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Research paper

Analysis of antibacterial and antioxidant potential of commercial essential oils from Tuzla markets

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Abstract

Essential oils are natural products that contain a wide range of biologically active organic compounds. Therefore, they are intensively researched as potential antioxidant, antimicrobial and antitumor preparations, which could to some extent replace the classic synthetic drugs used to treat various pathological conditions. In this paper, the antioxidant and antibacterial activity of selected essential oils available in local markets in the area of Tuzla was examined. The efficiency of neutralization of free radicals was determined by spectrophotometric method, while the antibacterial potential was determined by diffusion technique on reference bacterial strains. For essential oils that showed antibacterial activity, the minimum inhibitory concentration (MIC) was determined using a dilution technique. The results showed that commercial essential oils have a high ability to neutralize DPPH radicals in vitro. The diffusion technique confirmed the high antibacterial activity for the essential oils of tea tree, eucalyptus, immortelle and wormwood, while the essential oils of lemon and cedar showed extremely weak antibacterial activity.

Keywords: *Essential oil; Bioactivity; In vitro study; Antioxidants; Natural Antibiotics.*

1. Introduction

Essential oils, as natural sources of phenolic components, attract investigators to evaluate their activity as antioxidants according to protection against free radicals and pathogenic microorganisms (Nikolić et al. 2014). The resistance of some bacteria and fungi to commercial antibiotics and the possible negative effects of synthetic antioxidants are also increased interest in essential oil research. Studies on the health benefits of essential oils have focused mainly on their antioxidative (Sharififar et al. 2011), anti-inflammatory (Monteiro et al. 2007), lipid-lowering (Kim et al. 2011; Abdollahi et al. 2003), antidiabetic (Chung et al. 2010), and hepatic protective effects (Bak et al. 2012). Polyphenols, ubiquitous in plants, are amongst the most desirable phytochemicals because of their antioxidant activity. These components are known as secondary plant metabolites and also possess antimicrobial, antiviral, and anti-inflammatory properties and high antioxidant capacity (Ignat et al. 2011). Phenolic compounds, are considered to be the main ones responsible for the antibacterial action, reduce the proliferation of tumor cells, tumor volume or have a necrotic effect through their prooxidative activity. According to the ISO 9235 from the International Organization for Standardization (ISO), the essential oil can be obtained by distillation by any of its variants: hydrodistillation, steam distillation, or dry distillation and by mechanical processes.

Essential oil isolated from Lemon (Citrus limon) constituents are abundant in the terpenes, d-limonene, and l-limonene. Traces of phellandrene, pinene, and sesquiterpene are also present (Price, 1993). Lemon can accelerate the production of white blood cells, strengthen the immune system, and help in the digestion processes (Ziosi et al. 2010). It is effective in controlling nausea and vomiting along with its mood-elevating properties (Yavari et al. 2014).

The tea tree oil (Melaleuca alternifolia) majorly contained terpinene-4-ol, γ-terpinene, p-cymene, α-terpinene, 1,8-cineole, α-terpineol, and α-pinene (Benabdelkader et al. 2011). It is an immune booster due to terpinene-4-ol while cineole is responsible for antiseptic character (Modzelewska et al. 2005; Morcia et al. 2011). The tea tree essential oil has been shown to increase monocytic differentiation in vitro and reduce inflammation, therefore assisting the healing of chronic wounds. It also possesses antibacterial, anti-inflammatory, antiviral, insecticidal, and immune-stimulant properties and has demonstrated its ability to activate monocytes. In treatment of respiratory associated problems it has been used for tuberculosis, cough, bronchitis, asthma, and catarrh.

