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# A study of four antioxidant activities and major chemical component analyses of twenty-five commonly used essential oils

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**Synopsis**

Twenty-five essential oils and their major chemical components were screened for their possible antioxidant activities by assaying their DPPH free-radical scavenging activity (DFRS), total phenolic contents (TPC), trolox equivalent antioxidant capacity (TEAC), and ferric thiocyanate (FTC). Based on the TPC and TEAC assays, the essential oil ajowan is among the best essential oils studied. Furthermore, the DFRS and FTC assays reveal that the essential oils cinnamon bark extra and oregano are also among the best oils studied. More specifically, at a concentration of 1 mg $ml^{-1}$, the essential oils cinnamon bark extra and benzoin showed 93.75 ± 0.01% and 90.64 ± 0.01% DFRS, while the essential oils ajowan and oregano showed TEAC values of 4374.72 ± 0.01 and 4023.49 ± 0.01 μM of trolox per mg, respectively. In addition, the essential oils oregano and ajowan showed 29.17 ± 0.02% and 25.26 ± 0.03% FTC based on the assay results. At a concentration of 10 mg $ml^{-1}$, the essential oils ajowan and oregano showed 1845.20 ± 0.04 and 1665.36 ± 0.04 μg of TPC relative to GAE, respectively.

Two major chemical components of the essential oils cinnamon bark extra, ajowan, and oregano were *trans*-cinnamaldehyde (90.61%), eugenol (2.58%), carvacrol (61.20%), *p*-cymene (37.44%), thymol (77.09%), and *p*-cymene (10.01%). It is clear that phenolic compounds in the aforementioned essential oils yield a positive correlation with the DFRS, TPC, TEAC, and FTC assays.

## INTRODUCTION

Essential oils are commonly used in the food and aromatherapies industries and have increased the value of products and also enlightened the cosmetics industry. Other applications include, but are not limited to: antioxidant, antimicrobial (1), anti-inflammatory, anti-cholinesterase (2), anti-thrombotic (3), anxiolytic (4), EEG (5) and blood-pressure influential (6) properties. Essential oils are commonly considered to be volatile oils, which give rise to the rich fragrance found in aromatics. Many chemical components in natural

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plants have exhibited antioxidant properties, and they often contain similar, but low, levels of essential oils and therefore antioxidant components. The compositions of the essential oils from the same plants may be different due to (i) the age of the plant, (ii) the time of harvesting, and (iii) the extraction methods.

Many articles have shown that aging is closely related to the presence of free radicals (7). Free radicals have weak bonds with atoms, molecules, or ions and contain unpaired electrons in the outer shells. Those unpaired electrons try to (and usually do) capture electrons from the nearest stable molecule to gain stability. When the "attacked" molecule loses its electron, it becomes a free radical itself, initiating a chain reaction (8,9). Our skin inhibits the pressure of being oxidized from environmental pollution and ultraviolet rays (10) by using antioxidants contained naturally within the body. Many artificial antioxidants—such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ), and propylene glycol (PG)—are added to food as well as cosmetics to prevent free radical formation. However, due to safety concerns (11), natural antioxidant alternatives have become more reliable. Natural antioxidants/essential oils such as *Cleistocalyx operculatus* and *Artemisia scoparia* have strong DFRS (12,13), while *Psammogeton canescens, Pistacia lentiscus,* and *Myrtus communis* extracts contain high levels of phenolic compounds (14,15). The extracts *Rosmarinus officinalis* and *Nigella sativa* have shown good TEAC capacities (16), whereas the essential oils *Ocimum basilicumru* and *Dorystoechas hastate* inhibit linoleic acid peroxidation based on results from the FTC assay (17,18). These examples show that essential oils have favorable antioxidation properties.

Previously we studied forty-five essential oils from an Australian company (19,20) and compared their antioxidant activities. In order to explore the sources of essential oils for functional foods, and their applications in cosmetic products, and to investigate their antioxidant activities, DFRS, TPC, TEAC, and FTC assays were employed. Herein we have extended our studies by analyzing the antioxidant activities and the major chemical components of an additional twenty-five essential oils from Ayus GmbH (Baden, Germany).

