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Physico-chemical properties, fatty acid and essential oil composition and antimicrobial effect of cumin (*Cuminum cyminum* L.) seed and essential oil

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Abstract

Cr, Mn, Fe, Co, Ni , Cu, Cd, Pb, As and Zn contents of cumin seeds were found as 0.298, mg/kg, 16.208, 95.279, 0.101, 1.545, 7.458, 0.037, 0.051, 0.109 and 29.183, respectively. As physico-chemical properties of cumin essential oil, oil yield, refractive index, viscosity, and ransimat value were measured as 1.6%, 1.506, 3.37, and 2.25 (110 ºC-h; µs/cm-h). Cumin oil contained 65.12% oleic, 29.25% linoleic and 3.51% palmitic acids as major fatty acid. Twenty-eight constituents were identified in the oil of *C.cyminum* seed oil, representing 99.998% of the total oil. Cuminal (25.59%), (r)-(+)-1-phenyl-1-propanol (22.112%), 1-isopropylidene-3-n-butyl-2-cyclobutene (17.006%), gamma-terpinene (11.029 %), trans-beta-ocimene (7.952%) and β-pinene (5.81%) were established as major constituents of cumin seed essential oil. The highest antifungal activity was observed on mycelia growing of *Aspergillus parasiticus* NRRL 3357. Cumin oil showed weak effect on the mycelia growt of *Zygosacc haromycesrouxii*. *S.aureus* showed resistance against cumin oil when compared with results of *S.enteritis*, *E.coli*, *L.monocytogenes* and *E.coli* O157:H7. Cumin oil exhibited antimicrobial activity against the investigated test organisms.

Keywords: cumin seed, *C.cyminum*, oil, essential oil, heavy metals, fatty acids, essential oil composition, antimicrobial activity

1. Introduction

The family *Apiaceae* (formerly Umbelliferae) members are angiospermic temperate herbs with some approximately 300 genera and 3000 species. These plants have been identified with their properties to induce apoptosis, antimicrobial, cyclooxygenase inhibitory efficiencies and antitumor effects [1].Cumin is naturally found in Iran, Turkey, India, Pakistan, Argentina, China, Central America and in the countries bordering the Mediterranean Sea [2,3]. Cumin fruits are used as spice and some species economically important and are used as culinary herbs, flavoring agents in perfumery and cosmetics [4]. Plant essential oils have gained importance as alternative remedies for treatment of many infectious diseases and food preservatives [5]. All the cumin varieties are used as a stimulant, a carminative, an astringent, and as remedy against indigestion, colic, flatulence and diarrhea [6,7]. The antimicrobial properties of spice and essential oils have been known for a long time, and a number of studies of the antimicrobial effect of spices, essential oil and their components have been reported. Although several studies have been conducted regarding in vitro antibacterial and antifungal properties of plant essential oils and extracts [1,8,9]. The antimicrobial and antioxidant properties of plants and the aromatic products derived from them are due to the different chemical

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substances in their composition - essential oils and glyceride oils, alkaloids, flavonoids, tannins, glycosides and other compounds [10,11]. Medicinal plants have the ability to inhibit the growth of wide range of pathogenic microorganisms due to presence of essential oils [12]. The aim of this study was to determine physico-chemical properties, fatty acid and essential oil composition and antimicrobial effect of cumin (*Cuminum cyminum* L.) seed and seed essential oils compoition.

2. Materials and Methods

2.1.Material

Cumin (C*uminum cyminum*) seeds were provided from Eskişehir (Sivrihisar) district by Afyonkarahisar Medicinal and Aromatic Plant Cetrum. Cumin seeds were kept at +4 ºC till using.

2.1.1.Recovery of the essential oils: About 200 g ground sample of cumin seed was extracted with hydrodistillation by using a Clevenger-type apparatus for 2 h, and the oil was dried over anhydrous sodium sulphate [13].

2.1.2.Extraction of Oil: About 10 g of ground seed was refluxed with 250 ml of petroleum ether in a soxhlet apparatus. The oil was recovered by distilling th esolvent in rotary evaporator at 50ºC. Then the total lipid was kept at -18ºC until analysis.

