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In Vitro ACE2 and 5-LOX Enzyme Inhibition by Menthol and Three Different Mint Essential Oils

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Abstract

Mentha arvensis L., *M. citrata* L., and *M. spicata* L. (family Lamiaceae) essential oils, and their characteristic constituent, menthol, were evaluated *in vitro* for angiotensin converting enzyme 2 (ACE2) and 5-lipoxygenase (5-LOX) enzyme inhibitory activity. The chemical compositions of *M. arvensis*, *M. citrata*, and *M. spicata* essential oils were analysed both by GC-FID, and GC/MS; 82.0%, 38.1%, and 0.4% menthol were identified, respectively. *M. spicata* essential oil contained 88.2% carvone as its major component.

The enzyme inhibitory activities of the essential oils were evaluated using a fluorometric multiplate based enzyme inhibition kit; the ACE2 inhibitions produced by *M. arvensis*, *M. citrata*, and *M. spicata* essential oils were 33%, 22%, and 73%, while the 5-LOX inhibitions were 84%, 79%, and 70%, respectively. In addition, menthol also showed remarkable ACE2 inhibition of 99.8%, whereas the 5-LOX inhibition was 79.9%. As a result, menthol and the three different mint essential oils may have antiviral potential applications against coronaviruses due to their ACE2 enzyme inhibition and anti-inflammatory features. However, further *in vivo* studies are needed to confirm the safety and efficacy.

Keywords

Mentha, essential oil, ACE2, LOX, menthol

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Introduction

Mentha species, commonly known as mints, are perennial herbaceous plants with creeping stems, mostly spreading in moist and wet places.[1] Mints, an important resource for essential oils, are distributed all over the world, except Antarctica. The plants have widespread usage due to their aromatic, and medical properties.[2]

In a previous study conducted with *Mentha citrata* L. in Iran, menthone and menthol were found as major components of the essential oil and were evaluated in terms of antifungal activity.[3] In another study, more than 50% menthol was found in *M. citrata* essential oil and antibiofilm activity was reported.[4] The antiviral activity of *M. citrata* and *M. spicata* was also studied and remarkable results were obtained against Herpes simplex type-1 (HSV-1), and parainfluenza type-3 (PI-3).[5] In addition to the essential oil of *M. citrata*, the antiviral and anti-inflammatory effects of alcohol extracts were also determined.[6] Another study suggested that the carvone-rich essential oil of *M. spicata* L. has potential as a food preservative due to its antimicrobial properties.[7] *M. arvensis* L., another menthol rich species, is also remarkable for its antimicrobial activity.[8] Mint species are frequently used for the treatment of flu and colds, due to their broad antimicrobial potential.[9,10]

In this present study, the potential antiviral effects of three different *Mentha* essential oils were evaluated by *in vitro* ACE2 and 5-LOX enzyme assays. To the best of our knowledge, this study is the first comparative work with the three different

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Mentha essential oils, and menthol for *in vitro* ACE2, and 5-LOX enzyme inhibition potential.

Results and Discussion

Chemical Composition of Essential Oils

M. arvensis and *M. citrata* essential oil analyses showed a relatively high menthol percentage, while carvone was the major consituent of *M. spicata*. *M. arvensis* essential oil contained 82.0% menthol, 4.1% menthone, 3.5% isomenthone, and 2.7% menthyl acetate. The major components of *M. citrata* were menthol (38.1%), menthone (22.2%), neomenthol (8.5%), and isomenthone (7.8%), and of *M. spicata*, carvone (88.7%), limonene (3.5%), terpinolene (1.2%), and *p*-cymen-8-ol (0.8%). The percentage of menthol in *M. spicata* essential oil was found to be 0.4%, as shown in Table 1. In previous studies, it was observed that *M. citrata* and *M. arvensis* essential oils collected from different locations at different times were rich in menthol. Although the percentages of menthone and menthyl acetate are more variable, their presence as major components was determined for both essential oils.[11] On the other hand, *M. spicata* was found to be rich in carvone, but menthol could not be detected in some analyses.[12,13]

Overall, the chemical analyses of the commercial oils were in accordance with the literature data and standards.

Table 1. The Chemical Composition of Mint Essential Oils.