## MATERIALS AND METHODS

### MATERIALS

Butyl hydroxyl toluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 3,4,5-trihydroxybenzoic acid (gallic acid) were purchased from TCI (Shanghai, China). Ammonium thiocyanate ($NH_4SCN$), disodium hydrogenphosphate ($Na_2HPO_4$), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), sodium dihydrogenphosphate ($NaH_2PO_4$), and sodium carbonate ($Na_2CO_3$) were purchased from Showa (Tokyo, Japan). Folin-Ciocalteu's phenol reagent, eugenol, potassium persulfate, and linalool were purchased from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from MP (Eschwege, Germany). Linoleic acid, *p*-cymene, and thymol were purchased from Acros Organics (Geel, Belgium). *Trans*-cinnamaldehyde was purchased from Alfa Aesar (Karlsruhe, Germany). In addition, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (trolox), and carvacrol were purchased from Sigma (St. Louis, MO). The twenty-five essential oils (of 100% purity) were purchased from Ayus GmbH (Baden, Germany). All other chemicals and solvents

were of standard analytical grade and purchased from Echo Chemical Co. (Miaoli, Taiwan).

### GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

Analyses of the volatile compounds were tested using a Thermo GC-MS system (GC-MS Trace DSQ-Mass Spectrometer, MSD 201351, Thermo, Minneapolis, MN). An Equity$^{TM-5}$ capillary column (Supelco, USA) with a 30-m length and a 0.25-mm inside diameter was used with a 0.25-μm-thick film. The oven temperature was programmed as follows: isothermal at 40°C, followed by a 5°C temperature ramp every minute to 100°C, which was held for five minutes. Subsequently, the temperature was increased 5°C every minute to 250°C and held for 20 minutes. The carrier gas was helium (1 ml $min^{-1}$). The injection port's temperature was 250°C and the detector temperature was 250°C. Ionization of the sample components was performed in the EI mode (70 eV). The injected volume was 1 μl. The linear retention indices for all compounds were determined by co-injection of the samples with a solution containing a homologous series of C8-C22 n-alkanes (21). The individual components were identified by retention indices and comparison with compounds known from the literature (22). Their mass spectra were also compared with known, previously obtained, compounds or from the Trace DSQ-MASS spectral database (Thermo, USA).

### DPPH FREE-RADICAL SCAVENGING ASSAY (DFRS)

The antioxidant activities of twenty-five essential oils were assessed by measuring their ability to scavenge the (stable) radical, 1,1-diphenyl-2-picrylhydrazyl. The DPPH assay was performed as described by Gyamfi *et al.* (23) with the following modifications. Two milliliters of test sample in an ethanol solution (1 mg $ml^{-1}$) was added to 0.5 ml of $2.5 \times 10^{-4}$ M DPPH ethanol solution. The reactive mixture and the blank were incubated in the dark at room temperature for 30 minutes. Ethanol (99.9%) was used as the control while BHT was used as the positive control. For each sample, three replicates were recorded. The disappearance of DPPH was determined spectrophotometrically at 517 nm using a UV-vis spectrophotometer (SP-8001, Metertech Inc., Taipei, Taiwan). Inhibition of the free radical by DPPH as a percent (%) was calculated using the following equation: I % = [1 − (absorbance of sample at 517 nm /absorbance of control at 517 nm)] × 100%. The $EC_{50}$ values were calculated by linear regression analysis, which was defined as the effective concentration of the sample to obtain 50% antioxidant.

### DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content (TPC) was determined by the methods described by Kujala *et al.* (24) with the following modifications. The test samples were diluted to a suitable concentration for analysis. One half a milliliter from the test samples in an ethanol solution (10 mg $ml^{-1}$), was mixed with 1 ml of 1 N Folin-Ciocalteu's reagent and 1 ml of 7.5% $Na_2CO_3$ (w/v). After three hours of incubation at ambient temperature, the sediment

and solution were divided by using a centrifuge at 3000 rpm for eight minutes. BHT was used as the positive control. Three replicates were recorded for each sample. The supernatant was measured with a UV-vis spectrophotometer (SP-8001, Metertech Inc., Taipei, Taiwan) at 760 nm. Different concentrations of gallic acid (10-100 μg/ml) were determined by a calibration curve ($y = 45.318x - 0.0181$; $\gamma^2 = 0.999$; y is the concentration of gallic acid, x is absorbance). The results were shown as micrograms relative to gallic acid equivalents (GAE) per 10 mg of essential oil.

### TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC) ASSAY

The trolox equivalent antioxidant capacity (TEAC) assay was determined by methods described by Erkan *et al*. (16) with the following modifications. When ABTS and potassium peroxodisulfate were mixed in a pH-7.4 phosphate buffer solution (PBS), followed by incubation in the dark for 16 hours, a blue-greenish $ABTS^+$ solution results. The final concentrations were 7 mM and 2.45 mM. Next, 300 μl of $ABTS^+$ solution was diluted with PBS to 20 ml, and that solution was incubated in the dark for one hour. After 1mg $ml^{-1}$ of the test sample (20 μl with 1980 μl dilute solution) was mixed and allowed to react for ten minutes, the absorbance was measured at 730 nm by a UV-vis spectrophotometer (SP-8001, Metertech Inc., Taipei, Taiwan). Trolox (25–800 μM) was used in different concentrations (as standards) to create a calibration curve ($y = -3497.2x + 1466.4$; $\gamma^2 = 0.999$; y is the concentration of trolox, x is absorbance) to find the relative concentration of trolox in each sample. BHT was used as the positive control. For each sample, three replicates were recorded. The results were shown as μM of trolox per milligram of essential oil.

### FERRIC THIOCYANATE (FTC) ASSAY

The antioxidant activity analysis was performed by ferric thiocyanate according to the procedure reported by Osawa and Namiki (25) and Zainol *et al.* (26) with the following modifications. The test samples (1 mg) were dissolved in 1 ml of ethanol, and mixed with linoleic acid (2.5%, v/v), 99.9% ethanol (1 ml), and 2 ml of 0.05 M sodium phosphate buffer (pH 7). The solution was incubated at 40°C for 240 h. Next, 1.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate aqueous solution, and 0.1 ml of ferrous chloride solution (20 mM in 3.7% HCI) were sequentially added to 0.1 ml of sample solution. After ten minutes of stirring, the absorbance was measured at 500 nm by using the ELISA (Sunrise™, Tecan Group Ltd., Männedorf, Switzerland). A control was performed using linoleic acid without the essential oils. BHT was used as the positive control. For each sample, three replicates were recorded. Inhibition of linoleic acid peroxidation was expressed as a percentage and calculated using the following equation: Inhibition of linoleic acid peroxidation % = [1− (Abs increase of sample at 500 nm/Abs increase of control at 500 nm)] × 100%.

### STATISTICAL ANALYSIS

Data were presented as the mean ± standard deviation (S.D.) from three independent experimental determinations. Statistical analyses were performed using a one-way analysis

of variance (ANOVA). Differences were considered significant at $p < 0.05$. Data were calculated by employing the statistical software SPSS (version 13.0, SPSS Inc., Chicago, IL).

## RESULTS AND DISCUSSION

### ANALYSIS OF CHEMICAL COMPOSITION BY GC-MS

The name, scientific name, department, origin, extraction method, and extraction part for the twenty-five essential oils studied herein are listed in Table I. GC-MS was used to analyze the chemical components of the essential oils cinnamon bark extra, ajowan, and oregano. The GC-MS data revealed the presence of five chemical components, which are

