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Evaluation of the Composition and Antimicrobial Properties of *Mentha piperita* L. Leaf Powder in Italian Salad Dressing

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ABSTRACT

In this research, the chemical composition and antimicrobial activity of *M. piperita* L. (Lamiaceae) leaf powder in Italian salad dressing was investigated. Five concentrations (0.0%, 0.4%, 0.8%, 1.2% and 1.6 %) of *Mentha piperita* leaf powder were added to the salad dressing formula and the samples were subjected to microbiological analysis and sensory evaluation. The essential oil of dried and ground peppermint leaf was extracted using hydrodistillation method and analyzed by Gas Chromatography-mass Spectrometry analysis. The analysis of *Mentha piperita* resulted in the identification of some important components with antimicrobial effects such as neo menthol, p-Mentone, 1, 8-cineole. The general result of experiments clearly indicated that the microbial population of treated samples reduced by increasing the amount of peppermint powder. The sample containing 1.2% of *Mentha piperita* leaf powder was the most suitable one due to good sensory properties and low microbial population ($p<0.05$). It could be concluded that the antimicrobial activity of *Mentha piperita* powder as natural flavoring and food preservative in Italian salad dressing samples may be attributed to its antimicrobial components.
KEYWORDS: *Mentha piperita* L. leaf powder, Chemical composition, Antimicrobial properties, Italian salad dressing.

1. INTRODUCTION

Mentha piperita L. (Lamiaceae) commonly known as peppermint, is an aromatics additive have been used in food products in order to inhibit different microorganisms and to extend the shelf life of foods. The essential oils of *M. piperita* are responsible for the antimicrobial properties and can control microorganisms including fungus, Gram-negative and Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Enterococcus faecium, Klebsiellapneumoniae, Escherichia coli* [1, 5, 21]. The different parts of plants, herbs and spices have been used for many years for prevention of microbial spoilage [3, 4, 13]. The oil of peppermint contains some antimicrobial compounds such as menthol, menthone, tannins, phenols, steroids, flavonoids and volatile oils [6, 28].

Italian salad dressing, an O/W emulsion, is composed of water, oil, vinegar or lemon juice, salt, different herbs and spices. This semisolid food that maybe probably become one of the most popular sauces in the world, is used for meats and vegetables as a salad seasoning or a marinade [8, 17].

Fine and coarse emulsion are two pourable types of salad dressing that have different flavor and physico-chemical properties [7].

The different emulsion forms of Italian salad dressing are: creamy, golden, separating, and dry mix, which may be either of the separating or stable emulsion type. Golden and creamy Italian salad dressings are respectively a coarse and fine emulsion [16]. Salad dressings are produced in emulsified and separated forms. Italian dressings are the most popular separating salad dressings that must be must be shaken before use. The continuous and dispersed phase in this emulsion separated quickly after pouring [17, 20].

The aim of this research was to investigate the antimicrobial effects of *M. piperita* leaf powder on the microbial population, shelf life and sensory properties of salad dressing.

2. MATERIALS AND METHODS

2.1 Materials

Fresh *M. piperita* leaf was prepared from the Adonis Company, However, the *M. piperita* leaf were dried in oven at 40℃ for 7 to 8 hours. After crushing the leaves, the powder stored in air tight container for further analysis. The concentrations of *M. piperita* leaf powder (0.0%, 0.40%, 0.80%, 1.20% and 1.60%) were applied to the Italian salad dressing formula. Italian salad dressing was prepared from *M. piperita* leaf powder using the following formula: Soybean oil (56 %), white vinegar (28 %), Lemon Juice (5 %), salt (1 %) and *M. piperita* leaf powder (0.0-1.6%), xezantan gum (0.05%), whey protein powder (2.5%), and water (remaining) in

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accordance with codex standards of food additives. The dry ingredients were blended together and hydrated in all available water, lemon juice and vinegar and other materials for several minutes by using a laboratory mixer until obtaining the suitable emulsion of Italian salad dressing. Then produced samples were aseptically filled and stored at 4℃ in Sterile Wide Mouth Bottles.

2.2 Isolation of the essential oil (hydrodistillation method)

The essential oil of *M. piperita* obtained by using a Clevenger-type apparatus. First, fresh leaves were dried, ground and then subjected to water-distillation for 4 hours in three replications. The essential oil was derided over anhydrous sodium sulfate, filtered and stored in a dark jar at 4℃ prior to analysis by GC–MS.

