

Original Research Article

In Vitro Screening of Antibacterial and Antioxidant Activities of Essential oils from four Moroccan Medicinal Plants

ABSTRACT

Aims: Evaluation of antibacterial and antioxidant activities of essential oils of *Salvia officinalis*, *Mentha viridis*, *Eucalyptus globulus* and *Myrtus communis* from Ouezzane province

Study Design: *In vitro* evaluation of antibacterial and antioxidant activities of medicinal plants essential oils (EOs).

Place and Duration of Study: Department of Biology (Faculty of Sciences), between July 2015 and September 2016.

Methodology: Essential oils are extracted by hydro-distillation. Antibacterial activities are performed by Agar-Well diffusion method against *Staphylococcus aureus* CECT 976, *Staphylococcus aureus* CECT 994, *Listeria monocytogenes* serovar 4b CECT 4032, *proteus mirabilis*, *Staphylococcus aureus* MBLA, *Escherichia coli* K12, *Pseudomonas aeruginosa* and *Bacillus subtilis* 6633. Antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method.

Results: The yield of essential oils varies according to species; it is ranged between 2.5 and 0.9%. All essential oils tested showed a significant antibacterial activity against all strains tested with some variability in diameters of inhibition. The essential oil of *M. viridis* is the most active with inhibition diameter (ϕ = 23mm). *P. mirabilis* appears the most sensitive strain, while *P. aeruginosa* is the most resistant against essential oils. *M. communis* and *S. officinalis* have shown the ability to scavenge the DPPH radical at low concentrations with IC₅₀ values of 0.24 and 0.46 mg / ml respectively.

Conclusion: Our results are developers and showed antibacterial and antioxidant powerful. Furthermore, the applications of essential oils in food preservation would be an effective alternative.

Key words: *S. officinalis*, *M. viridis*, *E. globulus* and *M. communis*, Essential oil, antibacterial activity, antioxidant activity.

1. INTRODUCTION

The resistance to antibacterial agents increases dramatically and become a major problem of health and economy, eradicating the discovery of antibiotics and their use in clinical medicine. Indeed, this is already predicted by Alexander Fleming saying it is likely that bacteria in certain circumstances have become insensitive to non chemotherapist drug while developing characters for her to support the action of antibiotics. Today, the bacterial resistance to antibiotics emerges in an accelerated way since their introduction into clinical use [1]. Indeed, bacterial resistance correlates positively with the use of antibacterial drugs in clinical practice [1, 2].

Because, each use of an antibiotic may increase the selective pressure of a bacterial population thus enabling the survival of resistant bacteria and dead others that remain sensitive. Practically, we could arrive to a bacterial population that resist to all available antibiotics. Indeed, multidrug resistance has been found in some pathogens bacterial strains such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* VRSA and *Mycobacterium tuberculosis* [4, 5].

Oxidative stress is a phenomenon of chemical imbalance between the oxidized molecules and those reduced (Ref ???). The result is the regeneration of free radicals that are often shown as initiators involved in the pathogenesis of several diseases such as cancer, atherosclerosis, diabetes, etc. Synthetic antioxidants have often shown adverse effects on human health beyond their desired beneficial [6].

Faced of these situations, the search for natural alternatives to overcome bacterial resistance and undesirable effects of synthetic antioxidants is an urgent necessary. Furthermore, Medicinal and aromatic plants are rich in so-called secondary metabolites that can be used as antibacterial agents and effective antioxidants (Ref). Essential oils are volatile substances of very diverse chemical structures. They are now regarded as a potential source of molecules with multiple biological activities. Indeed, Many scientists postponed the benefit of essential oils as antibacterial and powerful antioxidant [7, 8, 9, 10, 11]. The antibacterial and antioxidant activities of essential oils and their chemical compositions are related to the botanical origin of the plant, environmental and agronomic conditions, stage of development of the plant and the method of extraction used [8].

