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Original Article

Quantitative determinations on commercial samples of Melissae folium and their antioxidant activity

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ABSTRACT

Background and Aims: *Melissa officinalis* L. (lemon balm) is a perennial herb. Melissae folium and their preparations have been used for their sedative, spasmolytic and antibacterial actions. The study was aimed to investigate the qualities and also to compare the antioxidant activity potentials of the drug samples available in herbal markets and pharmacies in Turkey
Methods: The percentages of the loss on drying and total ash were determined by gravimetric method and the percentage of total hydroxycinnamic derivatives was calculated by a spectrophotometric method according to European Pharmacopoeia. Drug samples were investigated for their potentials to scavenge the DPPH radical by using an *in vitro* method.
Results: The percentages of the loss on drying were found to be between 8.51-16.53 %; whereas total ash amounts were determined between 9.41-11.33%. The percentage of total hydroxycinnamic derivatives was found in the range of 4.45-12.97 %. The extracts of the samples were found to have DPPH radical scavenging activity with EC_{50} values ranging from 10.60 to 19.10 µg/ml.
Conclusion: In the assays for total ash and quantification of total hydroxy cinnamic derivatives all of the examined commercial samples were found to be compatible with standards in European Pharmacopoeia. Among the tested samples; a sample sold in pharmacy seems to have the best quality when its compared with the standards in European Pharmacopoeia.

Keywords: Melissae folium, European Pharmacopoeia, quality control analysis

INTRODUCTION

Melissa officinalis L., commonly known as lemon balm, is a perennial herb belonging to the Lamiaceae family. Preparations, which are introduced in folk medicine as infusion from dried *M. officinalis* leaves, are recommended against colds and are used in functional disorders of the circulation. Preparations of lemon balm have been used for their sedative, spasmolytic and antibacterial actions. They are, therefore, employed for gastrointestinal disorders of nervous origin, in psychosomatic cardiac disorders and against migraine (Wichtl & Bisset, 1994). The Lamiaceae are a promising source of natural antioxidants due to the large amount of phenolic acids found in many species of this family (Weitzel & Petersen, 2011; Barros et al., 2013). Rosmarinic acid (a hydroxycinnamic derivative), which is one of the main secondary metabolites (phenolic acids) in the leaves, is potent antioxidant. The rosmarinic acid is considered an analytical marker for *M. officinalis* (Petersen & Simmonds, 2003). In the monograph of European Pharmacopoeia 6th edition, the percentage of total hydroxycinnamic derivatives of the herbal drug is expressed as rosmarinic acid.

The purpose of this study is to compare some quality control parameters of the commercial samples sold in the pharmacy and herbal market in Turkey with respect to the methods available in European Pharmacopoeia. In this context, assays for loss on drying and total ash were carried out by the gravimetric method. The content of total hydoxycinnamic derivatives were determined (expressed as rosmarinic acid)

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by using a spectrophotometric method (European Pharmacopoeia 6th edition; Arnow, 1937; Vladimir-Knežević et al. 2011). Moreover, the antioxidant activity of the samples were examined by using the DPPH method, which is a widely used and simple method employed for the determination of antioxidant activity (Brand-Williams, Cuvelier & Berset 1995; Choi et al., 2002).

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-2-picrylhydrazyl) reagent and methanol were purchased from Sigma- Aldrich (Germany). All other reagents and solvents used were of analytical grade.

Sample Preparation

Dried commercial samples were purchased from herbal market and pharmacies in Turkey. A and D samples of Melissae folium were obtained from two different herbal markets; B and C samples were obtained from two different pharmacies. The drug specimens were finely powdered. Methanolic and ethanolic extracts of Melissae folium were used in the studies. For methanolic extract preparation; the pulverized sample was weighed (2 g) and extracted with methanol (20 mL) in an ultrasonic bath for 30 min, three times. The extraction was followed by filtration and the filtrate was evaporated by using a rotary evaporator (Choi et al 2002).

