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Mentha longifolia (L.) ssp. *longifolia* Essential Oil: Source of Natural Antioxidant and Antimutagen as Food Additive

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Abstract: This research was performed to control the antioxidant activity, mutagenicity and antimutagenic effect of *Mentha longifolia* (L.) ssp. *longifolia* essential oil (EO), which is considered as a possible ingredient when producing healthy food. The antiradical activity was established using DPPH (2,2-diphenyl-1-picrylhydrazyl radical) and β-carotene/linoleic acid bleaching assays. The total phenolic content in the EO was evaluated by Folin Ciocalteau method (FCR). Ames Salmonella/microsome mutagenicity assay was applied to detect possible mutagenic and antimutagenic behavior. Our observations reveal that the IC_{50} value for DPPH radicals was 5.27 ± 0.13 mg/mL. The total antioxidant efficiency increased with an increase in the concentration of the EO, and IC_{50} value 11.7 ± 0.21 mg/mL. The total of phenolics was 186 ± 8.9 mg/g gallic acid equivalent/EO. Also, any concentrations of the EO used did not show mutagenic action but exhibited strong antimutagenic effects at 10.0-4.0 µg/plate concentrations. This research proposes that because of the antioxidant and antimutagenic characteristics, the EO is very advantageous and significant to the company's manufacturing food additives.

Mentha longifolia (L.) ssp. *longifolia* Uçucu Yağı: Gıda Katkı Maddesi Olarak Doğal Antioksidan ve Antimutajen Kaynağı

AnahtarKelimeler
Antimutajenik,
Antioksidan,
Uçucu yağ,
Gıda katkı maddesi,
Mentha longifolia

Özet: Bu araştırma sağlıklı yiyecek üretirken olası bir katkı maddesi olarak düşünülen *Mentha longifolia* (L.) ssp. *longifolia* uçucu yağının (EO) antioksidan aktivitesi, mutajenitesi ve antimutajenik etkisini kontrol etmek için gerçekleştirilmiştir. Antiradikal aktivite DPPH (2,2-diphenyl-1-picrylhydrazyl radical) yöntemi ile ve β-karoten/linoleik asit ağartma tayini kullanılarak belirlenmiştir. Uçucu yağın toplam fenolik madde içeriği Folin Ciocalteau metodu (FCR) ile tahmin edilmiştir. Olası mutajenik ve antimutajenik davranışını belirlemek için Ames Salmonella/mikrozom mutajenite testi uygulanmıştır. Gözlemlerimiz DPPH radikalleri için IC_{50} değerinin 5.27 ± 0.13 mg/mL olduğunu açığa çıkarmıştır. Total antioksidan etkinliği uçucu yağ konsantrasyonunun artmasıyla artmıştır ve IC_{50} değeri 11.7 ± 0.21 mg/mL'dir. Toplam fenolik madde içeriği 186 ± 8.9 mg/g gallik asit eşdeğeri/uçucu yağ'dır. Ayrıca uçucu yağın herhangi bir konsantrasyonu mutajenik etki göstermemiştir, fakat 10.0-4.0 µg/plate konsantrasyonlarında güçlü antimutajenik etki göstermiştir. Bu araştırma antioksidan ve antimutajenik özelliklerinden dolayı EO'nun gıda katkı maddesi üreten şirketler için çok avantajlı ve önemli olduğunu göstermektedir.

1. Introduction

Lipid oxidation is a major cause for food quality deterioration and generation of off odours and off flavours, decreasing shelf life, altering texture and colour, and decreasing the nutritional value of food [1]. It can also generate potential toxic compounds through the activity of free radicals [2]. It has also been acknowledged that these toxic compounds endanger health and cause ailments related to

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digestion, aging, cancer, mutagenesis and cardiovascular disorders. The oxidative degradation of lipids is also known to harm biological membranes, enzymes and proteins, which will ultimately affect the human health [3].

Addition of antioxidants is considered the most efficient, suitable and inexpensive technique out of all the approaches used to regulate the lipid oxidation [4]. Due to their exclusive characteristics that help in enhancing the shelf-life of food items without causing any harmful impacts on the physical or nutritional characteristics, the antioxidants are recognized as a crucial part of the food additives [5]. But the synthetic antioxidants and/or food preservatives are still consumed in far less in the edible food products, owing to the severe health and safety concerns that appear to have hazardous outcomes and to be potential carcinogens [6]. Hence, there is a requirement to discover different supplied of safe, efficient and suitable natural preservers [7].