Morocco has a Mediterranean climate with his ecological and economic interest in aromatic and medicinal plants [12]. Some of these plants are have showed several pharmacological properties in previous studies around Moroccan regions [13, 14, 15]. Moreover, the province of Ouezzane (North-West of Morocco) is very rich in aromatic and medicinal plants that are poorly used and less valued. Indeed, there are only few studies undertaken by our laboratory for pharmacological enhancement of extracts of some plants in this region [16, 17, 18, 19]. In this context, we conducted a preliminary study for screening the antibacterial and antioxidant activities of essential oils from above medicinal plants selected and based on ethnobotanical study.

2. MATERIAL AND METHODS

2.1. Plant material and extraction procedure

Medicinal plants were collected from Ouezzane province (Table 1). The identification was done by Pr. ENNABILI Abdesalam (National Institute of Medicinal and Aromatic Plants, Taounate, Morocco). The collected materials were air dried at room temperature ($\approx 23^{\circ}\text{C}$) in the shade and subjected to hydrodistillation [36], using a Clevenger-type apparatus for 3 h, until total recovery of oil. The preparation of the essential oils was performed three times (3×150 g) and oils were dried with anhydrous sodium sulphate, weighed and stored at 4°C until use. The essential oil yield is determined from the dry matter. Yields are expressed in ml /100 g of dry matter [34].

$$R = \text{Pb/Pa} * 100$$

R: oil yield in %; Pb: weight of oil g; Pa: plant weight in g.

2.2. Antibacterial activity

2.2.1. Bacteria strains

To evaluate the antibacterial activity of essential oils, we have used the following bacteria: *Escherichia coli* K12 and *Staphylococcus aureus* MBLA (Laboratory of Food Microbiology, UCL, Belgium: MBLA), *Staphylococcus aureus* CECT 976, *Staphylococcus aureus* CECT 994, *Listeria monocytogenes* serovar 4b CECT 4032 and *Proteus mirabilis* (Spanish Type Culture Collection: CECT), *Pseudomonas aeruginosa* IH (Institute of hygiene, Rabat, Morocco: IH) and *Bacillus subtilis* 6633 (German Collection of Microorganisms : DSM). Strains are maintained on

an inclined agar medium at 4°C. Before use, the bacteria were revived by two subcultures in an appropriate culture medium: Lysogeny broth (LB) (Biokar Diagnostics, Beauvais, France) at 37°C for 18 to 24h. For the test, final inoculums concentrations of 10⁶ CFU/ml bacteria were used according to the National Committee for Clinical Laboratory Standards, USA (NCCLS 1999).

2.2.2. Agar-well diffusion assay

The principle of this technique is to estimate the bacteriostatic activity of the essential oils by measuring the growth inhibition zone of germs around wells. It is mostly used in a preliminary step to further study because it provides access to essentially qualitative results. Briefly, a basal layer was prepared by Muller-Hinton agar. After the agar plates were solidified, sterile 8 mm diameter cylinders were deposited. Six ml of LB medium in superfusion containing 0.8% agar were inoculated by a fresh culture of indicator bacterial strain (a final concentration was 10⁶ CFU mL⁻¹). After solidification, the wells were filled with 50 µl of essential oil. After incubation at appropriate temperature (37°C) for 24 h, all plates were examined for any zone of growth inhibition, and the diameter of these zones was measured in millimeters. All the tests were performed in triplicate.

2.3. Antioxidant Activity

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay

The ability of the plant extracts to scavenge DPPH free radicals was assessed using the standard method with some modifications [38]. In brief, Aliquots (3 mL) of various concentrations (0.1; 0.25; 0.5; 1; 2.5 mg/ml) of the essential oils samples diluted in methanol were added to 1ml of a 0.004% methanolic solution of DPPH. After an incubation period of 30 min in darkness at room at 23 ± 2 °C temperature (23 ± 2°C), the absorbance was recorded against a blank at 517 nm with a spectrophotometer. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control. Samples were analyzed in triplicate.

$$\% \text{ Inhibition} = \frac{\text{Abs (blank)} - \text{Abs (sample)}}{\text{Abs (blank)}} \times 100$$

Where Abs (blank) is the absorbance of the control and Abs (sample) is the absorbance of the sample.

