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Antioxidant Activity and Sun Protection Factor Assays of Commercial Essential Oils †

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Abstract: Aromatic plants have been used since antiquity as great potential source of therapeutics in folk medicine, and as preservatives in foods, because they contain many biologically active compounds. Among all, the essential oils (EOs) are an important group of secondary metabolites that, even if not essential for plant survival, are significant for their allelopathic effects, either negative or positive, on microbes and environment. From the chemical point of view, EOs are highly complex mixtures involving from several tens to hundreds of different types of volatile compounds such as terpenoids, oxygenated terpenes, sesquiterpenes, and hydrocarbons. EOs have been widely used for their virucidal, bactericidal, fungicidal, anticancer, antioxidant, antidiabetic activities and the biological properties of EOs are strictly linked to their chemical composition. This study was carried out on the following commercial EOs: bergamot (*Citrus bergamia*), bitter orange (*Citrus aurantium*), clove (*Eugenia caryophyllata*), eucalyptus (*Eucalyptus globulus*), fennel (*Foeniculum vulgare dulce*), helichrysum (*Helicrysum italicum*), lavender (*Lavandula officinalis*), lemon (*Citrus limon*), oregano (*Origanum vulgare*), palmarosa (*Cymbopogon martini*), star anise (*Illicium verum*), tangerine (*Citrus reticulate*), tea tree (*Melaleuca alternifolia*), turmeric (*Curcuma longa*), yin yang chinese (mix of *Eucalyptus aetheroleum*, *Cymbopogon citratus*, *Caryophylli aetheroleum*, *Mentha piperita*, *Pinus sylvestris*, *Salvia rosmarinus*, *Lavandula officinalis*, *Foeniculum vulgare*, *Salvia officinalis*, *Illicium verum*, *Mentha arvensis*, *Abies siberica*), yin yang japanese (*Mentha arvensis*), ylang ylang (*Cananga odorata*). The EOs were tested for determination in vitro of antioxidant activity (DPPH assay) and for sun protection factor (SPF) by means of UV-Vis spectrophotometry. These biological activities allowed us to evaluate their potential application as natural preservatives and active ingredients in foods, beverages, and cosmetics, as well as in galenic preparations. As results, amongst the seventeen EOs studied, clove showed the highest antioxidant activity with an EC50 of 0.36 μL/mL, followed by yin yang chinese (5.35 μL/mL), oregano (11.58 μL/mL), and ylang ylang (12.71 μL/mL). Moreover, higher SPF values were recorded for bergamot (9.74), star anise (9.28), fennel (9.10), bitter orange (8.96), ylang ylang (8.41), and clove (8.26). Overall, clove and ylang ylang EOs resulted the best potential candidates as natural preservatives, being showed the highest health-promoting values, because at the same time they have provided protection against oxidative stress, as well as fighting free radicals that may form after sun radiation exposure.

Keywords: essential oils; antioxidant activity; sun protection factor; natural preservatives

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1. Introduction

The recent awareness about the environment, healthcare, and the minor usage of synthetic chemicals, led to an increased interest in natural compounds and in developing new plant-based products. Thus, the use of plant extracts and their phyto-constituents as active ingredients is a modern ecological approach in foods, beverages, cosmetics as well as in other industrial formulations [1–3]. Furthermore, these products have no side effects, broad spectrum of action combined with high efficacy, and generally low prices [1,4].

In the plant kingdom, there are 400,000 known species of both aromatic and medicinal plants, of which about 2000 species come from nearly 60 botanical families of essential oils bearing plants [5,6].

Aromatic and medicinal plants have been used since antiquity in many cultures for their medicinal and therapeutic advantages, offering a variety of benefits from medicinal cosmeceuticals and dietary purposes to religious use. Many studies have talked over their uses linked to their chemical composition, since these plants are sources rich in biologically active compounds, mainly phenolics and essential oils (EOs).

The EOs are highly complex mixtures involving several tens to hundreds of different types of volatile compounds such as terpenoids, oxygenated terpenes, sesquiterpenes, and hydrocarbons. Chemical constituents are one of the factors that determine the characteristic aroma, the purity and therapeutic value of each EO [3,5,7]. The EOs well-known activities, virucidal, antibacterial, antifungal, anticancer, antioxidant, and antidiabetic have been extensively useful in medicinal and pharmaceutical productions, in cosmetic industries, as perfumery and fragrance, and in aromatherapy and food sectors, as additives and preservatives [3,5,7].

