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Antiviral activity of *Lavandula angustifolia* L. and *Salvia officinalis* L. essential oils against avian influenza H5N1 virus

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Nowadays, viral infection is considered a major cause of mortality all over the world such as covid-19 pandemic. In this context, searching for antiviral agents are major researchers interests. In this study, essential oils (EO) of *Lavandula angustifolia* (lavender) and *Salvia officinalis* (salvia) were subjected to combat avian influenza H5N1 virus. Laboratory trials were performed to identify Lavender and salvia EOs and evaluate their antioxidant, anti-inflammatory and antiviral activity against an avian influenza H5N1 virus. EOs were prepared by the hydrodistillation of air-dried plants and analyzed by GC-MS methods. The results revealed that salvia has the highest EOs yield 1.3% than lavender 1%. The dominant constituents of lavender EO were linalyl acetate and linalool while camphor and α-thujone were the dominant compounds of salvia. Both oils exhibited antioxidant activity in DPPH and ABTS and total antioxidant capacity assays. The results suggest the use of salvia and lavender EOs as effective natural anti-inflammatories, antioxidant and antiviral agents.

**1. Introduction**

Considering the COVID-19 pandemic which is responsible for a growing mortality all over the world, in the same time, the absence of effective antiviral agents with no side effects leading researchers to focus their interest to found new antiviral agents from natural sources.

Last decades, researchers have focused their attention to natural products, trying to identify compounds as a source of antiviral drugs with low toxicity to human beings and then optimized by synthetic campaigns to find more active molecule against virus. Presently, there are two main commercially groups of anti-influenza drugs licensed by the Food and Drug Administration (FDA). The neuraminidase inhibitor group that includes oseltamivir, zanamivir, and peramivir antiviral drugs while the second M2 ion channel blockers group that includes amantadine and rimantidine antiviral drugs. Oseltamivir orally administered and systemically bioavailable has been the main antiviral treatment in avian influenza H5N1 infections due to minimal systemic bioavailability of the inhaled zanamivir and the rapid emergence of resistance to the M2 inhibitors [1]. Due to the heavy use of the antiviral drugs without rules and virus selective pressure, several antiviral resistance strains of influenza A viruses have emerged (Govorkova et al., 2013).

EOs are famous for their therapeutically effects, they are found in secretory ducts, gland and secretory hairs [2]. They found in ten percent of the plant kingdom, Among all natural products, EOs showed very promising biological activities. They have been recognized for their antifungal, antibacterial, insecticidal and antioxidant [3,4]. They are widely used in medicine and in food preservation [5].

Recently, herbal antibacterial and antiviral products have attracted particular interest due to the antibiotic resistance acquired by some bacteria [6], growing concerns about food safety, and the potential
health effects of synthetic additives [7].

Lavender and salvia are perennial woody sub-shrubs in the family Labiatae/Lamiaceae widely used in therapeutic, cosmetic and food applications. They considered the most important plants cultivated all over the world as sources of EOs which have promising biological activities [8]. Salvia and lavender EOs have been used to treat diarrhea, ulcers, seizure, inflammation, paralysis, dizziness, tremor, rheumatism.

Analysis of the EO composition of several of their species showed 1,8-cineole and borneol were the main ingredients. However, several authors have documented significant species variation in the concentrations of these compounds and the presence of others in high concentrations [9,10]; Haznedaroglu et al., 2001). In addition, as with other aromatic plants, the EO composition of Salvia and lavender species is strongly influenced by environmental and genetic factors [11].

One of the most important activities of sage and Lavender EO is their antibacterial activity, which has caught the attention of many scientists [12]. The antibacterial activity of sage and lavender has been known decades ago [13] and is based on the presence of thujone, camphor and 1,8-cineole, three terpenes with well-documented antibacterial and anti-viral potential [14,15], cancer, and hyperglycemia [16].

The aim of the present work was to study the chemical composition of the EO of lavender and salvia and evaluate their antioxidant, anti-inflammatory, and anti-viral activities.