Ascorbic acid was used as positive control and the concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage plotted against the essential oil concentration.

Data Analysis

The statistical analysis was performed by a one-way ANOVA analysis of variance followed by Duncan's test, and results were considered to be statistically significant with a 95% confidence level ($p < 0.05$). The measurements of total phenolic compounds, total flavonoids, DPPH radical scavenging activity, and antibacterial activity were carried out for three replicates. The results are expressed as mean ± SD.

3. RESULTS AND DISCUSSION

3.1. Essential oil yield

The water content in *S. officinalis* is higher (67.32%), while *M. viridis*, *E. globulus* and *M. communis*, have respectively 37.29, 26.41 and 32.08 %. To carry out the steam distillation, the dry material is placed in a water-distiller with a water / plant material. The Table 2 summarizes the average yields of extracted essential oil. Yields

are significantly varied between the four plants. The highest yield was recorded in *M. viridis* 2.5%, while *M. communis* showed the weakest performance.

3.2. Antibacterial activity

The antibacterial activity of four essential oils was assessed against eight bacterial strains by using Agar well diffusion method. The results are expressed as diameters zone of inhibition. The antibacterial activity can be divided into three levels [39, 40]: (i) low activity (≤ 12 mm zone of inhibition), (ii) average activity ($12 \text{ mm} < \text{inhibition zone} < 20 \text{ mm}$) and (iii) high activity ($\geq 20 \text{ mm}$ zone of inhibition). Table 3 shows the results obtained. They show that essential oils from four plants have interesting antibacterial activity against bacterial strains tested (Table 3). This activity is depending on the essential oil ($p < 0.05$) and the nature of strain tested ($p < 0.05$). The difference in diameters of inhibition could be due mainly to the difference in the chemical composition of essential oils and their major compounds (Table 1). The greatest value of the inhibition zone (43 mm) is obtained with the essential oil of *S. officinalis* against *B. subtilis*. On other hand, *P. mirabilis* appears the most sensitive of the bacteria tested, whereas *P. aeruginosa* is the most resistant to essential oils tested. In general, the Gram- bacteria are more resistant against of antibacterial agents than Gram+ [16, 17, 18, 41]. The resistance of Gram- bacteria is allocated may be to the character of their hydrophylique membrane which blocks the permeation of hydrophobic molecules such as phenolic compounds [42]. For example, the cell wall of *E. coli* is rich in lipopolysaccharides (LPS) that prevent hydrophobic molecules to pass through the membrane [43]. The antibacterial activity of these essential oils has been reported in some studies. The myrtle oil showed a significant antibacterial effect against several pathogenic strains. This effect was attributed to the presence of compounds such as Myrtenyl acetate, 1,8-cineol, α -pinene and linalool [44]. Another work carried out by Nabavizadeh et al. revealed the bacteriostatic and bactericidal ability of this essential oil [45]. The essential oil of *S. officinalis* was reported be effective against certain bacterial strains [46, 47, 48]. The essential oil *E. globulis* has been shown effective active against pathogenic bacteria by several authors [29, 49, 50]. The essential oils of *M. viridis* are also studied for their antibacterial and antioxidant activity [51].

