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Ceylon cinnamon and clove essential oils as promising free radical scavengers for skin care products

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Abstract: Due to adverse effects of free radicals on human skin and increasing consumer demand for natural ingredients, essential oils from basil, Ceylon cinnamon bark, clove, juniper, lavender, oregano, rosemary, tea tree, thyme, and ylang-ylang were assessed for their antiradical activity. The oils were evaluated in the concentration range of 5—0.1 mg · mL^{-1}, in which the three reference synthetic antioxidants are most often added to mass-produced cosmetics. Among all examined samples, C. cinnamon oil at a concentration of 5 mg · mL^{-1} showed the strongest DPPH radical scavenging activity (0.41 mg · mL^{-1} IC50), followed by clove oil, BHA, α-tocopherol, and BHT (0.82, 0.84, 0.88 and 0.93 mg · mL^{-1} IC50), respectively. At the same concentration, the reduction power of C. cinnamon oil was higher (1.64 mg · mL^{-1} Trolox Eq.) than that of α-tocopherol and BHT (1.42 and 0.80 mmol · L^{-1} Trolox Eq., respectively) but lower than that of BHA (1.81 mmol · L^{-1} Trolox Eq.). Antiradical activity of the other eight essential oils was low or negligible. C. cinnamon oil and clove oil are promising antiradical agents for skin care but according to our GC-MS analysis, these oils contain 0.29 % of cinnamaldehyde, 0.03 % of linalool, 0.02 % of D-limonene, and 0.02 % of eugenol or 0.41 % of eugenol and 0.002 % of linalool, respectively, which are monitored contact allergens in cosmetics. Such a product is not be suitable for consumers allergic to these substances but for the vast majority of consumers it does not pose a risk in terms of allergic manifestations.

Key words: *Cinnamonum zeylanicum* bark oil, *Eugenia caryophyllus* oil, free radical scavengers, skin care products

Abbreviations: BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; C. cinnamon: Ceylon cinnamon; DPPH: 1,1-Diphenyl-2-picrylhydrazyl; EC: European Commission; EO/EOs: Essential oil/ Essential oils; FRAP: Ferric reducing antioxidant power; GC-MS: Gas chromatography-Mass spectrometry; IC50: Median Inhibition Concentration; INCI: International Nomenclature of Cosmetic Ingredients; RSA: Radical scavenging activity; SCCS: Scientific Committee on Consumer Safety; TPTZ: 2,4,6-Tris(2-pyridyl)-*s*-triazine; Trolox: 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid.

Introduction

Finding substances that are not only effective against excess free radicals but are acceptable to both — the environment and the demanding consumer, is one of the challenges in human health care. Free radicals are highly reactive and unstable compounds which, together with oxidants, can be both harmful and useful to the human body. They are endogenously produced as part of normal mitochondrial metabolism. At moderate or low levels, free radicals have beneficial effects and are involved in various physiological functions such as immune function (i.e. defense against pathogenic microorganisms), a number of cellular signaling pathways, mitogenic response and in redox regulation (Phaniendra et al., 2015; Marchi et al., 2022). However, at higher concentration, reactive free radicals can attack biomolecules (DNA, proteins, and lipids) and are also associated with various diseases such as cancer and atherosclerosis as well as degenerative processes of the central nervous system such as Alzheimer's disease and aging progression (Labat-Robert and Robert, 2014; Phaniendra et al. 2015; Marchi et al. 2022). In addition to endogenous sources, more and more free radicals come from exogenous sources (excessive exposure to UVA rays and other radiation, air pollution, cigarette smoke, etc.). The imbalance between production and accumulation of free radicals causes oxidative stress in human body, including the skin (Poljšak and Dahmane, 2012; Pizzino et al., 2017; Sudarikov et al., 2021). To either prevent these reactive species from formation or remove them before they can damage vital components of the skin cells, synthetic antioxidants such as α-tocopherol, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly incorporated into skin care products (Hatwalne, 2012; Ácsová et al., 2021).

However, BHA and BHT are under investigation due to concerns regarding their side effects, e.g. endocrine disruption, and their toxicity to aquatic life and high bioaccumulation potential. While BHA use in cosmetics is gradually limited, BHT is still present in various product categories (Wang et al., 2017; Yang et al., 2018; Ribeiro et al., 2019;

Hojerová and Martiniaková, 2021). For this reason and the increasingly consumer interested in natural substances in skin care products, extensive investigation of natural sources of efficient radical scavenging compounds in health research has received high attention.

Essential oils (EOs) derived from medicinal plants are a complex of volatile substances as by-products of plant metabolism providing the plant with a characteristic odour. Chemical composition and the corresponding bioactive properties of EOs are influenced by many factors, such as species, vegetation stage at the time of harvest, geographical origin — cultivar, as well as climatic conditions of cultivation, method of isolation, part of the plant from which it was isolated, etc. In addition to the characteristic aroma, many EOs have antibacterial, antifungal, anti-inflammatory and insecticidal effects (Arantes et al., 2019; Behbahani et al., 2020; Abelan et al., 2021). At present, the antioxidant capacity of EOs is of great interest in the food and cosmetics industry (Ribeiro et al., 2019; Tepe and Ozaslan, 2020).

With regard to possible use in skin protection products, ten natural essential oils have been evaluated for their ability to scavenge free radicals and reduce metal ions compared to three synthetic antioxidants (BHT, BHA and α-tocopherol) commonly added to mass-produced cosmetics (Hojerová and Martiniaková, 2021).

