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EFFECTS OF DRYING AND EXTRACTION METHODS ON ANTIOXIDANT PROPERTIES OF HORSEMINT (*Mentha longifolia* L.)

SUMMARY

Mentha longifolia has the widest natural geographic distribution of any menthe species, from Western Europe to central Asia and in southern Africa. The current study was conducted to determine the antioxidant capacity of extracts and essential oil taken from aerial portions of *M. longifolia* L. The plant materials were dried by three different techniques (shade, sun and oven drying). Collected horsemint shoots were grinded then for evaluating effect of different extraction methods (maceration by aqueous, ethanolic and hydroalcoholic solvents, soxhlet and essential oil by Clevenger) and drying technique (shade drying, sun drying and oven drying) were used. Total phenol content (TPC) and total flavonoid content (TFC) of the samples were measured. Furthermore, total antioxidant capacity (TAC) was evaluated by both phosphomolybdenum and DPPH% assays.

Soxhlet extraction of shade drying had highest TPC and TFC among other extracts a drying methods. In terms of antioxidant activity, essential oil obtained from Clevenger displayed high antioxidant capacity, resulting in a higher radical scavenging ability, which can be attributed to the lower temperatures in shade dried plant material and the higher stability of the extracted compounds. But by exposure the shoots to sun or high temperature (oven) decreasing of bioactive compounds amounts present in plant material were observed. There was a significantly positive correlation ($P<0.01$, $r>0.7$) between TPC and TFC in all drying techniques. Also a significant correlation was observed between TAC and %inhibition ($P<0.01$, $r>0.8$) in sun drying extracts. Extraction and drying the herbal material influences the bioactive compounds and antioxidant properties.

Keywords: *Mentha longifolia* L., extraction, antioxidant capacity, drying, total phenol content

INTRODUCTION

Mentha longifolia L. Hudson as a member of lamiaceae (mint family) is a perennial and aromatic herb that most of the time is observed in moist and nearly shaded places (Sher and Khan, 2007; Shinwari et al., 2011). The aerial parts and leaves alone are added as condiment in salads and cooked foods (Facciola, 1990). In Iranian folk medicine has been introduced as carminative, sedative of stomach pains and antispasmodic (Zargari, 1990). In herbal medicine assumed to be especially useful in construction of immune system and protecting against

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secondary infections. The plant is consumed for cure of cough, cold and grippe. Extraneously, the usage of wild mint is for curing of sores and inflammation of glands (VanWyk et al., 1997). Also, horsemint is usually used in Iran in the form of dried that is added to Iranian conventional cheese or as herbal tea. Lamiaceae herbs are very rich sources of antioxidant compounds (Shan et al., 2005) which could be beneficial in prevention of oxidative reactions in foods and plants organs.

The antioxidants are chemical compounds that have the capability to counteract free radicals by reducing and scavenging these radicals or their activities (Pisoschi and Negulescu, 2011). The key role of antioxidant compounds is demonstrated in many reports in dietary plants such as flavonoids, carotenoids, proantocyanidins, benzoic acids and derivatives and phenolic compounds (Capecka et al., 2005). The drying process can lead to degradation of bioactive compounds (Chan et al., 2009), however it can also release the bound bioactive compounds to result in high recovery yield (Do et al., 2014). The effects of different drying methods on the chemical content and biological activities of essential oils of *Salvia officinalis* and *Juniperus phoenicea* has been established (Do et al., 2014; Ennajar et al., 2010). Aromatic herbs and spices are most sensitive to drying techniques and this raised biological deterioration. Therefore a high quality product is achieved when the drying process is carried out carefully.

Drying techniques are necessary in conservation and post-harvest of herbs. By decreasing the moisture content less than 15%, drying inhibits from any microbial reaction. Factually, effective drying methods will increase the quality of dried material such as aroma and appearance by preventing any biochemical changes (Facciola, 1990). The most drying methods in medicinal herbs that have been used is drying in shade, then oven or low temperature drying. The ratio of varied ingredient is also will affected by drying techniques (Gulluce et al., 2007). Many reports have showed the effect of sample preparation, processing and extraction methods on the phenolics efficiency (Hajlaoui et al., 2009; Handa et al., 2016; Horwitz, 1984). It was also expressed that the drying methods affects chemical composition and antioxidant properties of yam flours to various limits (Hossain et al., 2010). Therefore, it is necessary to identify a suitable thermal drying method to not only retain the bioactive compounds, but also minimize the cost to producers.

