

Chemical Composition and *In vitro* Antioxidant and Antidiabetic Activities of *Eucalyptus Camaldulensis* Dehnh. Essential Oil

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The present study was designed to determine the composition of the essential oil of *Eucalyptus camaldulensis* Dehnh. leaves and to examine its *in vitro* antioxidant and antidiabetic activities. The chemical composition of the essential oil from *Eucalyptus camaldulensis* Dehnh. leaves was analyzed by GC/GC-MS, twenty-nine compounds representing 99.10% of the total oil were identified. The major components of the oil were *p*-cymene (68.43%), 1,8-cineole (13.92%), 1-(S)- α -pinene (3.45%) and R-(+)-limonene (2.84%). The antioxidant features of the essential oil were evaluated using inhibition of 2,2-diphenyl-1-picrylhydrazyl, hydroxyl, and superoxide radicals, inhibition of hydrogen peroxide and lipid peroxidation assays. We also studied α -amylase and α -glucosidase inhibition *in vitro* to assess the antidiabetic properties of the essential oil. Both α -amylase and α -glucosidase were inhibited by a non-competitive mechanism.

Keywords: Antioxidant activity, Essential oil, *Eucalyptus camaldulensis* Dehnh, GC-MS, α -amylase, α -glucosidase

INTRODUCTION

Aerobic organisms are in absolute need of molecular oxygen to survive, oxygen in high concentrations has toxic effects. The toxic effects are caused by reactive species called "oxygen radicals" [1]. Some of the reactive oxygen species (ROS), including hydrogen peroxide, singlet oxygen, hydroxyl and superoxide radicals, have positive roles in energy production in *in vivo* systems, phagocytosis, intercellular signal transfer, regulation of cell growth and the synthesis of important biological compounds [2]. Additionally, reactive oxygen species modify DNA and membranes by attacking the lipids, proteins, and carbohydrates in cell membranes and tissues [3].

The defense systems that work to prevent ROS damage in

the body are called antioxidant defense systems [4]. In the organism, the rates of production and removal of free radicals are in balance, known as oxidative balance. An increase in the rate of production or a decrease in the rate of removal disrupts this balance and increases the levels of ROS. This condition, which is called oxidative stress, indicates a serious imbalance between the production of free radicals and the antioxidant defense systems, resulting in tissue damage [5]. A relationship between ROS and many diseases, including myocardial infarction, neurological disorders, asthma, diabetes, rheumatoid arthritis, cancer and aging, has been observed [6].

Diabetes (Diabetes mellitus (DM)) is a disease that arises from total or partial insulin deficiency, and is characterized by high blood sugar (hyperglycemia). Uncontrolled high blood sugar increases the risk of paralysis, gangrene and coronary diseases owing to long-term complications such as kidney failure from nephropathia, nervous system disease

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(neuropathy), blindness due to retinosis, and foot ulcers [7,8].

As postprandial glucose control has been proven to be important for the prevention of diabetic complications, control of postprandial plasma glucose levels is critical for the early treatment of diabetes and for the reduction of chronic vascular complications. One of the therapeutic approaches to lower postprandial blood sugar is to delay the absorption of glucose by inhibiting carbohydrate hydrolysis enzymes such as α -amylase and α -glucosidase in the digestive organs. For this reason, efforts to develop new pharmacological agents focus on the inhibition α -amylase and α -glucosidase [9,10].

Essential oils are found in various parts of plants, such as the leaf, flower, root and body, and are stored in special oil cells and gates. The essential oils extracted from plants are indispensable materials in the pharmaceutical, food and cosmetics sectors. A great majority of the essential oils are used as fragrances in perfumes and as aromas in the food industry. The essential oils have a number of biological activities, including antibacterial, antifungal and antioxidant properties [11,12].

Myrtaceae is a plant belonging to the (Myrtle) family. The eucalyptus tree that grows in Southern Anatolia is known as 'Adana eucalyptus'. The material used in our study is the leaf of *Eucalyptus camaldulensis* Dehnh. (Myrtaceae). Its leaves are 15-30 cm long, 2-5 cm wide, hard, crisp and yellowish-green in color [13]. Several hundred species of *Eucalyptus* contain volatile oils which are classified into three types; medicinal, perfumery and industrial [14]. Some studies have demonstrated that leaf extract and essential oil of *Eucalyptus* spp. have antifungal, repellent, antibacterial, analgesic and anti-inflammatory activities [15,16]. In India, the leaf essential oil is traditionally used externally as a mosquito repellent and as an insecticide. In Spain, the essential oil of fruit and leaf is traditionally used in inhalation therapy for the treatment of colds and catarrh [17]. In France, Spain and Guetamala, hot water extract of the leaf is taken orally as a hypolycemic [17]. In Turkey, the oil of *Eucalyptus* smells of camphor, affecting the nervous system and providing a feeling of relief. Additionally, it is used locally to stop bleeding, can be applied by massaging to stop migraine headaches, can be chewed to treat respiratory tract congestion, chronic bronchitis, coughing, tuberculosis, gum and mouth diseases, and malaria, and can be applied as a dressing material to treat wounds and

inflammations [13].

