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1,1-Diphenyl-2-picrylhydrazyRadical-Scavenging Capabilities of 13 Essential Oils and Analysis of Volatile Components of the Most Effective Clove Essential Oil

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ABSTRACT

Objective: In this study,it is aimed to research the antioxidant activity of 13 essential oils and 4 synthetic standards, which are frequently used in aromatherapy.

Methods: The essential oils were determined by their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging abilities and the volatile components of the most effective essential oil were analyzed by GC-MS.

Results: Among the essential oils, the one with highest DPPH radical-scavenging activity was clove essential oil (Eugenia Caryophyllus Flower Oil) (IC_{50} 2.31 µg/mL). A low IC_{50} (half-maximal inhibitory concentration) value indicates high radical-scavenging ability. The IC_{50} values of DPPH scavenging of essential oils and standard antioxidants decreased in the following order: ylang-ylang, frankincense, thyme, cedarwood (23.11 µg/mL), ylang-ylang, eucalyptus, bergamot, rosemary, wintergreen, lemon, ginger, immortelle, myrrh, grapefruit (34.66 µg/mL), and patchouli (69.32 µg/mL). help_outlineThe analysis of clove essential oil with the highest DPPH activity was performed by gas chromatography–mass spectrometry, and it was determined that it was mainly composed of eugenol (81.80%) and caryophyllene (13.90%).

Conclusion: Clove can be used for medicinal and food preservation purposes as a natural antioxidant.

Keywords: Clove, DPPH, Essential oil, GC-MS

INTRODUCTION

Essential oils are named after the plant from which they are derived. These are aromatic, volatile liquids obtained by steam distillation from the leaves, fruit, bark, or root parts of plants. Essential oils are chemically pure volatile compounds, usually colorless or light yellow, that can easily crystallize. Essential oil components vary greatly, sometimes due to genetic reasons but also due to climate, precipitation, or geographic origin.[1,2]

Essential oils are composed of hydrocarbons (monoterpenes, sesquiterpenes, and diterpenes), oxygenated derivatives of terpenes (esters, aldehydes, ketones, alcohols, phenols, and oxides), phenylpropanoids, and nonterpenic substances according to their chemical components.[3-5]

Thanks to the secondary metabolites contained in essential oils, they show antispasmodic, irritating, antiseptic, antifungal, antiviral, antimicrobial, and antioxidant properties. In addition, since essential oils have free radical-scavenging activity, it is reported that they have a preventive effect against many types of cancer, including liver, breast, and colon cancer cells caused by cellular damage caused by these radicals.[4-7]

Essential oils are widely used in hygiene products such as soaps and detergents, in perfumery and dermatological cosmetics, and in insecticide products due to their aromatic scents. Medicinal and aromatic plants are used as antioxidants, for antimicrobial activities, and as flavoring components in herbal tea, food supplements, and additives.[8,9] It has also been experimentally shown that essential oils used in aromatherapy are effective for memory, mental balance, and emotion and increase work efficiency.[7]

Essential oils contain natural antioxidants and help reduce oxidative stress. Antioxidants neutralize the effects of harmful molecules known as free radicals and prevent cell damage .[10]

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Clove essential oil (Eugenia Caryophyllus Flower Oil) has been used for years as a topical anesthetic and sweetener. It is known to have antimicrobial, anti-inflammatory, and antioxidant activity, mostly in relation to the content of eugenol and other polyphenolic compounds. Other uses of clove have also emerged, such as an insect repellent or growth-promoting agent .[11]

Oxidative stress is a condition that occurs when cells produce more free radicals than normal or when the cells' antioxidant defense mechanisms are insufficient. Free radicals are reactive molecules that can damage cells and cause oxidative damage because oxygen molecules carry more electrons than normal.[12]

Normal physiological processes and environmental factors (cigarette smoke, air pollution, radiation, etc.) are among the causes of oxidative stress. Oxidative stress can damage cell functions by damaging cell components such as deoxyribonucleic acid, proteins, and lipids. As a result, it may be associated with the development of various diseases; for example, conditions such as cancer, cardiovascular diseases, neurodegenerative diseases, and aging may be associated with oxidative stress.[13,14]

Free radicals, known to have harmful effects on human metabolism, cause deterioration during the processing and storage of fatty foods. To prevent this situation, natural antioxidants are preferred over synthetic antioxidants due to their side effects.