An ethanolic extract was prepared according to European Pharmacopoeia 6th edition. The powdered plant material (0.20 g) was extracted with 50% ethanol (190 mL) under a reflux condenser in a boiling water bath for 30 min. The cooled extract was filtered, the filter rinsed with ethanol, and then the filtrate and rinsing solution was combined and diluted to 200.0 mL with 50% ethanol.

Procedures recorded in the European Pharmacopoeia 6th edition were used to determine the amounts of total hydroxycinnamic derivatives, found in samples. The assays for loss on drying and total ash were performed according to European Pharmacopoeia 8th edition. All the experiments were performed in triplicate.

Quantitative determination of total hydroxycinnamic derivatives, expressed as rosmarinic acid

Determination of hydroxycinnamic acid derivatives was performed according to the procedure described in European Pharmacopoeia 6th edition. Briefly, an aliquot of the ethanolic extract (1.0 mL) was mixed with 0.5 M hydrochloric acid (2 mL), Arnow reagent (10% aqueous solution of sodium nitrite and sodium molybdate, 2 mL) and 8.5% sodium hydroxide (2 mL) and diluted to 10.0 mL with water. The absorbance of the test solution was measured immediately at 505nm against blank. The content of total hydroxycinnamic derivatives was calculated and expressed as rosmarinic acid, according to the following expression: (%)=A×5/m, where A is the absorbance of the test solution at 505 nm and m is the mass of the sample, in grams. (European Pharmacopoeia 6th edition; Vladimir-Knežević et al., 2011)

DPPH radical scavenging activity

The samples were extracted with methanol and analyzed for antioxidant activity by DPPH (2,2-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995; Choi et al., 2002).

The free radical scavenging activities of the samples were measured using the stable DPPH radical, according to the method of Brand Williams. Briefly, 0.3 mM solution of DPPH in methanol was prepared and this solution (1 mL) was added to sample solution in methanol at different concentrations (2.5–100 µg/mL). The mixture was allowed to stand for 30 min in the dark, and the absorbance was then measured at 517 nm.

The capability to scavenge the DPPH radical (EC%) was calculated using the following equation: $EC\%=100-[Abs_{sample}- Abs_{blank}) x100/Abs_{cont}$), where Abs_{sample} is the absorbance obtained in the presence of the different extract concentrations and Abs_{blank} is that obtained in the absence of extracts. A methanol plus plant extract mixture was used as a blank. All the determinations were done in triplicate. Ascorbic acid was used as a positive control. The results are presented as mean±SD. EC_{50} correlation analysis was carried out with GraphPad Prism version 5.03 for Windows, GraphPad Software, San Diego California USA.

RESULTS AND DISCUSSION

In the context of quality control experiments, the loss on drying and total ash contents of the specimens were determined. The amounts of loss on drying were found to be between 8.51-16.53% and the total ash contents were found to be between 9.41-11.33% (Table 1). In the European Pharmacopoeia 8th the limit for loss on drying was 10% and for total ash was 12%.

The total ash contents of all the samples were compatible with the standard values in the monograph, however the content of loss on drying was higher than the limit value in three samples. This might be due to the storage conditions of the samples.

The content of total hydroxycinnamic derivatives was determined by a spectrophotometric method using the Arnow reagent (Vladimir-Knežević et al., 2011). The results are expressed as rosmarinic acid. In European Pharmacopoeia 6th edition, it is indicated that pharmacopeial grade Melissae folium contains at least 4% total hydroxycinnamic acid derivatives expressed as rosmarinic acid. In our study, the range of total hydroxycinnamic acid derivatives was found between 4.45-12.97% (Table 1) and the results were compared with the results of the previous published data.

Carnat, Carnat, Fraisse & Lamaison, (1998), reported that, the total hydroxycinnamic acid content was determined by a spectrophotometric method with the Arnow reagent and as a result total hydroxycinnamic acids based on the dry weight of the leaf were found as 11.29%.