Material and methods

Essential oils

Ten plant EOs (Tab. 1), antioxidant activity of which is currently discussed in literature (Olszowy1 and Dawidowicz, 2016; Wang et al., 2017; Mahomoodally et al., 2018; Behbahani et al., 2020), were selected for the study. All ten EOs were supplied by Calendula Inc. (Nová Ľubovňa, Slovak Republic) and used without any purification. Eight of these EOs were produced directly by Calendula company, while tea tree oil and ylang-ylang oil by Misitano & Stracuzzi SpA (Messina, Italy), all by steam distillation from parts of the plant or the whole plant as shown in Tab. 1.

Chemicals

Three synthetic antioxidants (α-tocopherol, purity ≥ 95.5 %, butylated hydroxyanisole, BHA ≥ 99 %, and butylated hydroxytoluene, BHT ≥ 99 %) supplied by Sigma-Aldrich (Darmstadt, Germany) were used as the reference compounds. 2,2-Diphenyl-1-picrylhydrazyl (‘DPPH ≥ 99 %), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox 97 %), and 2,4,6-Tris(2-pyridyl)-*s*-triazine

Tab. 1. Evaluated plant essential oils.

Essential oil	International Nomenclature of Cosmetic Ingredients name	Volatile oil from	CAS number
Basil	*Ocimum Basilicum Oil*	basil leaves	84775-71-3
Ceylon cinnamon bark	*Cinnamomum Zeylanicum Bark Oil*	inner bark of Ceylon cinnamon	84649-98-9
Clove	*Eugenia Caryophyllus Bud Oil*	dried flower buds of clove	84961-50-2
Juniper	*Juniperus Communis Wood Oil*	wood and twigs of juniper	84603-69-0
Lavender	*Lavandula Angustifolia Flower Oil*	lavandula flowers	8000-28-0
Oregano	*Origanum Vulgare Oil*	whole plant of wild marjoram	84012-24-8
Rosemary	*Rosmarinus Officinalis Leaf Oil*	flowering tops and leaves of rosemary	84604-14-8
Tea Tree	*Melaleuca Alternifolia Leaf Oil*	tea tree leaves	85085-48-9
Thyme	*Thymus Vulgaris Oil*	whole plant of thyme	84837-14-9
Ylang-ylang	*Cananga Odorata Flower Oil*	cananga flowers	83863-30-3

(TPTZ ≥ 98 %) were also obtained from Sigma-Aldrich (Darmstadt, Germany). UV-grade ethanol and UV-grade methanol were purchased from Fisher Slovakia (Bratislava, Slovak Republic), ferric chloride, acetic acid glacial, and sodium acetate from Centralchem (Bratislava, Slovak Republic).

Methods

Antiradical activity of samples was estimated by two electron transfer assays (DPPH and FRAP) using an Epoch Microplate Reader (BioTek Instruments, Winooski, USA) in 96-well ELISA plates (Biotech, Bratislava, Slovak Republic). All tested contained 5 $mg \cdot mL^{-1}$ of the respective compound, also concentration range of 5 to 0.1 $mg \cdot mL^{-1}$ was tested as it is the common concentration range of the three synthetic antioxidants (α-tocopherol, BHA and BHT) selected as references (positive controls) in this study in cosmetic products (Fiume et al. 2018; CIR 2019; L'Oréal 2020; Hojerová and Martiniaková 2021). Each test was done in five replicates.

Free radical (DPPH•) scavenging assay

The DPPH free radical scavenging activity (RSA) assay is based on the measurement of the ability of an antioxidant substance to scavenge radical diphenylpicrylhydrazyl and to reduce it to diphenylpicrylhydrazine with simultaneous colour change from purple to yellow measured spectrophotometrically. The method described by Brand-Williams et al. (1995) was used in this study. Briefly, EO (0.5 mL at 5 $mg \cdot mL^{-1}$ in ethanol) was added to 2 mL of 0.07 $mmol \cdot L^{-1}$ methanolic DPPH. After a 60 min incubation at ambient temperature in the dark, the absorbance of the mixture at 518 nm against methanol as a blank was read. Free radical (DPPH•) scavenging activity was expressed as percentage effect and calculated using the following equation (1):

$$RSA\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \qquad (1)$$

where $A_{control}$ and A_{sample} are the absorbance values of a mixture of ethanol + methanolic DPPH and a mixture of sample + ethanol + methanolic DPPH, respectively. IC50 value, the concentration of sample exhibiting 50 % free radicals scavenging activity, was also determined by non-linear regression analysis using the GraphPad Prism® version 7 software.

Then, for the three EOs (C. cinnamon, clove and thyme) with the highest RSA values, kinetic profiles of RSA were determined at reaction times of 0, 10, 20, 30, 40, 50, and 60 min and concentrations of 5, 2, 1, 0.5, 0.25, and 0.1 $mg \cdot mL^{-1}$.