2.2.Method

Essential oil, fatty acid composition, essential oil composition, heavy metals, refractive index, oxidatif stability, iodine value, antibacterial and antifungal effects agains tested microorganisms (*Escherichia coli ATCC 25922, Escherichia coli O157: H7, Listeria monocytogenes ATCC 7644, Salmonella enterica* subsp. *enterica serovar, Enteritis ATCC 13076, Staphylococcus aureus ATCC 2592, Candida zeyland* and *zygosaccharomyces rouxii*) of cumin seed essential oil and seeds were investigated. Also, fatty acid composition and iodine value of cumin oil were determined.

2.2.1.Refractive indexs: The refractive indexs of cumin (*Cuminum cyminum*) essential oil were measured by Abbe refractometer. For this purpose, essential oil sample of the plant taken with the help of a spatula was poured onto the prisma of refractometer and read at 20°C [14].

2.2.2.Oxidation stability: In this study, 3 ml of sunflower oil was used as a control group for oxidation stability. Then induction time of3 ml of sunflower oil + 0.3 ml of cumin (*Cuminum cyminum*) essential oil was detected with the rancimat instrument (Metrohm 743Rancimat instrument). The air flow rate set at 110 degrees was made at 10 L / h. The ultra-pure water having conductivity of 0.055 µs was used in the study [15].

2.2.3.Viscosity: Viscosity analysis of oil sample was measured according to method of Lazaridou [16].

2.2.4.Iodine value: 0.15 g of oil from the sample was weighed on a precision scale, 25 ml of Wijs reagent was added to the erlenmayer flask with the burette under the fume hood, and the solution was slightly agitated.10 mL of 2.5%. Mercury Acetate solution was added and mixed. The samples were kept at room temperature in a dark cabinet for 3 min and titrated.2-3 drops of starch indicator, 20 ml of Potassium iodide (KI) and 100 ml of purified water were added to the solution before the titration, respectively. Finally, the solution was titrated with 0.1 N Sodium Thiosulphate until white color conversion. At the same time, and witness titration was performed with the same reagents under the same conditions [17].

2.2.5. Determination of heavy metals: Plant seed samples were dried at 70 ºC in a drying cabinet with air-circulation until they reached constant weight. Later, about 0.5 g dried and ground sample was digested by using 5ml of 65% HNO_3 and 2 ml of 35% H_2O_2 in a closed microwave system (Cem-MARS Xpress) at 200 ˚C. The volumes of the digested samples were completed to 20 ml with ultra-deionized water and mineral concentrations were determined by inductively coupled plasma-optical emission spectroscopy (ICP-AES; (Varian-Vista, Australia). Distilled deionized water and ultrahigh-purity commercial acids were used to prepare all reagents, standards, and samples. After digestion treatment, samples were filtrated through whatman No 42. The filtrates were collected in 50 ml flasks and analysed by ICP-AES. The heavy metal contents of the samples were quantified against standard solutions of known concentrations which were analysed concurrently [18].

2.2.6. Composition of Essential Oil: The composition of cumin (C*uminum cyminum*) essential oil measured by gas-cromatography (Agilent GC). Gas Chromatography-Flame Ionization Detection (GC-FID) system was used in an attempt to determine essential oil components.

The essential oils obtained after the distillation were diluted with acetone in a ratio of 1:10 (v / v), and a volume of 0.1 µL was injected to colon (30m x 0.25mm x 0.25µm film)using a glass syringe. Helium was used in thesystem as carrier gas at 5 psipressure. Injection block temperature and FID detector temperature were 230°C and 250°C, respectively. The FID column temperature was set at 50 ºC and increased by 3 ºC per minute to a final temperature of 230 ºC. The sample was allowed to stand for 3 minutes at the start temperature and 15 minutes at the finish temperature. The proportional values of the components were determined as % with respect to the FID field without using the correction factor. Determination of essential oil components was done by Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent). Obtained spectra were defined by comparison with the spectra of the components in WILLEY and NIST libraries [19].