The hot air drying is widely used, but it lead to thermal harm and can strongly change the volatile compound and color of herbs (Hsu et al., 2003). Ambience temperatures and temperatures less than 50 °C are the best to preserve aromatic compounds (Ibáñez et al., 1999). However, the loss of phenolic compounds and antioxidant activity in hot air drying achieved up to 60% in comparison with freeze drying (Ji et al., 2012). It is recorded that oil yield in *Mentha longifolia* in dried state was three fold high than fresh state (Katalinic et al., 2006).

Extraction is an important step in the phytochemical processing for the discovery of bioactive constituents from plant materials. Also it is necessary to find the most effective solvent for the extraction of chemical compounds from a targeted material as previous studies have revealed that extraction solvents have a significant impact on extraction efficiency of bioactive compounds from plant materials (Katsube et al., 2004). It is necessary to consider that even though the maximum extraction yield is always procured to meet the requirements for functional activities, especially when they are sought to be applied in food or medicinal industries, the fitness and environmental risks must be taken into consideration when a solvent is chosen for extracting of herbs (Khan et al., 2011; Lim and Murtijaya, 2007). The polarity of the solvents of extraction influences the solubility of chemical constituents in the samples and therefore their extraction efficiency. Usage of water with other organic solvents by creating a polar medium is useful for extraction of polyphenols because the contact surface area between plant matrix and solvent and turgidity of herb substances will enhance the extraction efficiency (Lindsay and Astley, 2002).

In present study we evaluated the effects of some extraction methods and three drying types on antioxidant content and free radical scavenging activity of *M. longifolia* L. shoots. Also total phenol and flavonoid contents of various extracts and essential oil were evaluated.

MATERIAL AND METHODS

Plant material

The aerial parts of *M. longifolia* L. were gathered from the wild nature toward Hervi (Paveh county, Kermanshah province with 46º 14′ Longitude, 35º 7′ Latitude and 650 m Altitude) at March 2016. Plant material was disported into three portion. One portion was dried in front of direct sun; another portion was located in in a dark room, without radiating of direct sun to be dried and, while third portion was put in the oven to be dried (Mors, Model: LX-5340, Turkey) which was kept at 70 ºC, 1 hour.

Extraction of non-volatile compounds of the samples

The shade dried aerial parts of *M. longifolia* were powdered by a blender and then macerated in solvents that was included: Water, Hydro alcoholic (70% ethanol/ water) and ethanolic solvents according to 5% dry weight. Samples were kept in a dark room about 24 hours, then were sat on a shaker about 6 hours. Afterwards for the filtering of the extracts were used from a whatman filter paper No.1 and finally all samples were kept in sterile vessels impervious to air and light, in a refrigerator for further analysis.

Extraction of volatile compounds of the samples

About 20 g each of the dried shoots were separately hydro distillated for 3 h, and for this purpose was helped from a Clevenger-type apparatus. Also for complete isolation of process about 20 ml ethanol was added to apparatus. Because of sensitivity of oils to light, oxygen and temperature and changing of

their components in this conditions, so the isolated oils were kept immediately into a sealed dark vials for further analysis.

Extraction with soxhlet

About 20 g each of the dried shoots were separately combined with chloroform or n- hexan for 30 min to separate the non-polar compounds such as lipids. Then were filtered and plant materials were placed into a whatman filter paper in the apparatus. Isolation process was done with 150 ml ethanol 96% in 90 ºC.

Total phenol content (TPC)

For measuring of TPC was used from Folin-Ciocalteu method (Martysiak-Żurowska and Wenta, 2012). About 0.5 ml of the sample extract was admixed with 1ml of 7.5% (w/v) Na_2CO_3. Then 1 ml of Folin-Ciocalteu reagent and 1 ml of deionized water was added. Afterwards, incubating of the mixture was carried out at room temperature for 30 min. Its absorbance was recorded using spectrophotometer. Gallic acid was used as a reference standard and TPC being reported as mg Gallic acid equivalents per gram of dried weight (mg GA/g DW).