MATERIALS AND METHODS

Chemicals

Ascorbic acid, trichloroacetic acid (TCA), xanthine-oxidase, hydrogen peroxide (H_2O_2), methanol, n-hexene, n-butanol, hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), sulfuric acid (H_2SO_4), sodium phosphate (Na_3PO_4), ammonium molybdate, curcumin, potato starch, α -amylase, sodium potassium tartarate.tetrahydrate, sodium hydroxide (NaOH), Tris-HCl, 3,5-dinitrosalicylic acid (DNS), sodium chloride (NaCl), maltose, sodium disulphite ($Na_2S_2O_5$), glucose, buthylated hydroxytoluene (BHT), eisen (III) chloride ($FeCl_3$), etilen diamine tetra acetic acide (EDTA), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogene phosphate (NaH_2PO_4), dipotassium hydrogen phosphate (K_2HPO_4) sodium dihydrogene phosphate (KH_2PO_4) were obtained from Merck (Darmstad, Germany). Thiobarbituric acid (TBA), xanthine, nitroblue tetrazolium (NBT), 2,2,-diphenyl-picrylhydrazyl (DPPH), α -glucosidase, para-nitrophenol- α -D-glucopyranoside (pNPG) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Apparatus

Unicam UV spectrometer, electro.mag M₁₆ heater-stirrer, Gec avery weight, Memmert water-batch, Labor alliance heater-jacket, Jenway 3010 pH meter, electro.mag vortex, B. Braune homegenizatore and Centrifuge 5810 R centrifugatore were used in experimental procedures.

Collection of Plant Material

Eucalyptus camaldulensis Dehnh. leaves, collected in June 2006 from Adana in Turkey, were identified in the Biology Department of Cumhuriyet University by Dr. Erol DÖNMEZ. A voucher specimen of plant ED (14253) was deposited in the Herbarium Laboratory in the Biology Department at the University of Cumhuriyet (CU).

Extraction of Essential Oil

The leaves of *Eucalyptus camaldulensis* Dehnh. were shade-dried and subjected to water distillation for 3 h using a Clevenger-type apparatus (yield 0.254% v/w). The obtained

essential oil was stored at +4 °C until testing and analysis.

Gas Chromatography (GC)

The GC analysis of the essential oil was performed using a Trace-GC-ultra with an INNOWAX capillary column (length 60 m, inner diameter and film thickness 0.25 mm 0.25 µm, respectively.) Helium gas was used as the carrier at a flow rate of 1 ml min⁻¹. Injector and MS transfer line temperatures were set at 200 and 250 °C, respectively. The GC oven temperature was kept at 50 °C for 2 min, then increased to 250 °C at a rate of 5 °C min⁻¹ and held for 5 min. Diluted samples (1:100 v/v, in acetone) of 1.0 ml were injected manually in the splitless mode.

Gas Chromatography/Mass Spectrometry (GC/MS)

Analyses were conducted under the same column and conditions with GC interfaced with FINNIGAN Trace-DSQ mass spectrometer (ionization energy of 70 eV). The mass range was from an m/z of 50 to 450. Identification of compounds was based on comparisons of the relative retention indices and mass spectra with those of the Wiley and NIST library data standards of the GC/MS system.

Antioxidative Capacity

Hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity was carried out by measuring the hydroxyl radicals generated from the Fe³⁺/ascorbate/EDTA/H₂O₂ system [18]. The attack of the hydroxyl radical to deoxyribose leads to the formation of thiobarbituric acid reactive substances (TBARS) [19]. Various concentrations of the samples (essential oil and three major components) (in n-hexene) were added to a reaction mixture containing 3.0 mM deoxyribose, 0.1 mM FeCl₃, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H₂O₂ and 20 mM phosphate buffer (pH 7.4), at a final volume of 3.0 ml. The reaction mixture was incubated at 37 °C for 1 h. Then, 1 ml of thiobarbituric acid (TBA, 1%) and 1.0 ml of trichloroacetic acid (TCA, 2.8%) were added to test tubes where they were incubated at 100 °C for 20 min. After the mixtures cooled, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. The percentage inhibition (I) of deoxyribose degradation was calculated in the following way:

$$\%I = (A_0 - A_1/A_0) \times 100$$

where A₀ is the absorbance of the control reaction (containing all reagents except the test compound) and A₁ is the absorbance of the test compound.

Inhibition of superoxide radical. Superoxide radical generation by the xanthine/xanthine oxidase system was determined spectrophotometrically by monitoring the production of nitroblue tetrazolium (NBT) [20]. Various concentrations of the samples (in n-hexene) were added to a reaction mixture containing 2 nM xanthine, 12 nM NBT, 1.0 U ml⁻¹ xanthine oxidase, and 0.1 M phosphate buffer (pH 7.4), making up a final volume of 2.0 ml. After the incubation of the mixture at 25 °C for 10 min, the absorbance was read at 560 nm and compared with the control samples in which the enzyme was not included.