Ferric reducing power assay

The ferric reducing antioxidant power (FRAP) assay uses antioxidants as reductants in a redox-linked colorimetric reaction, where Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion reduction at low pH causes the formation of a coloured ferrous-probe complex from a colourless ferric-probe complex. Such colour change is measured spectrophotometrically. The reducing power of all samples in this study was determined according to the procedure of Benzie and Strain (1996) with slight modifications. In the FRAP assay, EO (20 µL at 5 and 2 $mg \cdot mL^{-1}$ in ethanol) in a well of a 96-well plate was mixed with 180 µL of the FRAP reagent (25 mL of 300 $mmol \cdot L^{-1}$ acetate buffer at pH 3.6, 2.5 mL of 10 $mmol \cdot L^{-1}$ 2,4,6-tri(2-pyridyl)-1,3,5-triazine in 40 $mmol \cdot L^{-1}$ HCl (TPTZ solution) and 2.5 mL of 20 $mmol \cdot L^{-1}$ $FeCl_3.6H_2O$). After incubation for 30 min in the dark and at 37 °C, absorbance of the mixture at 593 nm against a blank (freshly prepared FRAP reagent without the sample) was read. Absorbance data of the samples were compared with the Trolox standard data from the calibration curves and then expressed as $mmol \cdot L^{-1}$ Trolox Equivalent (Trolox Eq.).

Gas chromatography-mass spectrometry assay

Major constituents of four EOs were analysed using an Agilent 7890 A gas chromatograph (GC) connected with an Agilent 5975C mass selective (MS) detector (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separations were performed on a HP-5 ms capillary column (30 m × 0.25 mm; 0.25 µm film thickness; Agilent Technologies). Solution of EO (1 µL in hexane) was injected into the inlet (280 °C) operating in split mode 1 : 10. The GC was operated at a helium flow rate of 2 ml min^{-1} (pressure of 220 kPa) and programmed with an initial oven temperature of 80 °C (2 min hold), which was then increased from 10 °C min^{-1} up to 300 °C (8 min hold). The MS detector parameters were as follows: transfer line temperature of 250 °C, MS source temperature of 230 °C, quadrupole temperature of 150 °C, filament ionisation energy of 70 eV and *m/z* range of 28—600. Recording and evaluation of data was performed using the ChemStation software E 02/01/1177. Product identification was carried out by comparing mass spectra with a commercial database NIST (the National Institute of Standards and Technology), and Wiley Mass Spectral Library. Each sample was measured in triplicate.

Statistical analysis

Results were expressed as mean values ± standard deviation (SD), unless stated otherwise. Statistical comparison between sample means was determined by one-way analysis of variance (ANOVA) followed

by Bonferroni's test for comparison of EO with the reference of α-tocopherol. Differences were considered significant at $p < 0.05$(*) and $p < 0.01$(**).

Results and discussion

There are many different terms (antiradical activity, antioxidant activity, antioxidant capacity, antioxidant power, antioxidant ability) and numerous methods to assess the mechanisms of action of antioxidant compounds. However, none of these terms or of these, mostly non-specific, methods describe "total antioxidant activity" or cover all aspects of the antioxidant properties of a particular sample. The total antioxidant activity must reflect the capacity of both the lipophilic and the hydrophilic components and, at least for physiologically active compounds, hydrogen atoms transfer (radical quenching) and electron transfer (radical reduction) must be considered and distinguished (Alam et al., 2013; Ortuño et al., 2016; Brainina et al., 2019; Coy-Barrera, 2020; Echegaray et al., 2021).

Given the intended use of EOs in cosmetic products to protect human skin from free radicals generated in the external environment, the aim of this study was to determine their antiradical activity as one of the most important aspects of antioxidant activity.

DPPH radical scavenging activity of essential oils

A number of methods to evaluate the radical scavenging efficiency of antioxidants have been developed. The DPPH and ABTS methods are routinely used for this purpose as standard colorimetric methods easy to perform The DPPH assay was chosen in this study because the DPPH radical is more stable than the ABTS (2,2´-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) one. DPPH radical is stable since it is a nitrogen centred free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule (Gulcin et al., 2012; Olszowy and Dawidowicz, 2016; Akar et al., 2017; Gotmare, 2018). According to Chen et al. (2013), the DPPH method is the most widespread test of antiradical activity of plant extracts.

Radical scavenging potential of the samples was determined by measuring the degree of discolouration of DPPH solution. Results for ten EOs and three synthetic antioxidants at 5 $mg \cdot mL^{-1}$ after 60 min of incubation are given in Fig. 1. C. cinnamon essential oil demonstrated the strongest DPPH radical inhibitory activity of all examined samples, reaching up to 81 % RSA and 0.41 $mg \cdot mL^{-1}$ IC50. The second and third most effective EOs were clove oil and thyme oil (63 % RSA, 0.82 $mg \cdot mL^{-1}$ IC50 and 57 % RSA, 0.99 $mg \cdot mL^{-1}$ IC50), respectively, with RSA and IC50 values comparable to those of BHA, α-tocopherol, and BHT (73, 62, and 61 % RSA and 0.85, 0.88, and 0.93 $mg \cdot mL^{-1}$ IC50), respectively. Ylang-ylang oil (55 % RSA, 1.03 $mg \cdot mL^{-1}$ IC50) showed moderate DPPH radical scavenging activity. RSA values for tea tree, oregano, lavender, juniper, basil and rosemary oil were lower or much lower