2. Experimental

2.1. Plant material

The experiment was carried out at the National Research Centre, Dokki, Cairo Egypt. Aerial part of flowering salvia and lavender were obtained in the spring of 2020 from the El-Orman garden, Giza, Egypt. Both plants were identified by Plant Taxonomy Dept., National Research Centre (NRC), Egypt. Both plants were air dried at room temperature for about four days. The powdered samples were then kept frozen in a -35 °C freezer until used. All laboratory tests were conducted at the NRC, Cairo, Egypt.

2.2. Extraction and analysis of the EOs

Hundred grams aerial part of salvia and lavender were macerated in 500 ml of distilled water during 24 h before extraction. Plants were then submitted to Clevenger hydrodistillation during 3 h. The obtained EOs were dried over anhydrous sodium sulphate and after filtration stored at 4 °C until evaluation. The yield of extraction was evaluated according to Williams and Lasunzi [17]. The oils were then subjected to GC-FID and GC-MS analysis. The components of salvia and lavender EOs were identified by GC/MS, using GC Hewlett Packard model (5890) series II plus, equipped with a Carbowax 20 M capillary column (50 m × 0.32 mm x 0.32 microm i.d.), flame ionization detector (FID), helium as carrier gas at a flow rate of 1 ml/min, initial column temperature was 60 °C increased to 200 °C at a rate of 3 °C/min. and hold at 200 °C for 40 min, injector and detector temperatures were 200 and 250 °C, respectively. MS analysis was made using Hewlett Packard Mass Spectrometry model (5970); MS ionization voltage was 70 eV. Qualitative identification of the oil constituents was carried out by comparing their retention times and mass fragmentation patterns with those of the available authentic compounds in the data base of Kato Aromatic Company, El-Haranyia, Giza.

2.3. Determination of antioxidant capacity of EOs

Free radical scavenging capacity (DPPH, ABTS) for both extracts was determined according to Ye et al. [18] and Arnao et al. [19]. While, the total antioxidant activity was carried out according to Prieto et al. [20].

2.4. In-vitro anti-inflammatory activity

According to albumin denaturation method Rahman et al. [21], anti-inflammatory activity of EOs was determined. BSA (0.5g/100 ml water) was mixed with 0.05 ml sample containing 50,100,150,200 µg/ml of EOs or standard drug, and incubated for 20 min at 37°C. After cooling, samples were mixed with 2.5 ml phosphate buffer (0.1 M, pH 6.4) and absorbance was read at 255 nm against control sample i.e. 100% protein denaturation and compared with Diclofenac sodium. Percent inhibition calculated using following equation.

\[
\% \text{ inhibition} = 100 - \left( \frac{ODS - ODC}{ODS} \right) \times 100
\]

ODS = Optical density of sample
ODC = Optical density of control

2.5. Antiviral activity

2.5.1. Cytotoxicity assay

Two EOs were 10-fold serially diluted with Dulbecco’s Modified Eagle’s Medium (DMEM). The cyotoxic effect of each compounds were tested individually in Madin Darby Canine kidney (MDCK) cells using the 3-(4,5-dimethylthiazol -2-y)-, 5-diphenyltetrazolium bromide (MTT, Lonza) with minor modification as previously described by Moshmann [22].

2.5.2. Plaque reduction assay

Anti-viral activity of the EOs were tested using plaque reduction assay, Tobita et al., [23]. Six well plate were cultivated with MDCK, 24 h at 37°C then infected with the NRC isolated and characterize Ana/chick-en/egypt/M721/2013 (H5N1) Elshesheny et al. (2014). Tested viruses were diluted to 10² PFU/well, mixed with different concentrations of EOs and incubated for 1 h at 37°C then added to the cells after moving MDCK from the cell culture plates and inoculated with (100µl/well) of the tested EOs. 3 ml of DMEM medium supplemented with the overlay medium was added to the cell monolayer, left to solidify, and incubated...
for 3 day at 37°C until formation of viral plaques. Plates were satined with 0.1% crystal violet after fixing solution was added to cells. Control wells were incubated with MDCK. Plaques were counted and % reduction in plaques formation compared with control was recorded as follows:

\[
\% \text{ plaques reduction} = \frac{\text{UVC} - \text{TVC}}{\text{UVC}} \times 100
\]

3. Statistical analysis

All experiments were designed in a completely randomized design, with at least triplicate assays. Analyses of variance (ANOVA) and the LSD test were performed at level of \( P < 0.05 \) to evaluate the significance of differences among mean values. Data were analyzed using the MSTATC statistical computer package.