Hydrogen peroxide scavenging activity. The ability of the essential oil to scavenge hydrogen peroxide was determined spectrophotometrically as described previously [21]. Briefly, a solution of hydrogen peroxide (2 mM) was prepared in 0.17 M phosphate buffer (pH 7.4). Various concentrations of the samples (in methanol) were added to the reaction mixture containing 2 mM hydrogen peroxide. After a 10 min incubation at room temperature, the absorbance was read against a blank at 230 nm.

Lipid peroxidation inhibition assay. Assays for non-enzymatic lipid peroxidation were performed as described [22]. With minor changes. Rat liver (25% (w/v)) was homogenized with 40 mM Tris-HCl buffer (pH 7.0) in three strokes. The homogenate was centrifuged at 10,000 g for 120 min and the supernatant was used in the experimental studies. A 1 ml sample of the reaction mixture contained 100 µl of different concentrations of the essential samples (in n-hexene), 100 µl supernatant, 20 µl 1 mM FeCl₃ and 20 µl 1 mM ascorbic acid to induce hydroxyl radical generation. After an incubation period of 1 h at 37 °C, the extent of lipid peroxidation was measured by the TBA reaction. Then, 1 ml TBA and 1.0 ml 2.8% TCA were added and the test vials were heated to 100 °C for 20 min. After cooling, 2.5 ml n-butanol was added and the samples were centrifuged at 3500 rpm for 5 min. The absorbance was read at 532 nm.

DPPH assay. The DPPH assay was measured by following the bleaching of a purple methanol solution of DPPH [23]. Various concentrations of the samples (in methanol) were added to 5 ml of a 0.004% solution of DPPH

in methanol. After a 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm.

All experiments were replicated three times and all percentage inhibition values were calculated in line with the equality in the scavenging method for hydroxyl radical.

Effect on α -Amylase and α -Glucosidase

α -Amylase inhibitory assay. The bioassay method was adopted and modified from Sigma-Aldrich [24]. A starch solution (0.5% w/v) was obtained by stirring 0.125 g of potato starch in 25 ml of 20 mM sodium phosphate buffer (with 6.7 mM sodium chloride, pH 6.9) at 65 °C for 15 min. The enzyme solution was prepared by mixing 0.0253 g of α -amylase in 100 ml of cold distilled water. The colorimetric reagent was prepared by mixing a sodium potassium tartarate solution (12.0 g of sodium potassium tartarate, tetrahydrate in 8.0 ml of 2 M NaOH) and 96 mM 3,5-dinitrosalicylic acid solution. Both the control and samples (in n-hexane) were added to the starch solution and left to react with α -amylase solution under alkaline conditions at 25 °C. The reaction was measured over 3 min.

The generation of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction (corresponding to color change from orange-yellow to red) was detectable at 540 nm. In the presence of α -amylase inhibitors, less maltose would be produced and the absorbance value would be decreased. The α -amylase inhibition was expressed as a percentage of inhibition and calculated by the following equation:

$$\% \text{Inhibition} = 100 - \{[(\text{maltose})_{\text{test}}/(\text{maltose})_{\text{control}}] \times 100\}$$

Kinetic studies of *Eucalyptus camaldulensis* Dehnh. essential oil for α -amylase. A calibration curve was generated using the 3-5-dinitrosalicylic [DNS] colorimetric glucose assay method [25] with minor modifications. Briefly, 3 ml of 1% [DNS] reagent solution, (dinitrosalicylic acid (10 g), sodium disulphite (0.5 g) and sodium hydroxide (10 g) in 1 l distilled water) was added to 3 ml of glucose solutions of increasing concentrations (0.625-10 g l⁻¹). The test tubes were then capped and the mixture was heated to 90 °C for 5-15 min until a red-brown color developed. Then, 1 ml of a 40% potassium sodium tartarate solution was added. The test tubes

were cooled under tap water and the absorbance was measured at 575 nm.

Control experiments. All reagents were pre-incubated for 15 min at 37 °C in a water bath. To 0.5 ml of increasing concentrations of starch solution [(1-50 g l⁻¹) in 0.2 M sodium phosphate buffer at pH 7.0] 0.25 ml of buffer and 0.25 ml of α -amylase enzyme solution (30 μ g ml⁻¹ in 0.2 M sodium phosphate buffer containing 0.006 M NaCl, pH 7.0) were added. After 3 min, the reaction was stopped by the addition of 2 ml DNS reagent and heating in a boiling water bath at 90 °C for 10 min. Then, 1 ml of a 40% potassium sodium tartrate solution was added. The test tubes were cooled under tap water and the absorbance was measured at 575 nm.