Today, there are many bioanalytical methods that measure the antioxidant effect. One of them, 1,1-diphenyl-2-picrylhydrazyl (DPPH) removal test, is the most preferred, popular, and widely used method to determine antioxidant ability.[15]

1,1-Diphenyl-2-picrylhydrazyl, is a compound used to measure antioxidant activity and can react with free radicals. This compound is widely used in the DPPH radical-scavenging test, which is a test used to detect substances with antioxidant activity that have significant benefits for human health. DPPH has a purple crystal structure and can accept electrons because it is a free radical. Antioxidants neutralize DPPH radicals to form a colorless compound, and by measuring this change, their antioxidant activity can be determined.[16,17]

In our study, it was aimed to analyze the DPPH radical-scavenging activity of 13 of the essential oils known for their antioxidant properties and the most used in aromatherapy, and the essential oil analysis of the essential oil with the highest antioxidant effect by gas chromatography–mass spectroscopy (GC-MS).

METHODS

Plant Materials

Clove (Eugenia Caryophyllus Flower Oil), ylang-ylang (Cananga Odorata Flower Oil), eucalyptus (Eucalyptus Radiata Leaf Oil), bergamot (Citrus Aurantium Bergamia Fruit Oil), rosemary (Rosmarinus Officinalis Leaf Oil), wintergreen (Gaultheria Procumbens Leaf Oil), lemon (Citrus Limon Peel Oil), ginger (Zingiber Officinale Root Oil), immortelle (Helichrysum Italicum Flower Oil), myrrh (Commiphora Myrrha Oil), grapefruit (Citrus Paradisi Peel Oil), pelargonium (Pelargonium Graveolens Oil), and patchouli (Pogostemon Cablin Leaf Oil) essential oils were obtained from Elantra Pharmaceuticals Health Cosmetics Ltd. Co.

1,1-Diphenyl-2-picrylhydrazyl Radical–Scavenging Assay

Using the DPPH-scavenging technique, the free radical-scavenging capacity of the 13 essential oils and 4 standards was assessed.[18] The technique relies on antioxidants to remove DPPH free radicals. Standards and extracts were generated with concentrations ranging from 20 to 60 µg/mL. For each sample, 500 µL of DPPH (0.1 mM) was added to tubes. For 30 minutes, these tubes were kept in the dark at 25°C. At 517 nm, the measurements were taken. Samples of DPPH potentials were calculated and compared to standards. Finally, the half maximal inhibitory concentration (IC_{50}) values for each sample were determined. The decrease in absorbance demonstrates the sample's capacity to scavenge DPPH free radicals.[18]

Gas Chromatography–Mass Spectrometry

In the GC-MS study, a Agilent 6890N Network GC system, a 5977 mass spectrometer detector, an Agilent 7693 series autosampler, and an HP-5 MS column (30 m × 0.250 mm ID, film thickness 0.25 µm) were used. Helium was used as the carrier gas (0.8 mL/min). Chromatographic analysis was performed with a flow rate of 0.8 mL/min and an injection volume of 1 µL split mode (40:1). The GC oven temperature was kept at 60°C for 10 minutes, increased by 4°C, and fixed at 220°C for 10 minutes. It was incubated at 240°C for 1 minute at a rise of 1.0°C per minute. Injector and detector temperatures were chosen as 250°C and 300°C, respectively. Mass spectra were recorded at 70 eV scanning mode (35-450 m/z) and analyzed according to the peak area.

