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# *In vitro* ACE2 Enzyme Inhibitory Activity Evaluation of Different *Salvia* Essential Oils

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## ABSTRACT

In this present study, commertially available *Salvia triloba* L., *S.officinalis* L., and *S. sclarea* L. essential oils were evaluated for their *in vitro* angiotensin converting enzyme 2 (ACE2) inhibitory activity. The *Salvia* essential oils compositions were confirmed both by GC-FID and GC/MS. Main components of the *S. triloba* essential oil was characterized as 1,8-cineole (22.8%), camphor (17.2%), α-thujone (15.2 %), β-caryophyllene (11.4 %), and α-humulene (3%). Major constiutents were identified as α-thujone (28.5 %), camphor (20.6 %), 1,8-cineole (10.9%), α-humulene (5%), and camphene (4.9 %) in *S. officinalis* essential oil. Whereas, linalylacetate (56.8 %), linalool (21.1%), α-terpineol (6.1%), geraniol (5%), and β-caryophyllene (3.4%) were the major components of *S. sclarea* essential oil. The essential oils were evaluated using a fluorometric multiplate based enzyme inhibition kit, where the ACE2 inhibitions of *S. triloba, S. officinalis,* and *S. sclarea* essential oils were 50.1%, 60.5%, and 72.1% at a concentration of 20 µg/mL, respectively. As a result, further tests of *Salvia* essential oils supported by *in vivo* studies may have antiviral potential applications against coronaviruses due to ACE2 enzyme inhibitions.

**Keywords:** *Salvia*, coronavirus, ACE2, essential oil

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## INTRODUCTION

*Salvia* L., the largest genus of the Lamiaceae family, contains more than 900 species spread around the world, where some species are economically important, and as used as a spice and flavoring agent in food, condiments and beverages, also in cosmetic and pharma industries. *Salvia* essential oils are generally rich in 1,8-cineole and borneol content[1-4].

Ethnobotanical utilization of *Salvia* species are also common worldwide, *Salvia officinalis* L. is widely used in the treatment of cough, bronchitis and colds[5]. In addition, *Salvia sclarea* L. is also used to relieve the upper respiratory tract, especially in the treatment of upper respiratory tract infections[6]. Anatolian *Salvia triloba* L. (Synonym *Salvia fruticosa* Mill.) is commonly used against coughs and colds[7]. In addition, of *Salvia* essential oils are also used due to their antimicrobial and antiviral effects in aromatherapy[8-11].

The aim of the present study was to evaluate the *in vitro* ACE2 enzyme inhibitory potential of *S. triloba*, *S. officinalis*, and *S. sclarea* essential oils associated to their possible antiviral effect against coronavirus. The essential oil composition was identified by GC-FID and GC/MS, respectively.

## METHODOLOGY

### Chemicals and Plant Material

Commercial *S. triloba, S. officinalis,* and *S. sclarea* essential oils were kindly provided by Doallin Ltd., İstanbul, voucher samples are deposited at IMEF Herbarium (Herbarium No: IMEF 1146-1147-1148)

### GC-MS/GC-FID analysis

An Agilent 5975 GC-MSD system was used for GC/MS analyses. Whereas the Agilent 6890N GC system was used for the GC-FID. FID detector's temperature was set to 300°C. Concurrent auto-injection was applied in two identical columns with the same conditions in the GC/MS system. Relative percentages (%) were calculated using FID chromatograms (Figure 1-3). Relative retention times were used to characterize the essential oils chemical composition. This process was held either by authentic samples or analyzing relative retention index (RRI) of n-alkanes, along with GC/MS Library, MassFinder 3 Library, in-house "Başer Library of Essential Oil Constituents" [12].

### ACE2 Enzyme Inhibition Activity

The essential oils were dissolved initially using DMSO [< 1% (v/v)]. The *in vitro*

enzyme inhibition was performed according the manufacturer's instructions for the "Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit (Bio-Vision, K310)" and the enzyme inhibition of the essential oils were measured with Ex/ Em = 320/420 nm wavelength using a multimode microplate reader (SpectraMax i3). The enzyme inhibition of the essential oils were calculated by comparing with standards included in the kit and the percentage inhibition (%I) values were calculated as mean values resulting from triplicate data for all samples as previously reported [12].