Eucalyptus essential oil comprises of 1,8-cineole (eucalyptol) as the major compound and has other compounds such as cryptone, α-pinene, p-cymene, α-terpineol, trans-pinocarveol, phellandral, cuminal, globulol, limonene, aromadendrene, spathulenol, and terpinene-4-ol (Tyagi & Malik, 2011). Its oils have been used to regulate and activate various systems like the nervous system for neuralgia, headache, and debility. Throat infections, coughs, bronchitis, asthma, and sinusitis associated with the respiratory system have been taken care of by oils of this plant. Treatment of rheumatoid arthritis, muscle, and joint pains, and aches are well reported from the essential oils of this plant (Sadlon & Lamson, 2010). Eucalyptus oil has demonstrated its antioxidant, anti-inflammatory, anti-proliferative, and antibacterial activities, and researchers have proved its efficacy beyond doubt in the treatment of various metabolic and infectious diseases. Pine oils, derived by steam distillation of wood from pines, consist of a mixture of terpene alcohols. The major components in pine EOs are β-pinene, camphene, α-pinene, sabinene, 3-carene, myrcene, α-terpineol, terpinolene, limonene, bornyl acetate, caryophyllene, terpinene-4-ol, γ-muurolene, phellandrene, α-terpinene, thu-jene, γ-terpinene, p-cymene, germacrene D, and spathulenol.

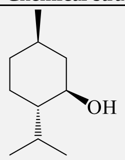
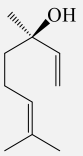
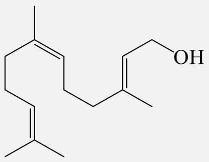
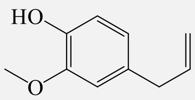
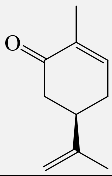
Cedarwood essential oil is steam distilled from the wood of the Cedar tree, of which there are several species. It is an important natural product for components used directly in fragrance compounding or as a source of raw components in the production of additional fragrance compounds. The oil is used to scent soaps, technical preparations, room sprays, disinfectants, and similar products. Essential oils obtained from different species of Cedrus, have been reported by their use in aromatherapy to obtain many clinical benefits traditionally ascribed to genitourinary, musculoskeletal and cutaneous systems (Mojay, 2004). Among important pharmacological properties that support their clinical use, we can name the anti-inflammatory and analgesic effects as well as immunomodulatory antioxidant, antibacterial and insecticidal activities.

Scientific studies reported various biological activity of immortelle (H. italicum) essential oil and extracts, such as antimicrobial, anti-inflammatory, antioxidant and antiviral (Viegas, 2014). Immortelle subspecies, is largely distributed in western Balkan countries, and its essential oils are dominated by α-pinene, neryl acetate and lower levels of γ-curcumene. Immortelle is known to contain ketones that help reduce the inflammatory process. Certain flavonoid ingredients of immortelle show antioxidant an activity closely related to its anti-inflammatory actions.

Extracts and essential oils of wormwood are used for healing various diseases (Ahmad et al. 2019). Antibacterial, antifungal and other bioactivities are characteristic of preparations from wormwood plants. Wormwood contains many phytochemical compounds: lactones, terpenoids (e.g., trans-thujone, γ-terpinene, 1,4-terpeniol, myrcene, bornyl acetate, cadinene camphene, trans-sabinyl acetate, guaiazulene, chamazulene, camphor, and linalool), organic acids, resins, tannins and phenols (Omer et al. 2007).

The pharmacological properties, antimicrobial as well as the antioxidant, of essential oils have been validated through various studies and their corresponding mechanisms of action have been reported in this paper. The aim of this study was to determine antioxidant and antibacterial activity of commercial essential oils from Tuzla markets.

Table 1: The Major Components of Essential Oils and Their Biological Activity

Compound	Chemical structure	Source	Activity
Menthol		Peppermint or mint oils	Antimicrobial, antioxidant, anti-inflammatory
Linalool		Lemon and cinnamon oil	Antimicrobial, antioxidant, insecticide
Farnesol		Rose oil, citronella oil	Antiseptic, antibacterial, anti-inflammatory
Eugenol		Clove oil	Antiseptic, antibacterial, antifungal, anaesthetic
Carvone		Caraway, spearmint oil	Aromatherapy and complementary medicine

