In vitro antibacterial activities of essential oils and extracts of six herbals against gram-positive and gram-negative bacteria

Mansour Amin1,2 · Sousan Akrami3 · Farkhondeh Haghparasty2 · Atiyeh Hakimi2

Accepted: 20 November 2022 / Published online: 13 December 2022
© The Author(s), under exclusive licence to Korean Society of Environmental Risk Assessment and Health Science 2022

Abstract
Object Considering the development of bacterial resistance to chemical antibiotics and their adverse effects on the environment and public health, there is a growing demand to replace them with plant-based derivatives or combine these green agents with antibiotics to give synergistic effects. In this study, the antimicrobial properties of essential oils and extracts of six medicinal plants from Ahvaz region, Iran, against 12 gram-positive and gram-negative bacteria were evaluated.

Methods The EOs and extracts were extracted using water distillation with Clevenger apparatus. We analyzed the chemical composition of essential oils obtained. The antimicrobial properties and determination of the minimum concentration of growth inhibition of herbals were investigated by the broth dilution technique.

Results All analyzed extracts and EOs showed antibacterial effects. The antimicrobial activity of *Oliveria decumbens* was strongest herbals with the least MIC ranges (0.008–0.1 mg/ml for EO, 0.9–20 mg/ml for extract), while the antibacterial effects of *Artemisia vulgaris* extract and *Glycyrrhiza glabra* EO with the highest MIC were weaker than the others. According to the effectiveness of plant extracts on bacteria, *Pseudomonas aeruginosa* was resistant to all extracts except *Oliveria decumbens*. In contrast, *Bacillus cereus* was more sensitive than other strains against analyzed EOs and extracts. In *Oliveria decumbens*, EO detected 18 components by GC/MS with accessible genuine standards, accounting for 98% of the oil content.

Conclusion It seems that due to the antimicrobial properties of the extracts and essential oils observed in this study, they can be used as an alternative to antimicrobial drugs after more extensive studies.

Keywords Antibacterial activities · Essential oils · Gram-positive bacteria · Gram-negative bacteria · Herbals · Phytochemicals

Introduction
Herbal essential oils (EOs) and extracts have been used for various purposes for thousands of years [1]. These aims vary from the use of rosewood and Cedar in perfumes to flavoring drinks with lime fennel or juniper berry oil, and the use of lemongrass oil in food preservation [2, 3]. The basis of food storage, herbal medicines, and natural treatments is the antimicrobial properties of plant essential oils and extracts [4]. Medicinal and aromatic plants are widely utilized in medicine to combat drug-resistant microorganisms, which are considered as one of the primary causes for the failure of therapy in infectious diseases. Medicinal plants are the primary source of novel medicines and may serve as an alternative to conventional medications [5, 6].

Traditionally, people in different areas of the globe utilize extracts and oils for six plants in this work for diverse purposes. Because different plants are rich in a wide range of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids, it is important to introduce the type of plant species. The essential oil of *Oliveria decumbens* contains antibacterial and antifungal properties as well as cytotoxic effects on several cancer cell lines [7]. Bioactive substances with varied antioxidant and antibacterial properties discovered in ethanolic extracts and essential oils of *Ocimum basilicum* have significant uses in nutraceutical and
pharmaceutical technologies [8]. Glycyrrhiza glabra has immunological effects in humans [9]; Artemisia vulgaris oil has good antimicrobial activities [10]; and finally, Silybum marianum herb extract has antibacterial and antiadherent/antibiofilm activity against gram-negative bacteria [11]. In Iran, numerous plant extracts and oils, notably for respiratory and gastrointestinal problems, are traditionally utilized as a medicinal plant.

Some investigations have been done only on one extract or one microorganism. While this information is valuable, the results are not directly comparable because each study has a different methodology such as the type of microorganisms, selection of plant extracts, and antimicrobial testing methods [12]. A review study consolidated and described the observed synergistic outcome between essential oils and antibiotics, and highlighted the possibilities of essential oils as the potential resistance modifying agent [13].