Owing to their antifungal/antimicrobial behavior, their antioxidant attributes or antimutagenic/antigenotoxic abilities, the EO, that are the secondary metabolites of plants, have always played their part in the packing of eatables [8,9,10]. In this perspective, EO could play a promising role either individually or as co-adjuvant in the development of food preservatives [11].

The EO of *M. longifolia* (L.) exhibited antimicrobial [12,13,14], antifungal [15], antiacetylcholinesterase [15,16], antidiarrheal [17] and antioxidant [12,13,15] properties. Therefore, the purpose of this work consists in determining the antioxidant, mutagenic and antimutagenic activities of *M. longifolia* ssp. *longifolia* EO in order to their effects on the storage of food products.

2. Materials and Method

2.1. Plant collection and EO isolation

M. longifolia (L.) Hudson ssp. *longifolia* were collected from wild populations growing locality in Adana (Turkey), in June 2012 and was identified by Dr. Olcay Ceylan, Department of Biology, University of Mugla Sıtkı Kocman, Mugla, Turkey. The dried and powdered samples were submitted to water-distillation method.

2.2. Antioxidant activity assay

2.2.1. DPPH radical scavenging activity

The ability of the EO to scavenge DPPH free radicals was measured by the method of Ebrahimabadi et al. [18] with a little modification. One milliliters amount containing diverse dilutions of EO was combined with 1 mL DPPH solution (0.2 mM). The mixture was stirred and left in a dark at 25ºC for half an hour. The absorbance was later measured at 517 nm. Butylated hydroxytoluene (BHT) and ascorbic acid were the two chemicals utilized as standard antioxidants. Free radical scavenging activity was calculated using the equation below:

$$RSA\ (\%) = [(Abs_c - Abs_s)/Abs_c] \times 100 \quad (1)$$

Here, RSA is radical scavenging activity, Abs_c is the optical density of the DPPH solution, and Abs_s is the optical density of the sample formulations. The oil concentration containing 50% radical inhibition activity (IC_{50}) was evaluated from the graph concerning the free radical scavenging action (%) with against the EO concentration. The results were demonstrated as IC_{50} ± standard deviation.

2.2.2. Inhibition of β-carotene/linoleic acid bleaching assay

The β-carotene bleaching potential of the EO was determined according to by Rauter et al. [19]. In short, 1 ml of β-carotene was combined with 200 mg of Tween 60 and 25 µl of linoleic acid. The combination was stirred and the chloroform was evaporated. Then distilled water (100 ml) was included to the combination and agitated. From this emulsion, 2.5 ml transferred into test tubes and 0.5 ml of the EO was added. The initial optical density of samples was determined after 1 min of vortexing at 470 nm. After incubation at 50 °C, the absorbance of every sample at 470 nm was measured after every 15 minutes, for the next 180 minutes. The antioxidant behavior of the oil was contrasted against the activity of BHT and ascorbic acid. The antioxidant behavior of the samples was assessed by applying the formula mentioned below:

$$Ln\ (Abs) = ln\ (Abs0) + R \times t \quad (2)$$

Here, R is the bleaching rate in the slope of ln (Abs) vs. time line, which can be assessed with the help of a linear regression, where t is used to denote the time in minutes.

$$AA\ (\%) = [(Abs_c - Abs_s) / Abs_c] \times 100 \quad (3)$$

Here, AA is antioxidant activity, Abs_c is the optical density of the β-carotene solution of dimethyl sulfoxide (DMSO) and Abs_s signifies the optical density of the β-carotene suspension of sample preparations. The results were illustrated as the IC_{50} ± standard deviation.

2.2.3. Determination of total phenolic content

As per Singleton et al. [20], FCR technique was applied to determine the total phenol content of the EO. Roughly, around 100 µl of FC reagents (0.2 N) was added to 200 µl of a suspension of EO (1 mg/mL), vortexed for 1 min, and after 3 min, the

mixture was neutralized with 2 mL of 5% aqueous Na_2CO_3 solution. The optical density of the samples was recorded to be at 760 nm after 2 hours. The same technique was implemented on the standards solution of Gallic acid to acquire a Standard curve. The total phenol content was articulated as milligram of Gallic acid equivalent per gram of the oil (mg/g GAE oil).