**Table I**
Essential Information for the Twenty-Five Essential Oils Studied

| No. | Name | Scientific name | Department | Origin | Extraction method | Extraction part |
|---|---|---|---|---|---|---|
| 1 | Ajowan | *Trachyspermum ammi* | Apiaceae | India | Distillation | Seed |
| 2 | Anis extra | *Pimpinella anisum* | Apiaceae | Turkey | Distillation | Whole plant |
| 3 | Bay laurel | *Laurus nobilis* | Lauraceae | Turkey | Distillation | Leaf |
| 4 | Benzoin | *Styrax benzoin* | Styracaceae | Indonesia | Solvent extraction | Resin |
| 5 | Cinnamon bark extra | *Cinnamomum verum* | Lauraceae | Sri Lanka | Distillation | Bark |
| 6 | Fir | *Abies sibirica* | Pinacea | Siberia | Distillation | Conifer |
| 7 | Frankincense | *Boswellia carterii* | Burseraceae | India | Distillation | Resin |
| 8 | Galbanum | *Ferula galbaniflua* | Apiaceae | Iran | Distillation | Resin |
| 9 | Ginger | *Zingiber officinalis* | Zingiberaceae | Vietnam | Distillation | Root |
| 10 | Grapefruit extra | *Citrus paradisi* | Rutaceae | Israel | Cold compression | Pericarp |
| 11 | Ho-oil | *Cinnamomum camphora* | Lauraceae | China | Distillation | Leaf |
| 12 | Jasmine (arab.) | *Jaminum sambac* | Floral-scented oils | India | Solvent extraction | Flower |
| 13 | Lavender Kashmir | *Lavendula angustifolia* | Lamiaceae | India | Distillation | Whole plant |
| 14 | Lemongrass extra | *Cymbopogon flexuosus* | Poaceae | Nepal | Distillation | Leaf |
| 15 | Lemonmint | *Mentha citrata* | Lamiaceae | India | Distillation | Whole plant |
| 16 | Litsea | *Litsea cubeba* | Lauraceae | China/Vietnam | Distillation | Fruit |
| 17 | Nutmeg | *Myristica fragrans* | Myristicaceae | Indonesia | Distillation | Fruit |
| 18 | Oregano | *Origanum vulgare* | Lamiaceae | Turkey | Distillation | Whole plant |
| 19 | Palmarosa | *Cymbopogon martini* | Poaceae | Nepal | Distillation | Whole plant |
| 20 | Patchouli | *Pogostemon cablin* | Lamiaceae | Indonesia | Distillation | Whole plant |
| 21 | Pepper extra | *Piper nigrum* | Piperaceae | Madagascar | Distillation | Fruit |
| 22 | Peppermint | *Mentha piperita* | Lamiaceae | India | Distillation | Whole plant |
| 23 | Sandalwood | *Santalum album* | Santalaceae | India | Distillation | Wood |
| 24 | Spikenard | *Nardostachys jatamansi* | Valerianaceae | Nepal | Distillation | Root |
| 25 | Wintergreen | *Gaultheria fragrantissima* | Ericaceae | Nepal | Distillation | Leaf |

summarized in Table II and account for 98.96% of total cinnamon bark extra essential oil. The major component (90.61%) was found to be *trans*-cinnamaldehyde (Table II), while the four other components were eugenol (2.58%), *p*-cymene (2.28%) β-caryophyllene (2.10%), and linalool (1.39%), respectively. There are two major chemical components, carvacrol (61.20%) and *p*-cymene (37.44%) in the essential oil ajowan (representing 98.64% of the oil). The essential oil oregano contains four major chemical components, namely thymol (77.09%), *p*-cymene (10.01%), linalool (9.59%), and carvacrol (2.06%), which represent 98.75% of the oil. In order to study the antioxidant activities of these major chemical components, these essential oils were purchased in their pure form.

