolic compounds found in the invasive plant species Carpobrotus edulis and contributes to the antibacterial (Elmarie van der and Johan, 2001) and antioxidant (Bouftira ., 2012). Properties of the plant *Quercetin* was detected in spring (2.5 mg g−1) and summer (2.7 mg g−1), but It was a very small amount. Quercetin is a flavonoid widely distributed in nature. It is a naturally occurring polar auxin transport inhibitor (Fischer ., 1997). *P-Coumaric acid* was not detected in winter and in other seasons; the amount was less than 0.7. The maximum *coumarin* was (4.96 mg g−1) observed in *Myrtus communis* during spring. Coumarin is also used as a gain medium in some dye lasers (Duarte, 2003). *Carvacrol* was not detected in winter and summer and in other seasons

the amount was less than 0.6 mg g−1. Many diverse activities of carvacrol such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, antiinflammatory, angiogenic, antiparasitic, antiplatelet, AChe inhibitory, antielastase, insecticidal, antihepatotoxic and hepatoprotective activities and uses such as feed additive in honeybee breeding and in gastrointestinal ailments have been shown (Baser, 2008). The results were identified *trans-ferulic acid* at different seasons and the most trans-ferulic acid activity was seen in cold seasons (8.0-8.46 mg g−1). These results might be seen due to the climate or stress which causes the activities of polyphenols (Gallic acid, Catechin, Chloregenic acid, Carvacerol, etc) to be different at seasonal variation. The variations in activities of polyphenols with respect to season might have been due to the influence of phonological status, and environmental conditions can influence the regulation of the biosynthesis of polyphenols. Previous investigations have demonstrated that harvesting season can alter the chemical composition of the essential oils of M. spicata, M. pulegium and Ocimum basilicum (Kofidis ., 2004; Hussain ., 2008). There are some reports in the literature on the qualitative and quantitative analyses of some Mentha essential oils from different countries(Gulluce ., 2007; Pandey ., 2003; Viljoen ., 2006), but we could not find a single report showing of polyphenolic compounds and antioxidant activity of Myrtus communis L. in Iran. The results are different from others and indicate that this study is by no means a repetition. These findings may be extended effect of seasonal variation on polyphenolic compounds of plants with the antioxidant activity. Furthermore, these achievements show that polyphenolic compounds producers and consumers, who utilize these compounds in pharmaceutical and cosmetic industries could benefit from this result.

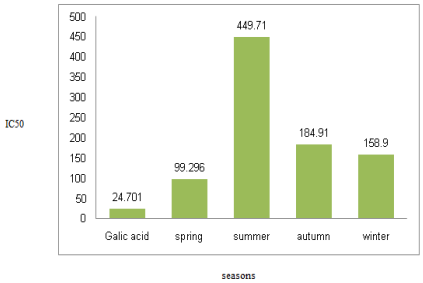

Figure 1. Effect of seasonal varation on antioxidant activity by DPPH assay

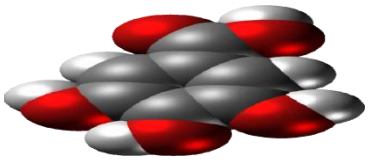

Figure 2. Chemical structure of Galic acid (Najafian and Rowshan, 2013)

Table1. Seasonal variation in Phenolic compounds. Different letters in superscript indicate significant differences within seasons and Phenolic compounds

Phenolic compounds	spring	summer	autum	winter
Gallic acid (mg/g)	21.04[a]	10.84[a]	13.03[a]	12.47[a]
Catechin (mg/g)	52.94[b]	31.13[b]	36.50[b]	83.14[b]
Chloregenic acid (mg/g)	-	-	0.10	-
Rutin (mg/g)	-	-	-	20.98
Quercetin (mg/g)	2.54	2.69	-	-
p-Coumaric acid (mg/g)	0.37	0.52	0.69	-
Coumarin (mg/g)	4.96[a]	3.84[a]	3.84[a]	3.94[a]
Carvacerol (mg/g)	0.53	-	0.25	-
Trans-ferulic acid (mg/g)	5.16[a]	4.27[a]	8.00[a]	8.46[a]
Ellagic acid (mg/g)	2.90	-	-	-

Conclusions

In conclusion, seasonal variation of secondary plant products especially polyphenolic compounds and antioxidant activity is an interesting research area which needs further studies with different aromatic plants essential oils constitute various components. Thus collection of the plants during spring seems the best choice for further use in the pharmaceutical and food industry.

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