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# Chemical Composition and Antioxidant Properties of Clove Leaf Essential Oil

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The antioxidant activity of a commercial rectified clove leaf essential oil (*Eugenia caryophyllus*) and its main constituent eugenol was tested. This essential oil comprises in total 23 identified constituents, among them eugenol (76.8%), followed by $\beta$-caryophyllene (17.4%), $\alpha$-humulene (2.1%), and eugenyl acetate (1.2%) as the main components. The essential oil from clove demonstrated scavenging activity against the 2,2-diphenyl-1-picryl hydracyl (DPPH) radical at concentrations lower than the concentrations of eugenol, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). This essential oil also showed a significant inhibitory effect against hydroxyl radicals and acted as an iron chelator. With respect to the lipid peroxidation, the inhibitory activity of clove oil determined using a linoleic acid emulsion system indicated a higher antioxidant activity than the standard BHT.

**KEYWORDS: Clove leaf essential oil; chemical composition; antioxidant properties; eugenol**

## INTRODUCTION

The clove tree [*Eugenia caryophyllus* (Spreng) Bullock and S. G. Harrison; syn. *Syzygium aromaticum* (L.) Merr., Myrtaceae] is a perennial tropical plant. It is used as a source for obtaining an essential oil, widely applied in medicine and cosmetics. The basic constituent of the oil is eugenol (2-methoxy-4-allylphenol), to which are attributed the antimicrobial and antioxidant properties of the oil. It is also used as a flavoring agent in food and cosmetic products and has pro-oxidant and antioxidant activities (*1*−*7*).

The addition of antioxidants to food products earns increasing popularity as a powerful means for extending the shelf-life of products and for decreasing the nutritional losses by preventing or slowing the oxidation process (*8*). The most commonly applied antioxidants in the food industry are synthetic phenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Their safety, however, is doubtful (*9*, *10*), and there has been a general desire to replace synthetic food additives with natural alternatives (*11*). Therefore, intensive research and utilization of natural antioxidants that may serve as potent candidates in combating carcinogenesis and aging processes have been done.

Sources of natural antioxidants are primarily plant phenolics that may occur in all parts of the plants, such as fruits, vegetables, nuts, seeds, leaves, roots, and barks (*12*, *13*). Plant phenolics are multifunctional and can act as reducing agents, metal chelators, and singlet oxygen quenchers (*14*, *15*).

Therefore, the aim of this study was to investigate an essential oil (rectified) of clove leaves with phenolic main compounds for their antioxidant properties to obtain further information about the above-mentioned effects.

## MATERIALS AND METHODS

**Materials.** The rectified clove leaf essential oil and all reference compounds, including eugenol (see **Table 1**), used in this study were placed at our disposal from Kurt Kitzing Co., Wallerstein, Germany.

**Chemical Composition of Essential Oil: Gas Chromatograpy (GC) Analysis.** GC/flame ionization detector (FID) analyses were carried out using a GC-14A with a split/splitless injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan), a GC-3700 with FID (Varian, Germany), and C-R1B-Chromatopac integrator (Shimadzu). The carrier gas was hydrogen (flow rate of 1.2 mL/min); injector temperature, 250 °C; detector temperature, 320 °C. The temperature program started at 40 °C during 5 min, increasing at 6 °C/min up to 280 °C for 5 min. The columns were 30 m × 0.32 mm bonded FSOT-RSL-200 fused silica, with a film thickness of 0.25 $\mu$m (Biorad, Germany), and 30 m × 0.32 mm bonded Stabilwax, with a film thickness of 0.50 $\mu$m (Restek, Bellefonte, PA). Quantification was achieved using relative percent peak area calculations (mean values of three repetitions), and compound identification was carried out partly using correlations between retention times (*16*−*20*).