Total flavonoid content (TFC)

For measuring of TFC was used from aluminum chloride method (Nguyen et al., 2016). To brief, 0.2 ml of the sample extract was added to 0.8 ml of deionized water, then were admixed with 1 ml of 2% (w/v) aluminum chloride solution (5% Acetic acid solution into methanol). Eventually, related mixture was kept 30 min. The absorbance of samples was measured at 430 nm using spectrophotometer. The standard curve of various concentration of quercetin (0, 1, 2, 4, 6, 8 and 10mg/l) were prepared. TFC was reported as milligram quercetin equivalent per gram of dried weight (mg QU/g DW) was recorded.

Antioxidant capacity

Reducing Power Assay

The phosphomolybdenum method was used for evaluating the total antioxidant capacity of the extracts (Nickavar et al., 2010). 0.3 ml of extract solution (1 mg/ml) from each extraction sample was picked up and then 3 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The obtained mixture was placed at 95 ºC for 90 min. Finally, the absorbance of the solution was measured at 695 nm against a blank. Ascorbic acid was applied for drawing of standard curve. Total antioxidant capacity of the extracts was evaluated as µg ascorbic acid equivalents per gram of dried weight (µg AA/g DW).

DPPH assay

The free radical scavenging activity of the plant extracts was assessed using 1, 1- diphenyl-2- picrylhydrazyl (DPPH) (Omidbaigi et al., 2004). 1 ml of the plant extract was admixed with 1 ml of methanolic DPPH 0.2 mM and left for 30 min in 25 ºC. The absorbance of admixture was recorded in 520 nm. Methanol without extract was applied as control and free radicals scavenging activity was measured using following formula:

$$\%\text{inhibition} = [\frac{(\text{OD control} - \text{OD sample})}{\text{OD control}}] \times 100$$

Where, %inhibition was free radical inhibition percent (antioxidant inhibition percent against free radical), OD control was absorbance value for control and OD sample was absorbance value for sample.

Statistical analysis

Results were represented as the means ± standard deviation of three replicate. The results statistically and difference between groups was accomplished by SPSS, version 19.0. The graphs was drew with the same software. For evaluating existence or non-existence significant difference between means were used from One-way analysis of variance (ANOVA) followed by Tukey HSD (high significant difference) multiple range test.

RESULTS

The extraction effects in terms of TPC, TFC, TAC and DPPH radical scavenging activity, obtained by Shade, Sun and Oven drying methods, were compared to each other.

Obtained results from analysis of variance table (Table 1) showed that the difference among various extracts and drying methods separately on Total phenol content, total flavonoid content, total antioxidant content and % inhibition was significant on 5% level. Furthermore, interaction between extracts and drying methods in Total phenol and flavonoid content wasn't significant ($P<0.05$).

Table 1. Analysis of variance (mean squares) of extraction and drying effects on some phytochemical characteristics of *Mentha longifolia* L.

	df	Total phenol content	Total flavonoid content	Total antioxidant capacity	% inhibition
Extract	4	37.326*	0.120*	18621.432*	610.442*
Drying	2	19.497*	0.016*	7363.588*	1310.766*
Extract × Drying	8	0.533ns	0.001ns	1493.288*	36.250*
Error	30	0.270	0.000	59.613	0.941

ns Non significant and * Significant difference at 5% level

Total phenol content determination

Sample plants are affected various extraction and drying methods. As shown in Figure 1, the extraction method had significant effect on drying method, so that the highest total phenols content (TPC) was identified in

extraction with soxhlet in shade drying that it's amount was 10.33 ± 0.58 mg GA/g DW and the lowest TPC was in Clevenger at oven drying amount of 3 ± 0.25 mg GA/g DW. Among the drying methods content of total phenol in shade drying was high in comparison of other methods. It is clear that in each drying methods, extraction with soxhlet had high TPC, so this method was most effective for extraction of TPC. The Clevenger method in shade drying and maceration of ethenolic solvent in oven drying had significant difference (*P<0.05*) with other methods; in sun drying maceration with hydro alcoholic solvent and ethanolic solvent didn't indicate significant difference (*P<0.05*). Analysis of variance table indicated difference in total phenol content between extracts and dryings was significant (*P<0.05*) level but between extract × drying was not significant.