2.3 Gas Chromatography-mass Spectrometry analysis conditions

In order to determination the chemical composition, analysis of the *M. piperita* essential was carried out using a Varian 3400 GC, equipped with a DB-5 non-polar capillary column (L = 30 m; ID = 0.25 mm; 0.25 µm film thickness) and a Saturn II mass selective detector. For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. The carrier gas was Helium. Injector and MS transfer line temperatures were set at 260 and 270℃, respectively. Column temperature was initially kept at 60℃, then gradually increased to 250 at 4℃/min. Diluted samples of 1.0 µl were injected manually.

2.4 Determination of antimicrobial activity

In order to assay the shelf life of prepared samples, a microbial analysis was conducted by the methods of colony count technique at 30 ºC after 0, 45, 90, 135 and 180 day storage periods at 4 °C [15]. Total viable counts were determined on Plate Count Agar (PCA, Merck) after incubating at 30 ºC for 72 hours. The results were reported as mean values standard deviation and compared with Guidance Note No. 3, 2001 [10].

2.5 Sensory evaluation

A sensory evaluation of samples was conducted after preparation and during storage at 4°C for 180 days. Sensory characteristics: taste, flavor, color, appearance and overall acceptability were evaluated by a 25-member panel on 10-point hedonic scale, with 1 being the lowest and 10 the highest according to Ranganna method [9].

2.6 Statistical analysis

Statistical analysis was performed by analysis of variance and Duncan's multiple range test procedures (SPSS, version 20). The analysis values are the mean of three replicate analyses.

3. RESULTS AND DISCUSSION

3.1 Identification of components by employing GC–MS

Table 1 shows the components of *M. piperita* essential oil. The chemical constituents of *Mentha piperita* oil were identified by comparison of their retention indices (KI) and mass spectra with authentic compounds, library data and the literature of the GC–MS system [2].

As shown in Table 1, the predominant compounds of *M. piperita* essential oil were: neo menthol (48.37%), p-Mentone (21.30%), 1, 8- cineol (6.39%), Limonene (4.10%), Isomenthol (3.72%), piperitone (3.24%), pulegone (2.73%), Camphor (1.61%), α-Pinene (1.58%), α- Caryophyllene (1.56%), and Trans-Dihydrocarvone (1.03%). This results indicated that the composition of *M. piperita* essential oil were consistent with the previously published studies but at different concentration and individual composition [11, 12, 25]. Gherman et al. found that the major composition of *M. piperita* identified from the mass spectra recorded were menthol (35%), Mentone (28%), isomenthone (8%), 1,8 cineole (7.5%), trans-sabinene hydrate (5%), limonene (3%), menthyl acetate (3%), b-caryophyllene (3.6%), germacrene (2.6%) [12]. Gavahian et al. studied the peppermint essential oil containing neo menthole(30.3%), menthofuran (15.5%), isomenthone (26.4%), 1,8 cineole (7.5%), limonene (7.5%), and pulegone (5.4%). They reported that extracted essential oils by hydrodistillation and steam distillation have approximately similar chemical compositions [11].

The highest amount of menthol in oil of *M. piperita* (56.4%) was reported from Sari province in Iran [28]. Also, the main component of the essential oils were mentone, menthol, menthofuran, pulegone, 1, 8-cineole, and menthyl acetate for first harvested [26].

Table 1. Chemical composition of *M. piperita* determined by GC-MS analysis (based on dry weight)

