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Original Research Article

Satureja myrtifolia (Boiss. & Hohen.) Lebanese wild plant, as a resource of natural antioxidants

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ABSTRACT

This study aims to evaluate total phenolic content (TPC) and *in vitro* radical scavenging activity (RSA) of *Satureja myrtifolia* collected from Lebanon. TPCs of *Satureja myrtifolia* were extracted by two polar solvents, namely methanol and water and subsequently determined spectrophotometrically. The RSA of both crude extracts were evaluated *in vitro* by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay. According to our findings, the obtained extracts exhibit relatively high levels of phenolic compounds which range from 343.12 ± 7.53 to 441.09 ± 10.70 mg GAE g⁻¹ in terms of dry weight (DW) depending on the solvent used. Moreover, both extracts exerted hydrogen-donating abilities in the presence of DPPH stable radical. However, the aqueous extract from the aerial parts of *S. myrtifolia* showed higher RSA capability. This study revealed that *S. myrtifolia* is a potential resource of biological active compounds which can reduce the risk of diseases and their noxious effects correlating with the antioxidant compounds.

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1. Introduction

In recent years, comprehensive investigations on natural products have gained much interest for the exploration of active compounds with antioxidant properties that can be applied in pharmacognosy and pharmaceutical sciences. The efficacy of herbal medicines in inflammatory and oxidant-related diseases has been previously reported (Hasani-Ranjbar et al., 2009; Rahimi et al., 2010). Oxidative stress plays a key role in the pathogenesis of aging and degenerative diseases, such as atherosclerosis, cardiovascular diseases, diabetes and cancer (Asmat et al., 2016; Kumar et al., 2017). Free radicals are degraded to non-reactive forms by enzymatic and non-enzymatic antioxidant defenses produced in the body and others supplied by the diet. Among these, extracts of different aromatic plants and their essential oils have been studied for their potential antioxidant capacities (Emami et al., 2010; Miguel, 2010; Chang et al., 2016; Mohammadhosseini, 2016;

Mohammadhosseini et al., 2016a; Mohammadhosseini et al., 2016b; Xu et al., 2017) which can be attributed to the presence of phenolic and volatile compounds contributing to the free radical scavenging activity (Edris, 2007).

The Lamiaceae family is one of the major and well-known sources of species containing large amounts of phenolic secondary metabolites because of its antioxidant properties. The genus *Micromeria* contains more than 60 plant species in the world and especially around the Mediterranean basin (Hilan et al., 2011). *Satureja myrtifolia* (Syn: *Micromeria myrtifolia* Boiss. & Hohen) is a perennial plant, belonging to the Lamiaceae family, which grows in rocky and limestone slopes exposed to sunlight (El-Beyrouthy et al., 2008; Zebib et al., 2015).

Located in the heart of the Mediterranean, Lebanon country, rejoices in a mild and sunny climate which is favourable for the growth of aromatic and medicinal plants. In Lebanon, *S. myrtifolia* plant, known as "Zoufa or Achnan Daoud", has been used in the traditional

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medicine for the treatment of pneumonia, respiratory infections, cough, stomachache, mouth ulcer, gastritis, and recommended as a cardi tonic, febrifuge, diuretic, and an internally expectorant agent, while the cooled infusion has been recommended as a gargle for laryngitis and as an external antiseptic remedy (El-Beyrouthy et al., 2008; Zebib et al., 2015). Generally, Lebanese people consume *S. myrtifolia* as an herbal tea infusion to prevent diseases.

In the literature, studies on *S. myrtifolia* species remain very poor. To our knowledge, to date only one study on the antioxidant properties (Formisano et al., 2014) and five studies on essential oil composition (Ozkan et al., 2007; Hilan et al., 2011; Formisano et al., 2014; Zebib et al., 2015; Carikçi, 2016) of *S. myrtifolia* have been published. However, it was shown that the essential oil of *S. myrtifolia* represents a lower RSA compared to the crude extracts in DPPH and ferric reducing antioxidant power (FRAP) methods (Formisano et al., 2014), e.g., methanolic extracts from the aerial parts of *S. myrtifolia* possess an RSA four times higher than that of the corresponding essential oil (Formisano et al., 2014). Hence, it is interesting to investigate the RSA of *S. myrtifolia* extracts.

In this context, this study aims to evaluate the TPC and the RSA of *S. myrtifolia* crude extracts and compare them with the only previous study (Formisano et al., 2014). It should be noted that water was selected in this study to provide a scientific evidence, if exists, for the Lebanese traditional people way, which widely consume the aerial parts of *S. myrtifolia* plant as tea infusion.