2.2.7.Determination of Fatty Acids: Fatty acid compositions for plant seed oil were determined using a modified fatty acid methyl ester method as described according to AOCS [20] (1989). The oil was extracted three times for 2 g air-dried seed sample by homogenization with petroleum ether. The oil samples (50-100 mg) was converted to its fatty acid methyl esters (FAME). The methyl esters of the fatty acids (1 µl) were analysed in a gas chromatography (HP 6890) equipped with a flame ionising detector (FID), a fused silica capillary column (60 m x 0.25 mm i.d.; film thickness 0.20 micrometer). It was operated under the following conditions: oven temperature program. 175 ºC for 7 min. Raised to 250 ºC at a rate 5 ºC/min and than kept at 250 ºC for 15 min); injector and detector temperatures, 250 and 250 ºC; respectively, carrier gas. nitrogen at flow rate of 1.51 ml/min; split ratio. 1/50 µl/min.

*2.2.8.Determination of Antimicrobiyal Activity a-Preparation of test microorganizms***:** *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Listeria monocytogenes* ATCC 7644, *Salmonella enteric* subsp. and *Entericas*erovarEnteritis ATCC 13076, *Staphylococcus aureus* ATCC 2592 species of bacteria used for the determination of antimicrobial activity and *Aspergillusparasiticus* NRRL 3357, *Aspergillusparasiticus* DSM 5771, *Zygosaccharomycesrouxii* ATCC 28253 fungal species were obtained from the culture collection of Namık Kemal University Agriculture Faculty Department of Food Engineering. In the study Nutrient Broth and Agar (NA, NB) for bacterial strains and Yeast Peptone Dextrose Broth and Agar (YPDB, YPDA) for yeast strains were used as a nutrient. Bacteria and yeast were transferred from the stock cultures into appropriate liquid medium and incubated for 24 hours at 37 and 28°C, respectively, to prepare fresh cultures. Antimicrobial effects of cumin essential oil on microorganisms were determined using two different methods (Disk diffusion and microdilution method).

***b-Disc Diffusion Method*:** The antimicrobial activity of cumin (*Cuminum cyminum*) essential oil was determined by disk diffusion technique according to the method recommended by National Committee for Clinical Laboratory Standarts (NCCLS 1997). Briefly; 100 µlequivalent cells from freshly tested microorganism cultures(108kob / mL, McFarland turbidity standard 0.5) were pipetted onto a petri containing YPD agar medium for yeast and Müller Hinton Agar (MHA) medium for bacteria and dispensed equally with the sterile bar. Sterile disks (Oxoid) of diameter 6 mm impregnated with 20 µl of oil and Dimethylsulfoxide (DMSO; negative control) were aseptically placed in the inoculated petris and allowed to incubate for 2 hours at 4°C and then at 37°C (bacteria) and 28°C (yeast) for 24 hours. At the end of incubation, the inhibition zone diameters were measured in millimeters with the help of caliper to determine whether bacteria or yeast were present. The experiments were carried out in duplicate. Gentamicin (10 µg/disc) for bacteria and Nystatin (10 µg/disc) for yeast were used as a positive control.

Minimum Inhibitory Concentration (MIC) of cumin essential oil (C*uminum cyminum*) was determined by microdilution method [21]. First of all, working buffer stock was prepared with oils dissolved in 10% DMSO andfiltered, at 1000 µg/mL concentration in test tubes containing 10 mL of MHB or 10 mL of YPDB. Working stocks were prepared in sterile tubes containing 10 ml of liquid medium by continuously reducing the medium (500, 250, 125, 62.5, 31.25, 15.62 and 7.8 µg/ml) from the buffer stock. To perform the MIC reading, 95 µlof MHB or 95 µl of YPDB were pipetted into each well of 96-well ELISA pots and 100 µl of the above-mentioned working stocks were added on them and mixed with pipet.5 µl of microorganism inoculum (from equivalent culture to 0.5 McFarland standard culture) was added to a volume of 195 µl, resulting in a total volume of 200 µl per well.

