In vitro ACE2 Enzyme Inhibition of Different *Salvia* Essential Oils

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INTRODUCTION

Salvia, 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 bewerages, also in cosmetic and pharma industries. *Salvia* essential oils are generally rich in 1,8-cineole and borneol content (Asgarpanah, 2021; Baser et.al, 1993; Baser et.al., 1997; Perry et.al., 1999; Putievsky et.al., 1990).

Ethnobotanical utilization of *Salvia* species are also common worldwide, *Salvia officinalis* L. is widely used in the treatment of cough, bronchitis and colds (Kamatau et.al., 2008). In addition, *Salvia sclarea* L. is also used to relieve the upper respiratory tract, especially in the treatment of upper respiratory tract infections (Parsaei et.al., 2016). Anatolian *Salvia triloba* L. (Synonym *Salvia fruticosa* Mill.) is commonly used against coughs and colds (Baytop, 1999). In addition, of *Salvia* essential oils are also used due to their antimicrobial and antiviral effects in aromatherapy (Najar et.al., 2021; Öztürk et.al., 2018; Sivropoulou et.al., 1997; Tseliou et.al., 2019).

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.

MATERIAL AND METHOD

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" (Demirci et.al., 2022).

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 (BioVision, 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 (Demirci et.al., 2022).

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).