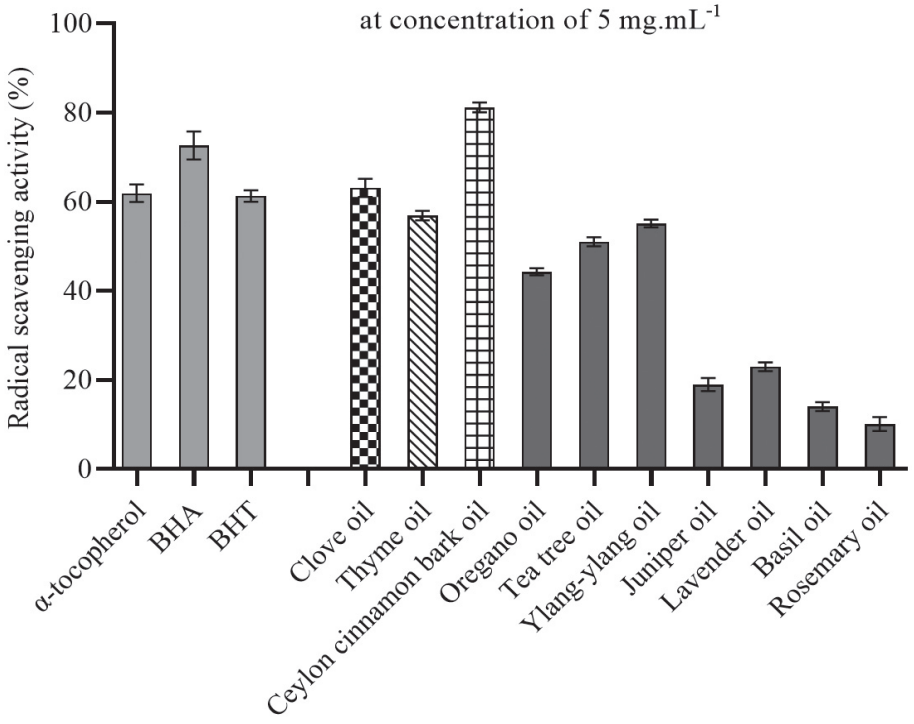


Fig. 1. DPPH free radical scavenging activity of ten EOs, α-tocopherol, BHA and BHT at 5 $mg \cdot mL^{-1}$ and 60 min. Data are given as the mean ± standard deviation (n = 5).

(51, 44, 19, 23, 14 and 10 %) than those of the three synthetic antioxidants. IC50 values for these EOs could not be calculated. In summary, the inhibition of DPPH radicals by EOs decreased in the order of C. cinnamon > clove > thyme > ylang-ylang > > tea tree > oregano > lavender > juniper > basil > > rosemary.

Kinetic profiles in the reaction time of 0, 10, 20, 30, 40, 50 and 60 min at six concentrations from 5 to 0.1 mg · mL^{-1} of C. cinnamon, clove and thyme, three EOs which proved to be the most effective of the evaluated ten oils in terms of RSA are illustrated in Fig. 2a–2c. For all three EOs, the most significant uptake of DPPH radicals was recorded during the first ten minutes of incubation at most concentrations evaluated. RSA values of C. cinnamon oil (Fig. 2a) at ≥ 2 mg · mL^{-1} began to stagnate after 20 min, which can be considered as the time when •DPPH was almost completely oxidised. Thyme oil showed increasing DPPH radical scavenging activity with increasing concentration throughout the incubation time (Fig. 2c) without stabilisation. In contrast, the kinetic dependence of clove oil (Fig. 2b) showed that with the increasing incubation time, the DPPH radical scavenging activity decreased, especially at the concentration ≥ 1 mg · mL^{-1}. Which might be due to the loss of the relationship between the degree of radical inhibition and the colour change of the evaluated solution at such high clove oil concentrations.

Free radical scavenging activity (expressed as inhibition percentage of DPPH radicals) of the three most effective EOs in the concentration range of 5–0.1 mg · mL^{-1} after 60 min of incubation compared to BHA, α-tocopherol, and BHT are presented in Fig. 3. Of all six examined samples, C. cinnamon oil showed the highest activity with statistical significance (Fig. 3) compared to α-tocopherol and BHT at concentrations of 5 mg · mL^{-1} and 2.5 mg · mL^{-1} and BHA at concentrations of