The objective of the current research was to develop directly comparable, quantitative antimicrobial data for extracts that have little information. This study focused on the effect of six plant species commonly used in our region, including Oliveria decumbens, Glycyrrhiza glabra, Rhamnus Ocimum basilicum, Abadan Ocimum basilicum, Silybum marianum, and Artemisia vulgaris on the most pathogenic bacteria that cause community-acquired and hospital-acquired infections.

Results

Antimicrobial activities

In this research, the antimicrobial effects of six plant species were evaluated against 12 bacterial strains. The results of the growth inhibition effect of plant extracts and essential oils on the studied bacteria are shown in Tables 1, 2, 3, 4. Among the plant EOs, Oliveria decumbens EO had the highest inhibitory activity and Glycyrrhiza glabra, Ocimum basilicum, and Ocimum basilicum essential oils had the least inhibitory effect on bacterial strains. However, all studied strains were sensitive against each essential oil. According to the effectiveness of plant extracts on bacteria, Pseudomonas aeruginosa was resistant to all extracts except Oliveria decumbens.

The antimicrobial activities of essential oils are displayed in Table 1. The results exhibited potential antibacterial activities of the essential oil against gram-positive bacteria: S. aureus with MIC of 0.25–4% (v/v) and MLC of 0.5–8% (v/v), and S. epidermidis with MIC of 0.25–4% (v/v) and MLC of 0.5–8% (v/v), E. faecalis with MIC of 0.5–4% (v/v) and MLC of 1–8% (v/v), C. diphtheriae with MIC of 0.5–4% (v/v) and MLC of 0.5–8% (v/v), B. cereus with MIC of 0.5–4% (v/v) and MLC of 1–8% (v/v); gram-negative bacteria such as E. coli with MIC and MLC from 0.25 to 8% (v/v), P. aeruginosa having MIC and MLC between 1 and 32% (v/v), and Shigella dysenteriae with MIC and MLC both equivalent to 0.5–4% (v/v).

The antimicrobial activities of extracts are displayed in Table 3. The results exhibited potential antibacterial activities of the essential oil against gram-positive bacterium: S. aureus with MIC of 2–8% (v/v) and MLC of 4–8% (v/v), and S. epidermidis with MIC of 1–8% (v/v) and MLC of 1–16% (v/v), E. faecalis with MIC of 2–8% (v/v) and MLC of 4–8% (v/v), C. diphtheriae with MIC and MLC both equivalent to 4–8% (v/v), B. cereus with MIC of 0.5–4% (v/v) and MLC of 1–8% (v/v); gram-negative bacteria such as E. coli with MIC and MLC from 1 to 16% (v/v), Shigella dysenteriae having MIC and MLC between 1 and 16% (v/v), and P. aeruginosa with MIC and MLC both equivalent to 2% (v/v).

Extraction yield and chemical composition of Oliveria decumbens EO

Oliveria decumbens EO and extract showed the strongest antimicrobial activity compared to other plants with significant differences. Therefore, additional analysis was done for this plant. The chemical compounds of O. decumbens were identified by GC/MS.

On a dry weight of three samples, the average yield of hydrodistilled O. decumbens EO in herbs was 0.30 ± 0.02%. The essential oil obtained was a pale, golden liquid with an odor that was lighter than water. The GC/MS analysis indicated that the Oliveria decumbens EO contained 18 constituents representing 98% of the total oil content (Table 5). Volatile oils (74.00%) were the primary groups of constituents in this oil. Monoterpene hydrocarbons (17.35%) and phenylpropenes (10.56%) were the compounds found in the highest concentrations in the oil of O. decumbens. Furthermore, the oil contained one alcohol with a non-terpenic structure, cis—ambrinol (0.22%).