The microplate was shaked in a rotary incubator for 30 seconds at 300 rpm and then it was incubated at 37 and 28°C for 24 hours. Microbial growth was determined by measuring the absorbance values against control at 600 nm with a microplate reader.The MIC value was taken as the lowest concentration of substance for which no microorganism development was observed [21].

2.3.Statistical Analysis

Standard deviations were calculated using a one-way ANOVA according to the significance levelinter averages of the data obtained as a result of the research, and each sample was evaluated as three parallel [22].

3. Results and Discussions

Some physico-chemical properties and heavy metals of cumin seed and essential oil are given in Table 1. Cr, Mn, Fe, Co, Ni, Cu, Cd, Pb, As and Zn contents of cumin seeds were found as 0.298, mg/kg, 16.208, 95.279, 0.101, 1.545, 7.458, 0.037, 0.051, 0.109 and 29.183, respectively. As physico-chemical properties of cumin essential oil, oil yield, refractive index, Viscosity, and ransimat value were measured as 1.6%, 1.506, 3.37, and 2.25 (110 ºC-h; µs/cm-h). Also, cumin seed contained 12.38% oil. Its iodine value was 110.90. The essential oil yields of cumin seeds ranged from 1.0–2.8 mL/100 g relative to the dried fruits [23-25]. *Chaudhty et al*. [23] reported that specific gravity and refractive index values of cumin essential oil were 0.928 and 1.503, respectively.

Fatty acid composition of cumin seed oil is given in Table 2. Cumin oil contained 65.12% petroselinic, 29.25% linoleic and 3.51% palmitic acids as major fatty acid. *Bettaieb et al*. [26] reported that tunisian cumin (*C.cyminum*) oil contained 55.90% petroselinic, 23.82% palmitic, 12.40% linoleic and 2.12% palmitoleic acids.

Constituents of essential oils of cumin seed are given in Table 3. Essential oil of cumin seed were analysed by gas chromatography-mass spectrometry (GC-MS). Essential oil was extracted by using a Clevenger type apparatus, and obtained 1.6% oil. Forty constituents were identified in the oil of *C.cyminum* seed oil, representing 99.998% of the total oil. Cuminal (25.59%), (r)-(+)-1-phenyl-1-propanol (22.112%), 1-isopropylidene-3-n-butyl-2-cyclobutene (17.006%), gamma-terpinene (11.029 %), trans-beta-ocimene (7.952%), beta-pinene (5.81%) and phellandrene (2.269%) were established as major constituents of *C.cyminum* seed essential oil. A Total of 20 major chemical components of *Cuminum cyminum* (cumin) of family Apiaceae were analyzed by GC-MS,and were found to be cuminaldehyde (36.67%) and caren-10-al (21.34%), beta-pinene (18.76%), γ-terpinene (16.86%), terpinen-4-ol (2.44%), α-thujene (1.88%), α-pinene (1.41%), *p*-cymene (0.30%), carbicol (0.19%) and alpha-terpineol (0.09%) in case of cumin essential oil [5]. Twenty five compounds were identified in the cumin oil-cuminaldehyde (30.834 %), 3-caren-10-al (17.223 %), β-pinene (14.837 %), γ–terpinene (11.928 %), 2-caren-10-al (8.228 %) and pcymene (6.429 %) (Teneva et al. 2016). Cumin (*Cuminum cyminum* L.) seeds contained 2 to 6.1% essential oils and 12-28% glyceride oils. The main components of cumin seed essential oil arecumaldehyde (15.7 - 63%, rarely to 79.8%), ρ-mentadien-1,3-al-7 (2.2 -27.4%), ρ-mentadien 1,4-al-7 (0.3 -17.4%), β-pinene (0.8 to 21%), ρ-cymene (4.2 – 23.2%), γ-terpinene (1.5 - 23.9%), caryophyllene, sabine hydrate, α-terpineol, 1,8-cineole and others [27-31]. The major constituents of the cumin seed essential oils were safranal (16.8%–29.0%), γ-terpinene (14.1%–19.6%), γ-terpinene-7-al (13.5%–25.5%), cuminaldehyde (17.5%–22.3%), β-pinene (6.8%–10.4%), and *p*-cymene (4.1%–8.8%) [25]. Compounds found in the cumin oil were α-pinene 0.9805%, β-pinene 16.2876%, Limonene 0.2307%, ɣ-terpinene 5.6551%, p-cymene 15.5464%, Dipentene 0.2102%, 1,8-cineol 2.2421%, cumin aldehyde 25.8749%, cuminyl alcohol 30.023%, perillaldehyde 0.6286% and α-terpineol 0.1929% and 2.1283% remain unidentified(*Chaudhry et al. 2012*). *C. cyminum* L. from Alborz mountain contained α-pinene (29.2%), limonene (21.7), 1,8-cineole (18.1%), linalool (10.5%), and α-terpineole (3.17) as the major compounds [24].