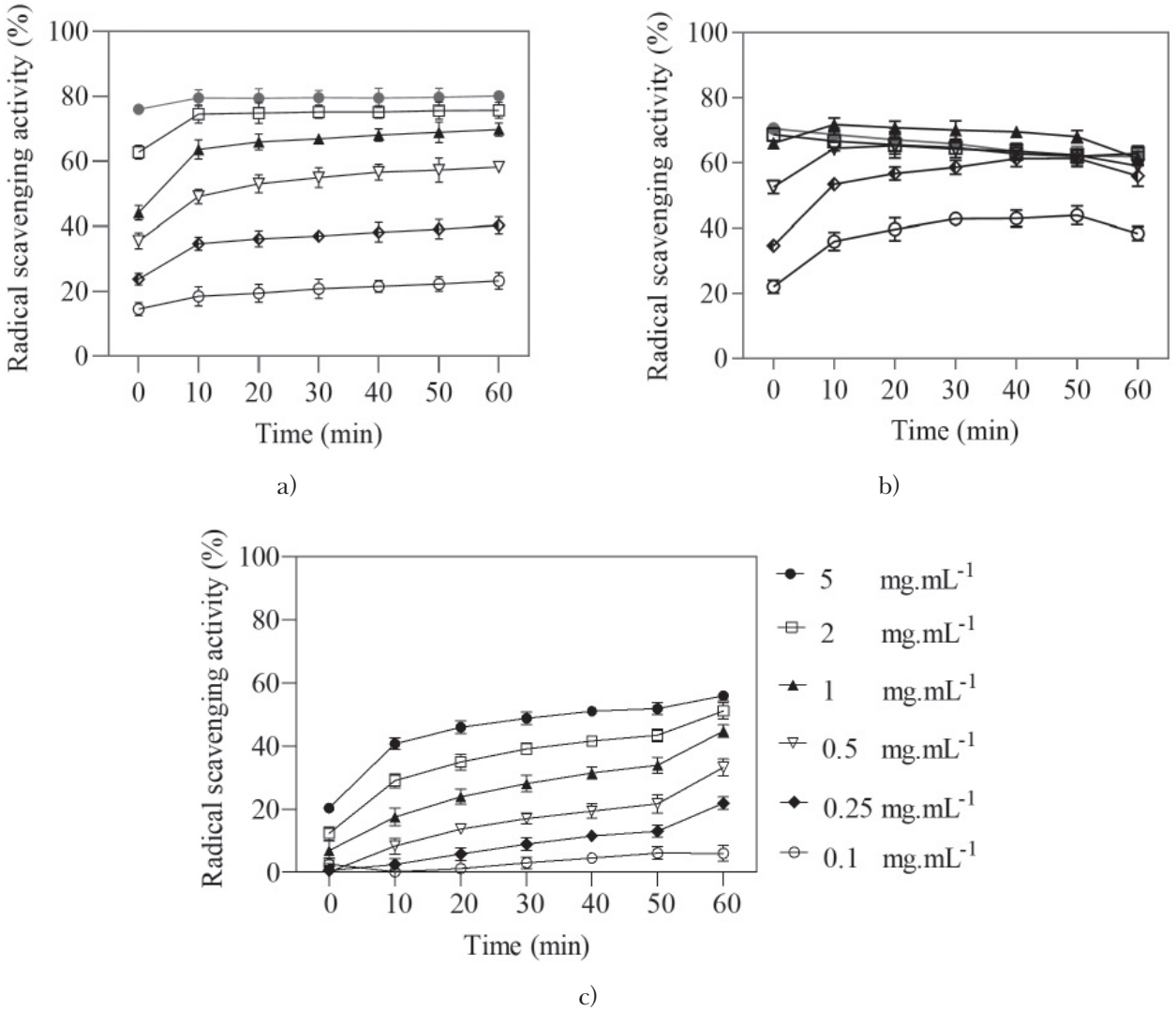


Fig. 2. Kinetic profile of DPPH radical scavenging by (a) Ceylon cinnamon bark oil, (b) clove oil and (c) thyme oil at 0.1–5 mg · mL^{-1}. Data are given as the mean ± standard deviation (n = 5).

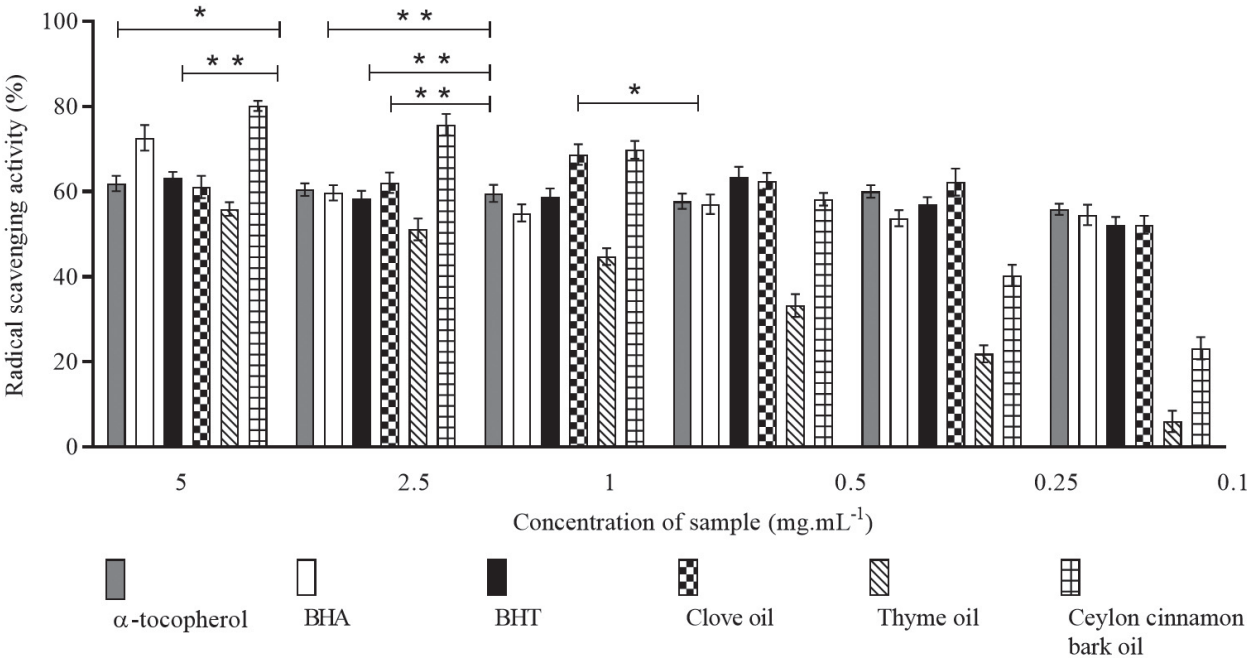


Fig. 3. Radical scavenging activity of α-tocopherol, BHA, BHT, Ceylon cinnamon bark oil, clove oil and thyme oil at 5—0.1 mg · mL^{-1} after 60 min of incubation. Data are given as the mean ± standard deviation (n = 5). Lines above the histograms indicate a statistically significant difference between the EO at a given concentration *vs.* α-tocopherol, BHA and BHT at $p < 0.05$(*), $p < 0.01$(**).