**GC/Mass Spectrometry (MS) Analysis.** For GC/MS measurements, a GC-17A with QP5000 (Shimadzu), SPME sleeve adapted to injector and Compaq-ProLinea data system (class5k software), a GC-HP5890 with HP5970-MSD (Hewlett-Packard, Palo Alto, CA), and ChemStation software on a Pentium PC (Böhm, Austria), a GCQ (Finnigan

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**Table 1.** Composition and Olfactoric Data of the Essential Leaf Oil of Clove

| compound[a] | RI[b] | percent[c] | odor description[d] | identification[e] |
|---|---|---|---|---|
| limonene | 1029 | 0.1 | fresh, herbaceous, lemon | reference |
| 1,8-cineole | 1032 | 0.1 | fresh (eucalyptus-like) | reference |
| *cis*-limonene oxide | 1137 | trace | fresh, mild, citrus note | tentative |
| *trans*-limonene oxide | 1142 | trace | fresh, warm, sweet orange | tentative |
| methyl salicylate | 1190 | 0.1 | minty, sweet, spicy | reference |
| methylchavicol | 1195 | 0.2 | sweet, anise note, minty | reference |
| chavicol | 1248 | 0.1 | anise-like, spicy, green minty | reference |
| eugenol | 1359 | 76.8 | spicy (clove cinnamon-like) | reference |
| methyleugenol | 1403 | trace | spicy (clove-like) | reference |
| *cis*-isoeugenol | 1407 | trace | spicy, weak clove note | reference |
| $\beta$-caryophyllene | 1415 | 17.4 | spicy woody, terpene note | reference |
| *trans*-isoeugenol | 1449 | 0.1 | spicy, weak clove note | reference |
| $\alpha$-clovene | 1452 | trace | mild spicy | tentative |
| *cis*-methylisoeugenol | 1455 | trace | clove-like, spicy, aromatic | reference |
| $\alpha$-humulene | 1458 | 2.1 | woody spicy | reference |
| *trans*-methylisoeugenol | 1491 | trace | clove note, mild spicy | reference |
| $\alpha$-farnesene | 1506 | 0.1 | floral oily, weak spicy | reference |
| eugenyl acetate | 1522 | 1.2 | clove-like, balsamic sweet | reference |
| *cis*-isoeugenyl acetate | 1566 | trace | weak clove note | tentative |
| caryophyllene alcohol | 1571 | 0.1 | woody spicy, terpene note | tentative |
| caryophyllene oxide | 1583 | 0.4 | spicy, green woody | reference |
| $\alpha$-humulene epoxide | 1607 | 0.1 | woody, terpene note | tentative |
| *trans*-isoeugenyl acetate | 1614 | trace | weak clove-like | tentative |

[a] In order of their relative retention indices. [b] Retention indices on an apolar OV5-type GC column. [c] Concentrations in percent peak area of GC/FID analysis (apolar column). [d] Odor description given in accordance to the published data (*2*, *28–31*) of aroma chemicals. [e] Compound identified using a reference component or only tentative.

Spectronex, Germany), and Gateway-2000-PS75 data system (Siemens-Nixdorf, Germany, GCQ software) were used. The carrier gas was helium (flow rate of 1.0 mL/min); injector temperature, 250 °C; interface heating at 300 °C; ion source heating at 200 °C; EI mode was 70 eV; and the scan range was 41−450 amu. For other parameters, see the description of GC/FID above. Mass spectra correlations were done using Wiley, NBS, NIST, and our own library as well as published data (*16*, *18*, *19*).

**Olfactoric Evaluations.** The sample and reference compounds were olfactorically evaluated by professional perfumers and aroma chemists, and the odor impressions were compared with published data (*2*, *28*−*31*) and our private databank of reference aroma chemicals.

**Scavenging Effect on the 2,2-Diphenyl-1-picryl Hydrazyl Radical (DPPH).** The radical scavenging ability was determined according to the method of Mensor et al. (*21*). A total of 1 mL from a 0.3 mM ethanol solution of the DPPH radical was added to 2.5 mL from the samples with different concentrations of clove oil and eugenol. The samples were kept at room temperature in the dark, and after 30 min, the optic density was measured at 518 nm. The optic densities of the samples, the control, and the empty samples were measured in comparison with ethanol. The synthetic antioxidants, BHT and BHA, were used as a positive control.