Antimicrobial activity of essential oil of cumin seeds are given in Table 4. The inhibitory effects of all the concentrations tested against microorganisms increased with higher levels. The highest antifungal activity was observed on mycelia growing of *Aspergillus parasiticus* NRRL 3357.

Cumin oil showed weak effect on the mycelia growt of *Zygosacc haromycesrouxii*. *S.aureus* showed resistance against cumin oil when compared with results of *S.enteritis*, *E.coli*, *L.monocytogenes* and *E.coli* O157:H7.

Table 1. Physico-chemical properties and heavy metals of cumin seed, oil and essential oil

Oil (%)	**Essential oil (%)**	**Viscosity (mPass-24˚C)**	**Ransimat value (110˚C-h) µs/cm-h**	**Iodine value**	**Refractive Index (nD)**
12.38 ±1.13	1.6 ± 0.221	3.37 ± 0.11	2.25 ± 0.11 * 2.69± 0.10**	110.90 ±2.56	1.506 ± 0.01
Heavy metal contents of cumin seed (mg/kg)					
Cr	**Mn**	**Fe**	**Co**	**Ni**	**Cu**
0.298	16.208	95.279	0.101	1.545	7.458
Ag	**Cd**	**Hg**	**Pb**	**As**	**Zn**
0.0	0.037	0.0	0.051	0.109	29.183

*Sunflower oil (Control); ** Sunflower oil + Cumin essential oil

Table 2. Fatty acid composition of cumin seed oil (%)

Fatty acids	Concentrations (%)
Myristic	0.04±0.01*
Palmitic	3.51±0.11
Palmitoleic	0.38±0.03
Margaric	0.05±0.01
Heptadecenoic	0.05±0.01
Stearic	0.08±0.01
Petroselinic	65.12±0.013
Linoleic	29.25±0.21
Linolenic	0.31±0.07
Arachidic	0.09±0.01
Ecosanoic	0.27±0.03
Behenic	0.02±0.01
Lignoseric	0.03±0.01
Nervonic	0.02±0.01

mean±standard deviation

Table 3 . Essential oil composition of cumin seed (%)

	RT	**Constituents**	**(%)**
1	6.298	α-pinene	0.594
2	6.359	α-thujene	0.215
3	8.095	β-pınene	5.81
4	8.393	sabinene	0.425
5	9.444	β-myrcene	0.534
6	9.535	l-phellandrene	2.269
7	9.935	α-terpinene	0.21
8	10.469	dl-limonene	0.499
9	10.718	1,8-cineole	1.252
10	11.878	ɣ-terpinene	11.029
11	12.684	trans-β-ocimene	7.952
12	20.475	(-)-camphor	0.51
13	21.274	linalool	0.118
14	22.072	ethanone, 1-(2-methyl-1-cyclopenten-1-yl)-	0.574
15	23.01	terpinene-4-ol	0.472
16	25.503	(-)-β-acoradiene	0.205
17	25.764	α- terpıneol	0.198
18	25.943	borneol	0.403
19	26.154	l-verbenone	0.363
20	28.654	cuminal	25.59
21	28.945	1-ısopropylidene-3-n-butyl-2-cyclobutene	17.006
22	29.122	(r)-(+)-1-phenyl-1-propanol	22.112
23	29.343	a-phellandrene epoxıde	0.224
24	33.23	carotol	0.375
25	33.698	p-mentha-1,4-dien-7-ol	0.374
26	34.258	cumic alcohol	0.412
27	35.356	carvacrol	0.256
28	35.785	18-crown-6	0.017
		Total	99.998