RRI	Compound	*M. arvensis*	*M. citrata*	*M. spicata*
1032	α-Pinene	0.4	0.8	0.1
1076	Camphene	-	-	tr
1118	β-Pinene	0.4	0.8	0.3
1132	Sabinene	0.1	0.2	0.1
1159	δ-3-Carene	-	-	0.6
1174	Myrcene	0.2	-	0.1
1176	α-Phellandrene	-	-	0.1
1188	α-Terpinene	-	-	0.3
1203	Limonene	1.2	3.4	3.5
1213	1,8-Cineole	-	0.2	0.1
1218	β-Phellandrene	-	-	0.1
1255	γ-Terpinene	-	-	0.4
1280	*p*-Cymene	-	0.1	0.5
1290	Terpinolene	-	-	1.2
1393	3-Octanol	0.2	-	0.4
1475	Menthone	4.1	22.2	0.1
1494	(Z)-3-Hexenyl isovalerate	0.1	-	-
1503	Isomenthone	3.5	7.8	tr
1535	β-Bourbonene	0.1	-	-
1553	Linalool	0.1	-	tr
1574	Menthyl acetate	2.7	5.5	0.1
1583	Isopulegol	0.4	-	-
1591	Bornyl acetate	-	-	tr
1604	Neomenthol	1.9	8.5	tr
1606	Iso-isopulegol	0.3	-	-
1624	*trans*-Dihydrocarvone	-	-	0.1
1632	Neoisomenthol	0.5	1.0	-
1638	Menthol	82.0	38.1	0.4
1675	Isomenthol	tr	1.6	-
1697	Carvotanacetone	-	-	0.1
1706	α-Terpineol	0.1	-	tr
1726	Germacrene D	0.2	-	-
1748	Piperitone	0.4	-	-
1751	Carvone	-	-	88.7
1864	*p*-Cymen-8-ol	-	-	0.8
	Total	98.9	90.2	98.1

RRI Relative retention indices calculated against *n*-alkanes.
% calculated from FID data.
tr Trace (<0.1%).

ACE2 Enzyme Inhibition

ACE2 enzyme inhibition assay was performed at concentrations of 20 μg/mL for essential oils, and 5 μg/mL for menthol. The essential oils were evaluated using a fluorometric multiplate based enzyme inhibition kit, where the *in vitro* ACE2 inhibition rates of *M. arvensis, M. citrata,* and *M. spicata* essential oils were 33.0 ± 0.13%, 22.1 ± 0.80%, and 73.2 ± 0.45%; whereas menthol inhibited the ACE2 enzyme by 99.8 ± 0.02% (Figure 1). Although the ACE2 enzyme inhibition of menthol is quite high, the fact that menthol-rich *M. arvensis* and *M. citrata* inhibited the enzyme at a lower rate than *M. spicata* may be due to the interaction with other substances present in the essential oils.

Mentha preparations are among the most frequently used in flu and cold infection cases.[14,15] They have also been the subject of some studies due to their antimicrobial effect.[16,17] *Mentha* species have antiviral potential, and successful results were obtained in studies with *M. citrata* essential oil against Type 1 and 2 Herpes simplex virus.[18]

As is well known, the COVID-19 pandemic is an important health issue with limited therapeutic and protective approaches. Since interaction of the coronavirus spike protein and ACE2 is necessary for the virus to infect, any agent that interrupts their interaction has therapeutic potential. The receptor-binding domain-based human monoclonal antibody, and recombinant human ACE2 protein (rhuACE2) have been targetted and attracted attention.[19,20] Therefore, as a result of the findings obtained in this study, especially based on the ACE2 enzyme inhibition values of *M. spicata* and menthol, it can be expressed that *M. spicata* and menthol may have potential in the prevention of coronaviruses, and antiviral effects.

5-LOX Enzyme Inhibition

The assay was performed at concentrations of 20 μg/mL for essential oils, and 5 μg/mL for menthol, as in the ACE2 assay. The 5-LOX inhibition results of *M. arvensis, M. citrata, M. spicata* essential oils, and menthol were 84.5 ± 0.14%, 79.0 ± 0.12%, 70.1 ± 0.34%, and 79.9 ± 0.43%, respectively as shown in Figure 2. Also NDGA was tested as a positive control, which showed 90.1 ± 0.02% 5-LOX enzyme inhibitory activity. In previous studies, essential oils of *M. citrata* subspecies and *M. spicata* were studied separately for their *in vitro* LOX