4. Results and discussion

4.1. Chemical composition

The results of GCMS analyses of lavender and salvia EOs are given in Table 1. The results revealed that salvia has the highest EOs yield 1.3% than lavender 1%. The major compounds in lavender EO were linalyl acetate 39.7%, linalool 33.6% and terpinen-4-ol 14.9% while the dominant components in salvia oil were camphor and α-thujone (23.94 and 22.68% respectively).

Data obtained in previous reports on salvia EO found that α-thujone varies between 3.5 and 56.9% and camphor between 3.5 and 36.9% [24, 25]. According to a study by Ben Khaleder et al. [26], salvia EO is rich in camphor, α-thujone, 1,8-cineole and β-caryophyllene and borneol. Ben Baarit et al. [27] reported that α-thujone, camphor and 1,8-cineole were the main components of salvia EO. In addition, Hayouni et al. [28] reported that 1,8-cineole, β-thujone, α-thujone, borneol, β-element, camphor and α-pinene were abundant salvia EO compounds.

Compared with previous GCMS studies for lavender EO, linalool and linalyl acetate have been reported as major components of lavender EO, but the rates varied significantly, especially in the range of 21–46% and 26–48%, respectively (EDQM, 2016). Fakhari et al. [29] found that Lavender EO contained 35.3% linalool and only 13.4% linalyl acetate, 26% plaques formation compared with control was recorded as follows:

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\[
\text{UVC} = \text{Untreated Viral Count}
\]

\[
\text{TVC} = \text{Treated viral count}
\]

Results expressed as \( \mu g \) ascorbic acid equivalents/ml. Results in columns marked with different capital letters and in rows with lower case letters are significantly different (p < 0.05).

| Tested sample               | Content (μg/assay) |
|-----------------------------|-------------------|
|                            | 5                 |
|                            | 10                |
|                            | 15                |

Lavender essential oils 28.7 ± 2.5a,b 32.9 ± 2.9c,b 37.6 ± 0.6c
Salvia essential oils 33.8 ± 2.5a,c 39.4 ± 1.1b,a 50.5 ± 5.2a,b
Ascorbic acid 29.3 ± 0.6a,b 45.9 ± 4.6b,a 84.2 ± 4.4b,a

Results in columns marked with different capital letters and in rows with lower case letters are significantly different (p < 0.05).

4.2. Antioxidant activity

The antioxidant activity of EOs is one of the most biological activities of major interest due to their activity in preserve foods from oxidants, as well as their activity in scavenging ROS which play a serious role in countless disease prevention such as cardiovascular disorders, cancer, diabetes, neurological diseases and immune system decline [38].

In our study, antioxidant activities of EOs of lavender and salvia were investigated using DPPH, ABTS, and total antioxidant capacity assays (Tables 2–4). Ascorbic acid showed the highest activity against DPPH and ABTS than salvia and lavender while salvia showed the highest activity in total antioxidant method and this may be due to other mode of action of salvia as antioxidant.

The antioxidant potential of the EOs of salvia and lavender was reported by several authors. Salvia EO showed the highest activity for chelating Fe²⁺ than BHT and ascorbic acid as well as inhibiting 5-lipoxigenase [39–41]. These activities were attributed to the presence of 1, 8-cineole, α-pinene and β-caryophyllene in the EOs. While the ROS scavenging activity of the lavender EO was shown by Refs. [40,42], who studied the inhibitory effect of lavender oil on fat oxidation reactions and lipid peroxidation in a linoleic acid model system. Chia-Wen et al. [43], Lu et al. [44], and Yang et al. [45] used DPPH assay to study the antioxidant powerful of lavender EO and found that lavender EO exhibited DPPH-scavenging activity similar to limonene.

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activities [36,47–56]. EOs of lavender exhibited antioxidant activity investigated using DPPH, FRAP, and ABTS [57]. The lavender EO resulting in scavenging of 50% of DPPH radical was 51.05 mg/ml [58] and 16.27 mg/ml (Pistelli) [57], and 27.7 mg/mL (Blazekovic). EC50 of salvia EOs in DPPH assay was 100 μg/ml [59]. Lavender thymol, carvacrol, p-cymene, linalool and eugenol have been demonstrated to possess in vitro antioxidant properties [60]. Thymol is able to act as electron donors.