Discussion

So far, wild or cultivated medicinal herbs have been used as important sources of medicine. Traditional botanical science offers valuable methods for discovering new medicinal herbs and herbal remedies [16, 17]. Today, the use and application of plants require comprehensive information about understanding their properties and characteristics [16]. The scientific study of medicinal plants as potential sources of new antimicrobial compounds is crucial [18]. The antibacterial action involved disruption of membrane potential, inner membrane permeabilization, blebbing, and leakage of cellular contents.
Several studies have been published on the examination of *O. decumbens* essential oil derived from various geographical locations. Most prior investigations [19, 20] identify thymol and carvacrol as main components of the essential oil, whereas γ-terpinene and myristicin were discovered as key compounds of *O. decumbens* oil gathered from Chaharmahal va Bakhtiari region, Iran [21]. In contrast to our findings, no carvacrol was identified in the essential oil of *O. decumbens* gathered in Lorestan region, Iran [22]. This demonstrates that changes in essential oil content can be caused by a range of factors such as the diversity of examined plant parts, geographical area, and plant collecting time. Despite the fact that some of these volatile chemicals may be found in essential oils from different families, the similarity of the primary components in essential oils from species in the same family indicates that they had significant chemotaxonomic significance [23].

The results of this study showed that the essential oils and extracts of these plant samples have antibacterial properties. Essential oils appear to have more antimicrobial effects than extracts. Antibacterial activity of different EOs and extracts of medicinal plants on different microorganisms in different regions has been reported.

In 2020, Khoshbakht et al. from Bandar Abbas examined *Oliveria decumbens*’ antibacterial activity of essential oil on seven microbial strains. MIC values were in the range of 0.0625—2 mg/ml. Chloramphenicol is an antibiotic and belongs to the class of antimicrobials against gram-positive and gram-negative bacteria, which inhibits protein synthesis. In study of Khoshbakht et al., the antibacterial activity of *O. decumbens* essential oils was higher than that of chloramphenicol used as positive control [7], while in this study, the minimum inhibitory concentration of essential oil and extract of *O. decumbens* was 0.008–0.1 mg/ml and 0.9–20 mg/ml, respectively. In line with previous research, the essential oil tested in our study had a significant antibacterial impact on the majority of the bacterial strains tested [19–21]. This effect is most likely owing to the presence of the phenolic chemical’s thymol and carvacrol, or to a synergistic action of these compounds [24, 25]. Contrary to other data, *O. decumbens* had antimicrobial activity against *P. aeruginosa* [24]. Also, the essential oil and extract of *O. decumbens* inhibited *H. pylori* significantly, with MIC = 0.25 and MIC = 1, respectively.

We discovered that *O. decumbens* essential oil and extract have substantial anti-*Helicobacter pylori* action. This is consistent with the herb’s historic usage in the treatment of gastrointestinal ailments [19]. According to a recent study, carvacrol has substantial anti-*Helicobacter pylori* activity; however, the addition of thymol reduces carvacrol’s anti-*Helicobacter pylori* activity [26]. As a result, more research is needed to identify the active principle responsible for this essential oil’s anti-*Helicobacter pylori* properties.

### Table 1

Antimicrobial activities (MIC and MLC (% v/v)) of herbal essential oils against bacteria

| Bacteria                    | Artemisia vulgaris | Silybum marianum | Abadan Ocimum basilicum | Ramhormoz Ocimum basilicum | Glycyrrhiza glabra | Oliveria decumbens |
|-----------------------------|-------------------|------------------|-------------------------|---------------------------|------------------|-------------------|
| **MIC and MLC (% v/v)**     |                   |                  |                         |                           |                  |                   |