2. Material and methods

The essential oils of tea tree (U-1), cedar (U-2), lemon (U-3), eucalyptus (U-4), immortelle (U-5) and wormwood (U-6) were purchased at a local market. Tea and cedar essential oil originates from Italy and France, while other oils originate from Bosnia and Herzegovina. Methanol and dimethyl sulfoxide were purchased from Semikem (Bosnia and Herzegovina). 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH), triphenyltetrazolium chloride (TTC) and other reagents used for antibacterial activity testing were purchased from Sigma Aldrich (United States).ž

2.1. DPPH radical inhibition assay

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method (Horozić et al. 2019). Lots of solutions were made in tubes by adding different volumes of essential oil supplemented with up to 2 mL of methanol. 0.5 mL of 0.5 mM DPPH solution were added and the samples were left to incubate for 30 minutes in a darkened room at a room temperature. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$[(Ac - As) / Ac] \times 100$

Where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution. The results are expressed as the IC_{50} value (µg/mL).

2.2. Analysis of antibacterial activity by diffusion technique

The *in vitro* antibacterial activities of the essential oils and standard antibiotic drugs were investigated using four Gram-positive bacteria (B. subtilis, S. aureus, L. monocytogenes and E. faecalis) and two Gram-negative bacteria (E. coli and P. aeruginosa). From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 10^7-10^8 CFU/mL). The strains were then placed on the surface of the nutrient substrate Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 100 µL of essential oil. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured.

2.3. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) for essential oils was assessed by the method of microdilation in Muller Hinton Broth (MHB), and in accordance with the guidelines given in Clinical and Laboratory Standards (CLSI M7-A7, 2006). Bacterial strain suspensions were adjusted to 0.5 membrane turbidity overnight. The essential oils were dissolved in dimethylsulfoxide (DMSO) and then diluted. 10 µL of dilute essential oil solution and 90 µL of MHB were mixed in the wells of a microtiter plate. Concentrations of test compounds ranged from 10 to 200 mg/mL. Microtiter plates were incubated for 24 hours at 37 °C. Bacterial growth was detected by adding 20 µL of 0.5% aqueous solution of triphenyltetrazolium chloride (TTC).

3. Results and discussion

3.1. Efficiency of free radical neutralization

Table 2 shows the results of neutralization of DPPH radicals expressed as IC_{50} value. Figure 1 graphically shows the ratio of oil concentration to the percentage of quenching, based on which the IC_{50} value was calculated. Wormwood oil showed the highest efficiency in removing free radicals, followed by cedar and tea tree essential oils. Other essential oils showed weaker antioxidant potency, with an IC_{50} range of 193.32-218.97 µg/mL. The mechanism of antioxidant action of essential oils is directly related to their chemical composition and is reflected in the donation of a hydrogen atom or electron to a free radical. Given that essential oils are rich in organic components containing an aromatic core and/or a hydroxyl group(s), their antioxidant activity is justified. Tested essential oils can certainly replace classic synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), dodecyl gallate (DG) and others, as their use is limited due to proven toxicity. Vitamin C was used as a control in this analysis, which showed better antioxidant potential than wormwood oil and other essential oils.

Table 2: Results of Antioxidant Activity of Essential Oils against DPPH Radicals

Essential oil	IC_{50} value [µg/mL]
U-1	142.50
U-2	123.93
U-3	193.32
U-4	218.97
U-5	202.63
U-6	91.78
Vitamin C	34.95

Studies of the removal of DPPH radicals by essential oils have been conducted by other scientists, and a deviation in the IC_{50} values obtained in this study has been found. The reasons for the different values are reflected primarily in the geographical origin of the sample used to obtain the essential oil, the different techniques for obtaining the essential oils, as well as the different storage and further processing conditions.

Msaada et al. (2015) analyzed the antioxidant activity of methanolic extracts of aboveground parts of wormwood, with better results compared to the values obtained for essential oils in this study. The obtained IC_{50} values ranged from 9.38±0.82-44.26±1.92 µg/mL, depending on the geographical origin of the sample. Methanol as an extraction agent is extremely suitable for the extraction of components with antioxidant activity, which may be the reason for better results for extracts compared to essential oils. The results of neutralization of DPPH radicals for other essential oils conducted by other scientists indicate better antioxidant capacity. It is important to emphasize that the tested oils were not commercial, but were prepared in the laboratory before analysis.