Component	RI [a]	% Composition	Identification [b]
α-Pinene	938	1.58	A, B, C
Sabemine	973	0.36	A, B, C
α-Myrcene	991	0.21	A, B, C
γ-Terpinene	1019	0.38	A, B, C
α-Terpinene	1017	0.02	A, B, C
Limonene	1025	4.10	A, B, C
1,8- cineol	1035	6.39	A, B, C
Cis-sabinene hydrate	1070	0.14	A, B, C
β-Ocimene	1037	0. 28	A, B, C
γ- Terpinene	1060	0.72	A, B, C
Linalool	1097	0.25	A, B, C
Camphor	1146	1.61	A, B, C
***p*-Mentone**	1155	21.30	A, B, C
Neo menthol	1168	48.37	A, B, C
isopinocampheol	1175	0.20	A, B, C
Terpinolene-4-ol	1177	0.63	A, B, C
Isomenthol	1182	3.72	A, B, C
α-Terpinol	1189	0.41	A, B, C
Trans-Dihydrocarvone	1193	1.03	A, B, C
Pulegone	1237	2.73	A, B, C
Carvone	1243	1.09	A, B, C
Piperitone	1254	3.24	A, B, C
linaly acetate	1257	0.86	A, B, C
Bornylacetate	1289	0.93	A, B, C
Methanol acetate	1294	6.04	A, B, C
α- Bourbonene	1383	0.28	A, B, C
α- Caryophyllene	1421	1.56	A, B, C
Sabinene hydrate	1431	0.17	A, B, C
α- Cadinol	1653	0.12	A, B, C

[a] *Retention indices on the DB-5 column*
[b] *A = Identification based on Retention time; B = identification based on co-injection with standard . compounds; C = identification based on comparison of mass spectra.*

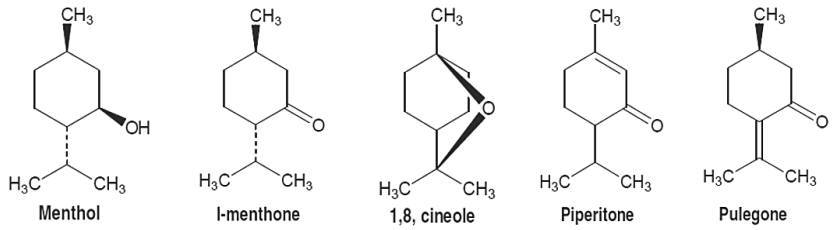

Figure1. Chemical structures of some components of *M. piperita* leaf Powder

3.2 Antimicrobial activities of *Mentha piperita* leaf Powder in treated samples

The total counts of bacteria of treated Italian salad dressing samples (filled at 2000×g in Sterile Wide Mouth Bottles) are presented in Table 2. Statistical significant differences on total viable count in all days of storage were found between the Italian salad dressing samples with *M. piperita* leaf and control sample ($P<0.05$). The total number of microorganism at first days indicated the *M. piperita* continuous reduction of microbial population in samples containing 0.40%, 0.80%, 1.20% and 1.60% other than the control sample. According to the obtained results, the highest antimicrobial activity belonged to the using 1.60% amount of *M. piperita* exhibited with 4.7×102 cfu g^{-1} after 180 day storage periods (Table 2).Regarding the existence of menthol and menthone in *M. piperita* leaf, the increased amount of *M. piperita* leaf powder leads to reduced microbial population. In the other words, the decreased microbial population was concomitant with increased antimicrobial properties which can be related to the effective active components such as menthol, menthone, isomenthone, piperitone, pulegone and dehydrocarvone. Synergistic effects of these components on the inhibition or elimination of bacterial pathogens have been mentioned more [1, 5, 14, 18, 19, 22].

According to previous experiments, menthol, as main constituent of *M. piperita*, has antiplasmid activity and can eliminate the resistance plasmids of bacteria during a special mechanism. Also, the lipophilic compounds of *M. piperita* might effect on cyclic hydrocarbons and modify the regular membrane fluidity, permeability and the activity of many membrane bound enzymes of bacteria. It has been proved that piperitone

can effect on *Staphylococcus aureus and* completely inhibit *Aspergillusflavus* at low concentrations and some components of *M. piperita* such as limonene can destroy cellular integrity and inhibit mitochondria respiratory activity, therefore represent antifungal properties. [5, 22, 24, 25].

As shown in Table 2, after 180 days, the reduction in total number of microorganism of samples containing 0.40 % and 1.60 % *M. piperita* powder were 30.27% and 56.88%, respectively. These results are accordance with a previous study in which exhibited the good antibacterial activity of the juices of *M. piperita* leaf and stem against some of bacteria [19, 22, 23, 27].

