Original Article

Anti-oxidant activity and major chemical component analyses of twenty-six commercially available essential oils

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Abstract

This study analyzed 26 commercially available essential oils and their major chemical components to determine their antioxidant activity levels by measuring their total phenolic content (TPC), reducing power (RP), β-carotene bleaching (BCB) activity, trolox equivalent antioxidant capacity (TEAC), and 1,1-diphenyl-2-picrylhydrazyl free radical scavenging (DFRS) ability. The clove bud and thyme borneol essential oils had the highest RP, BCB activity levels, and TPC values among the 26 commercial essential oils. Furthermore, of the 26 essential oils, the clove bud and ylang ylang complete essential oils had the highest TEAC values, and the clove bud and jasmine absolute essential oils had the highest DFRS ability. At a concentration of 2.5 mg/mL, the clove bud and thyme borneol essential oils had RP and BCB activity levels of 94.56% ± 0.06% and 24.64% ± 0.03% and 94.58% ± 0.01% and 89.33% ± 0.09%, respectively. At a concentration of 1 mg/mL, the clove bud and thyme borneol essential oils showed TPC values of 220.00 ± 0.01 and 69.05 ± 0.01 mg/g relative to gallic acid equivalents, respectively, and the clove bud and jasmine absolute essential oils had TEAC values of 809.00 ± 0.01 and 432.33 ± 0.01 μM, respectively. The clove bud and jasmine absolute essential oils showed DFRS abilities of 94.13% ± 0.01% and 78.62% ± 0.01%, respectively. Phenolic compounds of the clove bud, thyme borneol and jasmine absolute essential oils were eugenol (76.08%), thymol (14.36%) and carvacrol (12.33%), and eugenol...
1. Introduction

Free radicals are highly reactive molecules with unpaired electrons that can cause various oxidative stresses [1,2]. Oxidative stress involves the generation of reactive oxygen and nitrogen species. Such species have been implicated in aging and various pathological processes [3,4] because they damage the structures of cells, lipids, membranes, proteins, and DNA [5]. To reduce the damages caused by reactive species, butylated hydroxy anisole (BHA) and butylated hydroxyl toluene (BHT) are widely used as antioxidant additives; however, they have been extensively examined because of their potential toxicity [6,7]. Therefore, natural antioxidants have attracted increased interest because natural ingredients may be safer than synthetic ingredients [8].

Essential oils are natural, volatile complex compounds characterized by the odor of their corresponding aromatic plants, which synthesize them as secondary metabolites [9]. Numerous essential oils not only serve as food and cosmetic additives but also exhibit antimicrobial [10,11] and antioxidant properties [12]. In particular, phenolic compounds in essential oils are very effective free radical scavengers [13,14]. Factors such as reducing power (RP) [15], total phenolic content (TPC) [16], β-carotene bleaching (BCB) activity [17-19], trolox equivalent antioxidant capacity (TEAC) [20-22], and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging (DFRS) ability [23,24] have been evaluated to investigate the antioxidant or free radical scavenging abilities of foods, plant extracts, and essential oils.

The composition of essential oils substantially varies with different aspects, such as the manufacturer, harvesting time, and plant materials. However, because commercially available essential oils are used by people, it is essential to investigate whether these oils have good antioxidant activity (or their orders) as well as to elucidate the chemical components contributing to their observed antioxidant abilities. In this study, we studied more than 200 essential oils from Australian companies [25,26] and compared their antioxidant activities. To explore the sources of essential oils in functional foods, and their applications in cosmetic products and to investigate their TPC and antioxidant activities, factors such as RP, BCB activity, TEAC, and DFRS ability were evaluated. In addition, we assessed the antioxidant activities and analyzed the major chemical components of 26 essential oils obtained from Ayus GmbH (Baden, Germany).

2. Materials and methods

2.1. Raw materials and chemicals

DPPH, BHT, BHA and eugenyl acetate were purchased from TCI. (Shanghai, China) and 3,4,5-trihydroxybenzoic acid anhydrous (gallic acid) was purchased from Lancaster (England). The Folín–Ciocalteu phenol reagent (2N), eugenol, borneol, benzyl acetate, and potassium hexacyanoferrate were procured from Merck (Darmstadt, Germany). Moreover, 2,2’-azinobis (3-ethyl-benzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and β-carotene (type I, synthetic) were purchased from Sigma (St. Louis, MO, USA). Linoleic acid, thymol, L (+)-ascorbic acid, and benzyl benzoate were obtained from Acros Organics (Geel, Belgium). Sodium carbonate, polyoxyethylene sorbitan monopalmitate (TWEEN-40), sodium dihydrogen phosphate anhydrous, disodium hydrogen phosphate, and iron (III) chloride hexahydrate were procured from Showa (Tokyo, Japan). Carvacrol and p-cymene were purchased from SAFC (USA) and Fluka (Buchs, Switzerland), respectively. Trichloracetic acid (TCA) was obtained from Alfa Aesar (Karlsruhe, Germany). The 26 essential oils were purchased from Ayus GmbH (Baden, Germany) in their origin form. Trichloromethane and all other chemicals and solvents were of standard analytical grade and were procured from Echo Chemical Co. (Miaoli, Taiwan).