*MLC: Minimum lethal concentrations; MIC: Minimum inhibitory concentrations.*
In another study conducted in 2018 in Kazerun, *Glycyrrhiza glabra* extract had antibacterial effects on strains such as *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, and the MIC range was 6.25—100 μg/ml, The MIC for *B. cereus* strain was 12.5 mg/ml [27]. Also, in the present study, *Glycyrrhiza glabra* essential oil and extract showed antimicrobial effects on gram-positive and gram-negative bacterial strains, and MIC on *B. cereus* was 12 mg/ml. In the study of Manandhar et al. from Nepal, the antibacterial activity of *Artemisia vulgaris* extract on some gram-positive and gram-negative bacterial strains was investigated. The study results showed that the extract of this species did not have any effect on strains [10]. Also, in this study, *Artemisia vulgaris* extract did not affect *Pseudomonas aeruginosa* strain. In Turkey, Evren et al. studied the antimicrobial effect of *Silybum marianum* extracts. This species showed antimicrobial activity on gram-positive and gram-negative bacteria and created MIC with a range of 0.06–0.2 mg/ml [11]. In contrast, the extracts of this plant in our study showed antimicrobial activity with MIC of about 55–38 mg/ml. According to a study in Romania, the essential oil of plant species of *Ocimum basilicum* showed better effects on gram-positive bacteria [28]. Also, in our study, gram-positive bacterial strains were more sensitive to *Ocimum basilicum* extract and essential oil. Besides, in this study, essential oils and extracts of aerial parts of two types of basil species were investigated, and *Ramhormoz Ocimum basilicum* showed better antimicrobial effects than *Abadan Ocimum basilicum*. According to a study in 2020 in Cyprus, the effect of geographical conditions and altitude of the cultivation site can affect the type of compounds in the plant and their amount and thus antimicrobial activity, which due to the diversity of antimicrobial effect of different species in this study, our research consistent with the results of these researchers [29].

According to previous studies in different places and the findings of this study, it was found that the essential oils and extracts of the studied herbal species can show the antimicrobial effect on gram-positive and gram-negative bacteria and inhibit the growth with different MIC. This difference can be due to several reasons, including different methods of oil extraction, genetic factors, seasonal and environmental factors at the time of sampling. It seems that due to the antimicrobial properties of some extracts and essential oils that were observed in this study, they can be used as an alternative to antimicrobial drugs after more extensive studies.

### Table 2 Minimum inhibitory concentrations (μg/ml) for the growth of bacteria

| Bacteria                | Plants                        | Oliveria decumbens | Glycyrrhiza glabra | Ramhormoz Ocimum basilicum | Abadan Ocimum basilicum | Silybum marianum | Artemisia vulgaris |
|-------------------------|-------------------------------|--------------------|-------------------|---------------------------|-------------------------|------------------|------------------|
| *Staphylococcus aureus* | 22                            | 500                | 100               | 110                       | 50                      | 80               | 85               |
| *Staphylococcus epidermidis* | 20                        | 450                | 120               | 100                       | 50                      | 80               | 85               |
| *E. coli*               | 20                            | 400                | 110               | 95                        | 55                      | 76               |                  |
| *Pseudomonas aeruginosa* | 100                          | 1200               | 1100              | 1200                      | 200                     | 220              |                  |
| *Helicobacter pylori*   | 18                            | 420                | 230               | 220                       | 60                      | 75               |                  |
| *Acinetobacter baumannii* | 12                          | 420                | 220               | 240                       | 60                      | 80               |                  |
| *Enterococcus faecalis* | 20                            | 480                | 280               | 310                       | 55                      | 65               |                  |
| *Enterobacter cloacae*  | 22                            | 510                | 310               | 300                       | 65                      | 55               |                  |
| *Shigella dysenteriae*  | 16                            | 380                | 180               | 205                       | 45                      | 55               |                  |
| *Klebsiella pneumoniae* | 24                            | 480                | 350               | 365                       | 65                      | 85               |                  |
| *Corynebacterium diphtheriae* | 28                      | 560                | 120               | 180                       | 50                      | 75               |                  |
| *Bacillus cereus*       | 8                             | 210                | 84                | 95                        | 38                      | 50               |                  |

### Material & methods

**Medicinal plants and their collection**

The herbals used in this study were including *Artemisia vulgaris*, *Silybum marianum*, *Ramhormoz Ocimum basilicum*, *Abadan Ocimum basilicum*, *Glycyrrhiza glabra*, and *Oliveria decumbens*. During the flowering season, between December and April 2019, samples were gathered from several areas in Khuzestan (Table 6). The samples were thoroughly cleaned to remove any unusual flora, dust, or other pollutants.
Ethanolic extract preparation

Samples were washed, air-dried for 7–8 days, and ground into powder before being put in the flask of the Soxhlet apparatus for extraction using ethanol with increasing order of polarity to extract the phytoconstituents individually at 20 °C for 3–4 h. The extracts were then filtered using Whatman No.1 filter sheets. Following that, decreased pressure was used to evaporate and dry the filtrates, which were then kept at −20 °C in labeled, sterile, screw-capped vials.