2. Experimental

2.1. Plant material

The aerial parts (leaves, stems) of *S. myrtifolia* plant were harvested at the maturity stage during May/June 2016 from south of Lebanon country, South region, Kfar Hatta village (GPS data: elevation 350 m, longitude 35° 26'47.04"E, and latitude 33° 30'28.08"N) (Fig. 1). Plant samples were dried at summer ambient temperature (25-30 °C) away from the sun's rays and conserved for future use.

S. myrtifolia is an herb (20-60 cm), covered on all parts with glandular and non-glandular hairs. Stems are numerous, verticillasters globose-hemispherical and many flowered with purple pink color. Leaves are dorsiventral and hemispherical (Fig. 2). *S. myrtifolia* (Boiss. & Hohen) has been authenticated and cataloged in the herbarium of the Faculty of Agronomy (Holy Spirit University of Kaslik, Lebanon) under voucher specimen number MNV175a. Plant synonym (Taxonomy): *Micromeria myrtifolia* (Boiss. & Hohen.) Greuter & Burdet.

2.2. Extraction of total phenolic content



Fig. 1. Geographical map of Lebanon country showing study area (South) with the sampling site (kfar Hatta village).



Fig. 2. *Satureja myrtifolia* plant at flowering period (A) and at maturation stage (B).

Two solvents (water and methanol) were used for the extraction of TPC. 1.0-gram portions from the aerial parts of *S. myrtifolia* plant (leaves and stems) were powdered and extracted for 24 h with 20 mL of water or methanol (95%) at room temperature (30 °C), followed by rapid paper filtration through a Whatman No 0.45 mm filter paper. The resulting solutions were evaporated under the vacuum at 30 °C by Buchi Rotavapor R-200 to dryness. The residues were then dissolved in 3 mL of water or of methanol. The extraction yields (w:w) were: 24% (water) and 18.65%

Table 1

 Extraction yield, Total phenolic contents (TPC) and radical scavenging activity (RSA) of *Satureja myrtifolia* extracts.

Sample (<i>S. myrtifolia</i>)	TPC extraction yield (% w:w)	TPC (mg GAE g ⁻¹ DW) ^a	RSA IC ₅₀ (µg mL ⁻¹) TEs ^b	DPPH inhibition (%) (at 292.00 µg mL ⁻¹)
ME ^c	24.00	441.09 ± 10.70	155.00 ± 3.67	79.00 ± 1.45
WE ^d	18.65	343.12 ± 7.53	89.00 ± 1.95	80.00 ± 1.72
Trolox	-	-	14.60 ± 0.54	99.00 ± 1.71

^aDW: dry weigh; ^bTEs: Trolox equivalents; ^cME: Methanol extract; ^dWE: Water extract; ^eValues represent means±SD of three independent experiments carried out in triplicates.

(methanol).

2.3. Evaluation of total phenolic content

The TPC from both extracts were determined by a standard spectrophotometric technique using "Folin-Ciocalteu" reagent assay (Sánchez-Rangel et al., 2013). Accordingly, a volume of 20 µL of each sample solution and calibration solutions of gallic acid (50, 100, 150, 200 and 250 mgL⁻¹) were mixed with 1 mL of Folin-Ciocalteu reagent being diluted 10 times with water. Then, 8 mL of sodium carbonate (7.5% Na₂CO₃) was added to the obtained mixture and well stirred. After incubation for 30 min. at ambient temperature (20 °C), the absorbance was read at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. TPC was expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE g⁻¹ DW). All measurements were performed in triplicate. Data were expressed as means±standard derivation (±SD).

2.4. DPPH scavenging assay

The hydrogen atom donation ability of phenol compounds was measured on the basis of scavenging the 2,2'-diphenyl-1-picrylhydrazil free radical (Sánchez-Moreno et al., 1998; Dobravalskyte et al., 2013) with slight modifications. Accordingly, stock solutions of extracts were prepared to have a concentration of 1.0 mg/mL. Further dilutions were made to obtain concentrations of 10, 20, 40 and 60 µg/mL. Then, 50 µL of various concentrations of the extracts were added to 1950 µL of a 0.025 g L⁻¹ DPPH solution in methanol. The mixture was stirred and then maintained at ambient temperature (20 °C). After 30 min, the absorbance was measured at 510 nm. A sample containing methanol with all reagents (without extract) was used as blank. It is evident that the maximum absorbance (100% abs) relates to the DPPH alone. Trolox (concentrations: 5, 10, 25, 50 and 75 µM) was used as the standard. The measurements were carried out on 96-well microplates in triplicate for each sample and each concentration of standard. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula (equation 1):

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad (\text{eqn. 1})$$

Where A_{blank} is the absorbance of methanol, A_{sample} is the absorbance of the test compound (plant extract). Extract concentration providing 50% inhibition (IC₅₀) was calculated using the dose-inhibition curve in the linear range by plotting the extract concentration versus the corresponding scavenging effect (Jothy et al., 2011). Both DPPH scavenging and IC₅₀ values were negatively correlated to the concentration of the substrate; thus, the higher the antioxidant levels, the lower the DPPH scavenging and IC₅₀ values. The Trolox was used as positive control. All evaluations of RSA were performed in triplicate and expressed as Trolox equivalents (µg mL⁻¹ TEs). Data were expressed as means±standard derivation (±SD).