2.5 mg · mL^{-1} and 1.0 mg · mL^{-1}. Differences in RSA values of EOs and synthetic antioxidants at other concentrations evaluated were not statistically significant. Nanasombat and Wimuttigosol (2011) found that among the eight spice Eos in their study, C. cinnamon bark oil showed not only the highest antibacterial activity but also the strongest DPPH radical scavenging activity (61 % RSA) at 1 mg · mL^{-1}, which comparable to our result (63 % RSA) in Fig. 2a for the same concentration of C. cinnamon oil after 30 min of incubation. EI-Ghorab and EI-Massry (2013) studied cinnamon bark and clove bud oils obtained by supercritical fluid extraction and determined the DPPH radical scavenging activity of 47 % and 91 %, respectively, at the concentration of 500 ppm · mL^{-1} of these oils. In our study, RSA values of 58 % and 59 % were measured for C. cinnamon and clove oils, respectively, at the same concentration of 0.5 mg · mL^{-1}; however, it should be emphasised that these oils were obtained by steam distillation. Wang et al. (2017) determined the RSA value of 94 % and 69 % for clove and thyme oils, respectively, at 1 mg · mL^{-1} in the DPPH test, which are slightly higher values than our results (61 % and 45 % RSA) obtained at the same concentration.

Ferric reducing antioxidant power of essential oils

One possible way to counteract the action of free radicals is to reduce trace metal ions (especially from polluted environment), which are able to form reactive forms in the human body, especially on the skin surface. The FRAP method is an effective method for this assessment. Reducing power ability of test compounds exhibited a similar pattern as the radical scavenging assay.

From the FRAP values summarised in Tab. 2 it is clear that the reducing power (RP) of the test samples has a similar but not the same pattern as the radical scavenging assay. RP value of C. cinnamon oil at 5 mg · mL^{-1} is higher (1.64 mmol · L^{-1} of Trolox Eq.) than that of α-tocopherol and BHT (1.42 and 0.80 mmol · L^{-1} of Trolox Eq., respectively), but lower than that of BHA (1.86 mmol · L^{-1} of Trolox Eq.). At the same concentration, the RP values of oregano, tea tree, clove, thyme, and ylang-ylang oils were 1.22, 0.96, 0.76, 0.75, and 0.58 mmol · L^{-1} of Trolox Eq., respectively. Basil, juniper, lavender, and rosemary oils showed negligible RP values (Tab. 2). Olszowy and Dawidowicz (2016) determined the RP value of C. cinnamon bark oil at 0.5 mg · mL^{-1} to be twice that of thyme oil, which is comparable to our RP results for these oils at 5 mg · mL^{-1} (Table 2). Nanasombat and Wimuttigosol (2011) found by FRAP assay that C. cinnamon bark oil has the strongest reducing capacity of the eight spice essential oils studied.

Components of four most interesting essential oils

Essential oils are complex mixtures of volatile compounds with strong fragrance. To identify anti-

Tab. 2. Radical scavenging activity, IC50 and reducing power of ten natural essential oils and three synthetic reference antioxidant compounds.

Sample	Sample concentration [mg·mL^{-1}]			
	5	5	5	2
	DPPH assay after 60 min		FRAP assay after 30 min	
	RSA [%]	IC50 [mg·mL^{-1}]	TROLOX Equivalent [mmol·L^{-1}]	
α-tocopherol	61.90 ±2.79	0.88 ±0.01	1.42 ±0.04	1.36 ±0.03
BHA	72.65 ±1.84	0.84 ±0.02	1.86 ±0.02	1.81 ±0.02
BHT	61.30 ±1.84	0.93 ±0.05	0.80 ±0.03	0.72 ±0.03
Basil oil	14.05 ±1.34	‡	0.20 ±0.03	0.09 ±0.01
C. cinnamon oil	81.20 ±1.56	0.41 ±0.02	1.64 ±0.03	0.81 ±0.02
Clove oil	63.15 ±2.90	0.82 ±0.02	0.76 ±0.02	0.31 ±0.01
Juniper oil	19.00 ±2.12	‡	0.07 ±0.03	clouding/lack of colour
Lavender oil	23.00 ±1.41	‡	0.07 ±0.02	clouding/lack of colour
Oregano oil	44.30 ±1.13	‡	1.22 ±0.01	0.67 ±0.03
Rosemary oil	10.15 ±2.19	‡	0.02 ±0.01	clouding/lack of colour
Tea tree oil	51.05 ±1.48	‡	0.96 ±0.02	0.44 ±0.02
Thyme oil	56.95 ±1.48	0.99 ±0.02	0.75 ±0.01	0.73 ±0.02
Ylang-ylang oil	55.15 ±1.20	1.03 ±0.02	0.58 ±0.01	0.28 ±0.01

RSA: Radical Scavenging Activity.

IC50: Median Inhibition Concentration.