### DPPH FREE-RADICAL SCAVENGING ACTIVITY

The DPPH free-radical scavenging activity data of the twenty-five essential oils are listed in Table III. At a concentration of 1 mg $ml^{-1}$, the cinnamon bark extra essential oil showed 93.75 ± 0.01% DFRS. This DFRS activity was followed by the essential oils benzoin (90.64 ± 0.01%), nutmeg (86.88 ± 0.01%), spikenard (72.40 ± 0.01%), ajowan (70.30 ± 0.01%) and oregano (59.20 ± 0.01), respectively. The remaining essential oils had apparent DFRS values below 50%. The $EC_{50}$ value of cinnamon bark extra was 64.31 μg $ml^{-1}$ (Figure 1), followed by benzoin (292.46 μg $ml^{-1}$), nutmeg (481.26 μg $ml^{-1}$), spikenard (540.86 μg $ml^{-1}$), and ajowan (629.67 μg $ml^{-1}$). Compared to the positive control BHT, cinnamon bark extra essential oil showed the best DFRS (Figure 2).

The $EC_{50}$ values of the essential oils *Cleistocalyx operculatus* (12), *Artemisia scoparia* (13), *Satureja cuneifolia* (27), and *Thymus caramanicus* (28) are 807 μg $ml^{-1}$, 66 μg $ml^{-1}$, 65 μg $ml^{-1}$, and 263 μg $ml^{-1}$, respectively. Therefore, the DFRS of cinnamon bark extra (*Cinnamomum verum*) is better than that of *Cleistocalyx operculatus* and *Thymus caramanicus* and similar to that of *Artemisia scoparia* and *Satureja cuneifolia*.

**Table II**
Composition of the Essential Oils from Cinnamon Bark Extra, Ajowan, and Oregano

| | | | Peak area % | | |
|---|---|---|---|---|---|
| $R_t$ [a] | Compound[b] | M. f.[c] | Cinnamon bark extra | Ajowan | Oregano |
| 10.32 | *p*-Cymene | $C_{10}H_{14}$ | 2.28 | 37.44 | 10.01 |
| 12.53 | Linalool | $C_{10}H_{18}O$ | 1.39 | | 9.59 |
| 19.96 | *trans*-Cinnamaldehyde | $C_9H_8O$ | 90.61 | | |
| 20.92 | Carvacrol | $C_{10}H_{14}O$ | | 61.20 | 2.06 |
| 21.29 | Thymol | $C_{10}H_{14}O$ | | | 77.09 |
| 23.35 | Eugenol | $C_{10}H_{12}O_2$ | 2.58 | | |
| 25.45 | β-Caryophyllene | $C_{15}H_{24}$ | 2.10 | | |
| | Unknown | | 1.04 | 1.36 | 1.25 |

[a] $R_t$: Retention time (min).
[b] The components were identified by their mass spectra and retention indices (RIs) and by the Wiley and NIST mass spectral databases and previously published RIs.
[c] M. f.: Molecular formula.