3.3. Antioxidant activity

The results of the antioxidant activity tested by scavenging DPPH radical of essential oils are shown in Table 4. They are expressed in trapping percentage of DPPH radical. At the highest concentration (2.5mg / ml), essential oils from four plants (*S. officinalis*, *M. communis*, *E. globulis* and *M. viridis*) have proved a capacity reduction of DPPH free radical with percentages of trapping 87.26 ± 1.32 , 98.05 ± 2.12 , 53.29 ± 1.27 and 44.22 ± 0.74 mg/ml respectively. However this activity remains below that of ascorbic acid used as a positive control ($p < 0.05$). For each oil, the ability to reduce the DPPH radical is dependent on the concentration dependent. which results in a dose dependent effect. Therefore, the calculation of the effective concentration which reduces by 50% the initial concentration of DPPH (IC_{50}) is desirable to express the antiradical activity of essential oils studied (Table 4). This capacity was determined from the equation of percentage inhibition plotting the concentration of essential oils. The value of the antioxidant capacity 50 is inversely proportional to the antiradical effectiveness of the product tested. It is clear that the essential oil of *M. communis* showed great efficiency in reducing the DPPH radical ($IC_{50}=0.24$ mg/ml). This is

followed by essential oil of *S. officinalis* with an IC₅₀ of 0.46 mg/ml. This high activity may be explained by the presence in these two essential oils for a large proportion of phenolic compounds with antioxidant activity. These different activities are indeed related to the nature and proportion of the active compounds present in the different oils. Several studies have shown the antioxidant activity of essential oils from plants studied *M. viridis* [51], *S. officinalis* [46], *M. communis* [52] and *E. globulis* [49]. Our obtained results are in consonance with these works.

Conclusion

~~The results of screening of antibacterial and antioxidant activities of essential oils from four plants studied have clearly shown that these oils with interesting powers to inhibit the growth of bacterial strains tested and trapping free radicals with low doses. These results are promoters in research natural alternative to antibiotics and synthetic antioxidants which often showed side effects. Moreover, they suggest the use of such oils in food preservation against microbial spoilage as well as against oxidation. However, extensive studies must be carried downstream to potentially identify the active ingredients responsible for the effects found.~~

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

REFERENCES

1. Palumbi S.R. Humans as the world's greatest evolutionary force. *Science*. 2001; 293: 1786-1790.
2. Bronzwaer S.L., et al. A European study on the relationship between antimicrobial use and antimicrobial resistance. *Emerg Infect Dis*. 2002; 8: 278-282.
3. Goossens H, Ferech M, Vander Stichele R. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet*. 2005 ; 365 : 579-587.
4. Giedraitiene A, Vitkauskiene A, Naginiene R., Pavilonis A. et al. Antibiotic resistance mechanisms of clinically important bacteria. *Medicina (Kaunas)*. 2011; 47: 137-146.
5. Alekshun M.N, Levy S.B. Molecular mechanisms of antibacterial multidrug resistance. *Cell*. 2007; 12: 1037-1050.
6. Djeridane A, Yousfi M, Brunel JM, Stocker P. Isolation and characterization of a new steroid derivative as a powerful antioxidant from *Cleome arabica* in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants. *Food Chem Toxicol*. 2010; 48: 2599-2606.
7. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Contr*. 2007; 18: 414-420.