Incorporation of the inhibitor for α -amylase. A sample of 0.25 ml of essential oil of *Eucalyptus camaldulensis* Dehnh. (in hexane) was incubated with 0.25 ml of α -amylase enzyme solution for 15 min at 37 °C. The above-mentioned procedure was then repeated.

The Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) in the presence and absence of essential oil of *Eucalyptus camaldulensis* Dehnh. were determined using the Lineweaver-Burk equations.

α -Glucosidase inhibitory assay. A previously described bioassay method [26] was used to measure α -glucosidase inhibition by samples dissolved in n-hexane. All reagents were pre-incubated for 15 min at 37 °C in a water bath. Then, 0.20 ml of α -glucosidase enzyme solution (0.01 U in 50 mM sodium phosphate buffer with 100 mM NaCl, pH 6.9) was added to 0.1 ml of increasing concentrations of essential oil and incubated for an additional 15 min at 37 °C. Afterwards, 0.20 ml of the *para*-nitrophenol- α -D-glucopyranoside (pNPG) solution (2 mM pNPG in 50 mM sodium phosphate buffer, pH 6.9) was added and incubated for 30 min at 37 °C, and the reaction was stopped by the addition of 1 ml 0.1 M Na₂HPO₄. The test tubes were cooled under tap water and the absorbance was measured at 400 nm. The α -glucosidase inhibition was expressed as a percentage of inhibition and was calculated using the following equation:

$$\% \text{Inhibition} = 100 - \{[(\text{pNPG})_{\text{test}}/(\text{pNPG})_{\text{control}}] \times 100\}$$

Kinetic studies of *Eucalyptus camaldulensis* Dehnh. essential oil for α -glucosidase. A calibration curve was

generated using the pNPG standard [27]. Briefly, 1 ml 0.1 M Na₂HPO₄ solution was added to increasing concentrations of pNPG solution (4.00×10^{-4} - 0.018 mg ml⁻¹) and boiled for 10 min. The test tubes were cooled under tap water and the absorbance was measured at 400 nm.

Control experiments. All reagents were pre-incubated for 15 min at 37 °C in a water bath. Then, 0.20 ml of α -glucosidase enzyme solution (0.01 U in 50 mM sodium phosphate buffer with 100 mM NaCl, pH 6.9) and 0.10 ml buffer were added to 0.2 ml of increasing concentrations of pNPG solution [(0.125-2 mM) in 50 mM sodium phosphate buffer, pH 6.9]. After 3 min, the reaction was stopped by the addition of 1 ml 0.1 M Na₂HPO₄ and boiling for 10 min. The test tubes were then cooled under tap water and the absorbance was measured at 400 nm.

Incorporation of the inhibitor for α -glucosidase. An 0.10 ml sample of *Eucalyptus camaldulensis* Dehnh. essential oil (in hexane) was incubated with 0.01 U of α -glucosidase enzyme solution in 0.20 ml for 15 min at 37 °C. The above procedure was then repeated.

The Michaelis-Menten constant (K_m) and maximal velocity (V_{max}) in the presence and absence of essential oil of *Eucalyptus camaldulensis* Dehnh. were determined using Lineweaver-Burk equations.

Statistical analysis. For the essential oil or standard compounds, three samples were prepared for each assay. The data are presented as mean \pm standard deviation of three experiments. Statistical analyses were performed using the Sigma Plot graphing and statistical program and the Student's t-test. p values < 0.01 were regarded as significant. IC₅₀ values were calculated from the dose-response curve (Sigma Plot Graph and statistical Program 9.0) obtained by plotting the percentage of inhibition vs. the concentrations.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

As the biological activities of an essential oil are determined by the compounds contained within the oil, we first determined the chemical composition of the essential oil. A GC/GC-MS analysis identified 29 compounds that accounted for 99.10% of the content of eucalyptus essential oil, with *p*-cymene (68.43%), 1,8-cineole (13.92%), 1-(S)- α -

pinene (3.45%) and R-(+)-limonene (2.84%) as the main constituents (Table 1).

Leaf oils of *E. camaldulensis* Dehn. from Mozambique obtained by hydrodistillation and by supercritical carbon dioxide extraction were compared according to their major components. The major components of the oil obtained by hydrodistillation were 1,8-cineole (43%), α -pinene (5.5%), β -pinene (3.4%), *p*-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%). The extracts obtained by supercritical carbon dioxide extraction have higher amounts of alloaromadendrene and globulol, but have lower amounts of 1,8-cineole, α -pinene, β -pinene, and terpinen-4-ol [28]. Essential oil of *E. camaldulensis* Dehn. fruit which were obtained from four different areas in Turkey and the main compounds found in the volatile oils were: aromadendrene (6.45-15.02%), eucalyptol (0.17-12.61%), gamma-gurjunene (8.40-10.08%), terpinolen (1.98-8.39%), spathulenol (1.42-8.34%), α -pinene (0.85-6.81%), ledene (0.94-6.72%), and longifonene (0.07-6.22%) [29]. The results show that chemical composition of an essential oil can be affected by several factors; climate, obtained condition, etc. Essential oils of herbal origin have diverse biological activities which are caused by the monoterpenes that are their main components; herbal essential oils are composed mostly of monoterpenes, sesquiterpenes and their oxygenic derivatives [30].

