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COMPARATIVE STUDY ON THE EFFECT OF DIFFERENT METHODS OF DRYING ON PHENOLICS CONTENT AND ANTIOXIDANT ACTIVITY OF SOME EDIBLE PLANTS

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ABSTRACT

Antioxidants have important role in prevention and treatment of disorders and plants are considered as rich sources of radical scavenging compounds. Nowadays, many herbs are used in dried form which is possible to affect their antioxidant activity. In the present investigation, the effects of different drying methods on the antioxidant capacity and total phenolics content of some culinary plant species have been studied. Leaves of *Mentha piperita*, *Mentha spicata*, *Thymus vulgaris* and *Anethum graveolense* were dried in sun, shade, oven and microwave and extracted with methanol. Antioxidant activity of each extract was determined by DPPH free radicals and total phenolics content was measured by folin cio-calteus reagent. The results revealed that drying process reduced the antioxidant activity of *Anethum graveolense* whereas sun-drying and shade-drying increased the antioxidant activity of *Mentha piperita*, *Mentha spicata* and *Thymus vulgaris* (Lamiaceae). During sun-drying and shade-drying, total phenolics content increased for the three plants of Lamiaceae while oven- and microwave-drying reduced the phenolics content. Drying also declined the phenolics content of *Anethum graveolense* which gives the conclusion that some kind of correlation could be established between total phenolics content and antioxidant behavior for the three Lamiaceae and *Anethum graveolense*. Our study brought the view that diversity in the methods of drying could lead to different phenolics content and antioxidant behavior, suggesting that each plant needs a special drying method.

INTRODUCTION: Most edible herbs regarded as spices are traditionally prepared through different methods. Sometimes the fresh material is directly consumed along with the meal or the herbs might be air-dried or alternatively exposed to the direct sun light prior to use while oven and microwave drying are considered newer methods. People are used to dry spicy plant material in order to keep the herbs for future cooking as well as reducing the risk of bacterial or fungal contamination.

Many of culinary herbs which are used for flavoring food stuff contain antioxidants a part of which could be phenolic compounds.

Phenolic compounds are secondary metabolites that are synthesized by plants during development and in response to conditions such as infection, wounding and UV radiation. Approximately 8000 naturally occurring compounds belong to the category of "phenolics", all of which share a common structural feature: an aromatic ring bearing at least one hydroxyl substituent [1]. The term "plant phenolics" encompasses simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, tannins, lignans and lignins [2].

Antioxidant capacity of plant materials relies on the content and composition of phenolic compounds as well as other groups of natural substances. Compounds with antioxidant activity prevent the oxidative damage induced by free radicals and other reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [3]. Since many herbs are used as dried form, drying process may affect their phenolics content and antioxidant activity; therefore, it is necessary to determine the best method of drying to maintain the antioxidant effects and total phenolics content. The aim of the present study was to determine the influence of different methods of drying on the total phenolics content and antioxidant activity of *Mentha piperita*, *Mentha spicata*, *Thymus vulgaris* and *Anethum graveolense* which are commonly used as spices.

MATERIAL AND METHODS:

Chemicals: DPPH free radicals were purchased from Sigma (Steinheim, Germany). Folin Ciocalteus reagent was prepared from Merck (Darmstadt, Germany). All solvents were analytical grade (Merck, Germany).

Plant Material: *Mentha piperita*, *Mentha spicata* and *Thymus vulgaris* were obtained from Institute of Medicinal Plants, ACECR, Tehran, where a voucher specimen of each species is kept for further reference. *Anethum graveolense* was provided from the market.

Drying process: 5 grams of the plants leaves were used in each of the following processes; every experiment was carried out in triplicate.

1. **Sun-drying:** The plant materials were exposed to direct sunlight and were left to dry.
2. **Shade-drying:** The drying process was performed in a dark place away from sun exposure and in appropriate air flow.
3. **Oven-drying:** The species were kept in glass plates and placed in the oven. The oven temperature was adjusted to 80°C.
4. **Microwave-drying:** Glass plates containing the plant materials were placed in a microwave with the power strength adjusted to 100 W.

Duration of drying in each experiment has been reported in **table 1**.

TABLE 1. DURATION OF DIFFERENT METHODS OF DRYING

Drying Method → / Plant name ↓	Sun (day)	Shade (day)	Microwave (minute)	Oven (hour)
Mentha piperita	2	5	40	2
Mentha spicata	2	4	30	1.5
Thymus vulgaris	1.5	3	30	2
Anethum graveolense	2	4	30	2

Fresh Plant Extraction: 5 g of *Mentha piperita*, *Mentha spicata*, *Thymus vulgaris* and *Anethum graveolense* was extracted with methanol for 24 h using maceration method. The filtrate was transferred to a 50 mL volumetric flask and the plant residue was further extracted with methanol for another 24 h. It was then filtered and the filtrate was added to the previous one in the volumetric flask and diluted to the volume. The same process was carried out in triplicate for each species to offer three separate extracts.