8. Goodner KL, Mahattanatawee K, Plotto A, Sotomyaor J.A, Jordàn M.J. Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essential oils by GC–MS/GC–O. Ind. Crop Prod. 2006 ; 24 : 264-268.
9. Bouyahya A, Abrini J, Bakri Y, Dakka N. Les huiles essentielles comme agents anticancéreux: Actualité sur le mode d'action. Phytothérapie. 2016; 14. DOI: 10.1007/s10298-016-1058-z.
10. Bouyahya A, Bensaid M, Bakri Y, Dakka N, 2016. Phytochemistry and Ethnopharmacology of *Ficus carica*. International Journal of Biochemistry Researc & Review. 2016; 14: 1-12.
11. Bouyahya A, Jamal A, Edaoudi F, Et- Touys A, Bakri Y, Dakka N. *Origanum compactum* Benth: A review on phytochemistry and pharmacological properties. Medicinal and Aromatic Plants. 2016; 5. DOI: 10.4172/2167-0412.1000252.
12. Benabid A (2000). Flore et écosystème du Maroc. Evaluation et préservation de la biodiversité. (pp.154). Paris: Ibiss Press.
13. Talbaoui A, Jamaly N, Aneb M, Il Idrissi A, Bouksaim M, Gmouh S, Amzazi S, El Moussaouiti M, Benjouad A, Bakri Y. Chemical composition and antibacterial activity of essential oils from six Moroccan plants. Journal of Medicinal Plants Research. 2012; 6: 4593-4600.
14. Aneb M, Talbaoui A, Bouyahya A, EL Boury H, Amzazi S, Benjouad A, Dakka N, Bakri Y. In vitro cytotoxic effects and antibacterial activity of moroccan medicinal plants *Aristolochia longa* and *Lavandula multifida*. European Journal of Medicinal Plants. 2016; 16: 1-13.
15. Bouyahya A., Abrini J., Elbaoboua A., Bakri Y., Dakka N. Determination of phenol content and antibacterial activity of five medicinal plants ethanolic extracts from North-West of Morocco. J. Plant Pathol Microbiol. 2016; 7: 107-110.
16. Bouyahya A, El Moussaoui N, Abrini J, Bakri Y, Dakka N. Determination of Phenolic Contents, Antioxidant and Antibacterial Activities of Strawberry Tree (*Arbutus unedo* L.) Leaf Extracts. British Biotechnology Journal. 2016; 14: 1-10.
17. Bouyahya A, Abrini J, Khay EO, Charfi S., Boujida N, EL Harsal A, Talbaoui A, ET-Touys A, Bakri Y, Dakka N. *In vitro* Antibacterial of Organic Extracts from North-West Moroccan Medicinal Plant *Myrtus communis* (L.). British Biotechnology Journal. 2016; 16: 1-9.
18. Et-Touys A, Fellah H, Mniouil M, Bouyahya A, Dakka N, Abdennebi EH, Sadak A, Bakri Y. Screening of Antioxidant, Antibacterial and Antileishmanial Activities of *Salvia officinalis* L. Extracts from Morocco. British Microbiology Research Journal. 2016; 16: 1-10.
19. Eddouks M, Maghrani M, Lemhadri A, Ouahidi M.L, Jouad H. Ethnopharmacological survey of medicinal plants used for the treatment of diabetes mellitus, hypertension and cardiac diseases in the south-east region of Morocco (Tafilalet). Journal of Ethnopharmacology. 2002; 82: 97-103.

20. EL Hamsas EL Youbi A, Ouahidi I, EL Mansouri L, Daoudi A, Bousta D. Ethnopharmacological Survey of Plants Used for Immunological Diseases in Four Regions of Morocco. *European Journal of medicinal plants*. 2016 ; 13: 1-24.
21. El-Hilaly J, Hmammouchi M, Lyoussi B. Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco). *Journal of Ethnopharmacology*. 2003; 86: 149-158.
22. Fakchich J, Elachouri M. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *Journal of Ethnopharmacology*. 2014; 28: 76-87.
23. Lakhal H, Ghorab H, Chibani S, Kabouche A, Semra Z, Smati F, Abuhamdah S, Kabouche Z. Chemical composition and biological activities of the essential oil of *Salvia officinalis* from Batna (Algeria). *Der Pharmacia Lettre*. 2013; 5: 310-314.
24. Banafsheh N, Habib G, Amir R, Samira S, Saeed M, *In vitro* anti-leishmanial activity of methanolic extracts of *Calendula officinalis* flowers, *Datura stramonium* seeds, and *Salvia officinalis* leaves. *Chinese Journal of Natural Medicines*. 2014; 12: 423-427.
25. Amkaddem M, Bouajila J, Ennajar M, Lebrihi M, Mathieu F, Romdhane A. Chemical Composition and Antimicrobial and Antioxidant Activities of *Mentha (longifolia* L. and *viridis*) Essential Oils. *Journal of food science*. 2009; 74. doi: 10.1111/j.1750-3841.2009.01272.x.
26. Guzmán P, Fernández V, Graça J, Cabral V, Kayali N, Khayet M, Gil L, Chemical and structural analysis of *Eucalyptus globulus* and *E. camaldulensis* leaf cuticles: a lipidized cell wall region. *Frontiers in Plant Science*. 2014. 5. doi: 10.3389/fpls.2014.00481.
27. Bachir Raho G, Benali M, Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pac J Trop Biomed*. 2012; 2: 739-742.
28. Jouad A, Maghrani M, El Hassani R. H, Ed-douks M. Hypoglycemic Activity of Aqueous Extract of *E. globulus* in Normal and Streptozotocin-Induced Diabetic Rats, *J. Herbs, Sp. and Med. Plts*. 2003; 10: 462-464.
29. Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. *Curr Med Chem*. 2003; 10: 213-229.
30. Othman A, Ismail A, Ghani NA, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. *Food Chem*. 2007; 100: 1523-1530.
31. Shan B, Cai YZ, Brooks JD, Corke H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *Int J Food Microbiol*. 2007; 117: 112-129.