Table 2. Results of total bacterial counts (cfu g^{-1}) of treated Italian salad dressing during storage at 4°C[1]

Peppermint %	Storage periods(day)				
	0*	45*	90*	135*	180*
0.00	10.9 ± 0.17^{i}	7.4 ± 0.28^{g}	7.5 ± 0.36^{g}	8.5 ± 0.26^{h}	9.2 ± 0.70^{h}
0.40	9.2 ± 0.32^{h}	6.9 ± 0.65^{fg}	7.2 ± 0.70^{g}	7.3 ± 0.43^{g}	7.6 ± 0.20^{f}
0.80	8.8 ± 0.20^{h}	6.3 ± 0.36^{de}	6.9 ± 0.63^{fg}	7.1 ± 0.40^{g}	7.4 ± 0.51^{g}
1.20	6.2 ± 0.40^{def}	5.0 ± 0.25^{abc}	5.2 ± 0.32^{bc}	5.5 ± 0.26^{cd}	5.6 ± 0.36^{cde}
1.60	5.7 ± 0.20^{cde}	4.3 ± 0.62^{a}	4.5 ± 0.78^{ab}	4.6 ± 0.65^{ab}	4.7 ± 0.55^{ab}

[1]: Note: Means superscript (x 10^2), the values are expressed as means ± standard deviation.
[a- i] There is no significant difference between similar letters in each column (p<0.05).

3.3 Sensory evaluation of treated samples

In Table 3, Sensory analyses of Italian salad dressing samples contain different concentration of *M. piperita* leaf powder (0.00%, 0.40%, 0.80%, 1.20%, 1.60%, and 1.60%) are shown during the storage period for 180 days at 4°C. According to ANOVA GLM, peppermint leaf powder was found to have a significant effect on taste, color, flavor and overall acceptability of different samples. Also, there was no statistical difference in texture of samples among various peppermint percent ($P < 0.05$).

Regarding the flavor, the lowest score (8.02) was allocated to the control sample. Along with the increase in peppermint leaf powder content from 0% to 1.20%, flavor score improved considerably to 8.65.This proves the advantage of *M. piperita* leaf powder as the main flavoring component of Italian salad dressing. According to the results, taste, flavor and the overall acceptability scores of Italian salad dressing decreased as a result of the increase in the concentration of *M. piperita* leaf powder from 1.20% to 1.60%. It was a consequence of an increase in the content of Menthol [23, 27].

About the color score, the greenish color of Italian salad dressing is primarily provided by the *M. piperita* leaf powder. Also, by increasing in *M. piperita* leaf powder content from 0% to 1.60%, the color scores of Italian salad dressing increased as a result of the increase in the concentration of pigment compounds like as flavonoids, luteolin and rutin of *M. piperita* leaf powder from 8.01 to 8.20.

Table 3. Score means of the sensory evaluation in treated Italian salad dressing[1]

Peppermint %	Color*	Taste*	Flavor*	Texture	Overall acceptability*
0.00	8.01±0.06	8.03±0.02	8.02±0.16	8.03±0.81	8.10±0.10
0.40	8.03±0.02	8.20±0.08	8.15±0.07	8.10±0.06	8.33±0.07
0.80	8.13±0.05	8.35±0.05	8.20±0.10	8.05±0.11	8.50±0.13
1.20	8.17±0.03	8.61±0.03	8.65±0.17	8.11±0.08	8.82±0.04
1.60	8.20±0.05	8.10±0.07	8.04±0.05	8.15±0.07	8.15±0.11

[1]: Note: Means value of 25 measurements. The values are expressed as means ± standard deviation.
**: Statistically significant (p<0.05).*

Regarding the overall acceptability scores, the highest score (8.82) was allocated to the 1.20 % concentrations of *M. piperita* leaf powder in Italian salad dressing (23).So, the average data in Table 2 and 3 indicated that the sample contain 1.20 % *M. piperita* leaf powder was the best treated sample due to low microbial population and good sensory properties ($p< 0.05$).

4. Conclusion

This study could be concluded that the *M. piperita* leaf powder intensively decreased the microbial population and prolonged the shelf life of salad dressing samples. Thus, pepper mint powder can be used as a natural flavoring and preservative in foods and salad dressing. The total amount of neo menthol and p-Mentone

as antimicrobial component and the major constituents of *M. piperita* was 69.67%. Furthermore, *M. piperita* leaf powder can be used as a suitable natural antimicrobial agent and substitute for synthetic preservative in food industry.

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