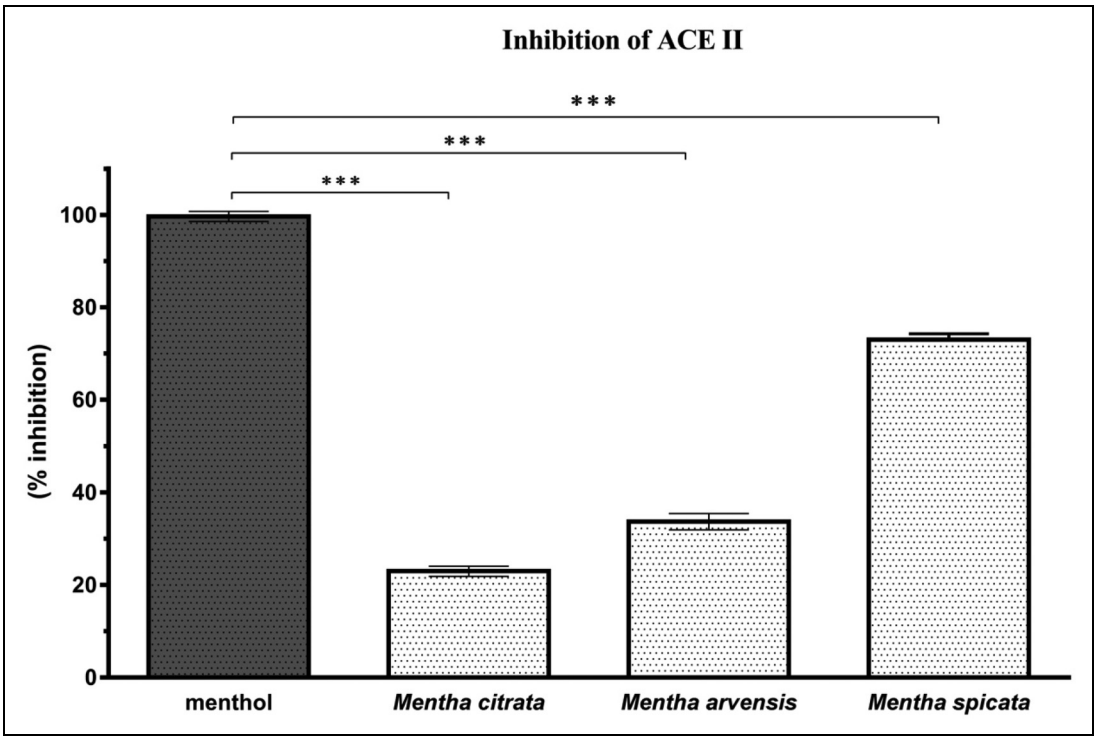

Figure 1. ACE II enzyme inhibition of *Mentha* essential oils (20 μg/mL), and menthol (5 μg/mL) (***$P < 0.0001$).

enzyme inhibition; anti-inflammatory activity was demonstrated for both.[21,22] Baylac and Racine tested *M. citrata* essential oil using the LOX method; effective results were reported.[23]

As is known, monocytes allow viruses to migrate to the tissues they infect and spread to all organs and tissues. Monocytes and macrophages infected with coronavirus can