32. Amensour M, Sendra E, Abrini J, Bouhdid S, Pérez-Alvarez JA, Fernández-López J. Total Phenolic Content and Antioxidant Activity of Myrtle (*Myrtus communis*) Extracts. Natural Product Communications. 2009; 4: 819-824.
33. Hayder N, Abdelwahed A, Kilani S, Ammar RB, Mahnoud A, Ghedira K, Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. Mutation Research. 2004; 564: 89-95.
34. Sepici A, Gurbuz I, Cevik C, Yesilada E. Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. Journal of Ethnopharmacology. 2004; 93: 311-318.
35. Amarti F, Satrani B, Ghanmi M, Farah A, Aafi A, Aarab L, El Ajjouri M, Chaouch A. Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus algeriensis* Boiss. & Reut. Et *Thymus ciliatus* (Desf.) Benth. du Maroc. Biotechnol. Agron. Soc. Environ. 2010 ; 14 : 141-148.
36. Bourkhiss M., Hnach M, Lakhlifi T, Bourkhiss B, Satrani B. Production et caractérisation de l'huile essentielle de la sciure de bois de *tetraclinis articulata* (vahl) masters. Afri. Sci. 2007; 79 : 4-11.
37. Kubola J, Siriamornpun S. Phenolic content and antioxidant activities of bitter melon (*Momordica charantia* L.) leaf stem and fruit fraction extracts *in vitro*. Food Chem. 2008; 110: 881-890.
38. Rota MC, Herrera A, Martinez RM, Stomayor JA, Jordan MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 2008; 19: 681-687.
39. Lv F, Liang H, Yuan Q, Li C. *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res. Int. 2011; 44: 3057-3064.
41. Wan J, Wilcock A, Coventry MJ. The effect of essential oils of basil on the growth of *Aeromonas hydrophila* and *Pseudomonas fluorescens*. J Appl Microbiol. 1998; 84: 152-158.
42. Inouye S, Yamaguchi H, Takizawa T. Screening of the antibacterial effects of variety of essential oils on respiratory tract pathogens, using a modified dilution assay method. J Infect. Chemother. 2011; 7: 251-254.
43. Sharif S, Singh M, Kim SJ, Schaefer J. *Staphylococcus aureus* peptidoglycan tertiary structure from carbon-13 spin diffusion. J Am Chem Soc. 2009; 131: 7023-7030.
44. Ben Hsouna A, Hamdi N, Miladi R, Abdelkafi S. *Myrtus communis* essential oil: chemical composition and antimicrobial activities against food spoilage pathogens. Chem Biodiver. 2014; 11: 571-580.
45. Nabavisedh M., Gholami A, Sheikhi R, Shokouhi M, Shams MS, Ghasemi Y. Chemical constituent and antimicrobial effect of essential oil from *Myrtus communis* leaves on