2.2. Gas chromatography-mass spectrometry

The volatile compounds were analyzed using a Thermo GC-MS system (GC-MS Trace DSQ-Mass Spectrometer, MSD 201351, Thermo, Minneapolis, MN, USA). An Equity™ 5 capillary column (length, 30 m; inside diameter, 0.25 mm; film thickness 0.25 μm; Supelco, USA) was used. The oven temperature was programmed as follows: isothermal at 40 °C, and then increased to 100 °C at 5 °C/min, and held for 5 min. Subsequently, the temperature was increased to 250 °C at 5 °C/min and held for 20 min. Helium (1 mL min⁻¹) was used as carrier gas. The injection port and detector temperatures were maintained at 250 °C. The sample components were ionized in electron ionization mode (70 eV). The injection volume was 1 μL of essential oil (100 ppm in ethanol [EtOH] 99.95%). The linear retention indices (RIs) for all compounds were determined by co-injecting the samples with a solution containing a homologous series of C8-C22 n-alkanes [25]. The individual components were identified comparing their RIs with those of known compounds reported in the literature, and by matching their mass spectra with those of the known compounds or the Trace DSQ-MASS spectral database (Thermo, USA).

2.3. TPC determination

The TPC was determined using a previously reported method [28] with some modifications involving the Folín–Ciocalteu reagent, and gallic acid was used as the standard. The reaction mixture included 0.5 mL of essential oil (10 mg/mL EtOH), 1 mL of Folín–Ciocalteu reagent, and 1 mL of Na₂CO₃ (7.5%)
solution. After a 2 h incubation at ambient temperature, the mixture was centrifuged at 3000 rpm for 10 min. The corresponding absorbance values were measured at 760 nm using a UV–Vis spectrophotometer (SP-8001, Metertech Inc., Taipei, Taiwan), and the mean values were obtained from triplicate experiments. The concentration levels of phenolic compounds were calculated according to the equation obtained from the standard gallic acid graph: Different concentrations of gallic acid (5–100 μg/mL) were used to create a calibration curve (y = 0.0184x + 0.016; r² = 0.9994; y, absorbance; x, gallic acid concentration).

### 2.4. RP determination

The RP was determined using a previously described method [27] with some modifications. The RP of essential oils was compared with that of BHA and L (+)-ascorbic acid. The reaction mixture containing 0.5 mL of essential oil (2.5 mg/mL EtOH) was mixed with phosphate buffer (0.25 mL, 2 mM, pH 6.6) and potassium ferricyanide (0.25 mL, 1%, w/v). The mixture was incubated at 50 °C for 20 min, followed by addition of 0.25 mL of TCA (10%, w/v). The upper layer of the solution (0.1 mL) was mixed with distilled water (0.25 mL) and FeCl₃ (0.75 mL, 0.1%, w/v), shaken vigorously and incubated in the dark at room temperature for 10 min. The corresponding absorbance values were measured at 700 nm in an enzyme-linked immunosorbent assay (ELISA) reader (Sunrise-Basic-Tecan), and the mean values were obtained from triplicate experiments. A higher absorbance of the reaction mixture indicated a higher RP. The percentage of RP complex formation was calculated using the following equation:

\[
\text{RP effect(%) =} \frac{\text{absorbance of sample at 700 nm}}{\text{absorbance of 2.5 mg/mL BHA at 700 nm} \times 100%}
\]

### 2.5. β-Carotene bleaching test

The antioxidant activity of essential oils was determined using the BCB method [29,30] with some modifications. Approximately 10 mg of β-carotene was dissolved in 10 mL of chloroform and, 0.2 mL of this β-carotene chloroform solution was pipetted into a boiling flask containing 20 μL of linoleic acid and 200 μL of Tween-40. Chloroform was eliminated using a rotary evaporator at 50 °C for 5 min. Subsequently 50 mL of distilled water was added to the residue, and vigorously agitated to form an emulsion, and 960 μL of this emulsion was added to a tube containing 40 μL of essential oil (2.5 mg/mL EtOH). The corresponding absorbance values were immediately measured at 460 nm against a blank (emulsion without β-carotene). The tubes were placed in a water bath at 50 °C and the oxidation of this emulsion was monitored spectrophotometrically at 460 nm using the ELISA reader (Sunrise-Basic-Tecan) over a 60 min period, and the mean values were obtained from triplicate experiments. The control samples contained 10 μL of distilled water instead of the essential oils. BHT was used as a synthetic reference. Antioxidant activity is expressed as an inhibition percentage with reference to the control after 60 min of incubation, according to the following equation [31]:

\[
\text{AA} = \left(\frac{\text{DRC} - \text{DRS}}{\text{DRC}}\right) \times 100%;
\]

\[\text{AA = antioxidant activity; DR}C = \text{degradation rate of the control} = \ln(\text{a/b}/60);\]

\[\text{DR}S = \text{degradation rate in the presence of the sample} = \ln(\text{a/b}/60);\]

\[a = \text{absorbance at 0 min, b = absorbance at 60 min.}\]