In a study by Aprotosoaie, Raileanu, Trifan, & Cioanca, (2013) total hydroxycinnamic acids expressed as g rosmarinic acid / 100 g dry weight in *Melissa officinalis* sample were found to be 4.15% using a spectrophotometric method.

Table 1. Quantitative determinations on lemon balm and its radical scavenging activity.

Sample code	Loss on drying % (±SD)	Total ash % (±SD)	Total hydroxycinnamic derivatives % (±SD)	Radical scavenging activity (EC_{50} µg/ml)* (±SD)
A	**12.08 (0.16)**	9.41 (0.10)	4.45 (0.09)	18.75 (3.46)
B	8.51 (0.09)	11.31(0.03)	8.85 (0.56)	17.60 (2.81)
C	**16.53 (0.29)**	10.05 (0.09)	12.97 (0.70)	10.60 (3.32)
D	**11.22 (0.17)**	11.33 (0.11)	4.97 (0.40)	19.10 (0.40)

All of the analysis were performed in triplicate. Loss on drying, total ash and total hydroxycinnamic derivatives are based on the dry weight of the leaf.
*EC_{50} means the effective concentration providing 50% effect. Concentration µg of dried Melissae folium extract/ml (final concentration)

In another study, the contents of hydroxycinnamic acid derivatives of *Melissa* samples were determined according to the assay methods instructions of the European Pharmacopeia 2008. The determined values ranged between 7.4 and 15.5% (Krüger, Schütze, Lohwasser & Marthe, 2010, Kittler et al., 2018).

For antioxidant activity, the methanolic extracts were analyzed by the DPPH method. The methanolic extracts of the samples were found to have DPPH radical scavenging activity with EC_{50} values ranging from 10.60 to 19.10 µg/mL (Table 1).

In the present study, the methanolic extract of Melissae folium showed a potent effect on scavenging the DPPH radical with a EC_{50} value similar to the results of previous studies on methanolic extracts of *M. officinalis* such as 13.74 µg/mL (López et al., 2007) and 24.3 µg/mL (Pereira et al., 2009)

Compatible results were obtained in antioxidant activity determinations on different extracts obtained from *Melissae officinalis* by using the DPPH method. In one study, an EC_{50} value of 9.76 dried sample mg/ml was calculated for the aqueous methanol (80%) extract of the plant (Karadağ, 2019).

In another study, the EC_{50} value for Melissae folium aqueous ethanol extract (70%) was calculated as 65.1 µg/mL (Benedec et al., 2015), while the EC_{50} value for aqueous ethanol extract (70%) was 512 mg trolox equivalent (TE)/g dw (dried weight) (Franco, Pugine, Scatoline, & Melo, 2018).

Low EC_{50} values indicate higher radical scavenging activity and therefore higher antioxidant activity. Phenolic acids are generally responsible for antioxidant activity. In the assay, for antioxidant activity, ascorbic acid was used as a standard (EC_{50} 3.31 µg/mL).

CONCLUSIONS

To the best of our knowledge, this is the first quality control study on commercial samples of Melissa folium sold in Turkey, revealing the quality of *Melissa officinalis* grown in our country and the herbal drug Melissae folium (its effective compounds, its antioxidant activity potential and quality properties such as moisture, ash). In the assays for total ash and quantitative determination of total hydroxy cinnamic acid derivatives, all of the examined commercial samples were found to be compatible with standards in the European Pharmacopoeia 6th and 8th. In contrast, the moisture contents of the samples were found to be higher than the values recorded in the European Pharmacopoeia 8th except one of the samples examined. This finding indicates that the sample was either not well dried or later absorbed moisture during packaging and transportation. Therefore, this study also pointed out that attention should be paid to moisture in the preparation and storage of herbal drugs.

Among the tested samples, sample B (a sample sold in pharmacy) seems to have the best quality with regard to the standards in the European Pharmacopoeia 6th and 8th .

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Conflict of Interest: The authors have no conflict of interest to declare.

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