- microorganisms involved in persistent endodontic infection compared to two common endodontic irrigants: An *in vitro* study. J. Conserv Dent. 2014; 17: 449-453.
46. Bozin B, Mimica-Dukic N, Samajlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. J. Agric. Food. Chem. 2007; 55: 7879-7885.
47. Khalil R, Li Z.G. Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria. African Journal of Biotechnology. 2011; 10: 8397-8402.
48. Abu-Darwish M.S, Ferreira IV, Cavaleiro C, Cruz MT, Al-bdour TH, Salgueiro L. Essential Oil of Common Sage (*Salvia officinalis* L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory potential. Bio Med Research International. 2013; 2013: 538940-538949.
49. Luís D, Neiva D, Pereira H, Gominho J, Dominique F, Duarte AP. Stumps of *Eucalyptus globulus* as a Source of Antioxidant and Antimicrobial Polyphenols. Molecules. 2014; 19: 16428-16446.
50. Siqueira Mota V., Teresa Turrini RN., de Brito Poveda V. Antimicrobial activity of *Eucalyptus globulus* oil, xylitol and papain: a pilot study. Rev Esc Enferm. USP. 2015; 49: 215-219.
51. Silva LF, Cardoso MG, Batista LR, Gomes MS, Rodrigues LMA, et al. Chemical Characterization, Antibacterial and Antioxidant Activities of Essential Oils of *Mentha viridis* L. and *Mentha pulegium* L. (L). American Journal of Plant Sciences. 2015, 6, 666-675.
52. Petretto GL, Maldini M, Addis R, Chessa M, Foddai M, Rourke JP, Pintore G. Variability of chemical composition and antioxidant activity of essential oils between *Myrtus communis* var. *Leucocarpa* DC and var. *Melanocarpa* DC. Food Chem. 2016; 197: 124-131.

Table 1: Plants used, family, common names, place of collection, part collected and some ethnopharmacological properties of plants tested

Plants species (family)	Trivial name	Place of collection	Part plant collected	Traditional use	Phytochemistry	Pharmacological properties
<i>Salvia officinalis</i> L. (Lamiaceae)	Salmia	Zoumi	flowering tops	Cardiac disease, hypertension and Diabetes [20] Asthma and Inflammation [21] Chill, rheumatism, cough [22] Diabetes, Cardiovascular diseases, Pathologies of the digestive system [23]	Polyphenols, flavonoids [19] α -Thujone, camphor, α -Pinene, β -Thujone, α -Thujone, 1,8-Cineole, camphor, viridiflorol [24]	Antioxidant and antibacterial activities anti-leishmanial activity [19, 25]
<i>Mentha viridis</i> L. (Lamiaceae)	Na'naa	Ain Beida	flowering tops	Cold, system digestive [22] Pathologies of the urinary system, Pathologies of the respiratory system and Dermocosmotology [23]	Carvone, 1,8-cineole, limonene [26]	Antibacterial activity of essential oil [13]
<i>Eucalyptus globulus</i> L. (Myrtaceae)	Kellito	Bni kolla	Leaves	Diabetes [20], Asthma [21]	9-Epoxy-18-hydroxyoctadecanoic acid 9,10,18-Trihydroxyoctadecanoic acid 9(7-10),16-Dihydroxyhexadecanoic acid	Antibacterial activity [28] Hypoglycemic activity [29]
<i>Myrtus communis</i> L. (Lamiaceae)	Rihan	Moukrisset	Leaves	Cardiac disease, hypertension and Diabetes [20], Cardiac weakness, digestive system [22], Pathologies of the digestive system allergy and diabetes [23]	α -Pinene, limonene, myrcene, p-Cymene, α -Caryophyllene Germacrene-D, Gallic acid, caffeic acid, syringic acid, vanillic acid, verulic acid [31, 32]	Antioxidant activity [33] Antibacterial activity [18] Anti-genotoxic effect [34] Hypoglycaemic effect