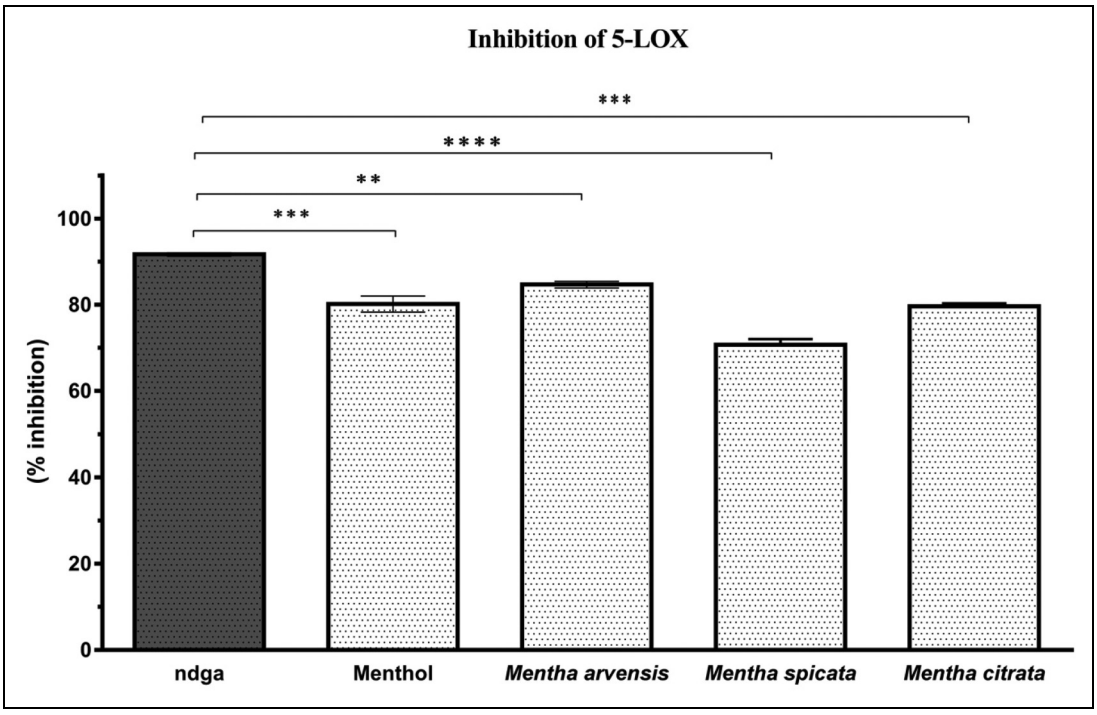

Figure 2. 5-LOX Enzyme inhibition of *Mentha* essential oils (20 μg/mL), menthol and nordihydroguaiaretic acid (ndga) (5 μg/mL) (**$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

produce numerous proinflammatory cytokines and chemokines that contribute to tissue inflammation and a systemic inflammatory response called a cytokine storm. Both tissue inflammation and cytokine storm play a fundamental role in the development of COVID-19-related complications, such as acute respiratory distress syndrome (ARDS), which is the cause of death in coronavirus patients. Therefore, evaluation of anti-inflammatory parameters in terms of coronavirus studies may be an approach.[20,24]

Thus, in the present study, both ACE2 and the 5-LOX enzyme inhibitory potential of *Mentha* essential oils were evaluated; the results and outcome were rather promising.

Conclusion

In this present study, three different *Mentha* essential oils, and menthol, the major component of *M. arvensis* and *M. spicata* oils, were evaluated for their potential *in vitro* 5-LOX and ACE2 enzyme inhibitory activities. To the best of our knowledge, the *Mentha* essential oils, and menthol are reported comparatively, in terms of *in vitro* ACE2 enzyme inhibition, for the first time. In addition, promising results were observed for both enzyme inhibitions, and compared with the major compound menthol. Based on the first *in vitro* experimental results, it can be suggested that especially *Mentha spicata* essential oil may be further evaluated as a potential antiviral tools against coronaviruses.

Experimental

Materials

Commercial essential oils were aquired from Doalinn Ltd, Şti. İstanbul. Menthol, NDGA, and LOX enzyme kits were obtained from Sigma-Aldrich (Germany); Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit was from BioVision (U.S.A).

GC-FID and GC/MS Analysis

An Agilent 6890N GC system was used for the GC-FID analyses, and an Agilent 5975 GC-MSD system for GC/MS. The temperature of the FID detector was set to 300 °C. Concurrent auto-injection was performed in two identical columns using the same conditions in the GC/MS system. Relative percentages (%) were calculated using FID chromatograms. Relative retention indices (RRI) were used to characterize the essential oil components, either with authentic samples or by comparison with the relative retention index (RRI) of *n*-alkanes, with commercial GC/MS Libraries such as MassFinder 3 Library, and the in-house "Başer Library of Essential Oil Constituents".[25]

ACE2 Enzyme Inhibition Assay

The test samples were initially dissolved in DMSO <1% (v/v). Enzyme inhibitions were performed accordancing the the manufacturer's instructions for "Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit (BioVision, K310)", and the enzyme inhibition of the samples was measured with Ex/Em = 320/420 nm wavelength in a multimode microplate reader (SpectraMax i3) in fluorescence mode. The enzyme inhibition of the test substances was calculated by comparing with standards provided in the kit. Inhibition % (%I) values were calculated for all samples resulting from triplicate data, as previously described.[26]

5-LOX Enzyme Inhibition Assay

5-Lipoxygenase (5-LOX) was measured by modifying the spectrophotometric method of Baylac and Racine.[23] The reaction was initiated by the addition of linoleic acid solution; the change of absorbance at 234 nm was observed for 10 min. All the kinetic experiments were performed in triplicate. The concentration of the tested essential oils was 20 μg/mL, and of the pure compounds 5 μg/mL. All tests and control assays were corrected by blank experimental data for non-enzymatic hydrolysis. The percentage of inhibition (%I) was calculated as the absorbance change per minute in enzyme activity (without inhibitor) compared to absorbance change per minute of the test sample. Nordihydroguaiaretic acid (NDGA) was used as the positive control. Experiments were performed in triplicates, and results are given as a mean, as previously reported.[26]

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Declaration of Conflicting Interests

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

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Trial Registration

Not applicable, because this article does not contain any clinical trials.

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