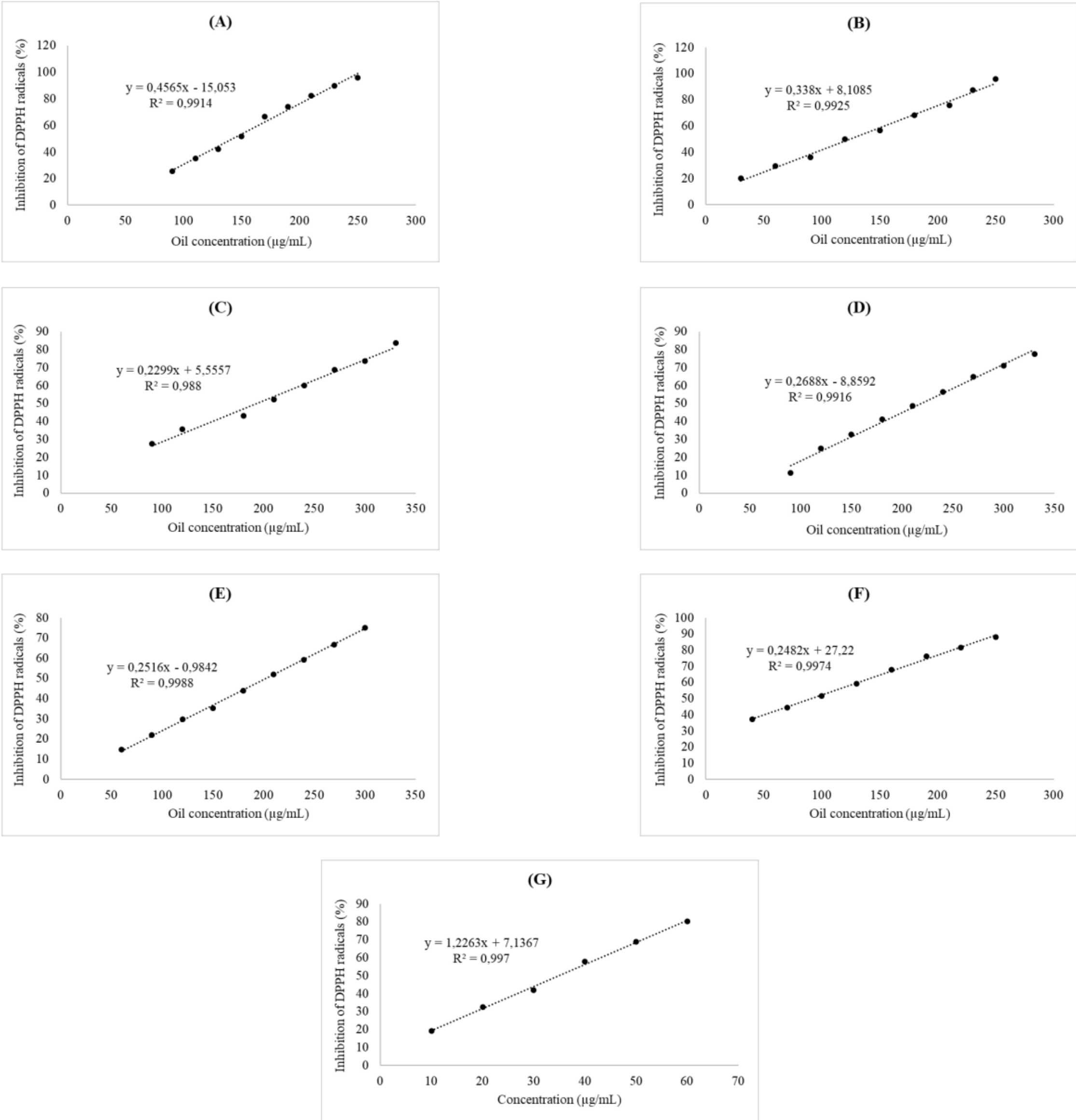


Fig. 1: Graphic Representation of the Dependence of Oil Concentration on the Percentage of Quenching of DPPH Radicals (A: Tea Tree, B: Cedar, C: Lemon, D: Eucalyptus, E: Immortelle, F: Wormwood and G: Vitamin C).