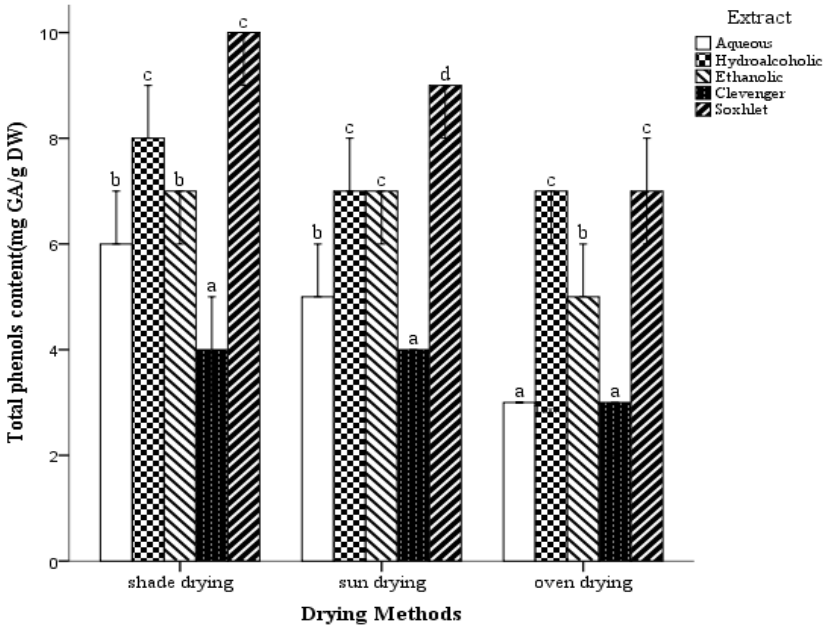

Figure 1. The effect of extraction and drying methods on total phenols content (TPC) of dried horsemint. Different letters over the columns showed significant difference (*P<0.05*) according to Tukey analysis.

Total flavonoid determination

As shown in Figure 2, the extraction of soxhlet was most suitable method for highest total flavonoid content (TFC). The soxhlet method in shade drying gave highest flavonoid content that was 0.59 ± 0.28 mg QU/g DW while the maceration using aqueous solvent had lowest amount of TFC was about 0.22 ± 0.1 mg QU/g DW. There was no significant difference (*P<0.05*) between maceration using hydroalcoholic solvent with ethnolic solvent in all drying tests. Similar to phenol amounts, flavonoid content in extraction with soxhlet had beneficial efficiency compare to other drying methods. Analysis of variance table

indicated difference in total phenol content between extracts and dryings was significant in 5% level but between extract × drying was not significant.

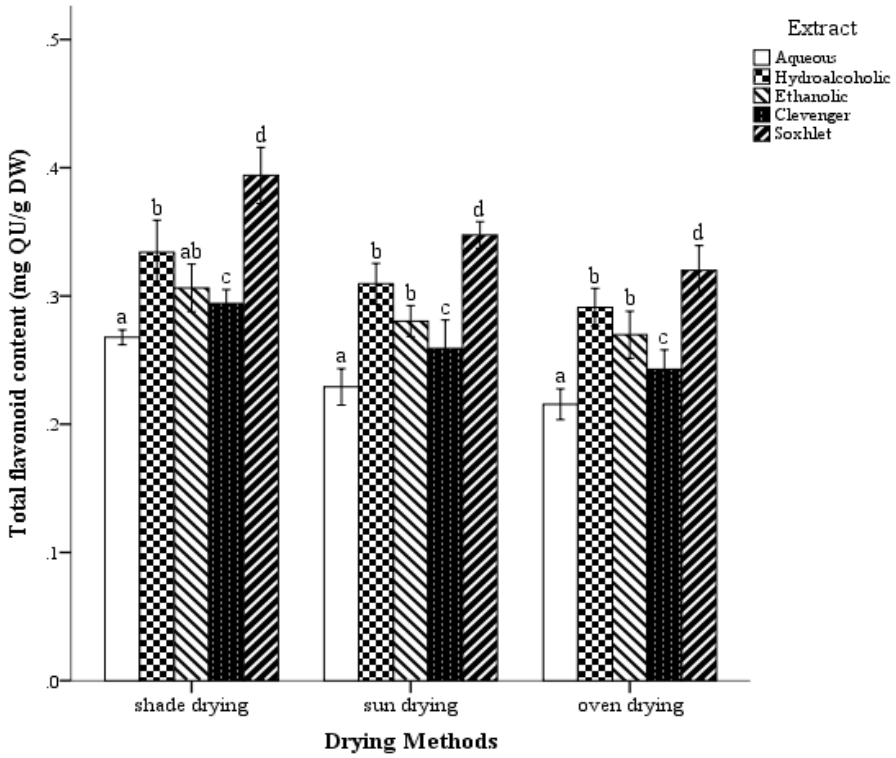

Figure 2. The effect of extraction and drying methods on total flavonoid content (TFC) of dried horsemint. Different letters over the columns showed significant difference (*P<0.05*) according to Tukey analysis.