**Table III**
DFRS, TPC, TEAC, and FTC Assays of the Twenty-Five Essential Oils

| No. | Name | DPPH free-radical scavenging activity (%)* | Total phenolic content (μg of GAE 10 $mg^{-1}$)* | TEAC (μM of trolox $mg^{-1}$) | Inhibition of linoleic acid peroxidation (%)* |
|---|---|---|---|---|---|
| 1 | Ajowan | 70.30 ± 0.01 | 1845.20 ± 0.04 | 4374.72 ± 0.01 | 25.26 ± 0.03 |
| 2 | Anis extra | 4.24 ± 0.01 | 15.51 ± 0.02 | 206.83 ± 0.02 | — |
| 3 | Bay laurel | 40.89 ± 0.01 | 28.23 ± 0.02 | 204.49 ± 0.01 | 4.89 ± 0.04 |
| 4 | Benzoin | 90.64 ± 0.01 | 90.53 ± 0.10 | 508.89 ± 0.01 | 18.59 ± 0.03 |
| 5 | Cinnamon bark extra | 93.75 ± 0.01 | 544.35 ± 0.01 | 1190.23 ± 0.01 | 10.46 ± 0.05 |
| 6 | Fir | — | 6.39 ± 0.02 | 88.58 ± 0.01 | — |
| 7 | Frankincense | 1.26 ± 0.01 | 10.01 ± 0.04 | 44.09 ± 0.01 | — |
| 8 | Galbanum | — | 9.56 ± 0.02 | 48.78 ± 0.01 | — |
| 9 | Ginger | 4.26 ± 0.01 | 16.72 ± 0.03 | 115.51 ± 0.01 | — |
| 10 | Grapefruit extra | — | 6.86 ± 0.03 | 79.22 ± 0.01 | — |
| 11 | Ho-oil | — | 4.59 ± 0.01 | 82.73 ± 0.01 | — |
| 12 | Jasmine (arab.) | 31.93 ± 0.01 | 47.75 ± 0.04 | 278.25 ± 0.04 | 13.70 ± 0.03 |
| 13 | Lavender Kashmir | — | 8.03 ± 0.04 | 121.36 ± 0.01 | — |
| 14 | Lemongrass extra | 16.90 ± 0.01 | 21.22 ± 0.03 | 99.12 ± 0.01 | — |
| 15 | Lemonmint | — | 8.58 ± 0.01 | 95.61 ± 0.01 | — |
| 16 | Litea | 3.69 ± 0.01 | 9.21 ± 0.02 | 89.75 ± 0.01 | — |
| 17 | Nutmeg | 86.88 ± 0.01 | 85.24 ± 0.04 | 363.71 ± 0.01 | 5.81 ± 0.05 |
| 18 | Oregano | 59.20 ± 0.01 | 1665.36 ± 0.04 | 4023.49 ± 0.01 | 29.17 ± 0.02 |
| 19 | Palmarosa | 5.13 ± 0.01 | 7.31 ± 0.02 | 97.95 ± 0.01 | — |
| 20 | Patchouli | 1.39 ± 0.01 | 22.16 ± 0.01 | 46.44 ± 0.01 | — |
| 21 | Pepper extra | 6.18 ± 0.01 | 10.84 ± 0.02 | 17.17 ± 0.01 | — |
| 22 | Peppermint | 1.79 ± 0.01 | 13.92 ± 0.01 | 176.39 ± 0.01 | — |
| 23 | Sandalwood | 1.91 ± 0.01 | 13.86 ± 0.04 | 89.75 ± 0.01 | — |
| 24 | Spikenard | 72.40 ± 0.01 | 58.44 ± 0.08 | 253.66 ± 0.01 | — |
| 25 | Wintegreen | 4.66 ± 0.01 | 7.01 ± 0.01 | 3847.87 ± 0.01 | — |

Values are mean ± S.D. (n = 3). The concentrations of each essential oil for DFRS, TPC, TEAC, and FTC assay were 1 mg $ml^{-1}$, 10 mg $ml^{-1}$, 1 mg $ml^{-1}$, and 1 mg $ml^{-1}$, respectively.
* Antioxidant activity has not been observed.

At a concentration of 1 mg $ml^{-1}$, the essential oil cinnamon bark extra and three of its main components (*trans*-cinnamaldehyde, eugenol, and *p*-cymene) were analyzed for their DFRS activity. In Figure 3, it is clear that the DFRS increases is in the following order: eugenol > cinnamon bark extra essential oil > *trans*-cinnamaldehyde > *p*-cymene.

Eugenol was the major chemical component attributed to the high DFRS value for the cinnamon bark extra essential oil. The other major components, *trans*-cinnamaldehyde and *p*-cymene, yielded DFRS values of 6.01% and 4.55%, respectively.

### TOTAL PHENOLIC CONTENT (TPC)

The TPC of the twenty-five essential oils studied herein were expressed as equivalents of gallic acid (GAE 10 $mg^{-1}$). The results are shown in Table III, and the essential oils were