2.3. Mutagenicity and antimutagenicity assay

The possible mutagenic/antimutagenic influences offered by EO were assessed on two histidine-dependant (His−) mutant sample strains of *Salmonella typhimurium* TA98 and TA100. Before the experiment, the strains were analyzed for spontaneous reversion and genetic integrity including UV sensitivity, ampicillin resistance, histidine requirement, crystal violet sensitivity, and dose-dependent cytotoxic effects were observed of the EO as presented by Mortelmans and Zeiger[21].

The mutagenic potential and antimutagenic influence of the EO was achieved by applying the plate/incorporation process as explained by Maron and Ames [22]. 4-nitro-o-phenylenediamine (4-NPD) (3 µg/plate) (for TA98) and sodium azide (NaN_3) (8 µg/plate) (for TA100) used as positive controls.

The tube containing DMSO was used to determine spontaneous reversion. After incubating for 72 h at 37 °C, the His^+ revertant colonies were determined. The mutagenicity of 4-NPD and NaN_3 as a positive control in the absence of the EO was described as 100% mutagenicity. The percentage inhibition was computed by applying the formula given below:

$$\% \text{ inhibition} = [1 - (A-C)/(B-C)] \times 100 \quad (4)$$

Here, A is the number of revertants present in each plate whilst mutagen and test samples are present, and B is the number of revertants in each plate in the positive control, while C is the total number of spontaneous revertants present in each plate.

2.4. Statistical analysis

All determinations of antioxidant activity were performed in triplicate. Each dose of mutagenic and antimutagenic activity was tested using triplicate plates in two independent experiments. The results were presented as the average and Standard deviation. The comparison of average values of each samples were analyzed with one-way ANOVA, followed by Tukey's test.

3. Results

3.1. Antioxidant activity and total phenolic content

The antioxidant action and the phenol content of EO, in this research were assessed by methods DPPH, β-carotene/linoleic acid bleaching and FCR assays. The results obtained from the EO displayed that the antiradical activity against DPPH free radical and IC_{50} values of 5.27 ± 0.13 mg/mL, while the ascorbic acid and BHT were 0.01 ± 0.03 mg/mL and 0.184 ± 0.01 mg/mL, respectively (Table 1).

The antioxidant action by β-carotene/linoleate model system of EO was examined and contrasted against ascorbic acid and BHT (Table 1). It was found that the IC_{50} of the EO was 11.7 ± 0.21 mg/mL, while those of BHT and ascorbic acid were 0.05 ± 0.12 mg/mL and 0.020 ± 0.2 mg/mL, respectively. The total phenolic content in EO was 186 ± 8.9 mg Gallic acid equivalent/g EO (Table 1).

3.2. Screening of mutagenic and antimutagenic activity

The EO in the nontoxic dose range of 10.0 – 2.0 µg/plate was observed for signs of mutagenicity and it was discovered that when different concentrations of EO were present, it did not alter considerably, in the number of revertants when compared to spontaneous mutation incidences (data not shown). Therefore, the *M. longifolia* ssp. *longifolia* EO seems to be non-genotoxic against TA98 and TA100 strains.

The EO in the nontoxic dose variety range of 10.0 – 2.0 µg/plate were also measured for antimutagenicity of two dissimilar types of genotoxic compounds on *S. typhimurium* TA98 and TA100 (Table 2). The report revealed that in the TA100 strain, the EO was strong efficient in decreasing the influence of NaN_3 genotoxicity, inhibition percentages range between 45.6 and 52.5% with respect to the observed level of the positive control. Maximum inhibition of TA98 was observed with concentrations of 10.0-4.0 µg/plate (46.7%-53.7%) against 4-NPD genotoxicity (Table 2). The EO displayed moderate antimutagenic action (30.8%) in opposition to 4-NPD genotoxicity at 2.0 µg/plate dose on the TA 98 strain.