Determination of total antioxidant capacity

Reducing power assay

The results in Figure 3 indicated supriority of clevenger in three drying method. Total antioxidant capacity (TAC) in clevenger of shade drying was highest (222.15 ± 10.08 µg AA/g DW) although the lowest TAC was 75.19 ± 4.5 µg AA/g DW in maceration with ethanolic solvent of oven drying.

In all drying types there were no significant difference (*P<0.05*) between three maceration using various solvents, moreover clevenger and soxhlet extraction in drying sample using shadesignificantly hadn't difference.Analysis of variance table demonstrated difference in total phenol content between extracts, drying and extract × drying was significant (*P<0.05*).

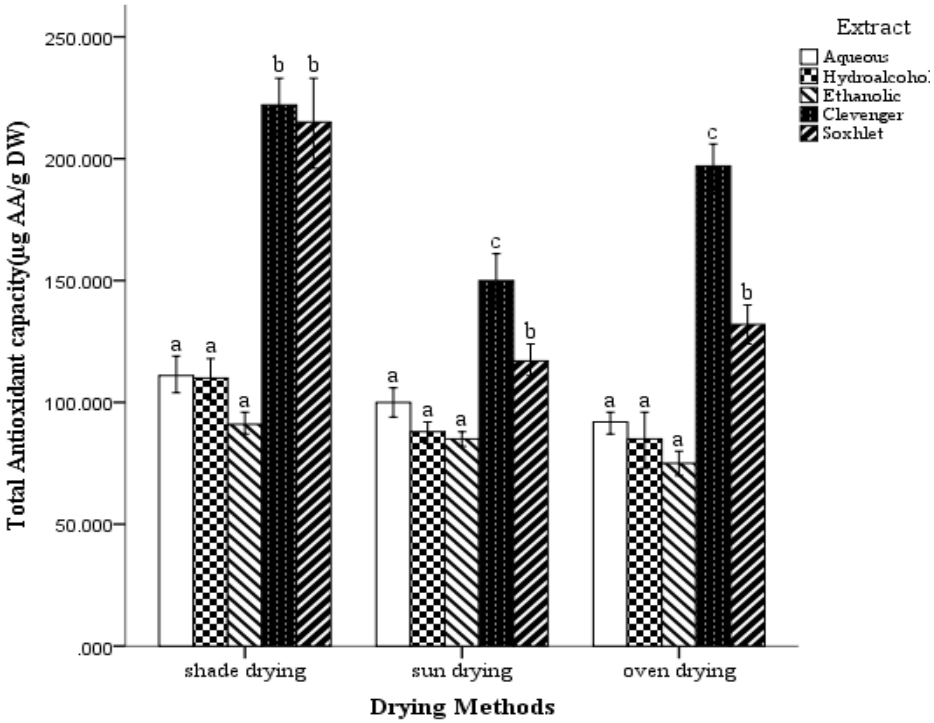

Figure 3. The effect of extraction and drying methods on total antioxidant capacity (TAC) of dried horsemint. Different letters over the columns showed significant difference ($P<0.05$) according to Tukey analysis.

The scavenging activity of DPPH radicals

As shown in Figure 4, similar results to antioxidant capacity, inhibition percent in essential oil obtained from Clevenger had high efficiency. Average antioxidant capacity for horsemint samples in this study were determined using DPPH free-radical scavenging assay. Among all drying and extraction that tested in this article Clevenger method of shade drying gave highest free radical inhibition percent (56.3 ± 0.56 %) but soxhlet in oven drying produced lowest (14.55 ± 1.32 %) inhibition percent.