4.3. Anti-inflammatory activity

In addition to the potentiality of EOs as a potent antioxidant, there is also evidence that EOs possess potent anti-inflammatory potential. The anti-inflammatory potential of EOs might be related not only to their potent antioxidant potential but also to their interactions with signaling cascades involving the expression of pro-inflammatory genes and transcription factors.

The anti-inflammatory activity of tested EOs is shown in Table 5. Standard shown the highest activity followed by salvia while lavender showed weak activity when compared with the slandered. The activity of salvia may be due to α-thujone [61–63].

As reported repeatedly in the literature, based on the chemica composition, EOS act anti-inflammatory by either affecting arachidonic metabolism or cytokines production Moreover, Eos modulate the pro-inflammatory gene expression, inhibit the cyclooxygenase and lip-oxygenase activities, and/or inhibit the production of tumor necrosis factor, interleukin, and prostaglandins [64–66]. Possible mechanism of EOs anti-inflammatory action are presented in figure (2).

Lavender and salvia EOs, showed strong anti-inflammatory activity by the inhibition of lipooxygenase activity [67]. The authors attributed the anti-inflammatory activity of salvia EO to the presence of 1,8-cineole [41]. While, the anti-inflammatory activity of Lavender EO was due the presence of linalool and its ability to inhibit PGE2 production, COX-2 expression and NO release [68]. Numerous studies showed that the EO constituents act synergistically because their main constituents when used alone have less activity than the EO. Nevertheless their activity can be changed due to several factors affecting on their main constituent ex. harvesting time; climatic conditions, plant part, type of extraction can be considered as responsible for fluctuations in their chemical compositions.

Table 5
Anti-inflammatory activity of tested essential oils (%).

| Tested sample         | Content (μg/assay) | Concentration (μg/ml) |
|-----------------------|--------------------|-----------------------|
|                       | 25                 | 50                    | 75                    |
| Lavender essential oils | 31.4 ± 0.5c        | 35.5 ± 0.2bc          | 58.5 ± 1.6c           |
| Salvia essential oils  | 60.1 ± 0.8bc       | 70.8 ± 1.0bc          | 74.5 ± 0.3bc          |
| Diclofenac sodium      | 81.7 ± 0.6bc       | 85.5 ± 0.2bc          | 88.5 ± 0.3bc          |

Results in columns marked with different capital letters are significantly different (p < 0.05).

4.4. Cytotoxicity

Cytotoxicity of EOs in respect of the MDCK cells that used for virus propagation is an initial step to determine its suitability for in vitro antiviral potential. The cytotoxicity of EOs were evaluated on MDCK cell lines using MTT. The results showed that the lavender EO could be considered cytotoxic with TC50 of 49 μg/ml, while EO of salvia was 150 μg/ml.

4.5. Antiviral activity

The viral diseases are a public health problem affecting the large people of the world. Medicinal plants can be used to treat diseases mainly viral infections. They are considered a cheap source for safe new pharmacological compounds [69]. The methanol extract and EO of Salvia sp. have a good antinfluenza viruses cells and herpes simplex viruses in MDBK cells [70, Ogutcu et al., 2008]. Tariq et al. [71] reported that the essential oils from plants possess antiviral activities against many DNA and RNA viruses, such as herpes simplex (HSV-1 and HSV-2), dengue type 2, influenza, poliovirus, Junin, and coxsackievirus B1 virus. Three essential oil combination (Coriandrum sativum L., Origanum dictumans L and Salvia fruticosa Mill), in extra-virgin olive oil shown to exhibit a direct antiviral activity against different types from influenza virus strains. They reduce the duration and severity of symptoms of patients with upper respiratory tract viral infections [72].

To the best of our knowledge, this is the first study on antiviral activity of lavender and salvia EOs in an endeavor to find an effective, safe and cheap antiviral drugs against avian influenza H5N1 viruses.