Dry Plant Extraction: The dried plants materials were ground (particle size 500µm). The extraction process was preformed thereafter just the same as the fresh plant extraction and in two stages with methanol.

Finally for each of the species fifteen separate extracts were prepared (three fresh plant extracts and twelve extracts of four drying methods). The extracts were subjected to further antioxidant and total phenolics content studies.

DPPH Radical Scavenging Assay: This method is one of the most extensively used antioxidant assays for plant samples which is based on scavenging of DPPH free radicals by antioxidant agents, that produces a decrease in absorbance at about 520 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color. This delocalization is also responsible for the deep violet color, characterized by an absorption band at about 520 nm [4, 5].

In the present study in order to determine DPPH radical scavenging activity of the extracts, 2 mL of a 100µM DPPH methanol solution was added to 2 mL of various concentrations of the extracts. The mixture was shaken vigorously and left to stand at room temperature for 30 min. Then, the absorbance of the solutions was measured at 517 nm and antioxidant activity calculated using the following equation:

Scavenging capacity % = 100-[(ABS of sample-ABS of blank)×100/ABS of control]. Methanol (2mL) plus plant extract solution (2mL) was used as blank, while DPPH solution (2mL) plus methanol (2mL) was used as negative control. Vitamin C was used as positive control as well. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration. The tests were performed in triplicate [6, 7].

Determination of Total Phenolics Content: Total phenolics content of the extracts was determined colorimetrically with the Folin-Ciocalteus assay[8]. 400 µL of the extracts, methanol or standard solution of gaillc acid (25, 75, 125, 200 and 250 mg/mL) was mixed with 3 mL of Folin-Ciocalteus reagent (10 fold diluted with distilled water) and allowed to stand at 22° for 5 minutes. 3 mL sodium hydrogen carbonate solution (6%) was added thereafter and the mixture was left to stand at 22° for 90 minutes. The absorbance was then measured at 725 nm with an UV-VIS spectrophotometer. The data for the total phenolics content were expressed as mg of gallic acid equivalents (GAE) per 1 g of the plants.

Statistics: Statistical analysis was carried out using one-way ANOVA test with GSTAT software.

RESULTS AND DISCUSSION: Antioxidant activity and total phenolics content of the fresh plant extracts along with the extracts obtained from different drying processes have been demonstrated in **figures 1 and 2**.

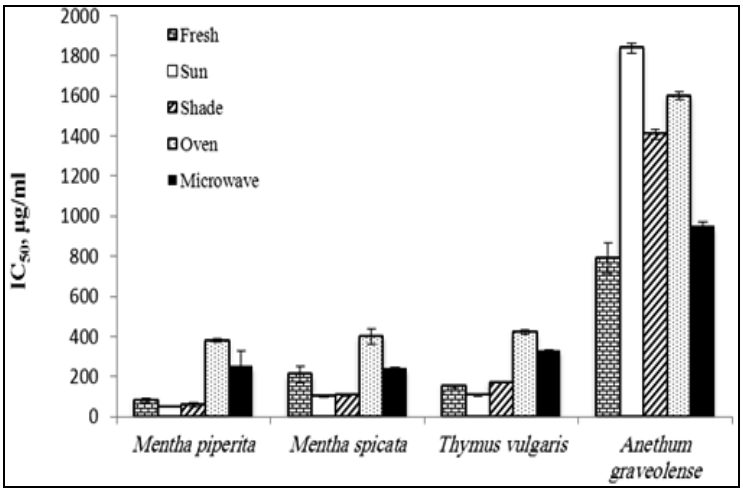

FIGURE 1: ANTIOXIDANT ACTIVITY OF FRESH AND DRIED *MENTHA PIPERITA, M. SPICATA, THYMUS VULGARIS* AND *ANETHUM GRAVEOLENSE*

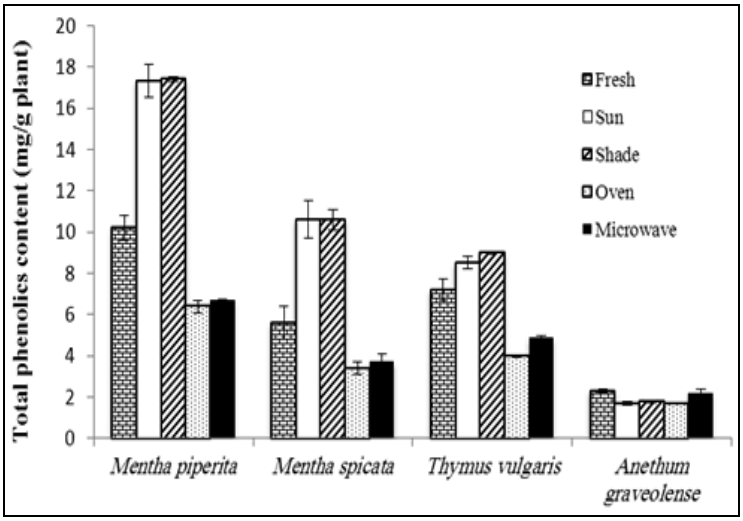

FIGURE 2. TOTAL PHENOLICS CONTENT OF FRESH AND DRIED *MENTHA PIPERITA, M. SPICATA, THYMUS VULGARIS* AND *ANETHUM GRAVEOLENSE*