**Detection of Hydroxyl Radicals by the Deoxyribose Assay.** The assay was performed as described by Halliwell et al. (*22*) with minor changes. All solutions were freshly prepared. A total of 1.0 mL of the reaction mixture contained 28 mM 2-deoxy-D-ribose (dissolved in 10 mM $KH_2PO_4/K_2HPO_4$ buffer at pH 7.4), a 500 $\mu$L solution of various concentrations of the clove oil or eugenol, 200 $\mu$M $FeCl_3$, 1.04 mM ethylenediaminetetraacetic acid (EDTA) (1:1, v/v), 10 mM $H_2O_2$, and 1.0 mM ascorbic acid. After an incubation period of 1 h at 37 °C, the extent of deoxyribose degradation was measured by the thiobarbituric acid (TBA) reaction. A total of 1.0 mL of TBA (1% in 50 mM NaOH) and 1.0 mL of trichloroacetic acid (TCA) were added to the reaction mixture, and the tubes were heated at 100 °C for 20 min. After cooling, the absorbance was read at 532 nm against a blank (containing only buffer and deoxyribose). The percentage inhibition was calculated by the formula: $I\,(\%) = 100 - (\mathrm{Abs_{sample}}/\mathrm{Abs_{control}}) \times 100$. The $IC_{50}$ value represented the concentration of the compounds that caused 50% inhibition of radical formation. Quercetin was used as a positive control.

**Evaluation of the Antioxidant Activity in the Linoleic Acid Model System.** Linoleic acid emulsions were prepared by mixing 0.285 g of linoleic acid, 0.289 g of Tween 20 as an emulsifier, and 50 mL of phosphate buffer (pH 7.2). The mixture was homogenized for 5 min according to Yen et al. (*15*). The antioxidant was added at the final concentrations of 0, 0.0025, 0.005, and 0.01 wt %/vol of oil; 0.01% BHT was used as a control. The mixture was incubated in an oven at 37 °C for 10 days. The course of oxidation was monitored by measuring the conjugated dienes (CD) formation and thiobarbituric acid reactive substances (TBARS). The antioxidant activity at the end of the assay time was expressed for each indicator as the reduction percent of peroxidation (RP %), with a control containing no antioxidant being 0%. RP % = [(peroxidation indicator value without antioxidant) − (peroxidation indicator value with antioxidant)/peroxidation indicator value without antioxidant)] × 100. A higher percentage indicates a higher antioxidant activity.

**Determination of CD Formation.** Aliquots of 0.02 mL were taken at different intervals during incubation. After incubation, 2 mL of methanol in deionized water (60%) was added and the absorbance of the mixture was measured at 233 nm. The CD concentration was expressed in milliliters per milligram in each sample. The results were calculated as $\mathrm{CD} = B \times \mathrm{vol/wt}$, where $B$ is the absorbance reading, vol denotes the volume (in milliliters) of the sample, and wt is the mass (in milligrams) of emulsion measured (*23*).

**Determination of TBARS.** A modified TBARS method was used to measure the antioxidant activity of oil in terms of the inhibition on lipid peroxidation. A total of 0.1 mL of sample was taken every day, from the emulsion, and the following were sequentially added: the TBA−TCA solution (20 mm TBA in 15% TCA). The mixture was heated in a 100 °C water bath for 15 min and cooled at room temperature. After 2 mL of chloroform was added, the mixture was mixed and centrifuged at 2000 rpm for 15 min. The chloroform layer was separated, and the absorbance of the supernatant was measured at 532 nm against a blank containing the TBA−TCA solution. Malonaldehyde standard curves were prepared by 1,1,3,3-tetramethoxypropane, and TBARS were expressed as milligrams of malonaldehyde per kilogram of dry matter. The data obtained at each point were the average of three measurements.