3. Results and Discussion

3.1. Phenolic compounds of *S. myrtifolia* extracts

Antioxidants from natural sources can improve the antioxidant system in body due to their scavenging characteristic of free radicals (Rahimi et al., 2010; Adewoyin et al., 2017). Polyphenols are considered to be the major contributors to the radical scavenging activity of medicinal plants, fruits and vegetables (Sulaiman et al., 2011). The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers, as well as metal chelating agents. It is important to indicate that *S. myrtifolia* plant was consumed by Lebanese people as a tepid infusion to prevent diseases. For this reason, water was selected as one of the used solvents to provide, scientifically, the evidence for the Lebanese traditional people consumption way of *S. myrtifolia* plant.

Therefore, in the present study, the TPCs from aqueous and methanolic crude extracts of *S. myrtifolia* were estimated and the respective results are given in Table 1. Results show that *S. myrtifolia* crude extracts contain high amounts of TPC. Methanolic extract contained more TPC (441.09±10.70 mg GAE g⁻¹) than the aqueous extract (343.12±7.53 mg GAE g⁻¹) from the aerial parts of *S. myrtifolia*. The effect of the solvent systems on the amount of TPC was significant, as well. The results presented here are in agreement with many previously published studies showing that polar solvents extract the highest antioxidants from

botanical materials (Dobravalskyte et al., 2012). It is evident that water ($\epsilon=78.4$) is more polar than methanol ($\epsilon=32.7$), but it is not strange that the aqueous extract of *S. myrtifolia* contains lesser TPC than methanolic extract. These results suggest that some polyphenols classes are poorly or partially extracted by water phase due to their limited degree of solubility affected by their diversified structures and configurations (Khan and Dangles, 2014). Compared with the previous data in the literature, TPC levels obtained in the extracts of *S. myrtifolia* were much higher than those reported in the only previous study (Formisano et al., 2014), in which TPC level was 221.80 ± 0.05 mg GAE/L. It should be noted that in our study, TPCs were extracted at 35 °C for 24 h, while the previous study showed a different extraction conditions (at 50 °C for 1 week). This significant difference in TPC amount can be attributed to the extraction conditions of phenolic derivatives. In fact, the recovery of phenolic compounds from plant materials is influenced by the duration of extraction, which affect polyphenol solubilization (Ignat et al., 2011; Azmir et al., 2013). In addition, long extraction times can increase the probability of oxidation of phenolics compounds which will decrease the yields and change the conformation of the extracted polyphenols (Dai and Mumper, 2010). In conclusion, the obtained results confirm that adopted conditions in this report for the extraction are more effective than those reported in the previous study (Formisano et al., 2014).

3.2. Radical scavenging activity of *S. myrtifolia* extracts

For more information about the RSA of TPC, both extracts were evaluated by using DPPH free radical scavenging activity assay. This method has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Compounds with radical scavenging capacity are able to reduce DPPH radical through the transfer of hydrogen atom to DPPH free radical. Interaction of antioxidant compounds with DPPH is based on electron or hydrogen atom shift to DPPH radical leading to its subsequent conversion to 1-1'-diphenyl-2-picrylhydrazyl (Anouar et al., 2013; Schaich et al., 2015). The reduction of DPPH radicals causes discoloration from purple color to yellow pale color accounting for the scavenging activity of the tested extracts. The decrease of absorbance of DPPH radicals was finally measured at 510 nm.

The results of RSA, expressed in $\mu\text{g mL}^{-1}$ TEs or in DPPH inhibition percents of aqueous and methanolic extracts at the same concentration ($292.00 \mu\text{g mL}^{-1}$ TEs) were 80.00 ± 1.45 and $79.00 \pm 1.67\%$, respectively while Trolox as the positive standard had an RSA of $99.00 \pm 1.72\%$ under the same condition (Fig. 3). The IC_{50} value for *S. myrtifolia* extracts determined from the linear range of the DPPH inhibition curve (Fig. 4).