### Statistical analysis

The statistical analysis was carried out using the GraphPad Prism, Version 7.02 (La Jolla, California, USA). *In vitro* data was expressed as mean ± standard deviation (Mean± SD). The statistical significance was analyzed by One-way ANOVA (followed by Dunnett's post hoc test) and Paired Samples T-Test. The $p<0.05$ was accepted as statistically significant.

## RESULTS and DISCUSSION

### GC-MS/GC-FID analysis

The phytochemical constituents of the *Salvia* essential oils were confirmed by using GC-FID and GC-MS. *S. triloba* essential oil contained the main constituents 1,8-cineole (22.89%), camphor (17.15%), α-thujone (15.18%), and β-caryophyllene (11.43%), respectively. Major components of *S. officinalis* were identified as α-thujone (28.46%), camphor (20.58%), 1,8-cineole (10.45%), and α-humulene (5%); whereas *S. sclarea* essential oil contained linalylacetate (56.8%), linalool (21.06%), α-terpineol (6.05%), and geraniol (15.18%) among others (Figure 1-3).

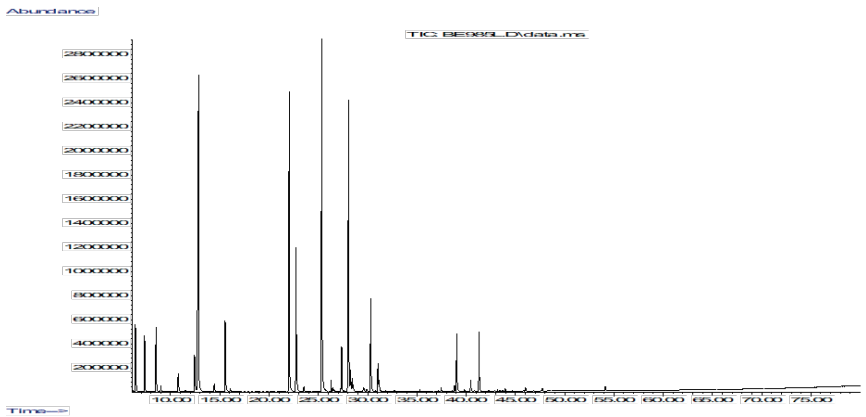


**Figure 1.** GC Chromatogram of *Salvia officinalis* essential oil

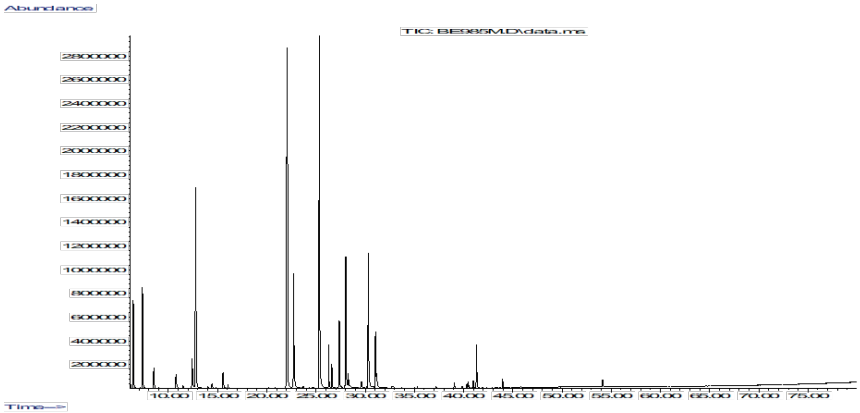


**Figure 2.** GC Chromatogram of *Salvia triloba* essential oil

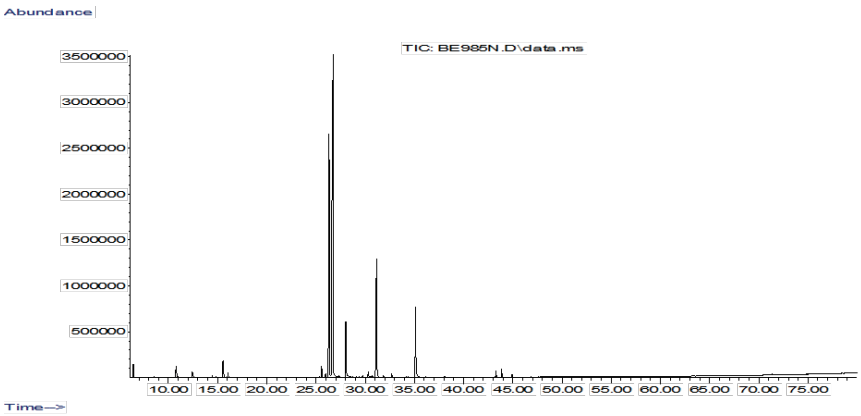


**Figure 3.** GC Chromatogram of *Salvia sclarea* essential oil

Recent literature on *S. triloba, S. officinalis,* and *S. sclarea* essential oils from Turkey show that major components of the species have similarity [13]. Compared to *Salvia officinalis*, *Salvia triloba* essential oil has a very low thujone content. *S. triloba* essential oil, known as Turkish sage, is a non-toxic alternative to *S. officinalis* as it does not contain thujone. In addition, *S. triloba* is richer in 1,8-cineol than *S. officinalis*. *S. sclarea*, on the other hand, has a rich content of linalool and linalyl acetate.

### ACE2 Enzyme Inhibition Activity

The enzyme inhibition assay was performed at a concentration of 20 µg/mL for all tested *Salvia* essential oils using a a fluorometric multiplate based enzyme inhibition kit, where the *in vitro* ACE2 inhibition rates of *S. triloba*, *S.*