No significant difference ($P<0.05$) were observed between maceration with ethanolic solvent and soxhlet in sun drying, maceration with aqueous and Clevenger; other extraction hadn't difference with each other. Surprisingly, in oven drying maceration method with aqueous had highest inhibition percent other than extraction methods of oven drying, also hadn't significant difference with Clevenger.

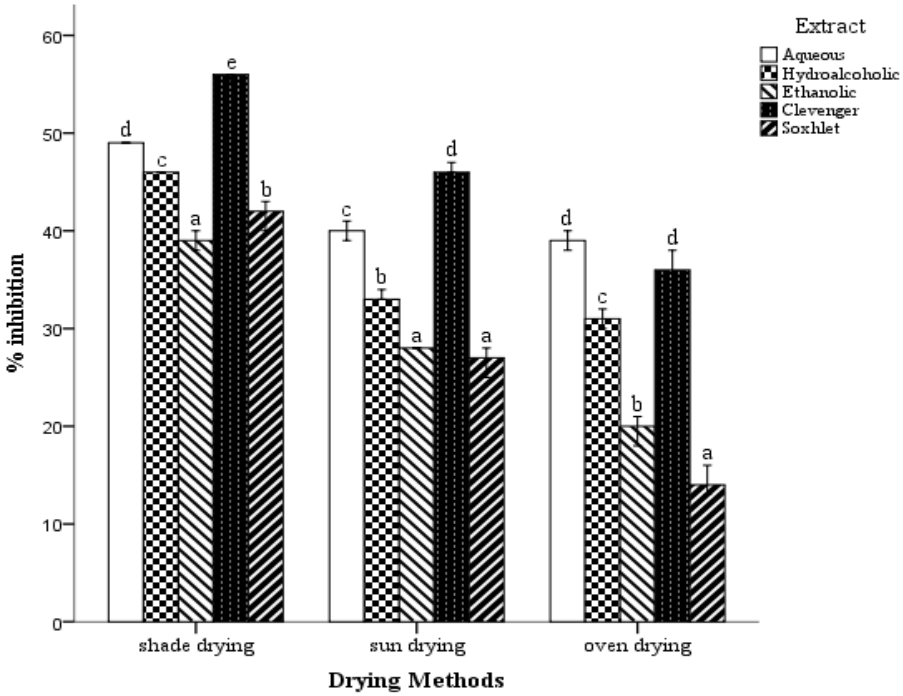

Figure 4. The effect of extraction and drying methods on free radical inhibition percent (DPPH) of dried horsemint. Different letters over the columns showed significant difference ($P<0.05$) according to Tukey analysis.

Correlation between measured factors

In order to better evaluation the relationship between total phenol and flavonoid contents, antioxidant capacity and DPPH radical scavenging activity Pearson correlation under extraction and drying conditions were done (Table 2).

In the case of the variable "Extract" for shade drying (Data not shown), correlation between extract, TFC and antioxidant capacity were positive significant ($P<0.01$). This showed that most of the flavonoid compounds extracted, contributed to the antioxidant capacity of shade drying extracts. The pair of extract-DPPH correlation was non-significant and negative that meaning antioxidant capacity assays of plant samples by phosphomolybdat method had efficiency better than DPPH method.

Under the "Extract" parameter of sun drying (Data not shown), there was a positive significant correlation ($P<0.05$) between extract and TFC. This can be resulted for the effect of sun on the antioxidant capacity of dried samples or extraction conditions.

In the case of "extract" in oven drying method (Data not shown), TFC positively and significant ($P<0.05$), DPPH radical scavenging activity significantly negative ($P<0.01$) were correlated.

Concerning the "TPC", it was positively significant (*P<0.01*) correlated with TFC for all three methods (shade, sun and oven drying extracts), while "TFC" was negative and mostly significant correlated with TAC and DPPH assays in all three drying types. Therefore, it could be concluded that there are other phenolic compounds different from flavonoids that contributed to the antioxidant capacity of extracts.

Table 2. Pearson correlation coefficients between different assays [a] under effect of extraction and drying conditions [b].