‡: IC50 cannot be calculated (RSA values are around or less than 50 %).

radical agents and potential skin allergens, the four essential oils (C. cinnamon, clove, thyme and ylang-ylang) that showed the highest antiradical activity in previous tests were analysed by GC-MS (Table 3). This study confirmed the major constituent of C. cinnamon (*Cinnamomum zeylanicum*) bark oil to be cinnamaldehyde (58.7 %), which gives cinnamon its flavour and aroma as well as its beneficial and harmful properties (will be discussed later). Cinnamon aldehyde is followed by linalool (5.8 %), eugenol (4.9 %), caryophyllene (3.8 %), D-limonene (3.6 %) and α-terpineol (1.1 %). These findings are comparable with the results of other authors (Wong et al., 2014; Nabavi et al., 2015; Weeratunga et al., 2015) for *Cinnamomum zeylanicum* bark oil, although small differences (depending on geographical origin, vegetative stage, growing season and processing) were recorded. It should be emphasised that the genus *Cinnamomum* (family *Lauraceae*) includes about 250—350 species worldwide and their composition varies considerably (Abdelwahab et al., 2017). To date, more than 300 volatile constituents of various cinnamon species essential oils have been identified (Wong et al 2014). For example, in a study by Abdelwahab et al. (2017), linalool (36.0 %), methyl eugenol (12.8 %), limonene (8.3 %), α-terpineol (7.8 %), terpinen-4-ol (6.4 %), γ-terpinene (3.5 %), α-terpinene (2.3 %) and 1,8-cineole (2.3 %) were identified in *Cinnamomum altissimum* Kosterm bark oil, which are marked differences compared to the composition of *Cinnamomum zeylanicum* bark oil. Kačániová et al. (2021) studied *Cinnamomum cassia* bark oil by GC–MS and identified cinnamaldehyde (61.6 %), trans-4-methoxycinnamaldehyde (13.8 %), cinnamyl acetate (5.4 %) and o-hydroxycinnamic acid (4.1 %) as the major constituents. In addition, it is necessary to distinguish cinnamon bark oil and more cost-effective cinnamon leaf oil which is also different in the composition (Yang et al., 2022). EO from C. cinnamon bark contains 65 to 80 % of cinnamaldehyde and 5 to 10 % of eugenol, while EO from C. cinnamon leaves contains only 1 to 5 % of cinnamaldehyde but up to 70 to 95 % of eugenol (Rao and Gan, 2014).

The major volatile constituent of clove oil is eugenol (82.4 %) which causes its specific odour as well as its beneficial and harmful properties (discussed later). The other three major constituents include caryophyllene (9.0 %), humulene (4.1 %) and eugenol acetate (1.6 %). These results are compatible with those obtained by other authors for this species, *E. caryophyllus,* (Chaieb et al., 2007; Amelia et al., 2017; Rodríguez et al., 2018). According to the DPPH assay of the selected essential oil components by Horvathova et al. (2014), eugenol showed the highest antioxidant activity compared to thymol.

Previous research by several authors (Nanasombat and Wimuttigosol, 2011; Sharma et al., 2016; Suryanti et al., 2018; Tanaka et al., 2019) has shown that the strong DPPH scavenging activity of C. cin-

namon oil and clove oil is mainly caused by cinnamaldehyde and eugenol, respectively, the primary components of these oils. Both cinnamaldehyde (3-phenyl-2-propanal) and eugenol (2-methoxy-4-(2-propenyl) phenol) belong to phenylpropanoid class of phytochemicals. Cinnamaldehyde bears an aldehyde group on the benzene ring linked via a three carbon chain, while eugenol has one hydroxy

Tab. 3. Main volatile constituents of four essential oils with the highest antiradical activity among the ten samples.

Class	Constituent	CAS number	C. cinnamon bark oil	Clove oil	Thyme oil	Ylang-ylang oil
			Constituent content (wt. %)			
Monoterpenes and their derivatives	α-Pinene	80-56-8	0.20	–	6.25	–
	β-Pinene	18172-67-3	0.27	–	0.94	–
	Tricyclene	508-32-7	–	–	2.03	–
	γ-Terpinene	99-85-4	0.68	–	0.15	–
	D-Limonene*	5989-27-5	3.63	–	–	–
	Cyclofenchene	488-97-1	–	–	1.48	–
	Pseudolimonene	499-97-8	–	–	0.69	–
	Carvestrene	38738-60-2	–	–	0.77	–
	Calarene	17334-55-3	–	–	–	0.24
Monoterpene alcohol and its derivatives	α-Terpineol	98-55-5	1.07	–	3.16	–
	Linalool*	78-70-6	5.79	0.31	5.49	9.97
	Geraniol*	106-24-1	–	–	–	7.54
	Cinnamyl alcohol*	4407-36-7	–	–	–	0.88
Sesquiterpenes and their derivatives	β-Caryophyllene	87-44-5	3.76	8.97	1.08	–
	α-Humulene	6753-98-6	–	4.05	0.13	3.31
	γ-Bisabolene	13062-00-5	–	0.28	–	–
	α-Amorphene	20085-19-2	–	–	–	9.40
	β-Cubebene	13744-15-5	–	–	–	1.96
	β-Copaene	18252-44-3	–	–	–	0.40
	α-Farnesene	502-61-4	–	–	–	10.33
	β-Cadinene	523-47-7	–	–	–	2.63
	Cedrol	77-53-2	–	–	–	0.33
	tau-Muurolol	19912-62-0	–	–	–	1.18
Phenol and its derivatives	Eugenyl acetate	93-28-7	–	1.58	–	–
	Eugenol*	97-53-0	4.88	82.43		0.66
Polycyclic sesquiterpene	Caryophyllene oxide	1139-30-6	0.31	0.29	0.52	
Diterpenes	Germacrene D	37839-63-7	–	–	–	6.53
	α-Cadinol	481-34-5	–	–	–	1.40
Aldehydes	Benzaldehyde	100-52-7	0.21	–	–	–
	Cinnamal*	104-55-2	58.72	–	–	–
	o-Methoxy cinnamal	1504-74-1	0.70	–	–	–
Terpenoidic phenols	Thymol	89-83-8	–	–	42.13	–
	Benzene, 1-methoxy-4-methyl	104-93-8	–	–	–	1.70
	Phenylethyl alcohol	60-12-8	–	–	–	
	Acetic acid, phenylmethyl ester	140-11-4	–	–	–	4.75
Other class	Other compounds		qs 100	qs 100	qs 100	qs 100