Antioxidant Activity of the Essential Oil

The role of antioxidants in preventing various degenerative and age-related diseases, which are caused by oxidative stress, is becoming increasingly clear through experimental, clinical and epidemiological studies; thus, the antioxidants have started to gain more importance. However, since it has been stated that synthetic antioxidants cause liver damage and have carcinogenic properties, the focus has shifted towards natural antioxidants [31].

The IC₅₀ (concentration of essential oil for 50 percent inhibition) values of the essential oil and positive standards for the reactive oxygen species and DPPH inhibition are presented in Table 2.

Hydroxyl radical scavenging activity was examined by measuring the hydroxyl radicals generated from the Fe³⁺/ascorbate/EDTA/H₂O₂ system. It was determined that essential oil of eucalyptus possesses hydroxyl radical

Table 1. GC-MS Analysis of the *Eucalyptus Camaldulensis* Dehnh. Essential Oil

Compound	^a RT	^b RI	^c %
5-Amino-4-cyano-3-(4-ethylaminobutyl) pyrasole	8.62	785	0.01
1-(S)- α -pinene	13.78	915	3.45
Trance-oxobycycle[3.3.0]oct-7-en-2-on,4-methoxy-7-methyl	14.62	932	0.03
α -Methyl-benzenmethanol	16.22	964	0.24
2- α -Pinene	16.60	972	0.24
Bicyclo[3.1.0]hex-2-en, 4-methylene-1-(1-methylethyl)	17.15	1237	0.84
1-Phellandrene	18.33	1006	1.35
α -Terpinene	18.78	1014	1.30
R-(+)-limonene	19.40	1026	2.84
1,8-Cineole	19.77	1033	13.92
γ -Terpinene	20.78	1052	0.77
2-Methylprop-1-enyl-cyclohexa-1,5-dien	21.05	1057	0.21
<i>p</i> -Cymene	21.67	1068	68.43
α -Terpinolene	21.98	1074	0.19
<i>p</i> -Ment-1-en-3,8-diol	23.30	1099	0.09
3-Methyl-2-(2-penthenyl)cyclopentanone	25.39	1138	0.03
Linalool oxide fraction (2)	26.33	1156	0.18
α -Thujone	26.87	1166	0.34
Linalool oxide fr (1)	27.12	1171	0.10
α -Campholen aldehyde	28.11	1189	0.07
L-Linalool	28.56	1198	0.13
Trance-cyclohexanone, 5-methyl-2-(1-methylethenyl)	29.27	1112	0.08
2-Cyclohexane-1-on, 6-methyl-3-(1-methylethyl)	29.71	1220	0.09
(R)-2-Cyclohexane 1-on, 2-methyl-5-(1-methylethenyl)	30.28	1232	0.04
4-(1-Methylethyliden) cyclohexanone	30.63	1239	0.31
1,4-Terpineole	30.74	1241	2.56
Trance-pinocarveol	32.23	1270	0.77
<i>p</i> -Ment-1-en-8-ol	32.89	1283	0.45
Trance-pinocarvilacetate	33.00	1296	0.04
Total			99.10

^aRetention time (min). ^bRetention index relative to (C₈-C₁₆) n-alkanes. ^c%, Relative proportions of the essential oil components expressed as percentages obtained by GC-MS.

scavenging activity greater than BHT and curcumin, which were used as positive controls. Curcumin's hydroxyl radical scavenging activity was greater than BHT. Because ascorbic acid was a component of the assay, it was not used as a positive standard for hydroxyl radical scavenging. Although IC₅₀ value of 1,8-cineole was observed, IC₅₀ values of *p*-cymene and 1-(S)- α -pinene were not determined. 16.98%

(1.11 μ l ml⁻¹) and %28.75 (2.42 μ l ml⁻¹) were the highest hydroxyl radical scavenging inhibition percentages and concentrations of *p*-cymene and 1-(S)- α -pinene, respectively.

Essential oil and positive standards were also found to scavenge superoxide radicals (O₂⁻) generated by a xanthine and xanthine oxidase system. Similar to hydroxyl radical scavenging activity, the essential oil possessed the greatest

Table 2. Antioxidant Activity of the *Eucalyptus Camaldulensis* Dehnh. Essential Oil