In nature, EOs play very important roles in plant defense and signaling processes. For instance, they are involved in defense mechanisms against insects, herbivores, and microorganisms, including attraction of pollinating insects and fruit-dispersing animals, water regulation and allelopathic interactions [8].

Nowadays, large quantities of EOs are produced globally for the industries of fragrances and flavors, cosmetics, as well as for phytomedicine and aromatherapy. Demand comes mostly from the following markets: food and beverage (35%), fragrances, cosmetics and aromatherapy (29%), household (16%), and pharmaceutical (15%) [9].

For all these reasons, this work aims to study different commercial EOs, through the chemical screening of protective and health-promoting compounds, in order to evaluate their potential application as natural preservatives and active ingredients in replacement of chemical additives in foods, beverages, cosmetics as well as in pharmaceutical formulations. In particular, were investigated seventeen commercial EOs (bergamot, bitter orange, cloves, eucalyptus, fennel, helicrysum, lavender, lemon, oregano, palmarosa, star anise, tangerine, tea tree, turmeric, yin yang chinese, yin yang japanese, ylang ylang), testing in vitro two activities: the antioxidant and the sun protection factor (SPF).

2. Materials and Methods

2.1. Reagents and Standards

All reagents and solvents were of analytical grade or otherwise stated. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (USA).

2.2. Essential Oils

EOs of 13 plants were purchased from the following companies:

- bergamot from *Citrus bergamia* (peels; origin: Italy; A&N Fascì);
- bitter orange from *Citrus aurantium* (peels; origin: Ivory Coast; Essenthya);
- clove from *Eugenia caryophyllata* (buds; origin: Sri Lanka; Primavera);
- eucalyptus from *Eucalyptus globulus* (leaves and twigs; origin: Spain; Phoenix Pharma);
- fennel from *Foeniculum vulgare dulce* (seeds; origin: Italy; Primavera);

- helicrysum from *Helicrysum italicum* (flowers; origin: Italy; FresiAromi);
- lavender from *Lavandula officinalis* (flowers; origin: Bulgaria; Primavera);
- lemon from *Citrus limon* (peels; origin: Italy; A&N Fascì);
- oregano from *Origanum vulgare* (flowering plants; origin: Spain; Primavera);
- palmarosa from *Cymbopogon martini* (flowering plants; origin: India; Essenthya);
- star anise from *Illicium verum* (fruits and seeds; origin: Vietnam; Primavera);
- tangerine from *Citrus reticulate* (peels; origin: Italy; Oleolio)
- tea tree from *Melaleuca alternifolia* (leaves and twigs; origin: Australia; Naturando);
- turmeric from *Curcuma longa* (rhizomes; origin: Madagascar; Essenthya);
- yin yang chinese constituted by a mix of EOs (*Eucalyptus aetheroleum*, *Cymbopogon citratus*, *Caryophylli aetheroleum*, *Mentha piperita*, *Pinus sylvestris*, *Salvia rosmarinus*, *Lavandula officinalis*, *Foeniculum vulgare*, *Salvia officinalis*, *Illicium verum*, *Mentha arvensis*, *Abies siberica*; origin: China ; Best of Nature);
- yin yang japanese from *Mentha arvensis* (whole plant; origin: Japan; Best of Nature);
- ylang ylang from *Cananga odorata* (whole plant; origin: Madagascar; Essenthya).

2.3. In Vitro Antioxidant Activity Assay

The antioxidant activity of EOs was evaluated the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according Blois procedure [10]. Briefly, 1.35 mL of 60 μM DPPH radical in methanol were added to different EO concentrations. The decrease in absorbance at 517 nm was continuously determined until absorbance stabilization. The radical scavenging activity percentage (%RSA) of DPPH discoloration was calculated according to the formula:

$$\%\boldsymbol{RSA} = \frac{(\boldsymbol{A_{DPPH}} - \boldsymbol{A_s})}{\boldsymbol{A_{DPPH}}} \times \mathbf{100} \quad (1)$$

where A_S was the absorbance of the solution when the EO was added and A_{DPPH} was the absorbance of the DPPH solution. The extract concentration (EC) necessary to achieve a 50% of radical DPPH inhibition (EC_{50}) was obtained by plotting the *RSA* percentage as function of extract concentrations and was expressed as mg/mL, as reported by Vella et al. [2].