**Statistical Analysis.** All experimental data (in triple repetition) were included in an approximation model through polynomic dependences from the fourth order. For all cases, the plural correlation coefficient $R^2$ was determined. The level of the concentration, which corresponds to 50% of inhibition, was calculated according to this approximated

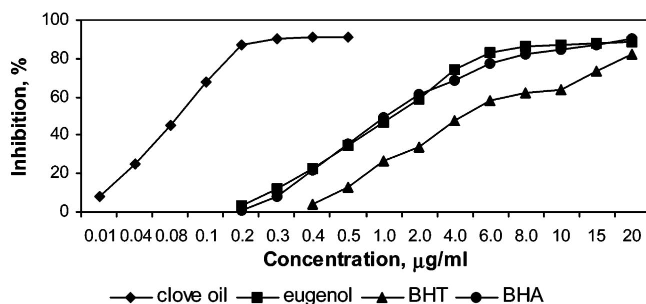


**Figure 1.** Scavenging effects on the DPPH radical of clove oil, eugenol, BHT, and BHA.

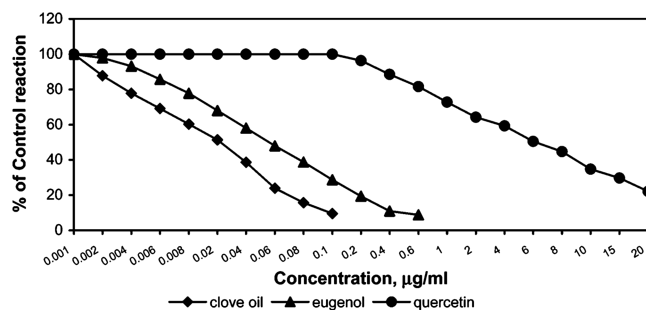


**Figure 2.** Action of clove oil, eugenol, and quercetin on the hydroxyl radical-dependent degradation of deoxyribose.

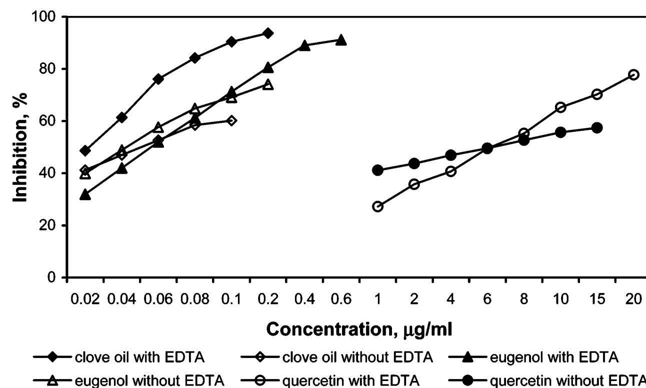


**Figure 3.** Metal-chelating activity of clove oil, eugenol, and quercetin on the deoxyribose degradation by $OH^{\cdot}$.

dependence for which $R^2$ is at a maximum. The mathematical analysis of the data is carried out with the specialized software MatLab 5.3/6.0.

## RESULTS AND DISCUSSION

**GC and GC/MS Analyses.** A total of 23 components were identified as constituents of the essential leaf oil of clove (see **Table 1**), comprising about 99% of the total oil composition. The main components were found to be eugenol (76.8%), followed by $\beta$-caryophyllene (17.4%), $\alpha$-humulene (2.1%), and eugenyl acetate (1.2%). Further constituents were found to be in quantities below 0.5%. The characteristic and pleasant odor of the essential clove oil is determined by main and minor compounds, in accordance to the odor impressions of the single constituents presented in **Table 1**.