Sample	Hydroxyl ^a IC ₅₀ (µl ml ⁻¹)	Superoxide ^a IC ₅₀ (µl ml ⁻¹)	Hydrogen peroxide ^a IC ₅₀ × 10 ⁴ (µl ml ⁻¹)	Lipid peroxidation ^a IC ₅₀ (µl ml ⁻¹)	DPPH ^a IC ₅₀ (µl ml ⁻¹)
Essential oil	0.268 ± 0.015	0.069 ± 0.006	5.612 ± 0.22	0.055 ± 0.004	4.096 ± 0.724
Curcumin	13.56 ± 1.19	8.655 ± 0.222	3.70 ± 0.10	1.220 ± 2.013	9.60 ± 0.46
Asc. acid	nt	92.450 ± 4.14	11.20 ± 0.56	nt	17.57 ± 2.86
BHT	32.00 ± 1.61	62.301 ± 0.903	6.984 ± 0.337	3.022 ± 1.346	23.750 ± 0.500
<i>p</i> -Cymene	-	-	-	0.422 ± 0.160	-
1,8-Cineole	2.25 ± 0.12	-	-	0.020 ± 0.221	-
1-(S)- α -Pinene	-	-	-	0.328 ± 0.780	-

^aValues are expressed as mean ± SD.

capacity to scavenge superoxide radicals. The superoxide radical scavenging activity of standards decreased in the following order: curcumin, BHT and ascorbic acid, respectively. IC₅₀ values of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were not observed. 38.88% (0.340 µl ml⁻¹), 42.20% (0.238 µl ml⁻¹) and 26.67% (1.136 µl ml⁻¹) were the highest superoxide radical inhibition percentages and concentrations of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene, respectively.

It was seen that essential oil had lower hydrogen peroxide scavenging activity than the curcumin being used as a positive control. Essential oil was found to have a higher hydrogen peroxide scavenging activity than the other positive controls, BHT and ascorbic acid. The hydrogen peroxide scavenging activity of BHT was similar to that of the essential oil and greater than ascorbic acid. Similar to superoxide scavenging activity IC₅₀ values of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were not observed. The highest hydrogen peroxide inhibition percentages and concentrations for *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were 25.51% (0.98 µl ml⁻¹), 26.81% (1.22 µl ml⁻¹) and 20.85% (1.95 µl ml⁻¹), respectively.

Epidemiological studies have shown that increased lipid peroxidation occurs in plasma lipoproteins, erythrocyte membrane lipids and various tissues in diabetes [32]. Essential oil was found to effectively inhibit non-enzymatic lipid peroxidation in rat liver homogenates more effectively than the positive standards, as indicated by decreased thiobarbituric acid-reactive substance (TBARS) formation. It was observed that curcumin was more active than BHT in inhibiting lipid peroxidation. IC₅₀ values of *p*-cymene, 1,8-cineole and 1-(S)-

α -pinene were observed for lipid peroxidation inhibition. The lipid peroxidation inhibition activity of three major components of essential oil increased in the following order: *p*-cymene, 1-(S)- α -pinene and 1,8-cineole.

In the presence of antioxidants, the characteristic purple color of DPPH lightens. By spectrophotometrically following the essential oils, which are prepared by dissolving in methanol, the bleaching of a 0.004% DPPH solution was used to determine the inhibition of DPPH radical formation. The results showed that essential oil possessed the greatest reducing activity of all samples. Among the positive standards, ascorbic acid had the highest DPPH radical reducing activity followed by curcumin. The lowest activity was observed for BHT. IC₅₀ values of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were not observed for DPPH radical scavenging activity. 37.65% (125 µl ml⁻¹), 42.65% (125 µl ml⁻¹) and 40.37% (125 µl ml⁻¹) were the highest DPPH inhibition percentages and concentrations of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene, respectively.

The components *p*-cymene, 1,8-cineole and 1-(S)- α -pinene share a monoterpene structure. Terpenes, including monoterpene, constitute the largest class of secondary metabolites. There are diverse studies which maintain that *p*-cymene has low or no antioxidant activity [33]. Earlier studies have indicated that 1-(S)- α -pinene and 1,8-cineole have antioxidant activities, too [34].

Antioxidant activity of essential oil can be attributed to the presence of some components that have antioxidant activity [35]. Although the amounts of these compounds are

Table 3. Inhibitory Activity of Eucalyptus Essential Oil Against α -amylase and α -glucosidase

Sample	Concentration ($\mu\text{l ml}^{-1}$)	%Inhibition of α -amylase ^a	Concentration ($\mu\text{l ml}^{-1}$)	%Inhibition of α -glucosidase ^b
Essential oil	0.093	22.35 \pm 1.28	0.150	20.05 \pm 1.28
	0.186	32.00 \pm 0.78	0.350	35.28 \pm 0.98
	0.372	48.22 \pm 1.05	0.634	52.15 \pm 0.89
	0.465	52.11 \pm 0.06	0.975	60.35 \pm 0.10
	0.559	54.05 \pm 0.56	1.400	69.00 \pm 1.16
	0.745	55.00 \pm 2.00	1.904	80.36 \pm 2.05
			2.536	88.54 \pm 0.08
		3.174	93.25 \pm 2.64	
<i>p</i> -cymene	0.115	36.50 \pm 1.50	1.308	50.00 \pm 0.18
1,8-cineole	0.075	43.23 \pm 2.57	1.118	50.00 \pm 0.97
1-(S)- α -pinene	0.075	32.22 \pm 1.73	1.420	50.00 \pm 0.65