*officinalis*, and *S. sclarea* essential oils were 50.07 ± 2.99%, 60.45 ± 1.82%, and 72.12 ± 0.90%, respectively as also illustrated in Figure 4.

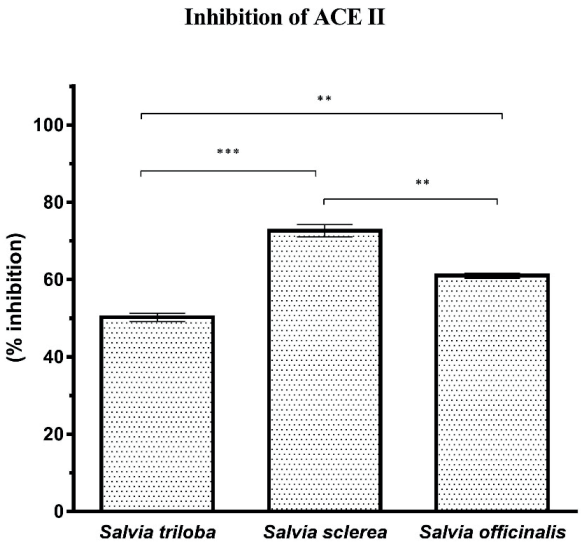


**Figure 4.** ACE2 Enzyme Inhibition of *Salvia Essential* Oils (at 20 µg/mL concentration)

ACE2 was proved to be the receptor for the SARS-CoV, the human respiratory coronavirus NL63, and the novel coronavirus 2019 nCoV/SARS-CoV-2[14].It is also known that previous studies identified that ACE2 is the essential and important receptor for coronavirus types to enter into the cell [15-16]. The highest ACE2 enzyme inhibition was observed in *S. sclarea* essential oil. *S. sclarea* was found to be effective against many viruses and human pathogenic microorganisms in previous studies [17-18]. In another detailed study on *S. sclarea* essential oil, it was revealed that the essential oil has a better antimicrobial effect compared to food preservatives. In addition, in this study, it was shown that the essential oil mechanism of action of was by damaging the cell membrane and impairing the cell membrane permeability. It is also thought to cause the release of macromolecular substances, even substances such as ATP and DNA, inside the cell. In general, the antimicrobial-antiviral effect of the essential oil of *Salvia sclarea* is not only attributable to a single pathway, but is thought to involve a series of events both on the cell surface and in the cytoplasm [19]. Due to the relevance of the prevention of coronavirus and the recovery process of the disease with ACE2, the results of the study can also be associated with the COVID-19 pandemic [20]. Based on all this information and the ACE2 enzyme inhibition of *S. sclarea* essential oil, it can be said that this essential oil may also

be effective against coronavirus.

Its effects on DNA and deformation of the cell membrane make its effectiveness against viruses possible. To the best of our knowledge, this is the first report on the ACE2 enzyme inhibition evaluation of *Salvia* essential oils. Further studies will be continued to evaluate the antiviral potentials of these essential oils, especially *S. sclarea*.

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