3.2. Antibacterial activity

The antibacterial activity of the essential oils obtained by the diffusion technique is shown in Table 3, and the values of the minimum inhibitory concentration for the samples that showed antibacterial activity are shown in Table 4. Tea tree, eucalyptus and wormwood oil proved to be the most effective in inhibiting the growth of the tested bacteria. Immortelle oil showed slightly weaker results, while lemon and cedar essential oils showed the lowest antibacterial efficiency. All tested oils most effectively prevented the growth of L. monocytogenes, with a range of inhibition zones of 13-38 mm. The weakest activity of essential oils, compared to other bacteria, was recorded in E. coli and E. faecalis. The mechanism of antibacterial action of essential oils is attributed to their action on the cytoplasmic membrane, resulting in loss of integrity and increased permeability. Essential oils showed a better bactericidal effect on Gram-positive bacteria than Gram-negative. This could be attributed to the structure of the bacterial membrane because Gram-negative bacteria possess an outer membrane with the presence of lipopolysaccharide molecules, which provide a hydrophilic surface. Accordingly, gram-negative bacteria are relatively resistant to hydrophobic antibiotics (Ze-Hua et al. 2019). A 1 mg/mL solution of Ciprofloxacin, used as a control, showed larger zones of bacterial growth inhibition (>40 mm). The results of the minimum inhibitory concentration test showed that L. monocytogenes and S. aureus are the most sensitive to the action of essential oils. In general, Gram-positive bacteria proved to be more sensitive than Gram-negative bacteria. The obtained results indicate that the essential oils of tea tree, eucalyptus, immortelle and wormwood are potential bacterial inhibitors with a broad antibacterial spectrum, but the mechanisms of antibacterial action need to be further studied.

Table 3: Antibacterial Activity of Essential Oils Determined by Diffusion Method

Bacteria	ATCC	Inhibition zone [mm]					
		U-1	U-2	U-3	U-4	U-5	U-6
E. coli	25922	16	-	-	13	11	16
P. aeruginosa	27853	16	-	-	17	-	16
S. aureus	25923	26	-	17	27	17	25
B. subtilis	6633	11	-	-	20	15	22
E. faecalis	51299	16	-	-	16	15	18
L. monocitogenes	19118	30	13	20	30	17	38

Table 4: Minimum Inhibitory Concentrations (MIC) of Essential Oils Based on the Dilution Method

Bacteria	ATCC	Minimum inhibitory concentration [mg/mL]					
		U-1	U-2	U-3	U-4	U-5	U-6
E. coli	25922	75	-	-	100	100	70
P. aeruginosa	27853	75	-	-	50	-	80
S. aureus	25923	35	-	50	20	60	30
B. subtilis	6633	90	-	-	50	90	40
E. faecalis	51299	75	-	-	60	90	80
L. monocitogenes	19118	20	160	30	20	70	20

4. Conclusion

In vitro analyzes of the antioxidant and antibacterial activity of commercial essential oils have given very good results. The samples showed exceptional efficacy in neutralizing DPPH radicals, with a relatively close IC_{50} value for tea tree, cedar and wormwood essential oils, while other oils showed weaker antioxidant potential. The antibacterial action of the essential oils of tea tree, eucalyptus, immortelle and wormwood was recorded in all bacterial strains, while the essential oils of lemon and cedar showed significantly weaker antibacterial activity. Based on the obtained results, it is obvious that commercial oils have significant antioxidant and antibacterial activity and represent a good basis for use in therapy, as an alternative to classic synthetic drugs with antioxidant and antibacterial activity.

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