RESULTS

The DPPH free radical-scavenging activities of essential oils and positive antioxidants, such as α-tocopherol (1.14 µg/mL; $r^2 = 0.9377$), Trolox (1.14 µg/mL; $r^2 = 0.9377$), BHA (butylated hydroxyanisole) (1.33 µg/mL $r^2 = 0.9526$), clove (2.31 µg/mL; $r^2 = 0.9136$), BHT (butylated hydroxytoluene) (3.85 µg/mL; $r^2 = 0.9535$), ylang-ylang (34.66 µg/mL; $r^2 = 0.9281$), eucalyptus (34.66 µg/mL; $r^2 = 0.9722$), bergamot (34.66 µg/mL; $r^2 = 0.9889$), rosemary (34.66 µg/mL; $r^2 = 0.9019$), wintergreen (34.66 µg/mL; $r^2 = 0.9453$), lemon (34.66 µg/mL; $r^2 = 0.9777$), ginger (34.66 µg/mL; $r^2 = 0.8340$), immortelle (34.66 µg/mL; $r^2 = 0.8770$), myrrh (34.66 µg/mL; $r^2 = 0.9049$), grapefruit (34.66 µg/mL; $r^2 = 0.9452$), pelargonium (34.66 µg/mL; $r^2 = 0.9551$), patchouli (69.32 µg/mL; $r^2 = 0.9889$) (Table 1, Figures 1 and 2).

Table 1. The IC_{50} (µg/mL) Values for $DPPH^{\cdot}$ Scavenging of Essential Oils and Standard Antioxidants

	$DPPH^{\cdot}$ Scavenging	
Antioxidants	IC_{50}	r^2
α-Tocopherol	1.14	0.9377
Trolox	1.14	0.9377
BHA	1.33	0.9526
Clove	2.31	0.9136
BHT	3.85	0.9535
Ylang-ylang	34.66	0.9281
Eucalyptus	34.66	0.9722
Bergamot	34.66	0.9889
Rosemary	34.66	0.9019
Wintergreen	34.66	0.9453
Lemon	34.66	0.9777
Ginger	34.66	0.8340
Immortelle	34.66	0.8770
Myrrh	34.66	0.9049
Grapefruit	34.66	0.9452
Pelargonium	34.66	0.9551
Patchouli	69.32	0.9889

BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; $DPPH^{\cdot}$, 1,1-diphenyl-2-picrylhydrazyl radical.

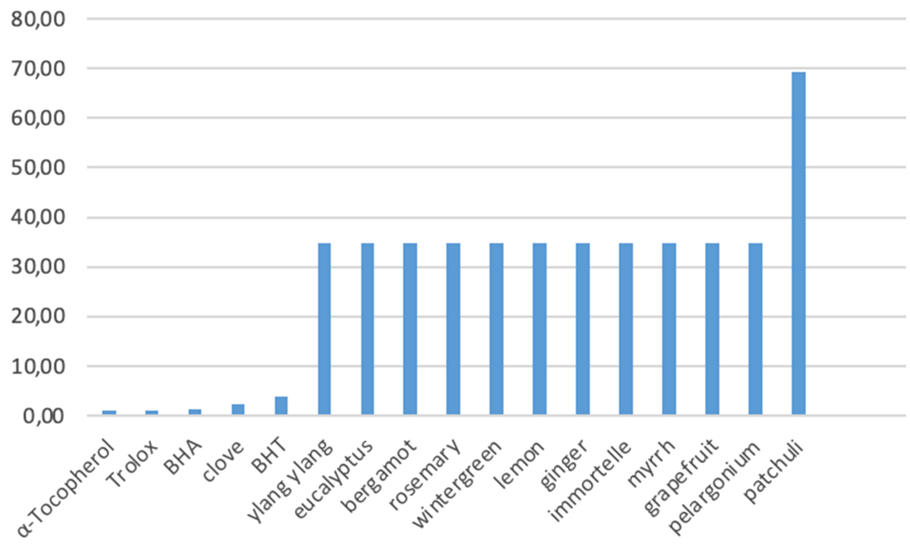

Figure 1. Comparison of DPPH•-scavenging abilities of essential oils and positive controls. DPPH•, 1,1-diphenyl-2-picrylhydrazyl radical.