### 2.6. TEAC determination

The TEAC was determined using a previously described method [22] with some modifications. This test is based on the reduction of ABTS⁺ radical cations by antioxidants. The ABTS⁺ cations were prepared by mixing a 7 mM stock solution of ABTS and 2.45 mM potassium persulfate (final concentration). This mixture was allowed to stand for 12–16 h until the reaction was complete and the absorbance was stable. For TEAC measurements, 1980 μL of ABTS⁺ solution was mixed with 20 μL of essential oil (1 mg/mL EtOH) and incubated in the dark at room temperature for 1 h. The mean values were obtained from triplicate experiments. After the mixture had achieved balance, the absorbance of the solution was measured at 730 nm with a UV–Vis spectrophotometer, and the mean values were obtained from triplicate experiments.

Decolorization of the assay was linear with increasing trolox concentrations. Different concentrations of trolox (12.5–800 μM) were used to construct a calibration curve (y = -0.0003x + 0.2507; r² = 0.9990; y, absorbance; x, trolox concentration). The results are expressed as μM trolox equivalent/1 mg of essential oil.

### 2.7. DFRS assay

The antioxidant activity of essential oils was measured in terms of their hydrogen donating or radical scavenging abilities by using the stable DPPH method [32] with some modifications [33]. A reaction mixture containing 0.4 mL of essential oil (1 mg/mL EtOH) and 0.1 mL of DPPH (2.5 × 10⁻⁴ M) was vigorously shaken and incubated in the dark at room temperature. The reduction of the reaction mixture was assessed by measuring the absorbance values at 517 nm after 30 min by using the ELISA reader (Sunrise-Basic-Tecan). The mean values were obtained from triplicate experiments. EtOH (99.9%) and BHT served as the control and positive control, respectively. Inhibition of the free radicals by DPPH (%) was calculated using the following equation:

\[
\text{Scavenging effect} = \left(1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right) \times 100%
\]

EC₅₀ values, calculated through linear regression analysis, and were defined as the effective concentrations of samples sufficient to obtain 50% antioxidant activity. The samples with best results were used to process with EC₅₀ and BHT.

### 2.8. Statistical analyses

Data are presented as means ± standard deviations (S.D.) from three experimental determinations. Statistical analyses were performed using a one-way analysis of variance. A p value of
<0.05 was considered statistically significant. TPC and TECA data were obtained using SPSS (version 13.0, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Chemical composition analyses through GC-MS

Table 1 lists the essential information of the 26 essential oils. GC-MS was performed to analyze the chemical components of the clove bud, jasmine absolute, and thyme borneol essential oils (Table 2). The GC-MS data revealed that four components accounted for 99.49% of the total content of the clove bud essential oil. Eugenol was the major component (76.08%), followed by α-pinene (12.57%), β-caryophyllene (9.98%) and α-caryophyllene (0.86%). A total of 17 compounds (accounting for 97.85% of the total oil content), namely 1α-pinene, camphene, β-pinene, p-cymene, ν-limonene, crithmene, linalool, camphor, borneol, terpine-4-ol, terpeneol, methyl thymyl ether, bornyl acetate, thymol, carvacrol, β-caryophyllene, and δ-cadinene were observed in the thyme borneol essential oil. The three most abundant compounds were borneol (28.39%), thymol (14.36%), and carvacrol (12.33%). The jasmine absolute essential oil comprised 16 compounds (representing 98.90% of the total oil content), namely benzyl alcohol, linalool, benzylacetate, indole, eugenol, jasmone, α-farnesene, cis-3-hexenyl benzoate, methyl jasmonate, benzyl benzoate, methyl palmitate, isophytol, farnesyl acetate, ethyl linolenate, trans-phytol, and squalene. Of these 16 compounds, benzylacetate (32.30%) and benzyl benzoate (22.94%) were most abundant. The major pure chemical components of these essential oils were purchased to assess their antioxidant activity.