Table 4. Antimicrobial activity of cumin essential oil

	S. enteritis ATCC 13076	*E. coli* ATCC 25922	*L. monocytogenes* ATCC 7644	*S. aureus* ATCC 2592	*E. coli* O157:H7
DD*	12.45	16.99	12.87	11.25	14.85
MIC**	125	125	125	250	125

*DD: disc diffusion method (ml);**MIC: minimum inhibition concentrations (µg/ ml).

Aspergillus parasiticus NRRL 3357	*Aspergillus parasiticus* DSM 5771	*Zygosacc haromycesrouxii* ATCC28253
46.85	47.12	62.42

Inhibition zome:mm

The MIC values of *S.enteritis*, *E.coli*, *L.monocytogenes* and *E.coli* O157:H7 were found similar. The antifungal activities of the *C. cyminum* L. essential oil against *Aspergillus flavus* PICC-AF39, *Aspergillus flavus* PICC-AF24, *Aspergillus parasiticus* NRRL-2999, and *Aspergillus niger* were qualitatively and quantitatively assessed according to the inhibition zone diameter as well as the MIC and MFC values [24]. The four fungi species exhibited high diameters of growth inhibition (35, 55, 37 and 38 mm). At a dose of 10 µl of the oil per disc per petri plate, the *C. cyminum* L. essential oil was particularly effective against *Aspergillus parasiticus* NRRL-2999, *Aspergillus niger*, *Aspergillus flavus* PICC-AF24, and *Aspergillus flavus* PICC-AF39 with diameters of inhibition of approximately 25, 35, 23 and 23 mm, respectively, on the second day of incubation. At the end of thirtieth day, the diameter of inhibition was approximately 13, 9, 12, and 13 mm for *Aspergillus parasiticus* NRRL-2999, *Aspergillus niger*, *Aspergillus flavus* PICC-AF24, and *Aspergillus flavus* PICC-AF39 respectively [24]. Cumin esential oil demonstrated remarkable antibacterial activity against *Salmonella typhi* with an inhibition zone diameter of 54 with identical MIC value of 12.5 µl/ml [5]. The antimicrobial activity of essential oils against pathogenic (*Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 8739, *Salmonella* sp. (clinical isolate), *Staphylococcus aureus* ATCC 6538P, *Proteus vulgaris* G) microorganisms by disc-diffusion method was examined. Gram-positive bacteria were more sensitive to the oils (inhibition zones being between 8 and 12.5 mm) and the minimum inhibitory concentration was more than 600 ppm; Gram-negative bacteria were less sensitive [32].

Many researchers have reported a potential antimicrobial activity exhibited by cumin seed essential oil [29, 33-36]. The antibacterial activity of the essential oils was individually evaluated against four positive-Gram (*Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Listeria monocytogenes*) and two negative-Gram ones (*Proteus vulgaris* and *Salmonella typhimurium*) using disc diffusion and serial dilution methods, and the inhibition zones and MIC values for bacterial strains which were sensitive to the EOs of green cumin were in the range of 8.2–33.2 mm and 31–250 µg/mL, respectively [25]. Our findings suggest that, the antibacterial activity of cumin essential oil may probably be due to their major chemical constituents. Results partially showed differences with previous studies on essential oil compositions of *Cumin* seeds. These differences can be probably due to location, climatic factors, soil structure and genetic factors. Further studies will be on biofunctional properties of cumin seeds derivetives such as extract, oleoresin, infusion and decoctions in *in vitro* and/or *in vivo*. Some factors such as collection time, plant maturity, drying conditions, mode of distillation, geographic and climatic factors play a role in the essential oil composition of plants.

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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