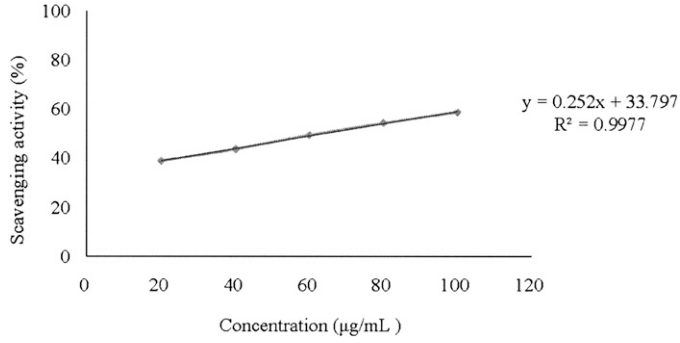


**Figure 1.** DPPH free-radical scavenging activity in different concentrations for cinnamon bark extra essential oil.

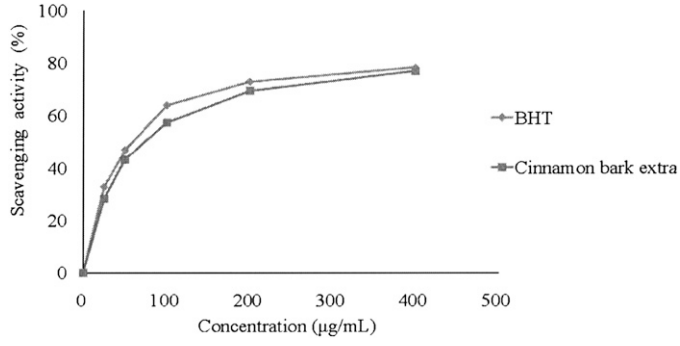


**Figure 2.** Concentration-dependent effect of DPPH free-radical scavenging activity of cinnamon bark extra essential oil and BHT.

found to have various phenolic levels, which ranged from 4.59 to 1845.20 µg of GAE 10 $mg^{-1}$. For each 10 milligrams of essential oil, ajowan had the highest TPC (1845.20 ± 0.04 g of GAE), followed by oregano (1665.36 ± 0.04 µg of GAE) and cinnamon bark extra (544.35 ± 0.01 µg of GAE). The lowest TPC values were given by the essential oils, ho-oil (4.59 ± 0.01 µg of GAE) followed by fir (6.39 ± 0.02 µg of GAE).

The TPC of the essential oils *Psammogeton canescens* (14), *Pistacia lentiscus, Myrtus communis* extract (15), and *Satureja cuneifolia* (27) are 340, 4830, 3070, and 1855 µg of GAE, respectively. Therefore, the TPC of the essential oil ajowan (*Trachyspermum ammi*) is better than that of *Psammogeton canescens,* similar to that of *Satureja cuneifolia,* but lower than that of *Pistacia lentisus* or *Myrtus communis* extract.

### TROLOX EQUIVALENT ANTIOXIDANT CAPACITY (TEAC) ASSAY

The TEAC assay was used to analyze the concentration of the twenty-five essential oils relative to that of a standard sample of trolox (25-800 µM), and the results are shown in Table III. At a concentration of 1 mg $ml^{-1}$, ajowan was the best essential oil (4374.72 ± 0.01 µM of trolox $mg^{-1}$) based on the TEAC assay results. The TEAC assay results for

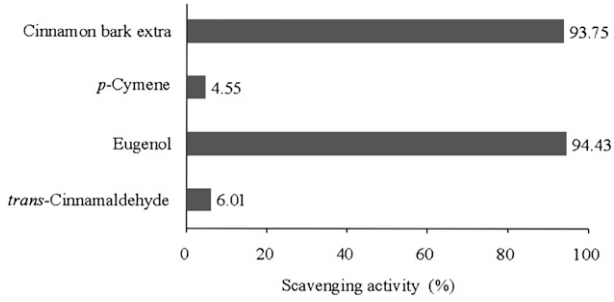


**Figure 3.** DPPH free-radical scavenging activity from three major chemical components of cinnamon bark extra compared with its essential oil at a concentration of 1 mg $ml^{-1}$.

oregano, wintergreen, and cinnamon bark extra were 4023.49 ± 0.01, 3847.87 ± 0.01, and 1190.23 ± 0.01 μM of trolox $mg^{-1}$, respectively. The TEAC assay of pepper extra essential oil was 17.17 ± 0.01 μM of trolox $mg^{-1}$, which was the lowest reported from the TEAC assay.