3.2. TPC determination

The clove bud essential oil had the highest TPC (220 ± 0.01 mg/g of gallic acid equivalents [GAE]), followed by the thyme borneol (69.05 ± 0.01 mg/g GAE), chamomile (56.30 ± 0.01 mg GAE), ylang ylang complete (26.27 ± 0.01 mg GAE), jasmine absolute (18.39 ± 0.02 mg/g GAE), and vetiver bourbon (16.87 ± 0.01 mg/g GAE) essential oil at a concentration of 1 mg/mL. By contrast, the rosewood essential oil had the lowest TPC (3.30 ± 0.01 mg/g GAE), followed by the petitgrain bigarade (6.56 ± 0.02 mg/g GAE) essential oil at a concentration of 10 mg/mL (Table 1).

In a previous study, the TPC values of cinnamon leaf and thyme red essential oils [25] were 420 and 270 mg/g, respectively. At a concentration of 5 mg/mL, the TPC values of the cinnamon bark, origanum, and thyme wild essential oils [26] were 658.4, 1107.2 and 275.50 mg GAE, respectively. The extracts of Anthemis arvensis, Artemisia campestris [34], and Achillea santolina [35] had TPC values of 32.32, 20.38, and 104.66 mg/g GAE, respectively. Therefore, the TPC values of the cinnamon bark and origanum essential oils are higher than that of the thyme borneol essential oil, whereas, The TPC value of the clove bud essential oil is higher than those of the thyme wild essential oil, and the A. arvensis, A. campestris, and A. santolina extracts.

3.3. RP determination

As presented in Table 1, the RP assay revealed that the clove bud and thyme borneol essential oils had the highest RP among the 26 essential oils. At a concentration of 2.5 mg/mL, the clove bud and thyme borneol essential oils showed 103.56 ± 0.06% and 24.64 ± 0.03% of RP relative to BHA, respectively. The rosewood (1.39 ± 0.01%) and verbena lemon (1.50 ± 0.01%) essential oils had the lowest RP.

To compare the RP levels of the love bud essential oil, thyme borneol essential oil, BHA, BHT, and L (+)-ascorbic acid, the solutions were diluted from 0.1 to 0.5 mg/mL. Fig. 1 presents the experimental results. The RP ranked as follows: clove bud essential oil > BHA > L (+)-ascorbic acid > BHT > thyme borneol essential oil.

At a concentration of 0.1 mg/mL, the absorbance value associated with the RP of the clove bud essential oil was 0.364, which is comparable to the reported value. At a concentration of 0.1 mg/mL, the absorbance value of Origanum compactum essential oil [36] was 0.105. At a concentration of 0.2 mg/mL, the absorbance value of Periploca laevigate [37] and Vitex agnus-castus L. essential oils [38] were 0.15 and 0.103, respectively. The absorbance value of Psammogeton canescens [39] and Thymus capitatus essential oils [40] were 0.568 and 1.2, respectively. Therefore, the RP level of the clove bud essential oil is higher than those of the O. compactum, P. laevigate, and V. agnus-castus L. essential oils, but it is lower than those of the P. canescens and T. capitatus essential oils.

At a concentration of 2.5 mg/mL, the clove bud, and thyme borneol essential oils and five pure chemical components (eugenol, eugenyl acetate, borneol, thymol, and carvacrol) were used to evaluate the reduction of Fe³⁺ to Fe²⁺. Their corresponding RP levels were 94.56 ± 0.05%, 24.64 ± 0.03%, 97.52 ± 0.01%, 3.79 ± 0.01%, 1.47 ± 0.01%, 96.63 ± 0.04% and 88.41 ± 0.06%. The RP ranked as follows: eugenol > thymol > clove bud essential oil > carvacrol > thyme borneol essential oil > eugenyl acetate > borneol (Fig. 2). Therefore, eugenol and thymol were the major reducing components in the clove bud and thyme borneol essential oils, respectively.

3.4. BCB test

The clove bud and thyme borneol essential oils had the strongest BCB activity (94.58 ± 0.11% and 83.87 ± 0.10%, respectively, Table 1; Fig. 3), followed by the rose blossoms absolute (79.10 ± 0.08%), jasmine absolute (71.48 ± 0.01%) and labrador tea (70.59 ± 0.06%) essential oils. The eucalyptus rakiata, rosewood, spruce black, and lantana essential oils did not exhibit significant BCB activity.