Regarding the antiviral activity, the oil samples of lavender and salvia EOs were evaluated at different subcytotoxic concentrations to determine their antiviral activity against H5N1 virus. As shown in Fig. 1, both tested oils showed promising inhibition against H5N1 virus with IC50 0.11 ± 0.01 μg/ml and 0.41 ± 0.02 μg/ml for lavender and salvia EOs, respectively. Amoros et al. [73] reported that selectivity index values (SI = TC50/IC50) for lavender and salvia EOs were 445.4 and 1000, respectively.

As Saddi et al. [74] as well as Astani et al. [75], reported, the antiviral potential of EOs depend on denaturing viral protein and/or glycoprotein. Where, EOs interfere virus membrane then mask viral compounds and inhibit specific process in the replication cycle. Consequently preventing viral cell diffusion.

Worth to emphasize is fact that EOs act mostly act on enveloped viruses, but not inside the cell. Compounds of EOs can also act synergistically with traditional antiviral drugs. For example, eugenol in a concentration of 30–120 μg/mL, increased the effect of low doses of Aciclovir [76,77].

Reichling et al. [78] combined the most significant results about antiviral potential of EOs published previously. EOs have broad spectrum antiviral activity against DNA viruses like HSV (herpes simplex virus) type 1 and 2, and RNA viruses like NDV (Newcastle disease virus), Junin...
virus, and SARS-CoV (severe acute respiratory syndrome-associated coronavirus).

According to the literature, Salvia EO has antiviral activity against SARS-CoV (RNA virus) with IC\textsubscript{50} 870 mg/mL. It is worth noting that the overriding clinical feature of SARS is the rapidity with which many patients develop symptoms of acute respiratory distress syndrome (ARDS) [79].

The mechanism of antiviral activities of EOs have been well-documented (Fig. 3). The mechanism of EOs as antiviral agents including a) inhibiting viral replication by inhibiting viral polymerases, b) preventing viral uncoating, c) block binding to host cell, d) inhibit viral infectivity, e) inhibit proteases, f) inhibit viral host receptors [80]. Numerous results demonstrate that EOs interfere with free viruses by masking the viral proteins or by modifying the virus envelope structure, which are important for viral entry into the host cells. Moreover, The EOs were observed to display a striking decrease in virus infectivity. The high antiviral action was achieved by rendering the free virus particles inactive and acting on virus particles tends to be the most common

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**Fig. 2.** Possible mechanism of anti-inflammatory actions of EOs.

**Fig. 3.** Possible mechanism of antiviral actions of EOs.
mechanism of action. Additionally, EOs were found to exhibit anti-influenza A/WS/33 virus by reduction in visible cytopathic effects of the virus. However, EOs from clary sage, marjoram and anise, were found to possess anti-influenza activity. Interestingly, linalool was found to be the most common constituent found in these three plants EOs [81].

In silico results conducted by Sharma and kaur [82] indicated that eucalyptus EO effectively bound to COVID-19 proteinase through hydrophobic interactions, ionic interactions and hydrogen bond. While, *Anomoides verticillata* EO was reported to be a potent inhibitor against angiotensin converting enzyme 2, a receptor of COVID-19, through Pi-H bonding [83-90].

5. Conclusion

The search for antiviral agents in EOs has been dramatically increased due to COVID-19 pandemic. Additionally, the search for anti-inflammatory agents in EOs was also increase due to their anti-inflammatory activity and their ability to savage free radicals, preventing lipid peroxidation, and chelating metal ions. In this context, The antiviral, anti-inflammatory activities of EOs has been target of this investigation. In this study, the chemical composition, cytotoxicity, antiviral, anti-inflammatory and antioxidant potential of lavender and salvia EOs are evaluated. The outcomes revealed that lavender and salvia EOs are dominated by camphor, α-thujone, sclareol, linalool, linalyl acetate, and α-thujone and terpinen-4-ol. The biological studies of EOs demonstrated a promising antioxidant, anti-inflammatory and antiviral activity. These findings supported the use of these EOs as good candidates for the development of new antiviral agents (particularly against avian influenza HSN1 virus) which could be a good source of pharmaceutical industries. Future research is encouraged to become aware of the predominant compounds that are responsible for the therapeutic activities.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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