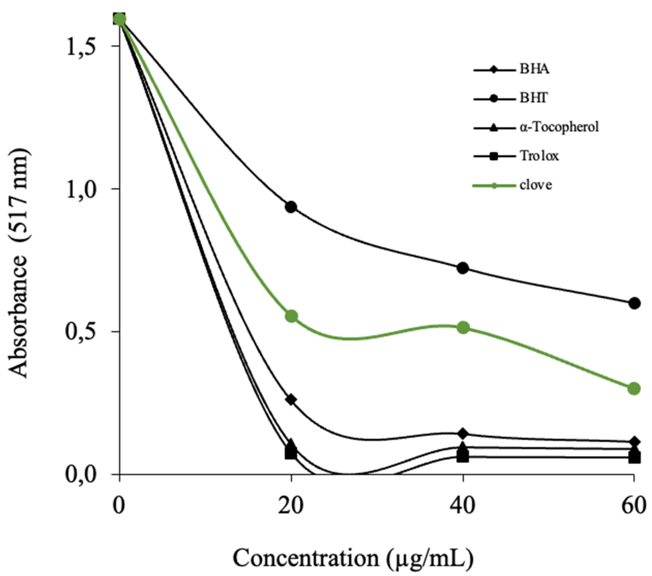

Figure 2. DPPH•-scavenging ability of clove and positive controls. DPPH•, 1,1-diphenyl-2-picrylhydrazyl radical.

The analysis of the clove essential oil was carried out by GC-MS. In the resulting chromatogram, the representative and characteristic components are defined. The ratios of these components specified by the integrator are shown in Table 2. This creates the chromatographic profile of the essential oil. According to the results of the analysis, eugenol was determined to be 81.80% and caryophyllene was determined to be 13.90%. It is thought that eugenol may be responsible for antioxidant and other biochemical and pharmacological activities.

Table 2. GC-MS Analysis of Clove Essential Oil

Peak	Compounds	RT (Minutes)	Area%
1	Chavicol	23.685	0.51
2	Eugenol	30.375	81.80
3	Caryophyllene	34.287	13.90
4	α-Humulene	36.496	3.60
5	Cadinene δ	40.541	0.19
	Total identified		100

RT, retention time.

DISCUSSION

In this study, the DPPH radical-scavenging abilities of the 3 most commonly used oils in aromatherapy treatments were determined and compared. In our study, clove oil had a very high radicalscavenging effect compared to BHT and other oils used as positive controls. Half maximal inhibitory concentration was used to indicate the practicality of antioxidant testing using DPPH radicals. The lower the IC_{50} values, the higher the DPPH radical-removing ability of the antioxidants. In this context, the IC_{50} value is widely used in biochemistry to compare the radical-scavenging capacities of different antioxidants. When the previous studies are examined, the IC_{50} values of the DPPH radical-scavenging activity of the 13 essential oils we studied are as follows: clove 1.8 µL/mL DPPH IC_{50},[19] ylang-ylang 1.30 ± 0.03 µL/mL,[20] eucalyptus > 10 000 mg/L,[21] bergamot 1040 ± 0.9 µg/mL,[22] rosemary 77.6 µL/mL,[23] wintergreen 34.16 mg/mL,[24] lemon 16 mg/mL,[25] ginger 675 µg/mL,[26] immortelle 438.9 ± 15.6 µg/mL,[27] myrrh 11.33 mg/mL,[28] grapefruit 22.06 ± 0.92 mg/mL,[29] pelargonium 83.26 ± 0.01 µg/mL,[30] patchouli IC_{50} = 1.31 µg/mL.[31]

Clove essential oil is popularly used for sore throat and toothache, due to its beneficial effects on the oral–dental mucosa. It is generally used in mouthwash as a mouthwash between 1% and 5%.[32,33] In our study, the clove essential oil contained 81% eugenol, and the oil's effect is thought to be due to this active ingredient. The effects of essential oils can vary depending on the amount and type of phenolic compounds in their content. In addition, the content of essential oils can vary according to environmental factors such as soil, climate, and temperature in the environment where the plants grow.[2]

With a general perspective, the DPPH radical-scavenging activity of 13 essential oils and synthetic antioxidants (BHT, BHA, α-tocopherol, and Trolox) most commonly used in aromatherapy was determined in our study. Essential oil components were determined by GC-MS analysis of the most active clove. It is possible that clove essential oil can be used as a radical scavenger in the food or pharmaceutical industries. For further studies, the antioxidant mechanisms of clove and major eugenol should be explained by different methods.

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Informed Consent: There is no need to obtain patient consent.

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