At a concentration of 2 mg/mL, the BCB activity levels of the clove bud and thyme borneol essential oils were 94.02% and 75.21%, respectively. Hence, the BCB activity levels of the clove bud essential oil is more favorable than those of the V. agnus-castus L. (86.17%) [35], Thymus caramanicus (79.03%) [41], Satureja spicigera (81.7%), Satureja cuneifolia (93.7%) [42] and Marrubium globosum subsp. (79.85%) [43] essential oils, respectively. However, the thyme borneol essential oil has a lower BCB activity level than the aforementioned five essential oils.
| No. | Name              | Department | Scientific name                  | Origin    | Extraction part | Extraction method | TPC (10 mg of GAE g⁻¹)ᵃ | RP (% of BHA)ᵇ | BCB antioxidant activity (%)ᵇ | TECA (μM of troclox/mg)ᵇ | DFRS (%)ᵇ |
|-----|-------------------|------------|----------------------------------|-----------|-----------------|-------------------|-------------------------|---------------|--------------------------------|--------------------------|------------|
| 1   | Cedarwood atlas   | Pinaceae   | Cedrus atlantica                 | Morocco   | Wood            | Distillation      | 68.21 ± 0.01           | 18.61 ± 0.02 | 27.23 ± 0.04                   | 1.79 ± 0.01              | 1.89 ± 0.01 |
| 2   | Chamomile         | Asteraceae | Matricaria recutita              | Egypt     | Flowers         | Distillation      | 56.30 ± 0.01           | 17.96 ± 0.03 | 62.41 ± 0.07                  | 8.16 ± 0.01              | 8.14 ± 0.01 |
| 3   | Cardamom          | Zingiberaceae | Elettaria cardamomum          | Ecuador   | Fruit/seeds     | Distillation      | 13.24 ± 0.01           | 2.23 ± 0.01  | 11.29 ± 0.02                  | 1.42 ± 0.01              | 1.42 ± 0.01 |
| 4   | Clove bud         | Myrtaceae  | Eugenia caryophyllus            | Madagascar | Bud             | Distillation      | 220.00 ± 0.01           | 94.56 ± 0.06 | 94.58 ± 0.11                  | 80.09 ± 0.01             | 94.13 ± 0.01 |
| 5   | Eucalyptus rakiata | Myrtaceae  | Eucalyptus rakiata              | Australia | Leaves          | Distillation      | 18.39 ± 0.02           | 19.97 ± 0.01 | 71.48 ± 0.01                  | 354.56 ± 0.02             | 78.62 ± 0.01 |
| 6   | Jasmine absolute  | Oleaceae   | Jasminum officinale             | Morocco   | Flowers         | Solvent extraction| 15.58 ± 0.01           | 2.19 ± 0.01  | 34.34 ± 0.01                  | 24.56 ± 0.01             | 11.6 ± 0.01 |
| 7   | Myrtle (lemon)    | Myrtaceae  | Backhousia citriodora           | Australia | Leaves          | Distillation      | 14.58 ± 0.01           | 2.19 ± 0.01  | 34.34 ± 0.01                  | 24.56 ± 0.01             | 11.6 ± 0.01 |
| 8   | Neroli bigarade   | Rutaceae   | Citrus aurantium                | Morocco   | Flowers         | Distillation      | 14.46 ± 0.01           | 5.05 ± 0.01  | 8.14 ± 0.03                   | 24.56 ± 0.01             | 1.26 ± 0.01 |
| 9   | Niaouli, extra    | Myrtaceae  | Melaleuca quinquenervia         | Madagascar | Leaves          | Distillation      | 4.25 ± 0.01            | 5.05 ± 0.01  | 43.42 ± 0.05                  | 13.40 ± 0.01             | 7.23 ± 0.01 |
| 10  | Petitgrain bigarade | Rutaceae  | Citrus aurantium                | Paraguay  | Leaves          | Distillation      | 4.39 ± 0.01            | 5.05 ± 0.01  | 43.42 ± 0.05                  | 13.40 ± 0.01             | 7.23 ± 0.01 |
| 11  | Ravensara         | Lauraceae  | Ravensara aromatica            | Madagascar | Leaves          | Distillation      | 11.49 ± 0.01           | 4.99 ± 0.01  | 10.84 ± 0.02                  | 24.56 ± 0.01             | 3.15 ± 0.01 |