	Shade drying extracts				Sun drying extracts				Oven drying extracts			
	TPC	TFC	TAC	DPPH	TPC	TFC	TAC	DPPH	TPC	TFC	TAC	DPPH
TPC	1	0.744**	-0.057^{ns}	-0.686**	1	0.793**	-0.810**	-0.890**	1	0.885**	-0.624*	-0.766**
TFC		1	0.347^{ns}	-0.494^{ns}		1	-0.808**	-0.692**		1	-0.365^{ns}	-0.814**
TAC			1	0.511^{ns}			1	0.863**			1	0.523*
DPPH				1				1				1

[a] TPC, total phenol content, TFC, total flavonoid content, DPPH radical scavenging activity, TAC total antioxidant activity.
[b] Number of replicates

^{ns} Non significant
* Significant at *P<0.05*
** Significant at *P<0.01*

Hence, this results is useful to clarify the relationship between the extraction technique and drying methods based on TPC, TFC, TAC and DPPH (% inhibition). Pearson correlation showed that TPC was only one technique that had a significantly high correlation with the concentration of TFC.

DISSCUSION

Herbs with strong antioxidative properties are a complete supply of phytochemicals (OrphAnides et al., 2013). It is important that should be attentioned to antioxidative performance of extracts and antioxidant activity depend not only on the extraction techniques, but also on the processing prior to extraction, the harvesting time, its geographic origin, the quality of initial plant, its storage conditions (Papageorgiou et al., 2008; Pham et al., 2015).

Although by performing of drying microbial activities and biochemical changes will been prevented, simultaneously, it is possible that losses of aromas or forming of new aromas in effect of oxidation and esterification processes increase. This alterations will affect on appearance, aroma content and herb quality. Furthermore, drying techniques destroys bioactive compounds may be antioxidant or had other health-improving properties (Pirbalouti et al., 2013). It is

noteworthy that the flavonoid content was lower than the respective Total phenol content in all the cases in our study, This is probably due to flavonoids are considered to be restricted in distribution and composition in different plant materials in comparison with the overall phenolic compounds (Pisoschi and Negulescu, 2011) that confirms our results. As the major groups of compounds acting as initial antioxidant free radical annihilator, phenolic compounds playing a key role (Pulido et al., 2000). It was reported that obtained essential oil yields (v/w on dry weight basis) were highest in both landraces that dried in shade, afterwards the freeze dried of purple landrace and the fresh green landrace that confirmed the results of present study, the Clevenger showed high essential oil yield especially in total antioxidant capacity and %inhibition. It is worth noting that different plant substances used in these experiments were related to plants may their physiology had been different, which is determine by their genetic makeup (Quispe-Condori et al., 2008). Thus drying method can affect the chemical composition of essential oils but it depends on plant material used.

The results of present study were showed that extraction with various methods in each three drying methods had phenol and flavonoid content and antioxidant capacity but highest amount among different drying method was in samples that dried in shade and the best extraction method in each drying methods was determined. The usage of heating deactivates enzymes fastly and at the same time, they may destroy heat-sensitive phenolic compounds (Rabeta and Lai, 2013). The reduction of TPC value in our study after exposure the plant materials under sun may be possibly caused by the enzymatic reaction during the process (Rabeta and Lai, 2013). Moreover, it also causes enzymes degradation and loss of antioxidant enzyme activities. New works also exhibited that the stability of phenolic compounds in herbal infusions will affected by temperature (Rafiee et al., 2011) that is in lined with our results that total phenol content of plant material had been dried with oven was lower than other methods, similar results was observed for total flavonoid content.

In addition to chemical content, the biological activity of the extracted substances was change by changing of method of drying herbal material; As well as the essential oil of *Mentha longifolia* L. Hudson dried in shade has exhibited the highest antioxidant activity and dried samples in laboratory in the oven has the lowest antioxidant property (Riehle et al., 2013) that similar results was obtained in our study. It was showed that most oil content in savory was in temperature of 45 ºC oven, shade and sun drying methods, respectively (Rocha and Melo, 2011); this results is disagree with the results of our study that the most oil content was in shade, sun and 70 ºC oven, respectively. In present study by enhancing drying temperature certainly reduced the essential oil content of all samples. The essential oil content of sage and thyme (*Thymus vulgaris*) dried in oven at 60 ºC were reduced in higher temperature (Sefidkon et al., 2006). Furthermore, similarly results with our results was reported (Riehle et al., 2013; Sellami et al., 2015) that the extracts of herbs dried in the laboratory oven (1.13 ± 0.11 m/mol Fe2+/mg of the dry extract and EC50 = 0.033 ± 0.001 mg/mL) has