						[35]
--	--	--	--	--	--	------

289
290
291

Table 2: Humidity of plants tested and their yield of essential oil

Species	% of humidity	Yield (%)
<i>Salvia officinalis</i> (L.)	67.32	2.1
<i>Mentha viridis</i> (L.)	37.29	2.5
<i>Eucalyptus globulus</i> (L.)	26.41	1.2
<i>Myrtus communis</i> (L.)	32.08	0.9

292

293

Table 3: Antibacterial activity of four essential oils against strains tested

Bacterial strains	Inhibition zones diameter* (mm)			
	<i>M. viridis</i>	<i>S. officinalis</i>	<i>E. globulis</i>	<i>M. communis</i>
<i>S. aureus</i> MBLA	18±1.34	14±0.81	13±0.46	11±0.34
<i>S. aureus</i> 976	09±1.9	07±0.6	8±2.33	08±1.3
<i>L. monocytogenes</i>	21±3.11	10±1.55	9±1.42	14±0.89
<i>S. aureus</i> 994	07±0.66	NA	07±0.98	NA
<i>B. subtilis</i> 6633	15±0.80	23±0.00	NA	NA
<i>E. coli</i> K12	09±0.65	NA	NA	NA
<i>P. aeruginosa</i> IH	NA	NA	NA	09±0.75
<i>P. mirabilis</i>	19±0.41	16±0.83	14±3.21	21±2.23

294

295

296

*Diameter of inhibition zone produced around the well. The values are the average of three replicates and the final cell density is about 10⁶ CFU/ml.
 NA: no active

Table 4: Antioxidant properties of Ascorbic acid and essential oils from *S. officinalis*, *M. viridis*, *E. globulus* and *M. communis* as determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging.

Essential oils	Antiradical activity (%)						IC ₅₀ (mg/ml)
<i>S. officinalis</i>	EO concentration (mg/ml)	0.1	0.25	0.5	1	2.5	0.46
	Scavenging effect on DPPH (%)	<u>23,25</u>	<u>31,73</u>	<u>53,94</u>	<u>67,49</u>	<u>87,26</u>	
<i>M. viridis</i>	EO concentration (mg/ml)	0.1	0.25	0.5	1	2.5	> 2.5
	Scavenging effect on DPPH (%)	<u>3,05</u>	<u>9,37</u>	<u>23,25</u>	<u>31,75</u>	<u>44,22</u>	
<i>E. globulus</i>	EO concentration (mg/ml)	0.1	0.25	0.5	1	2.5	2.37
	Scavenging effect on DPPH (%)	5,53	8,35	16,75	26,398	53,29	
<i>M. communis</i>	EO concentration (mg/ml)	0.1	0.25	0.5	1	2.5	0.24
	Scavenging effect on DPPH (%)	29,23	53,244	67,234	93	96,05	
Ascorbic acid	Ascorbic acid concentration (µg/ml)	30	60	125	250	500	0.027
	Scavenging effect on DPPH (%)	<u>97,32</u>	<u>97,23</u>	<u>98,42</u>	<u>97,87</u>	<u>98,52</u>	

Table 4: Scavenging effects of EO s from *S. officinalis*-----and Ascorbic acid on DPPH radicals

EOs	Percentages of scavenging DPPH Radicals (%)	Remarks
<i>S. officinalis</i>		
<i>M. viridis</i>		
<i>E. globulus</i>		

<i>M. communis</i>		
Ascorbic acid		

Table 5:

EOs	Antioxidant capacities on DPPH radicals (IC50 mg/ml)	Remarks
<i>S. officinalis</i>		
<i>M. viridis</i>		
<i>M. communis</i>		
<i>E. globulus</i>		
Ascorbic Acid (positive control)		