The TEAC assay of *Rosmarinus officinalis* extract, *Nigella sativa* essential oil (16), *Oxytropis halleri,* and *Lathyrus binatus* extract (29) revealed 15700, 2500, 34, and 158 μM of trolox $mg^{-1}$, respectively. Therefore, the TEAC of the essential oil ajowan (*Trachyspermum ammi*) is better than that of *Nigella sativa, Oxytropis halleri* or *Lathyrus binatus* and worse than that of *Rosmarinus officinalis* extract.

### FERRIC THIOCYANATE (FTC) ASSAY

The inhibition of linoleic acid peroxidation for the FTC assay of the twenty-five essential oils is listed in Table III. At a concentration of 1 mg $ml^{-1}$, the FTC assay of the twenty-five essential oils was in the range of 0–29.17%. According to the results, oregano was found to have the strongest FTC (29.17 ± 0.02%). This FTC activity was followed by that of ajowan (25.26 ± 0.03%), benzoin (18.59 ± 0.03%), jasmine (arab.) (13.70 ± 0.03%), cinnamon bark extra (10.46 ± 0.05%), nutmeg (5.81 ± 0.05%), and bay laurel (4.89 ± 0.04%) essential oils. The other essential oils showed negligible FTC assay values.

*Hygrophila auriculata* extract (30) at 1 mg $ml^{-1}$ of concentration shows 55.29% inhibition of linoleic acid peroxidation. Therefore, the FTC of oregano (*Origanum vulgare*) essential oil is worse than that of the *Hygrophila auriculata* extract.

At a concentration of 1 mg $ml^{-1}$, the essential oil oregano and four of its main components (thymol, *p*-cymene, linalool, and carvacrol) were analyzed via the FTC assay. Figure 4 clearly shows that the inhibition of linoleic acid peroxidation has the following order: carvacrol > oregano essential oil > thymol > *p*-cymene and linalool.

Carvacrol and thymol were the major components of the oregano essential oil attributed to the high inhibition of linoleic acid peroxidation. *P*-cymene and linalool showed negligible FTC assay values. We have studied the DFRS, TPC, and TEAC antioxidant activities of 23 esters, 14 aldehydes, 10 ethers, 14 phenols, 14 monoterpenols, and 10 monoterpenes as chemical components. In the future, we will use these data to make a

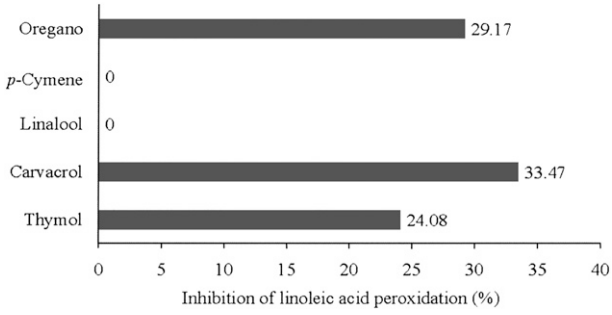


**Figure 4.** Inhibition of linoleic acid peroxidation of the FTC assay from four major chemical components of oregano compared with its essential oil at a concentration of 1 mg $ml^{-1}$.

table via easy calculations (percentage of the chemical component multiplied by the values of the different kinds of antioxidant activity), to obtain the antioxidant activity of each essential oil.

## CONCLUSION

This study examined the antioxidant activities and major chemical components of twenty-five commonly used essential oils using the DFRS, TPC, TEAC, and FTC assays. Eugenol, of the essential oil cinnamon bark extra, yielded the best DFRS assay values. Carvacrol, of the essential oil ajowan, showed the best results from the TPC and TEAC assays. Thymol, of the essential oil oregano, showed the strongest inhibition of linoleic acid peroxidation via the FTC assay. Those components with high phenolic contents revealed great antioxidation properties and contained a positive correlation with the DFRS, TPC, TEAC, and FTC assays. Our research shows that cinnamon bark extra, ajowan, and oregano essential oils have the potential to be developed into antioxidant ingredients for functional foods and cosmetic products.

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