| 12  | Geranium rose     | Geraniaceae | Pelargonium roseum             | Morocco   | Leaves          | Distillation      | 10.11 ± 0.02           | 3.18 ± 0.01  | 42.96 ± 0.05                  | 17.89 ± 0.01             | 4.40 ± 0.01 |
| 13  | Rosemary cineol   | Lamiaceae  | Rosmarinus officinalis          | Morocco   | Whole flower plant | Whole flower plant | 10.11 ± 0.02           | 3.18 ± 0.01  | 42.96 ± 0.05                  | 17.89 ± 0.01             | 4.40 ± 0.01 |
| 14  | Rosewood          | Lauraceae  | Aniba roseodora                 | Brazil    | Wood            | Distillation      | 107.01 ± 0.01          | 17.17 ± 0.03 | 61.12 ± 0.06                  | 83.44 ± 0.01             | 0.94 ± 0.01 |
| 15  | Marjoram sweet    | Lamiaceae  | Origanum majorana              | Egypt     | Whole           | Distillation      | 85.51 ± 0.03           | 13.44 ± 0.01 | 63.15 ± 0.06                  | 112.33 ± 0.01            | 0.63 ± 0.01 |
| 16  | Tea-tree          | Myrtaceae  | Melaleuca alternifolia         | Australia | Leaves          | Distillation      | 69.05 ± 0.01           | 24.64 ± 0.03 | 83.87 ± 0.10                  | 159.00 ± 0.01            | 68.55 ± 0.01 |
| 17  | Thyme borneol     | Lamiaceae  | Thymus satureoides             | Morocco   | Whole flower plant | Whole flower plant | 63.57 ± 0.07           | 8.61 ± 0.01  | 60.71 ± 0.06                  | 147.89 ± 0.01            | 62.26 ± 0.01 |
| 18  | Basil tropical    | Lamiaceae  | Ocimum basilicum               | Tanzania  | Whole flower plant | Whole flower plant | 16.87 ± 0.02           | 9.85 ± 0.01  | 45.70 ± 0.05                  | 111.22 ± 0.01            | 19.81 ± 0.01 |
| 19  | Vetiver bourbon   | Poaceae    | Vetiveria zizanioides          | Madagascar | Roots           | Distillation      | 26.27 ± 0.01           | 6.38 ± 0.01  | 44.50 ± 0.04                  | 432.33 ± 0.01            | 38.52 ± 0.01 |
| 20  | Ylang ylang       | Anonaceae  | Cananga odorata                | Madagascar | Flowers         | Distillation      | 26.27 ± 0.01           | 6.38 ± 0.01  | 44.50 ± 0.04                  | 432.33 ± 0.01            | 38.52 ± 0.01 |
| 21  | Spruce black      | Pinacea   | Picea mariana                  | Canada    | leaves          | Distillation      | 2.18 ± 0.01            | 2.18 ± 0.01  | 20.67 ± 0.03                  | 19.00 ± 0.01             | 1.5 ± 0.01  |
| 22  | Ammi visnaga      | Apiaceae   | Ammi visnaga                   | Morocco   | Whole flower plant | Whole flower plant | 80.02 ± 0.13           | 6.86 ± 0.01  | 79.01 ± 0.08                  | 140.11 ± 0.01            | 55.98 ± 0.01 |
| 23  | Rose blossoms     | Geraniaceae | Rose centifolia               | Morocco   | Flowers         | Solvent extraction| 43.50 ± 0.01           | 4.71 ± 0.01  | 56.78 ± 0.01                  | 16.78 ± 0.01             | 3.15 ± 0.01 |
| 24  | Lantana           | Verbenaceae | Lantana camara                | Madagascar | Flowers         | Whole flower plant | 17.79 ± 0.01           | 1.50 ± 0.01  | 16.78 ± 0.01                  | 16.78 ± 0.01             | 3.15 ± 0.01 |
| 25  | Verbena lemon     | Verbenaceae | Lippia citriodora             | Paraguay  | Whole flower plant | Whole flower plant | 25.82 ± 0.02           | 4.63 ± 0.01  | 70.59 ± 0.06                  | 17.89 ± 0.01             | 8.02 ± 0.02 |
| 26  | Labrador tea      | Ericaceae  | Ledum groenlandicum           | Canada    | Distillation    | Distillation      | 25.82 ± 0.02           | 4.63 ± 0.01  | 70.59 ± 0.06                  | 17.89 ± 0.01             | 8.02 ± 0.02 |