shown the lowest antioxidant capacity. Such results reveals value of methods that plant is dried prior of preparation. DPPH is a deep-purple colored stable free radical, in the hydrogen or electron donation process its color changes from purple to yellow and becomes a stable diamagnetic molecule (Señoráns et al., 2000). Because antioxidant has the electron and hydrogen donating ability, the discoloration degree of mixture indicates the scavenging power of the antioxidant determines by degree of mixture discoloration (Serra Bonvehí et al., 2001). Loss of other bioactive properties causes frequently in reduction of antioxidant properties (Hajlaoui et al., 2009). It was reported that IC50, defined as the concentration of sample extract necessary to obtain an activity of 50%, from lowest to highest was as follows: *M. piperita*, *M. pulegium*, *M. rotundifolia*, *M. longifolia*, *M. spicata*, respectively (Shan et al., 2005). When are able to obtain extracts that have high efficiency along with least changes in active properties of the extracts, it's said to be an extraction technique (Sher and Khan, 2007). According to accomplished investigation in present study, extraction with soxhlet had highest TPC and TFC but the most total antioxidant capacity and %inhibition was in essential oils obtained by Clevenger.

The antioxidant properties of the herbal material varies by application of different methods of extraction. For example, in one study because of highest content of total phenols (113.8 ± 2.0 mg of gallic acid/g of the dry extract) and flavonoids in dried herbs by shade was showed highest antioxidant capacity measured by two methods the ferric decreasing antioxidant property (FRAP) and DPPH assays (2.76 ± 0.15 m/mol Fe^{2+}/Mg of the dry extract and EC50 = 0.022 ± 0.001 mg/ml). These differences on ABTS and DPPH data for the same samples can be attributed to differences in the polarity of the solvents that affect the main mechanisms of electron transfer involved in both assays. This means generally the electron transfer reaction is the base of DPPH assay, and the interactions between antioxidants-DPPH% radicals are also determined by the structural form of the antioxidants. Thus, decreasing the number of DPPH% molecules in correspondence to the number of accessible hydroxyl groups in the antioxidant compound is result of very fastly reaction of some substances with DPPH% (Shinwari et al., 2011). A study to evaluate the antioxidant activities of the essential oil in methanol extract of *M. Longifolia* was reported (Stanisavljević et al., 2012). In both applied assays (inhibition of free radical 2, 2‐diphenyl‐1‐picrylhydrazyl (DPPH) and β carotene/ linoleic acid systems), the extract showed significant activity in comparison with the essential oil. Other studies explained that the chief cause of more antioxidant influence of methanol extract than the essential oil is the phenolic compounds (Suja and Mohanasundari, 2016) that confirmed our study. In another study, for finding a newly potential resource of natural antioxidants the free radical‐scavenging potential (1, 1‐diphenyl‐2‐picrylhydrazyl scavenging activity) of nine *Mentha* spp. was evaluated. The methanolic extracts of *M. longifolia* exhibited high antioxidant activity (79%) (Sulieman et al., 2011).

A significantly high correlation (r=0.7, P<0.01) between TPC and TFC; between TAC and %inhibition (r=0.8; P<0.01) was obtained in present study. Earlier studies found no agreement in correlation between total phenolics and antioxidant activity. Therefore some reports showed a strong positive correlation (Van Wyk et al., 1997; Venskutonis, 1997), other studies (Wong et al., 2006; Xiangyang et al., 2010) reported a poor correlation. In our study also there was a negative correlation between TPC and total antioxidant capacity. In another study (Zargari, 1997), the quantitative analysis of phenol and flavonoid content of the extracts highly correlated that was in lined with observed correlation in our results.

CONCLUSION

The current study found that Shade drying showed superiority over the other drying methods. Soxhlet extract from dried samples in shade demonstrated to had high total phenol and flavonoid content and good antioxidant capacity, also in terms of total antioxidant capacity and %inhibition assays essential oil that obtained by Clevenger in the plant material dried in shade had highest TAC and DPPH radical scavenging activity. DPPH was the best assay for estimating amount of free radical scavenging activity in comparison with TAC assay by phosphomolybdenum method. Our results demonstrated a significantly positive correlation (r=0.7, $P<0.01$) between total phenol and flavonoid content. Furthermore, total antioxidant capacity and DPPH radical scavenging activity showed positive correlation (r=0.8, $P<0.01$).

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