*Fragrance allergen according to the EU Cosmetics Regulation 1223/2009 (EC, 2020).

and one methoxy group directly attached to the ring. The effect of cinnamaldehyde and eugenol on DPPH is thought to be due to their hydrogen donating ability. Gulcin (2012) reported that eugenol exhibits higher radical scavenging activity compared to cinnamaldehyde because it easily donates a hydrogen atom to the hydroxyl (OH) moiety directly attached to the benzene ring. Although in our study, more eugenol was detected in clove oil (82.4 %) than in C. cinnamon oil (4.9 %), C. cinnamon oil showed slightly better antiradical activity. This can be explained by the fact that EOs are phytochemical complexes of aromatic components acting synergistically, the significant antioxidant activity being attributed mainly to the presence of phenolic and polyphenolic substances. Thus, eugenol, cinnamaldehyde (58.7 %), o-methoxy cinnamal (0.7 %) and other constituents of C. cinnamon oil are assumed to work together to quench the DPPH radical. According to a study by Suryanti et al. (2018), o-methoxy cinnamal in particular is capable of relatively high antiradical activity. Thymol (42.1 %) is a dominant substances of thyme (*Thymus vulgaris)* oil (Tab. 3), followed by α-pinene (6.3 %), linalool (5.5 %), α-terpineol (3.2 %), tricyclene (2.0 %), cyclofenchene (1.5 %), β-caryophyllene (1.1 %) and other compounds. Ahmad et al. (2014) determined up to 60.2 % of thymol in the *T. vulgaris* oil from a different geographical location, which confirms the variability of this EO composition. α-Farnesene (10.3 %) was determined as the main compound of ylang-ylang oil (Tab. 3) followed by linalool (10.0 %), α-amorphene (9.4 %), geraniol (7.5 %) and germacrene D (6.5 %).

Other beneficial properties of cinnamon and clove oils for cosmetic purposes are their antimicrobial activities, which have already been well established in many studies (Nanasombat and Wimuttigosol, 2011; Sharma et al., 2016; Kačániová et al., 2021). Tanaka et al. (2019) in a ten-week *in vivo* and *in vitro* study indicated that topical application of cinnamaldehyde restored UVB-mediated downregulation of cutaneous structural collagen, suggesting that cinnamon oil could protect the skin also from UVB-induced photoaging.

However, the use of EOs in dermal applications has also its downsides and is not without risk. The Scientific Committee on Consumer Safety (SCCS), a body of the European Commission (EC), has warned that contact allergy to fragrances is relatively common affecting 1 to 3 % of the general population (SCCS, 2011). The EC (2020) has therefore issued a list of the 25 most allergic compounds, among which are several components of essential oils. The most common symptoms of allergic contact dermatitis caused by an allergen present in EOs are itching, redness and flaking, which usually occur at the site of contact with the EO but may extend beyond this area (Weintraub et al., 2015; Sarkic and Stappen, 2018).

To ensure that an allergic consumer is adequately informed, these substances (regardless of whether they originate from a chemical synthesis or from nature) must appear on the label of the cosmetic product when present in the finished formula at certain concentrations (tresholds: 0.001 % in leave-on cosmetics, e.g. face cream, after shave-lotion, and 0.01 % in rinse-off cosmetics, e.g. shaving cream (EC, 2020)). This information makes it possible to avoid buying and using an allergen-containing product. Thus, if C. cinnamon oil or clove oil is used in a cosmetic product as an antiradical and flavour component at the concentration of 5 $mg \cdot mL^{-1}$ (0.5 % w/v), according to the results of our GC-MS analysis, the product will contain the following amounts of contact allergens: 0.29 % cinnamaldehyde, 0.03 % linalool, 0.02 % D-limonene and 0.02 % eugenol or 0.41 % eugenol and 0.002 % linalool, respectively (Table 3). In case of leave on products, all identified allergens have to be listed on the packaging in the list of ingredients according to the International Nomenclature of Cosmetic Ingredients (EC, 2020). This product would not be suitable for consumers allergic to these substances. It should be emphasised that the vast majority of mass-produced cosmetic products contain several allergens while not posing a risk of allergic manifestations in a significant part of the population (Hojerová and Martiniaková, 2021).

Conclusion

The present study indicates that C. cinnamon and clove essential oils could be applied as natural alternatives and effective substituents of synthetic antioxidants in cosmetic products due to their remarkable antiradical activity at 5 $mg \cdot mL^{-1}$ (DPPH scavenging activity of 81 % and 63 %, respectively, and Ferric reducing antioxidant power of 1.64 and 0.76 Trolox Eq. In $mmol \cdot L^{-1}$, respectively). However, if such concentration of C. cinnamon oil or clove oil is used in skin care products, their content of approx. 0.29 % of cinnamaldehyde or 0.41 % of eugenol must be indicated on the packaging of the final product as a warning to consumers with allergies.

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