2.4. In Vitro Sun Protection Factor Determination

In vitro SPF was determined according to the COLIPA standards [11] by measuring the percent transmittance across the UV spectrum (ranging from 290 to 320 nm) weighted by the erythemal factors at different wavelengths, by using the following equation:

$$\boldsymbol{SPF} = \boldsymbol{CF} \times \sum_{\mathbf{290}}^{\mathbf{320}} \boldsymbol{EE}\ (\boldsymbol{\lambda}) \times \boldsymbol{I}\ (\boldsymbol{\lambda})\ \times \boldsymbol{Abs} \quad (2)$$

where *CF* = correction factor (=10), *EE* (λ) = erythemal effect spectrum, *I* (λ) = solar intensity spectrum, and *Abs* = absorbance values of samples.

Equation (2) obtained by Mansur et al. [12] was applied to calculate the SPF, using the *EE* (λ) × *I* (λ) values determined by Sayre et al. [13], as reported in Table 1.

Table 1. Values of *EE* (λ) × *I* (λ) used in the SPF calculation.

Wavelength (nm)	*EE* (λ) × *I* (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0837
320	0.0180

For the determination of SPF, 1% *v*/*v* EOs solutions were prepared in ethanol, and from this stock solution, 0.1% working concentrations were obtained. The absorbance of the sample solutions were acquired by UV-visible spectrophotometer in the range of 290–320 nm, every 5 nm interval, using ethanol as blank [14].

3. Results and Discussion

Problems with chemically synthesized preservatives and the growing demand of consumers for natural food additives and in cosmetic formulations have turned attention to plant-derived natural compounds such as EOs.

In this study, determination in vitro of antioxidant activity (DPPH assay) and sun protection factor (SPF) were carried out on the following seventeen commercial EOs: bergamot, bitter orange, clove, eucalyptus, fennel, helicrysum, lavender, lemon, oregano, palmarosa, star anise, tangerine, tea tree, turmeric, yin yang chinese, yin yang japanese, and ylang ylang.

The principle of scavenging the stable DPPH radical is extensively used to determine the antioxidant capacity of EOs. In particular, the assay was based on the ability of a potential antioxidant compound to reduce the radical DPPH, aging as a hydrogen donor.

In this study, EOs of bergamot, cloves, fennel, helicrysum, lavender, lemon, oregano, palmarosa, star anise, tea tree, turmeric, yin yang chinese, and ylang ylang were able to inhibit 50% of the radical scavenging activity of DPPH, as showed in Table 2. On the contrary, bitter orange, eucalyptus, tangerine, and yin yang japanese revealed no antioxidant activity.

As results, amongst the seventeen EOs studied, clove showed the highest antioxidant activity with an EC_{50} of 0.36 μL/mL, followed by yin yang chinese (5.35 μL/mL), oregano (11.58 μL/mL), and ylang ylang (12.71 μL/mL). Furthermore, turmeric displayed a moderate antioxidant activity with 24.99 μL/mL, while the remaining EOs (bergamot, fennel, helicrysum, lavender, lemon, palmarosa, star anise, and tea tree) revealed weak antioxidant activity, with values ranging from 54.81 μL/mL to 950.52 μL/mL, as reported in Table 2.

Table 2. Antioxidant activity (expressed as EC_{50}) of EOs**.**

Essential Oil	EC_{50} (μL/mL)
Bergamot	128.09 ± 0.63
Bitter orange	n.d.
Clove	0.36 ± 0.02
Eucalyptus	n.d.
Fennel	90.86 ± 0.14
Helicrysum	373.48 ± 0.52
Lavender	665.54 ± 0.50
Lemon	760.68 ± 0.77
Oregano	11.58 ± 0.22
Palmarosa	950.52 ± 0.71
Star anise	500.57 ± 0.33
Tangerine	n.d.
Tea tree	54.81 ± 0.24
Turmeric	24.99 ± 0.44
Yin yang chinese	5.35 ± 0.13
Yin yang japanese	n.d.
Ylang ylang	12.71 ± 0.17

n.d. = not detected.