^aAll determinations were carried out in triplicate and averaged. The α -amylase inhibitory activity (%) was defined as the percent decrease in the maltose production rate over the control. ^bAll determinations were carried out in triplicate and averaged. The α -glucosidase inhibitory activity (%) was defined as the percent decrease in the pnpg rate over the control.

relatively low in oil, possible antagonistic and synergistic effects of compound in the oil could also be taken into consideration [36]. Hydroxyl, superoxide and DPPH scavenging activity of *Eucalyptus camaldulensis* Dehnh. essential oil was found to be significantly higher than the main components of oil and standard compounds. Higher scavenging activity of essential oil could be explained by the assumption that synergistic effect of components in essential oil or minor compounds could have radical scavenging activity. In the hydrogen peroxide scavenging assay, essential oil demonstrated more effective scavenging activity than major components and ascorbic acid which was used as standard compound. Hydrogen peroxide scavenging activity of essential oil was similar to BHT but lower than curcumin. Lipid peroxidation inhibition property of essential oil was lower than 1,8-cineole but higher than the other two main components and standards. This shows that 1,8-cineole antagonistically impresses other components in oil.

Antidiabetic Activity of the Essential Oil

The role of reactive oxygen species in diabetes has been a

widely discussed issue since the 1980's, and there has recently been an increase in studies on the connection between oxidative stress, diabetes and diabetic complications [37]. It has been observed that in diabetes, as a result of the increase in oxidative stress, the production of free radicals increases, but the production of antioxidants decreases. Thus, increased free radical concentration is considered as one of the important complications of diabetes [38].

Diabetes is a disease that plays a role in the pathology of many other diseases and is prevalent worldwide. Controlling postprandial blood sugar is a measure for the prevention of diabetic complications. Thus, the inhibition of α -amylase and α -glucosidase, which are carbohydrate digestion enzymes, is important. In Table 3, α -amylase inhibition percentages, which are observed for different concentrations of the essential oil, and the three major components are presented. The highest α -amylase inhibition occurred with 55.00 \pm 2.00% eucalyptus essential oil. The IC₅₀ (concentration of essential oil for 50 percent inhibition of α -amylase) value of eucalyptus essential oil was determined to be 0.435 \pm 0.003 $\mu\text{l ml}^{-1}$. IC₅₀ values of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were not observed

for α -amylase inhibition. In Table 3, the highest α -amylase inhibition percentages of the three major components are presented.

Since α -glucosidase inhibitors cause a decrease of the glucose absorption speed and the postprandial blood glucose level, α -glucosidase inhibition is an important target for the regulation of Type II diabetes [39]. The percentages of α -glucosidase inhibition for various concentrations of the essential oil are presented in Table 3.

The highest α -glucosidase inhibition by the eucalyptus essential oil was $93.25 \pm 2.64\%$. The IC_{50} (concentration of essential oil for 50 percent inhibition of α -glucosidase) value of eucalyptus essential oil was determined to be $0.548 \pm 0.006 \mu\text{l ml}^{-1}$. IC_{50} values of *p*-cymene, 1,8-cineole and 1-(S)- α -pinene were observed for α -glucosidase inhibitions. α -Glucosidase inhibitions property of the three major components of essential oil decreased; 1,8-cineole, *p*-cymene and 1-(S)- α -pinene, respectively. The highest α -glucosidase inhibition percentages and concentrations for *p*-cymene, 1,8-cineole and 1-(S)- α -pinene, were 79.22% ($4.361 \mu\text{l ml}^{-1}$), 82.20% ($3.489 \mu\text{l ml}^{-1}$) and 60.02% ($1.815 \mu\text{l ml}^{-1}$), respectively.

Because of were not determined IC_{50} values of major components for α -amylase inhibition activity, α -amylase inhibition activity of essential oil was higher than three major components. Similar results were obtained for α -glucosidase inhibition property of essential oil. Since IC_{50} value of essential oil was lower than that of the major three components, it was concluded that essential oil inhibition activity of α -glucosidase was higher than that of the components. These results imply that the main components synergize each other or other components in essential oil. A feature of monoterpenes may influence their inhibition activity of α -amylase and α -glucosidase. Further investigations can determine which features such as position and number of double bonds, position of adjacent group(s) or cyclic components, favor α -amylase and α -glucosidase inhibitor.

Catalytic studies for α -amylase (Fig. 1) and for α -glucosidase (Fig. 2) were performed using the Lineweaver-Burk equations. It was observed that both the maximal velocity (V_{max} , y-intercept) and the Michaelis-Menten constant (K_m , slope of the trend lines) values decreased with increasing concentrations of essential oil. This means that the essential oil acted as a non-competitive inhibitor of α -amylase.