Essential oil extraction

Essential oils were extracted by hydro-steam distillation using the Clevenger equipment from fresh, clean, weighed aerial parts of flowers, flowering branches, seeds, and rhizomes (5 kg) (Table 6) and collected and stored in dark sterile vials. Briefly, 100 to 150 g of each plant was dried in the shade, ground, and placed in a distillation flask (1L), which was linked to a steam generator through a glass tube and to a condenser. This was recovered in a funnel tube. Essential oil aromatic molecules were released from the plant material and evaporated into hot steam. The heated steam forced the plant material to release the essential oil without burning it. The steam containing the essential oil was then sent through a cooling system to condense it. The steam was applied for 3 h. The essential oil was extracted once the recovered mixture had been settled. The produced essential oil was dried by filtering the supernatant essential oil through anhydrous Na2SO4. The density was determined then gathered in tighter vials and refrigerated. Several dilutions of the oils were done using dimethyl sulfoxide (DMSO) for the antibacterial activity test.

Gas chromatography-mass spectroscopy

An Agilent gas chromatograph with a flame ionization detector was used to examine the Oliveria decumbens essential oil (FID). Helium was employed as the carrier gas in a 30 m DB-5 capillary column at a flow rate of 1 mL/minute. The temperatures of the injector and detector were the same (280 °C). The column temperature was designed to rise from 50 to 135 °C at 5 °C/min (1 min), 225 °C (5 min), and 260 °C (10 min). Injection volume was 1.0 μL (split ratio, 1:25).

Mass spectrometry was performed under the same circumstances as chromatography, using a Thermo Quest apparatus with a quadrupole detector. The column was connected to the mass spectrometer’s ion source. At 70 eV, mass units ranging from 10 to 900 were measured. The retention indices (RI) of a single molecule were obtained by co-injection with a homologous sequence of n-alkanes (C9-C22) under the
same conditions as (the retention indices were calculated using Van del Dool and Kartz’s [14] generalized equation). Data were analyzed for ANOVA using the MSTAT-C program, and mean separation was conducted using the LSD test at the p 0.05 level of significance.
Microorganisms and their maintenance

Twelve standard strains, including Staphylococcus aureus (S. aureus) PTCC 1113, Escherichia coli (E. coli) PTCC 1533, Bacillus cereus PTCC 1015, staphylococcus epidermidis PTCC 1435, Pseudomonas aeruginosa PTCC 1558, Helicobacter pylori PTCC 5211, Acinetobacter baumannii PTCC 1855, Enterococcus faecalis PTCC 1237, Enterobacter cloacae PTCC 1003, Shigella dysenteriae PTCC 1188, Klebsiella pneumoniae PTCC 1290, and Corynebacterium diphtheria ATCC 27,010, were obtained from the Persian Type Culture Collection. The subculture of bacteria using a panel of laboratory control strains was obtained from the Persian Type Culture Collection. All bacteria were stored in trypticase soy broth containing 25% (v/v) glycerol for the time of the investigation.

Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC)

The broth dilution technique was used to determine the MIC and MLC of bacteria and Candida species, as published by the Clinical and Laboratory Standard Institute [15]. The inoculum was created by diluting colonies in salt solution at a concentration of 0.5 McFarland, which was then spectrophotometrically validated at 530 nm. Using 96-well plates, the sensitivity test was performed in LB broth and RPMI-1640 medium. The oil solutions were diluted to concentrations ranging from 16% (v/v) to $5 \times 10^{-4}$% (v/v). Following shaking, 100 L of each oil dilution and 100 L of bacterial/yeast solution at 106 CFU/mL were added to each well and incubated at 37 °C for 24 to 48 h. The MIC value was established by the lowest dose of essential oil that reduced bacterial growth noticeably after overnight incubation. In order to determine the MLC value, 10 μL was seeded on Mueller Hinton agar and Sabouraud Dextrose agar and the plates were incubated for 24 to 48 h at 37 °C. The lowest concentration that lowers the viability of the original microbial inoculum by 99.9% was defined as the minimal lethal concentration (MLC). Each experiment was carried out in triplicate and three times. For each dilution series, one well containing bacterial suspension without oil solutions was considered as positive control and one well containing oil solutions without bacterial suspension was considered as negative control.

Statistical analysis

Microsoft Excel and SPSS version 22 statistical software were used to evaluate descriptive data (IBM Corporation, Armonk, NY, USA). The findings are given in the form of descriptive statistics in terms of relative frequency.

Conclusion

The findings of this study showed that all essential oils and extracts of the studied plant species have strong antimicrobial activity. Among the species, Oliveria decumbens extract and EO had the most inhibitory effect. These differences are related to the composition of EOs and extracts, as well as the susceptibility of the bacterial strains against different plants. It is noteworthy that in recent years, there have been many reports of bacterial resistance to antibiotics, and measures should be taken to combat them. Therefore, conducting additional research on the studied plants in this research and their active ingredients can be helpful in this regard. These plant extracts could be a potential source of new antibacterial agents.

Acknowledgement

This study was financially supported by a grant (no: OG-95127) from the Research Affairs, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran and the Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. We are grateful to the Research Affairs of the Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran for the financial support of this study.

Declarations

Conflict of interest Mansour Amin, Sousan Akrami, Farkhondeh Haghpastary, Atiyeh Hakimi declare that we have no conflict of interest.

Ethical statement This project was confirmed by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (No. IR.AJUMS.REC.1395.479).
References

1. Yang F, Dong X, Yin X, Wang W, You L, Ni J (2017) Radix Bupleuri: a review of traditional uses, botany, Phytochemistry, pharmacology, and toxicology. Biomed Res Int. https://doi.org/10.1155/2017/7597956

2. Orchard A, van Vuuren S (2017) Commercial essential oils as potential antimicrobials to treat skin diseases. Evid Based Compl Alternat Med 2017:4517971

3. Kumar S, Kumar A, Kumar R (2019) Himalayan (Himachal region) cedar wood (Cedrus deodara) Pinaceae essential oil, its processing, ingredients and uses: a review. J Pharm Pharmacol 8(1):2228–38

4. Bhavaniramya S, Vishnupriya S, Al-Aboody MS, Vijayakumar R, Cazella LN, Glamoclija J, Soković M, Gonçalves JE, Linde GA, Evren E, Yurtcu E (2015) In vitro effects on biofilm viability referred to Imam Khomeini Hospital Ahvaz. Iran. J Curr Biomed Rep 1(1):23–6

5. Akrami S, Abouali R, Olapour MM, Abady RH, Yousafi-Azarvand A (2020) Bacterial etiology and antibiotic susceptibility pattern of female patients with urinary tract infection referred to Imam Khomeini Hospital Ahvaz. Iran. J Curr Biomed Rep 1(1):23–6

6. Khoshbakht T, Karami A, Tahmasebi A, Maggi F (2020) The variability of thymol and carvacrol contents reveals the level of antibacterial activity of the essential oils from different accessions of Oliveria decumbens. Antibiotics 9(4):170

7. Rezzoug M, Bakchiche B, Gherib A, Roberta A, Kilincarslan Ö, Mamamadov R, Bardaweel SK (2019) Chemical composition and bioactivity of essential oils and Ethanolic extracts of Ocimum basilicum L. and Thymus algeriensis Boiss. & Reut. from the Algerian Saharan Atlas. BMC Complement Altern Med 19(1):1