The in vitro SPF measurement represents an admissible and fast tool to narrow in vivo experiments and related risks to UV exposure. SPF determination is a useful test for screening ingredients widely employed in food and cosmetic fields. In particular, this methodology may be useful as a rapid control tool during the production processes of food additives or supplements and cosmeceutical products, in the analysis of the final products and may give important information before proceeding to in vivo tests [14]. The higher the SPF, the more protection is offered by phyocostituent against UV light. In fact, EO if correctly mixed in food as natural preservatives and in cosmeceutical formulations, should absorb UV radiations (290–400 nm) in a such manner that confers the matrices capability to prevent skin damages and to counteract other health problems related to free radicals formed by sun exposure [14].

In this study, the highest SPF value was recorded for bergamot with 9.74, followed by star anise (9.28), fennel (9.10), bitter orange (8.96), ylang ylang (8.41), and clove (8.26) respectively, as depicted in Figure 1.

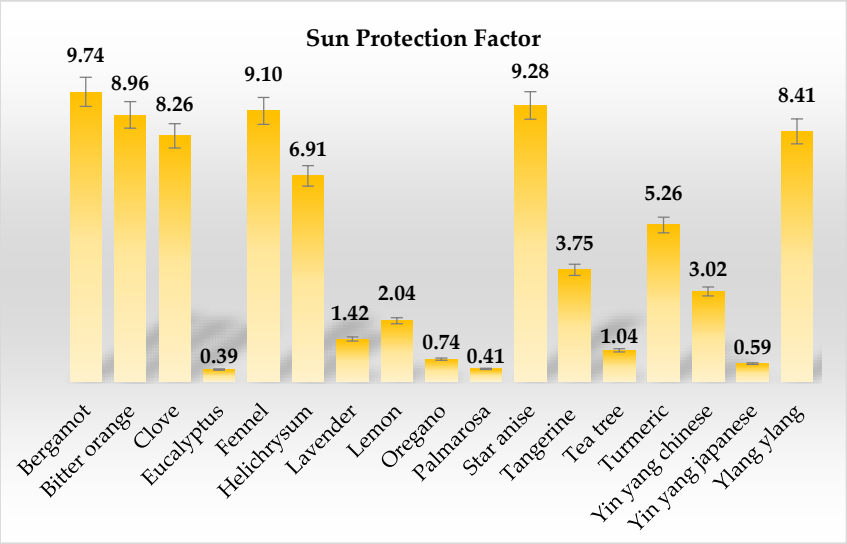

Figure 1. Sun Protection Factor (SPF) values of EOs.

On the other hand, helicrysum, turmeric, tangerine, and yin yang chinese EOs showed minor SPF values, respectively of 6.91, 5.26, 3.75, and 3.02. Further, it was observed that eucalyptus, lavender, lemon, oregano, palmarosa, tea tree, and yin yang japanese EOs possessed very low sun protection factors, about around 2 or less.

Generally, the knowledge of antioxidant activity and SPF calculation may help for selection of the best EO chemical profile, since biological activities are linked with them and, therefore, their quality and application.

Moreover, the growing interest on underutilized cultivars to be devoted to food and cosmetic market, according to the emergent demands of new applications, could be explored by means of the routine study of their EO biological activities, i.e antioxidant activity and SPF property, as reported in this research.

4. Conclusions

The increasing demand of natural phytocostituents from EOs can be due to their reduced side effects compared to chemical counterpart, their broad spectrum of action combined with a high efficay, and their generally low costs.

Overall, in this study clove and ylang ylang EOs resulted the the most effective candidates as natural preservative to use as source of health-promoting compounds, providing at the same time protection against oxidative stress, as well as fighting free radicals that naturally tend to form with sun exposure.

It can be concluded that the combined antioxidant activity and SPF property of EOs can provide synergistic protective effect in food additive or in cosmeceutical formulation.

EOs may be recognized and appreciated as antioxidants capable to act in the food sector as natural preservatives, thus avoiding the potential negative effects on human health of synthetic ones. Moreover, EOs may also be valuable for increasing the shelf life of foodstuffs, drinks, and cosmetics as it can be used as antioxidant agents in order to prevent natural oxidation and deterioration.

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