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ᵃ Mean ± standard deviation (n = 3).
ᵇ Mean 1 mg of GAE g.
At a concentration of 2.5 mg/mL, the BCB activity levels of the clove bud essential oil, thyme borneol essential oil, eugenol, eugenyl acetate, borneol, thymol, and carvacrol were 94.58% ± 0.01%, 83.87% ± 0.04%, 96.61% ± 0.03%, 40.43% ± 0.14%, 9.48% ± 0.15%, 90.36% ± 0.06%, and 88.13% ± 0.03%, respectively. BCB activity decreased in the following order: clove bud essential oil > eugenol > thymol > carvacrol > thyme borneol essential oil > eugenyl acetate.

### Table 2 – Chemical compositions of clove bud, thyme borneol, and jasmine absolute essential oils.

| Peak number | Rt (min) | Compound | M. wt. | M. f. | Content in samples (%) |
|-------------|---------|----------|--------|-------|------------------------|
| 1           | 7.79    | 1R-α-Pinene | 136    | C10H16 | Clove Bud 4.39 |
| 2           | 8.2     | Camphene   | 136    | C10H16 | Clove Bud 7.92 |
| 3           | 8.98    | β-Pinene   | 136    | C10H16 | Clove Bud 0.57 |
| 4           | 10.32   | p-Cymene   | 134    | C10H14 | Clove Bud 5.76 |
| 5           | 10.45   | α-Limonene | 136    | C10H16 | Clove Bud 0.62 |
| 6           | 10.57   | Benzyl alcohol | 108  | C6H5O | Thyme borneol 1.79 |
| 7           | 11.32   | Crithmene  | 136    | C10H16 | Clove Bud 0.51 |
| 8           | 12.51   | Linalool   | 154    | C10H18O | Thyme borneol 7.72 |
| 9           | 14.9    | Benzyl acetate | 108  | C8H18O2 | Thyme borneol 32.30 |
| 10          | 14.15   | Camphor    | 152    | C10H18O | Thyme borneol 0.76 |
| 11          | 15.04   | Borneol    | 154    | C10H18O | Clove Bud 28.39 |
| 12          | 15.55   | Terpene-4-ol | 154  | C10H18O | Thyme borneol 1.12 |
| 13          | 16.18   | Therpinone | 154    | C10H18O | Clove Bud 7.72 |
| 14          | 18.85   | Methyl thymyl ether | 164 | C10H18O | Clove Bud 4.04 |
| 15          | 20.68   | Bornyl acetate | 196  | C10H16O2 | Clove Bud 1.58 |
| 16          | 20.88   | Indole     | 117    | C6H11N | Thyme borneol 2.34 |
| 17          | 20.93   | Thymol     | 150    | C10H16O | Clove Bud 14.36 |
| 18          | 21.3    | Carvacrol  | 150    | C10H16O | Clove Bud 12.33 |
| 19          | 23.42   | Eugenol    | 164    | C10H18O | Clove Bud 76.08 |
| 20          | 24.74   | Jasnone    | 164    | C10H18O | Clove Bud 0.87 |
| 21          | 25.45   | α-Caryophyllene | 204 | C15H14O | Clove Bud 9.92 |
| 22          | 26.49   | α-Caryophyllene | 204 | C15H14O | Clove Bud 0.86 |
| 23          | 27.99   | α-Farnesene | 204  | C15H14O | Clove Bud 2.18 |
| 24          | 28.45   | β-Cadinene | 204    | C15H14O | Clove Bud 0.80 |
| 25          | 28.49   | Eugenyl acetate | 206  | C10H18O3 | Clove Bud 12.57 |
| 26          | 29.64   | cis-3-Hexenyl Benzoate | 204 | C10H18O2 | Clove Bud 1.35 |
| 27          | 31.55   | Methyl Jasnone | 224  | C10H18O3 | Clove Bud 0.88 |
| 28          | 34.24   | Benzyl Benzoate | 212  | C10H18O2 | Clove Bud 22.94 |
| 29          | 37.52   | Methyl palmitate | 270  | C18H36O | Clove Bud 0.65 |
| 30          | 37.97   | Isophytol   | 296    | C18H36O | Clove Bud 6.59 |
| 31          | 39.57   | Farneyl acetate | 264  | C20H36O2 | Clove Bud 3.22 |
| 32          | 40.86   | Ethyl linolenate | 306  | C20H36O2 | Clove Bud 2.04 |
| 33          | 41.09   | trans-Phytol | 296  | C20H36O | Clove Bud 7.71 |
| 34          | 55.59   | Squalene   | 410    | C30H50O | Clove Bud 5.13 |

*Rt*: retention time (min).

The components were identified by comparing their mass spectra and retention indices (RIs) to those of the Wiley and NIST mass spectral databases and the previously published RIs.

M. wt.: molecular weight.

M. f.: molecular formula.

At a concentration of 2.5 mg/mL, the BCB activity levels of the clove bud essential oil, thyme borneol essential oil, eugenol, eugenyl acetate, borneol, thymol, and carvacrol were 94.58% ± 0.01%, 83.87% ± 0.04%, 96.61% ± 0.03%, 40.43% ± 0.14%, 9.48% ± 0.15%, 90.36% ± 0.06%, and 88.13% ± 0.03%, respectively. BCB activity decreased in the following order: clove bud essential oil > eugenol > thymol > carvacrol > thyme borneol essential oil > eugenyl acetate.

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Fig. 1 – RP of BHA, BHT, and L(+)-ascorbic acid and clove bud and thyme borneol essential oils at concentrations of 0.1 – 0.5 mg/mL.

Fig. 2 – RP of five pure chemical components, BHT, and clove bud and thyme borneol essential oils at a concentration of 2.5 mg/mL.
acetate. Therefore, eugenol and thymol are the major components contributing to the high BCB activity levels of the clove bud and thyme borneol essential oils, respectively.

### 3.5. TEAC determination

The clove bud and ylang ylang complete essential oils had the highest TEAC among the 26 essential oils. At a concentration of 1 mg/mL, the clove bud and ylang ylang complete essential oils were determined to be 809.00 ± 0.01 and 432.33 ± 0.01 μM of trolox equivalent. The jasmine absolute, chamomile, and thyme borneol essential oils were 354.56 ± 0.01, 186.78 ± 0.01, and 159.00 ± 0.01 μM of trolox equivalent, respectively. However, the cardamom, eucalyptus rakiata and spruce black essential oils showed negligible TEAC results.