8. Hong YK, Wu HT, Ma T, Liu WJ, He XJ (2009) Effects of Glycyrrhiza glabra poly saccharides on immune and antioxidant activities in high-fat mice. Int J Biol Macromol 45(1):61–64

9. Manandhar S, Luitel S, Dahal RK (2019) In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. J Trop Med 2019:1895340. https://doi.org/10.1155/2019/1895340

10. Evren E, Yurtcu E (2015) In vitro effects on biofilm viability and antibacterial and antiadherent activities of silymarin. Folia Microbiol 60(4):351–356

11. Ca zella LN, Glamoclija J, Soković M, Gončalves J, Colauto NB, Gazim ZC (2019) Antimicrobial activity of essential oil of Baccharis dracunculiflora DC (Asteraceae) aerial parts at flowering period. Front Plant Sci 10:27

12. Yap PS, Yiap BC, Ping HC, Lim SH (2014) Essential oils, a new horizon in combating bacterial antibiotic resistance. Open J Med Microbiol 8:6

13. Adams RP (2007) Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, 4th edn. Allured Publishing Corporation, Carol Stream, IL

14. Clinical and Laboratory Standards Institute (2021) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard-Ninth Edition: M07-A9.

15. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H (2018) Medicinal plants: past history and future perspective. J Herbmed Pharmacol 7(1):1–7. https://doi.org/10.15171/jhp.2018.01

16. Kianifar J, Azadbakhht M, Azadbakhht M, Davoodi A (2020) Ethnobotanical study of medicinal plants used in skin diseases in the area Alamut-Qazvin. Iran J Med Plant Res 4(72):121–132

17. Cheesman MJ, Ilanko A, Blonk B, Cock IE (2017) Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution? Pharmacogn Rev 11(22):57

18. Ami n G, Sournaghi MS, Zahedi M, Khanavi M, Samadi N (2005) Essential oil composition and antimicrobial activity of Oliveria decumbens. Fitoterapia 76:704–707

19. Mahboubi M, Feyzabadi MM, Haghj, Hosseini H (2008) Antimicrobial activity and chemical composition of essential oil from oliveria decumbens vent Iran. J Med Arom plants 24:56–65

20. Hajimehdpoor H, Samadi N, Mozaffarian V, Rahimifard N, Shohei S, Pirali Hamedani M (2010) Chemical composition and antimicrobial activity of Oliveria decumbens volatile oil from West of Iran. J Med Plants 1:39–44

21. Amiri H, Lari Yazdi H, Dosti B, Samsamnia F (2011) Essential oil composition and anatomical study of oliveria decumbens vent Iran. J Med Arom plants 26:513–520

22. de SS Quintans J, Soares BM, Ferraz RP, Oliveira AC, da Silva TB, Menezes LR, Sampaio MF, Prata AP, Moraes MO, Pessoa C, Antonioli AR (2013) Chemical constituents and anti cancer effects of the essential oil from leaves of Xylopia laevigata. Planta medica 29(02):123–30.

23. Didry N, Dubreuil L, Pinkas M (1993) Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. Pharmazie 48:301–304

24. Xu J, Zhou F, Ji BP, Pei RS, Xu N (2008) The antibacterial mechanism of carvacrol and thymol against Escherichia coli. Lett Appl Microbiol 47:174–179

25. Falsafi T, Moradi P, Mahboubi M, Rahimi E, Montaz H, Hamed B (2015) Chemical composition and anti-Helicobacter pylori effect of Satureja bachtitarica Bunge essential oil. Phytomedicine 22:173–177

26. Jafari-Sales A, Bolouri P (2018) Evaluation of the antimicrobial effects of Glycyrrhiza glabra l. on some gram positive and gram negative pathogenic bacteria in laboratory conditions. Jorjani Biomed J 6(4):78–84

27. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

28. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

29. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

30. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

31. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

32. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

33. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

34. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

35. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

36. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

37. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

38. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

39. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

40. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409

41. Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. J Food Drug Anal 25(2):403–408

42. Chrysargyris A, Mikallou M, Petropoulos S, Tzortzakis N (2020) Antibiotics 9(7):409