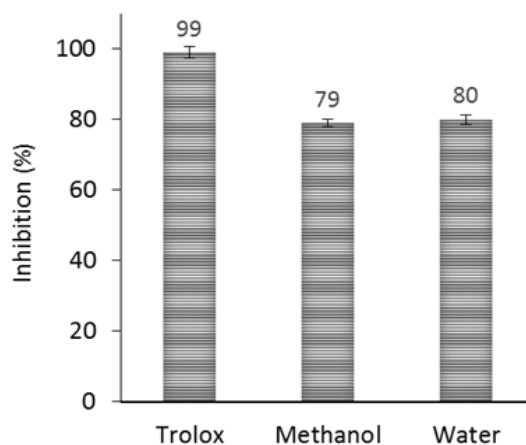


Fig. 3. DPPH inhibition (%) of *Satureja myrtifolia* extracts compared to standard (Trolox) at a concentration of $292.00 \mu\text{g mL}^{-1}$. Values are mean \pm SD of three independent experiments.

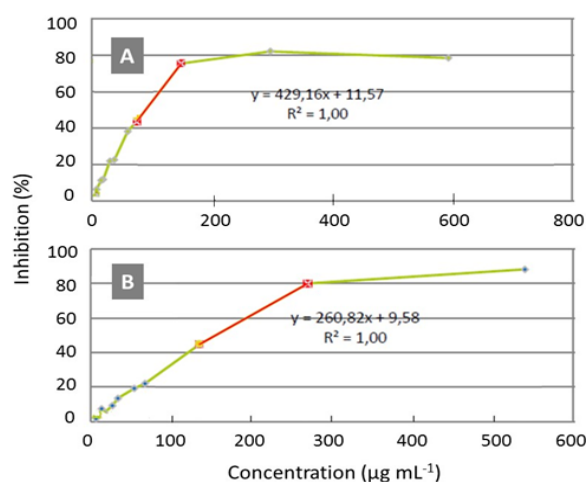


Fig. 4. DPPH inhibition profile (%) and IC_{50} of *Satureja myrtifolia* aqueous extract (A) and methanolic extract (B) determined from linear range of curve.

The obtained results show that the RSA of *S. myrtifolia* aqueous extract was higher than that of methanolic extract (Table 1). This result is in agreement with previously studies of Lamiaceae species in which aqueous extract of *Calamintha glandulosa* showed a higher RSA compared with the methanolic extracts (Ćavar et al., 2013) and higher than that of hexane, ethyl acetate, butanol extracts of selected Algerian Lamiaceae (Boulekbache-Makhlouf and Madani, 2014). These results could be explained by the presence of more hydro-polyphenols in the aqueous extract having more biological activities than those present in methanol extract, leading to an increase in RSA. Also, the RSA can be affected by the solubility and the quality of polyphenols, which governed by the polarity of the solvents used. By comparing with the RSA of Trolox at the same dose, *S. myrtifolia* aqueous extract has an RSA six times less, while the RSA of methanolic extract is about 10 times lower than that of Trolox (Table 1).

These data are interesting since the comparison was made between a crude extract, a complex mixture of compounds characterized by very different levels of biological properties, and Trolox, as a pure and strong antioxidant standard. With these results, it is possible to establish that the pharmacological effects attributed to *S. myrtifolia* in Lebanese folk medicine are in part due to the same radical scavenging activity that has been observed in other species of *Satureja* (Güllüce et al., 2003; Eminagaoglu et al., 2007; Ozkan et al., 2007; Hajhashemi et al., 2012). In addition, it is noticeable that aqueous and methanolic extracts of *S. myrtifolia* species contain biological active compounds which can reduce the risk of diseases and their side effects which correlated with the antioxidant compounds.

4. Concluding remarks

The present study provides some information on TPC and RSA of *S. myrtifolia* wild plant grown in Lebanon. The findings of this report showed that both extracts (aqueous and methanolic) contain valuable biological active components, such as antioxidants. The mean level of TPC reached 441.09 ± 10.70 mg GAE g^{-1} in terms of dry weight (DW) for the methanolic extract of *S. myrtifolia*.

Considerable antioxidant abilities of these extracts have been shown in DPPH test. By comparing with RSA of Trolox (14.60 ± 0.54 $\mu g mL^{-1}$) at the same dose, *S. myrtifolia* aqueous extract has an RSA six times less (89.00 ± 1.95 $\mu g mL^{-1}$ TEs), while the RSA of methanolic extract is about 10 times lower (155.00 ± 3.67) than that of Trolox. These data are interesting since the comparison was made between a crude extract, a complex mixture of compounds characterized by very different levels of biological properties, and Trolox, as a pure and strong antioxidant standard.

In addition, scientific data obtained from the aqueous extract of this herbal plant provides an evidence for the Lebanese traditional folk medicine, which use the aerial parts of *S. myrtifolia* as tea infusions to prevent diseases.

Finally, we hope that this study would help Lebanese community for attaining a protection program for *S. myrtifolia* natural sites and encourage local people to cultivate it. Also, we wish that the extensive studies on this species will provide clues about their possible pharmaceutical exploration in the field of oncology.

Conflict of interest

The authors declare that there is no conflict of interest.

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