**DPPH-Radical-Scavenging Activity.** DPPH is a free-radical compound and has been widely used to test the free-radical-scavenging ability of various samples (*24*−*26*). **Figure 1** shows the scavenging activity of clove oil, eugenol, BHT, and BHA on DPPH radicals at various concentrations. The greatest inhibitory activity observed was in the case of clove oil, reaching as high as 91.2% at 0.5 $\mu$g/mL, while for eugenol, BHT, and BHA, a concentration at 20 $\mu$g/mL was needed to achieve the inhibition of DPPH radicals of 88.7, 82.4, and 90.0%, respectively. The concentration of clove oil resulting in a 50% inhibition of the free radical, $IC_{50}$, was 0.08 $\mu$g/mL, while for eugenol, the corresponding concentration was 1.26 $\mu$g/mL. $IC_{50}$ values were within statistical significance, $p \leq 0.01$, and within high regression coefficients, $R^2 = 0.981$ (for clove oil) and $R^2 = 0.999$ (for eugenol). The standards BHT and BHA had $IC_{50}$ values of 4.41 $\mu$g/mL ($R^2 = 0.998$) and 1.12 $\mu$g/mL ($R^2 = 0.996$), respectively.

**Hydroxyl-Radical-Scavenging Activity.** Among all oxygen radicals, the hydroxyl radical ($OH^{\cdot}$) is the most reactive and damages various biomolecules. Hydroxyl radicals were generated in a reaction mixture containing ascorbate, hydrogen peroxide, and iron(III)−EDTA at pH 7.4 and were measured by their ability to degrade the sugar deoxyribose (*22*, *27*). Clove oil showed high hydroxyl-radical-scavenging activity that intensified with the increase of the concentration, reaching 93.7% at 0.2 $\mu$g/mL (**Figure 2**). The observed degree of $OH^{\cdot}$ inhibition was greater than that of eugenol, which was found to be 91.2% at a concentration of 0.6 $\mu$g/mL. Substantially weaker was the antioxidant activity of quercetin, 77.8%, at 20 $\mu$g/mL. The three studied antioxidants arranged by their antioxidant effect (expressed as $IC_{50}$) in descending order were as follows: clove oil, 0.02 $\mu$g/mL ($R^2 = 0.999$); eugenol, 0.06 $\mu$g/mL ($R^2 = 0.989$); and quercetin, 4.61 $\mu$g/mL ($R^2 = 0.834$). The same analytical method could also be applied for studying the inhibitory power of clove oil against the metal-ion-dependent generation of $OH^{\cdot}$, not only for assaying the ability of capturing already formed radicals. When $Fe^{+3}$ ions are added to the reaction mixture as $FeCl_3$ instead of the EDTA complex, some of the iron ions form a complex with deoxyribose. The $Fe^{+3}$ may be subsequently reduced by ascorbate to $Fe^{+2}$, which, in turn, may remain bound to deoxyribose and further react with $H_2O_2$. The reaction generates the necessary $OH^{\cdot}$ that immediately triggers the degradation of deoxyribose in a site-specific manner. Only molecules that are able to chelate Fe ions and render them inactive may inhibit the degradation of deoxyribose. **Figure 3** (without EDTA) shows that clove oil, eugenol, and quercetin are scavengers of $OH^{\cdot}$ and manifest chelative properties, most strongly expressed in the case of clove oil. Similar to most radicals, $OH^{\cdot}$ can be neutralized by a hydrogen atom. The capture of $OH^{\cdot}$ by clove oil is attributed to the hydrogen-donating ability of the phenol eugenol, which is found in a high concentration (76.8%) in the essential oil.

**Evaluation of the Antioxidant Activity in the Linoleic Acid Model System.** For the purpose of evaluating the antioxidant activity of clove oil, an emulsion of linoleic acid was used as a model system. Clove oil was tested for its ability to inhibit the generation of hydroxyperoxides at the early stages of the oxidation of linoleic acid, as well as for its inhibitory potential after the emergence of secondary oxidized products, such as aldehydes, ketones, or hydrocarbons. The analyses of those properties were carried out by using two indicators, corresponding to the different degrees of lipid peroxidation, CD formation and TBARS.