Dill (*Anethum graveolense*) is a common cooking spice in Iran which is usually used as dried. The fresh extract of dill showed significantly more antioxidant activity than dried extracts ($P<0.05$). The total phenolics content decreased with drying simultaneously. The microwave-dried extract exhibited the highest antioxidant activity and total phenolics among the drying methods which suggested the least loss of antioxidant material through this method of drying hence, microwave-drying might be a good alternative to shade-drying which is traditionally used for culinary usage.

Mentha piperita, Mentha spicata and *Thymus vulgaris* are aromatic plants of Lamiaceae family. Comparing the IC_{50} of these three species suggested a somewhat similar profile of antioxidant activity and also total phenolics content for the different extracts. In all the three species, the sun and shade-dried extracts showed significant lower IC_{50} thus higher antioxidant activity ($P<0.05$). Drying increased the antioxidant activity and total phenolics content of the three Lamiaceae species suggesting the possibility of new compounds formation with antioxidant activity. However, further investigations are necessary. Oven-drying and secondly microwave-drying seem to palliate the antioxidant activity during the drying process meanwhile decreasing the total phenolics content because of decomposition of compounds due to heating (figures 1 and 2).

In the present study total phenolics content seem to correlate with the antioxidant capacity of the extracts. Since correlation coefficients are greater than 0.8 then a considerable correlation could be proposed for all species (**table 2**).

TABLE 2. CORRELATION COEFFICIENTS BETWEEN ANTIOXIDANT ACTIVITY (IC_{50}) AND TOTAL PHENOLICS CONTENT OF THE PLANTS.

S. No.	Scientific name	Correlation coefficient (r)
1	*Mentha piperita*	-0.8426
2	*Mentha spicata*	-0.8811
3	*Thymus vulgaris*	-0.9334
4	*Anethum graveolense*	-0.9694

Coming to a conclusion, due to differences in plant constituents, a same method of drying should not be carried out in all plant species and according to our results for some species the dried plants might possess more antioxidant properties than the fresh ones.

The effect of drying methods on the phytochemical content of plant materials has been studied during previous investigations. The impact of drying, packaging and storage conditions on the retention of β-carotene and ascorbic acid, of savoy beet and amaranth leaves has been determined. Higher losses of β-carotene and ascorbic acid were observed in solar-drying as compared to cabinet-drying hence in order to protect these compounds it would be better to dry them away from sun light [9].

Evaluating the effect of various methods of drying (sun-drying, air-drying and oven-drying) on the content and chemical quality of the essential oil of *Mentha longifolia* has also been studied and it was reported that the essential oil underwent significant chemical transformation in its monoterpenoids when the leaves were dried by the three different methods. Due to the significant reduction of the potentially harmful pulegone and menthone by oven-drying, it was suggested that the herb should be oven-dried or cooked before consumption in order to reduce toxicity [10].

A study about the influence of drying methods on essential oil content of *Rosmarinus officinalis* revealed that the highest essential oil percentage was obtained from the shade-dried leaves while the lowest percentage belonged to the stems which were oven-dried [11]. According to Mohd Zainol *et al*., air-oven drying seemed to degrade the flavonoids content of *Centella asiatica* more than vacuum-oven drying and freeze-drying[12]. Chan et al. evaluated the effects of different drying methods (microwave-, oven-, sun-, air- and freeze-drying) on the antioxidant capacity of leaves of *Alpinia zerumbet*, *Etlingera elatior*, *Curcuma longa* and *Kaempferia galanga*.

The first three methods decreased the total phenolics content and antioxidant capacity while freeze-drying resulted in significant gains in total phenolics content of *A. zerumbet* and *E. elatior* leaves. After one week storage, antioxidant property of freeze-dried *E. elatior* leaves remained significantly higher than those of fresh control leaves[13]. Comparing the phenolic content of sun-dried *Eugenia uniflora* leaves with the air-dried ones has demonstrated that significantly lower content of phenolic compounds exist in the sun-dried leaves [14].

Investigating about the influence of drying methods on the phenolic content of *Azadirachta indica* has been carried out by Sejali and Anuar to show that the shade-dried leaves possessed higher phenolics content compared to the oven-dried leaves [15]. The effect of different drying methods on flavonoids, phenolics, oleanolic acid, ursolic acid, amygdalin, chlorophylls and carotenoids of *Eriobotrya japonica* Lindl. flowers were investigated. The results demonstrated that microwave-drying was more efficient and low-cost method and superior to oven drying [16].

Regarding the results of our study and other investigations reveals that each plant needs a special drying method to show the best antioxidant activity and highest total phenolics content. It seems that because of similarities in the secondary metabolites of a family, a particular drying method might be used in a plant family but it needs more investigations.

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