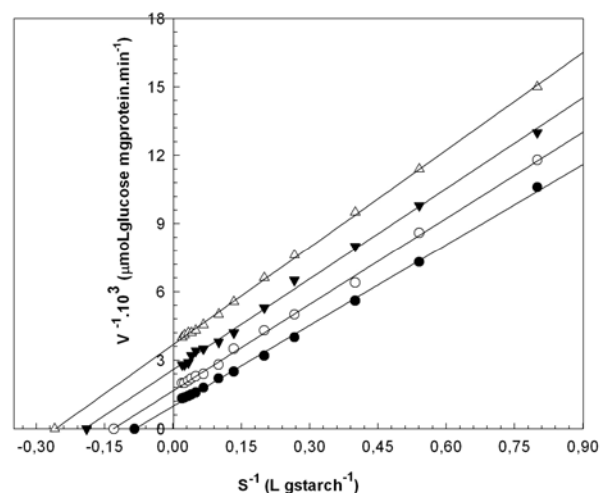


Fig. 1. Lineweaver-Burk plots derived from the inhibition of α -amylase by essential oil of *Eucalyptus camaldulensis* Dehnh. α -amylase was treated with each stated concentration of starch solution ($1\text{-}50 \text{ g l}^{-1}$) in the absence and presence of essential oil. The concentrations of essential oil were: (●) no inhibitor; (○) $0.187 \mu\text{l ml}^{-1}$; (▼) $0.425 \mu\text{l ml}^{-1}$ and (Δ) $0.745 \mu\text{l ml}^{-1}$.

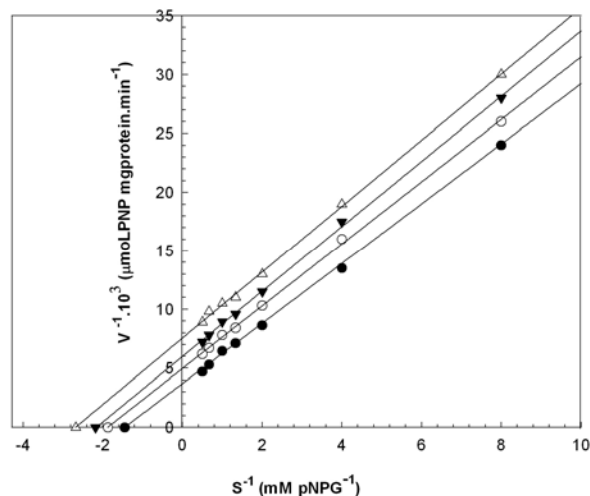


Fig. 2. Lineweaver-Burk plots derived from the inhibition of α -glucosidase by essential oil of *Eucalyptus camaldulensis* Dehnh. α -glucosidase was treated with each stated concentration of pNPG ($0.125\text{-}2 \text{ mM}$) in the absence and presence of essential oil. The concentrations of essential oil were: (●) no inhibitor; (○) $0.125 \mu\text{l ml}^{-1}$; (▼) $0.330 \mu\text{l ml}^{-1}$ and (Δ) $0.550 \mu\text{l ml}^{-1}$.

Non-competitive inhibitors do not compete with substrate to bind the active region of the free enzyme. They bind to enzyme-substrate complexes, resulting in enzyme-substrate-inhibitor complexes. For this reason, inhibition cannot be overcome by increasing the concentration of substrate. When the concentration of essential oil was plotted against $1/V_{\max}$ (observed), the K_i value was determined as $0.29 \pm 0.15, \mu\text{l ml}^{-1}$ via the Least Squares Method. It was observed that both the maximal velocity (V_{\max}) and the Michaelis-Menten constant (K_m) values decreased with increasing concentrations of essential oil. This means that, similar to α -amylase inhibition, the essential oil inhibited α -glucosidase via non-competitive inhibition. When the concentration of essential oil was plotted against $1/V_{\max}$ (observed), the K_i was determined to be $0.48 \pm 0.05 \mu\text{l ml}^{-1}$ via the Least Squares Method.

CONCLUSIONS

Plants have been used for the treatment of diseases for a very long time. Because of the harmful side-effects of synthetic products and the fact that plants are easily accessible, interest in drugs of herbal origin has significantly increased. Scientific research is being conducted all over the world to determine whether plants that are traditionally used to treat various diseases are actually appropriate for their intended use. This is the first detailed documentation of antioxidant and antidiabetic activities of the essential oil of *Eucalyptus camaldulensis* Dehnh. All experimental data indicate that the essential oil of eucalyptus has not only antioxidant effects by scavenging reactive oxygen species, but also an antidiabetic effect by inhibiting α -amylase and α -glucosidase. Since it is known that free radicals cause diabetes, it is stated that this essential oil can be effective in the treatment of diabetes, not only through the inhibition of enzymes, but also by scavenging reactive oxygen species. In accordance with these results, in vivo studies have to be undertaken to investigate the efficacy of the essential oil in the treatment of diabetes.

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