Table 1.DPPH radical scavenging activity, total antioxidant activity and total phenol content of the *M. longifolia* ssp. *longifolia* EO

Samples	Test Systems		
	DPPH[a] IC_{50} (mg/mL)	β-carotene–linoleic acid[a] IC_{50} (mg/mL)	Total phenols GAE / (mg/mL)
Essential oil	5.27 ± 0.13	11.7 ± 0.21	186 ± 8.90
BHT[b]	0.184 ± 0.01	0.05 ± 0.12	Ns[c]
Ascorbic acid[b]	0.01 ± 0.03	0.020 ± 0.20	Ns[c]

[a]IC_{50} values represent the means ± standard deviation. [b] Reference compounds. [c]Not studied. Significance compared to control at p<0.000

Table 2. Antimutagenicity of the EO of *M. longifolia* ssp. *longifolia* against *S. typhimurium* TA98 and TA100

Test items	Concentration	Number ofrevertantcolonies			
		TA98		TA100	
		Mean±SE	Inhibition (%)	Mean±SE	Inhibition (%)
Negative control		8 ± 4.07[a]		32 ± 17.75[a]	
Positive control					
4-NPD	3.0 μg/plate	308 ± 68.6			
NaN_3	8.0 μg/plate			655 ± 29.9	
Essential oil	10.0 μg/plate	143 ± 19.10[b]	53.7	311 ± 35.79[c]	52.5
	8.0 μg/plate	148 ± 16.60	51.9	320 ± 25.25	51.0
	6.0 μg/plate	150 ± 33.72	51.0	321 ± 35.70	51.0
	4.0 μg/plate	164 ± 39.31	46.7	325 ± 24.80	50.4
	2.0 μg/plate	213 ± 28.10	30.8	356 ± 05.51	45.6

[a]Values expressed are means ± standard error of two independent experiments, each performed in triplicate. Anova for mutagenicity inhibition (%) and EO concentrations was performed using the SPSS. [b]$P< 0.001$. [c]$P< 0.009$

4. Discussion and Conclusion

To get over the problems concerning stability, like fats and oils, synthetic antioxidants are commonly combined to avoid the oxidative degradation of lipids inside of foodstuffs [23]. But presently, their use in foods has heavily criticized due to potential health risk corresponding to toxic effects and to be potential carcinogens [6, 24]. As a result, natural antioxidants gained from plants are received a great deal of interest, as they can be biologically active constituent with various health endorsing influences [7].

EO showed the scavenging activity with DPPH free radical scavenging assay (IC_{50}: 5.27 ± 0.13 mg/mL). According to the β-carotene bleaching tests the IC_{50} values of the EO were found to be 11.7 ± 0.21 mg/mL (Table 1). In other studies, IC_{50} values of *M. longifolia* EO against DPPH free radical is determined as 10.700 μg/mL [12] and >8000 mg/L [13]. Similarly in a study, according to the β-carotene bleaching test the EO of M. *longifolia* ssp. *longifolia* was displayed only 24% inhibition at 2 mg/ml [12].

The antioxidative efficiency of natural resources was mainly caused because of the phenolic compounds [25]. Phenolic compounds were recounted to be of great importance in inhibiting autoxidation of oils [26]. *M. longifolia* ssp. *longifolia* EO can be declared very beneficial and a good resource of phenol antioxidants. The total phenolic content in EO was 186 ± 8.9 mg Gallic acid equivalent/g EO (Table 1).

Products of lipid oxidation have been implicated as having toxic, carcinogenic, mutagenic properties [24]. For this reason, in this study we researched mutagenic potential and antimutagenic activity of EO.

In this study, EO of *M. longifolia* ssp. *longifolia* did not display mutagenic activity. In a study *M. longifolia* extract has shown the ability to counteract the 2-amino-3-methylimidazo- quinolidine (IQ) and nitrosopiperidine (NP) mutagenic effects [27]. Similarly, apigenin derivatives isolated *M. longifolia* ssp. *longifolia* did not display mutagenic activity [28, 29].

In the present experimental conditions, the EO was effective antimutagens against two different types of genotoxic compounds. The current results reveal that the antimutagenic action in Ames test which might be ascribed in portion to influential radical scavenger related to the EO.

The antimutagenic activity of the *M. longifolia* was previously assayed by scrutinizing the organic extracts from the various parts of the plant [27, 30]. The *M. longifolia* extracts revealed an effectual antimutagenic action regarding NP, IQ [27] and NaN_3 [30]. In another study, luteolin [31] and apigenin derivatives [28, 29] were isolated from *M. longifolia* extracts show significant antimutagenic activity. But the antimutagenic action of the *M. longifolia* EO has not been researched.

Even though the food industry having variety preservers, it is still facing a shortage of powerful food preservers to maintain the quality of the foodsand make them safety. This EO could possibly be consumed as a food component to substitute the synthetic food antioxidants and as mutagen-protective food component. It can also help in encouraging health welfare, and reduce the chance of acquiring cancer and similar severe ailments that are usually linked with lipid metabolism problem.

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