The TEAC values of the clove bud and ylang ylang complete essential oils are comparable to those of the essential oils from Diospyros tesselleria (989 μM), Diospyros melannida (818 μM), Cassine orientalis (584 μM) and Coffea macrocarpa (447 μM) [44]. The TEAC values of the Byrsonima crassifolia leaf extracts, Davilla kunthii leaf extracts and Dalbergia subcymosa bark extracts [45] were 347.1, 282.9, and 197.4 μM, respectively. The TEAC values of the essential oils from D. tesselleria and D. mellanida are higher than those of the clove bud and ylang ylang complete essential oils. Moreover, the TEAC values of the essential oils from C. orientalis and C. macrocarpa are lower than that of the clove bud essential oil. In addition, the values of B. crassifolia leaf extracts, D. kunthii leaf extracts and D. subcymosa bark are lower than that of the ylang ylang complete essential oil.

### 3.6. DFRS assay

Table 1 presents the DFRS data. At a concentration of 1 mg/mL, the DFRS abilities of the clove bud, jasmine absolute, and thyme borneol essential oils were 94.13 ± 0.01%, 78.62 ± 0.01% and 68.55 ± 0.01%, respectively. The basil tropical and rose blossoms absolute essential oils showed DFRS abilities of 62.26% ± 0.01% and 55.98% ± 0.01%, respectively. The DFRS activity of the clove bud essential oil is higher than that of BHT (94.13% ± 0.01% vs 83.29% ± 0.04%). Other essential oils showed an apparent DFRS ability of <50%. However, the eucalyptus rakiata, marjoram sweet, spruce black, ammi visnaga, and verbena lemon essential oils exhibited negligible DFRS abilities.

The EC50 values of the clove bud, jasmine absolute, and thyme borneol essential oils were 7.81, 299.60, and 655.49 μg/mL, respectively. These values are considerably different from those reported for the essential oils from cinnamon bark (53 μg/mL), origanum (36 μg/mL) [40], Petigrain mandarin (79.84 μg/mL), geranium (66.45 μg/mL) [46], Vetiveria zizanioides (7790 μg/mL) [47], and Zataria multiflora (2220 μg/mL) [48]. Although the clove bud essential oil had the highest DFRS ability in this study, this level is lower than those reported in most reports. The EC50 values of the jasmine absolute and thyme borneol essential oils are lower than those of the essential oils from V. zizanioides and Z. multiflora. The DFRS abilities of the essential oils can be ranked as follows: clove bud > cinnamon bark > origanum > P. mandarin > geranium > jasmine absolute > thyme borneol > Z. multiflora > V. zizanioides.

The DFRS abilities of the clove bud essential oil, jasmine absolute essential oil, thyme borneol essential oil, eugenol, thymol, carvacrol, and eugenyl acetate were 95.13% ± 0.01%, 78.62% ± 0.01%, 68.55% ± 0.01%, 86.64% ± 0.01%, 63.37% ± 0.01%, 58.96% ± 0.01% and 45.91% ± 0.02%, respectively, at a concentration of 1 mg/mL (Fig. 4). However, borneol, benzyl acetate, and benzyl benzoate did not exhibit significant DFRS abilities.

After evaluating the antioxidant activity levels of pure chemical components of essential oils, we can obtain the antioxidant activity of each essential oil through simple calculations (percentage of each chemical component in the total oil content multiplied by its corresponding antioxidant activity index).
activity). In addition, according to the antioxidative activity levels of these essential oils, we can apply for a patent on the essential oil composition with free radical scavenging ability by developing a complex essential oil or a simple lotion formulation that includes 0.1% of the complex essential oil.

4. Conclusions

This study examined the antioxidative activity levels and major chemical components of 26 commonly used essential oils by evaluating several factors, namely TPC, RP, BCB activity, TEAC, and DFRS ability. Eugenol of the clove bud essential oil showed the most favorable results in all evaluations. Thymol and carvacrol of the thyme borneol essential oil showed good results in the RP, TPC, and BCB assays. A small percentage of eugenol from the jasmine absolute oil also showed good DFRS ability. The chemical components with phenolic contents showed excellent antioxidant properties and were positively correlated with RP, TPC, BCB activity, TEAC, and DFRS ability. Our study results reveal that the clove bud, thyme borneol, and jasmine absolute essential oils have potential for use as antioxidants in functional foods and cosmetic products. Furthermore, the antioxidant activity of individual essential oils can be obtained by using these study data in simple calculations (percentage of each chemical component in the total oil content multiplied by its corresponding antioxidant activity).

Conflicts of interest

The authors declare no competing financial interest.

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