**Determination of CD Formation.** It was determined that the peak in CD formation was reached on the 5th day following the incubation of linoleic acid (**Figure 4A**). At a concentration of 0.0025%, clove oil expressed an inhibitory effect on lipid peroxidation, comparable to that of BHT at a concentration of 0.01%, namely, 56.5% for clove oil compared to 58.7% for BHT. More significant was the antioxidant activity of 0.005%

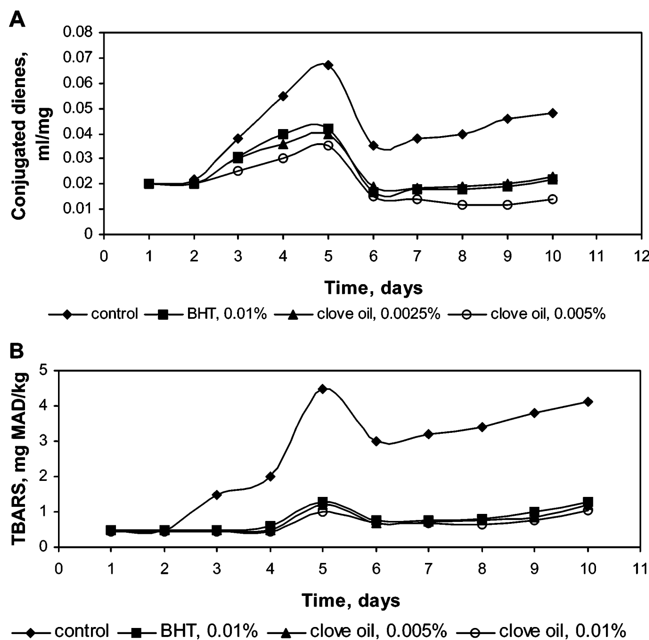


**Figure 4.** Effect of clove oil on (A) CD and (B) TBARS in a linoleic acid/water emulsion system.

clove oil, reaching as high as 73.91%, thus substantially exceeding the antioxidant effect of the standard.

**Determination of TBARS.** With the second indicator used, TBARS, peak accumulation of malonaldehyde was observed once again on the 5th day of linoleic acid storage (**Figure 4B**), suggesting that the process in concern ran almost analogically to the formation of CD. The antioxidant action of 0.005% clove oil was absolutely similar to that of the standard BHT at a concentration of 0.01%; the inhibition of lipid peroxidation reached 77.4% in the case of clove oil and 76.5% for BHT. It should be noted that achieving a degree of process inhibition comparable to that of BHT called for the concentration twice as high as that necessary for the inhibition of CD formation. This finding could most likely be attributed to the differences in the activation energy of the two processes and was considered as additional proof for the conclusion that CD formation ran parallel to the formation of the degradation products. The highest concentration of clove oil applied, 0.01%, resulted in a significantly stronger inhibition of the process, reaching as high as 80.6%.

In conclusion, the antiradical activity of clove oil against the DPPH radical was found to be significantly higher than those of eugenol, BHT, and BHA, manifested at a concentration many times lower than the standards. The inhibitory power of clove oil against $OH^{\cdot}$ was also exhibited at lower concentrations when compared to that of eugenol and quercetin. Clove oil manifested considerable chelate-generating potential against $Fe^{+3}$ as well, resulting in the prevention of the initiation of hydroxyl radicals. The clove oil was found to inhibit effectively the CD formation and the generation of secondary products from lipid peroxidation, both actions performed in concentrations lower than those of the standard BHT. The stage of generation of secondary products from lipid autoxidation was also inhibited; i.e., clove oil could be applied as an antioxidant even at the later stages of lipid oxidation. The current study shows that clove oil possesses significant antioxidant capacity and can readily be used as a natural preservative to minimize or prevent product losses emerging from oxidative processes.

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