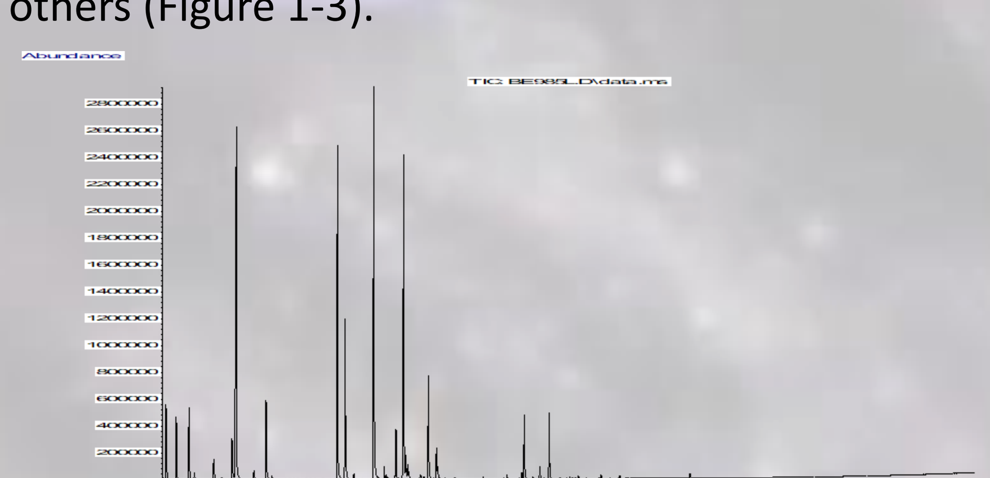

Figure 1. GC Chromatogram of *Salvia officinalis* essential oil

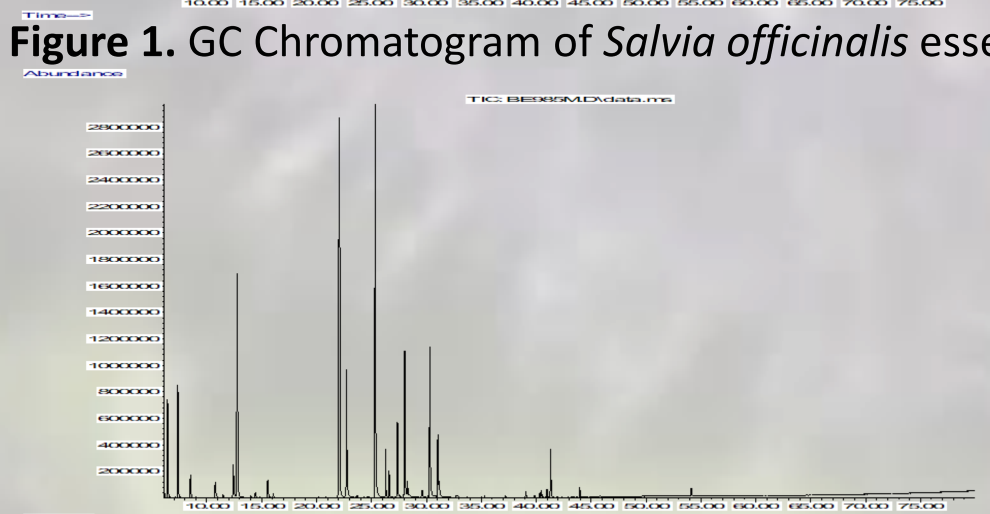

Figure 2. GC Chromatogram of *Salvia triloba* essential oil

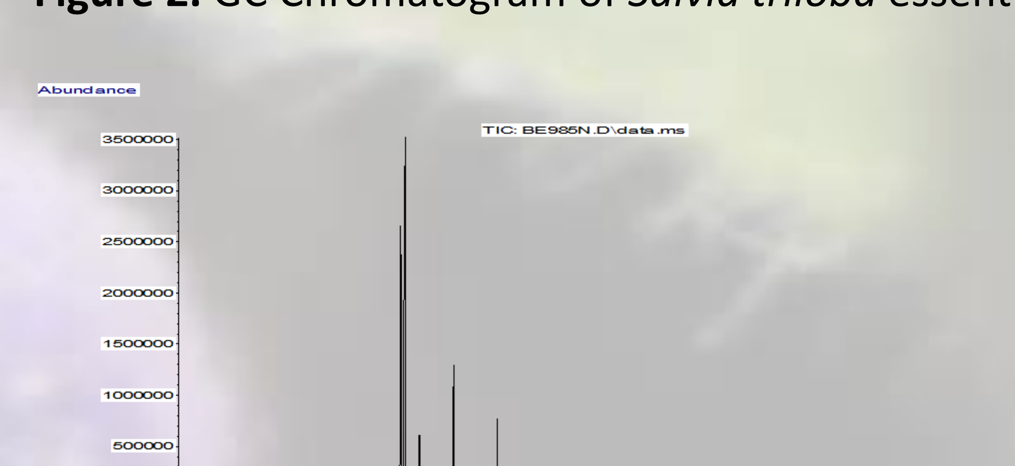

Figure 3. GC Chromatogram of *Salvia sclarea* essential oil

Recent literature on *S. triloba, S. officinalis*, and *S. sclarea* essential oils from Turkey show that major components of the species have similarity (Karik et al., 2018). 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.

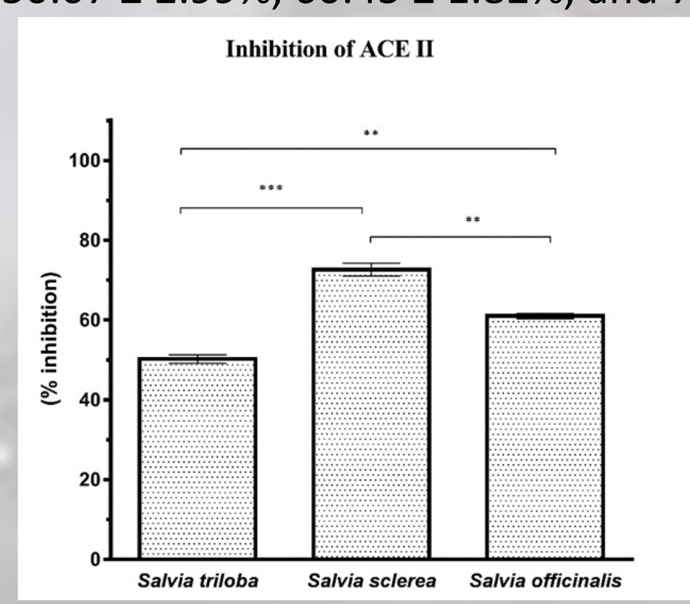

Figure 4. ACE2 Enzyme Inhibition of *Salvia* Essential Oils (at 20 µg/mL concentration)

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 (Nadjib, 2020; Öğütçü et.al., 2008). 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 (Cui et.al., 2015). 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 (Liu et.al., 2020). 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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