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Antibacterial effect of Turkish black cumin (*Nigella sativa* L.) oils

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RESUMEN

Efecto antimicrobiano de los aceites de comino negro turco.

Se ensayaron un total de cinco aceites diferentes de comino negro turco (*Nigella sativa* L.), que se utilizan habitualmente en alimentos para darles sabor, ayudar a la conservación o por sus efectos terapéuticos, para estudiar sus propiedades antimicrobianas a concentraciones de 0.5%, 1.0%, y 2%. Para ello se utilizó el método de difusión en agar, frente a veinticuatro microorganismos patógenos, causantes de alteraciones o bacterias ácido lácticas (LAB). Todos los aceites ensayados mostraron actividad antimicrobiana contra todos los microorganismos ensayados, siendo las concentraciones del 2% las concentraciones más eficaces. *Aeromonas hydrophyla* fue el microorganismo mas sensible a todas las concentraciones mientras que *Yersinia enterocolitica* fue la más resistente. Generalmente las bacterias acido lácticas tuvieron más resistencia que los gérmenes patógenos y las bacterias que causan alteraciones. En consecuencia, el aceite de comino negro turco se puede utilizar como agente antimicrobiano en productos alimenticios para evitar su alteración.

PALABRAS-CLAVE: Actividad antimicrobiana - Comino negro - Nigella sativa.

SUMMARY

Antibacterial effect of Turkish black cumin (*Nigella sativa* L.) oils.

A series of five different oils from Turkish black cumin (*Nigella sativa*) used in foods mainly for their flavour, preservation and natural therapies were screened for their antibacterial effects at 0.5%, 1.0% and 2.0% concentrations using the agar diffusion method against twenty four pathogenic, spoilage and lactic acid bacteria (LAB). All tested oils showed antibacterial activity against all the bacteria used in the assay. The oils at 2.0% concentration were more effective than of the other concentrations. The most sensitive bacterium against all of the oil concentrations was *Aeromonas hydrophila*, while the most resistant was *Yersinia enterocolitica*. Generally, lactic acid bacteria had more resistance than pathogenic and spoilage bacteria against black cumin oils. Consequently, black cumin oil may be used as an antimicrobial agent in food products to prevent spoilage.

KEY-WORDS: Antibacterial activity - Black cumin - Nigella sativa.

1. INTRODUCTION

Nigella sativa L. (family *Ranunculaceae*) is commonly known as black cumin or black seed. The seed or its oil is used as a carminative, diuretic, lactagogue and vermifuge (Akgul, 1989; Ali and Blunden, 2003). The dried seeds from black cumin are also used for sprinkling on bread or flavouring foods, especially bakery products and cheese (Ustum et al., 1990; Takruri and Daneh, 1998). *Nigella sativa* seeds contain 36-38% fixed oils, proteins, alkaloids, saponin and 0.4-2.5% essential oil (Ali and Blunden, 2003).

The antioxidant, antibacterial and antifungal activities of spices and their derivatives have been investigated by some researchers (De et al., 1999; Sagdic et al., 2002; Sagdic, 2003). Many bioactive properties have been attributed to black cumin seed, fixed oil and/or essential oil, including antibacterial (Akgul, 1989; Hanafy and Hatem, 1991; Farrag et al., 2000), antifungal (Akgul, 1989; Khan et al., 2003) and antioxidant activities (Burits and Bucar, 2000).

In the present study, an attempt has been made to investigate the antibacterial characteristics of five different Turkish black cumin samples. The present communication deals with the investigation of seventeen spoilage and/or pathogenic bacteria, and seven lactic acid bacteria (LAB). This study will help to establish a traditional role for black cumin as preservative, antiseptic and disinfectant.

2. MATERIALS AND METHODS

2.1. Black cumin samples

Five samples of black cumin (*Nigella sativa* L.) seeds which were grown in five different regions (A: Antalya region; B: Erzurum region; C: Kayseri region; D: Konya region; E: Tekirdag region) of Turkey were used in the study and purchased from different retail groceries in Istanbul, Turkey.

2.2. Bacterial strains

In this study, the seventeen spoilage and/or pathogenic bacteria from a total of twenty-four bacteria used for testing antibacterial activity were *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* FMC 19, *Bacillus subtilis* IMG 22, *Corynebacterium*

xerosis UC 9165, *Enterobacter aerogenes* CCM 2531, *Enterococcus faecalis* ATCC 15753, *Escherichia coli* DM, *Escherichia coli* O157:H7 KUEN 1461, *Klebsiella pneumoniae* FMC 5, *Listeria monocytogenes* Scott A, *Mycobacterium smegmatis* RUT, *Proteus vulgaris* FMC 1, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* EU, *Salmonella typhimurium, Staphylococcus aureus* Cowan 1 and *Yersinia enterocolitica* EU. These bacteria were supplied by the Department of Biology, Sutcu Imam University, Kahramanmaras-Turkey. Additionally, seven LAB including *Streptococcus salivarius* ssp. *thermophilus* S51, *Lactobacillus delbrueckii* ssp. *bulgaricus* A42, *Lb. casei* ssp. *casei* K64, *Lb. paracasei* ssp. *paracasei* A27, *Leu. pseudomesenteroides* E83, *Leu. gelidum* E26 and *Weissella paramesenteroides* E95 were obtained from Dr. O. Sagdic (Department of Food Engineering, Erciyes University).

2.3. Preparation of black cumin oils

One hundred g of each black cumin was ground in an omnimixer and extracted for 10 h in a Soxhlet extractor with 500 ml n-hexan (Merck-Darmstadt, Germany) at 70°C. The fixed oils were pooled and concentrated in a rotary evaporator (Buchi Rotavapor-RE 111), and then kept in small (10 ml) sterile bottles under refrigerated conditions until use (Ozcan, 1998).

2.4. Determination of antibacterial activity

All test bacteria in nutrient or MRS broths (Merck-Darmstadt, Germany) were enumerated using the serial dilution method. Final cell concentrations were 10^6-10^7 cfu/ml. 200 µL (1%) of the bacterial suspensions was seeded on 20 mL of nutrient or MRS agars at 43-45 °C. The prepared bacterial cultures were poured onto petri plates (9 cm diameter), and then agars were allowed to solidify. The agar diffusion method was used to detect the antibacterial activity of the fixed oils. The wells at 4 mm diameter were cut in nutrient or MRS agars. 50 µl of a solution with 0.5%, 1.0% and 2.0% concentrations of the oils in absolute methanol (Merck-Darmstadt, Germany) were added into the wells on nutrient or MRS agars. Absolute methanol was also used as control. The plates were incubated at a suitable temperature for 18-24 h (Aureli et al., 1992; Ozkan et al., 2003; Baydar et al., 2004). The diameter (mm) of inhibition zones of the oils was measured by compass. All tests were carried out in triplicate.

3. RESULTS AND DISCUSION

The antibacterial activities of black cumin samples against a total of twenty four bacteria (spoilage and/or pathogenic and LAB) are shown in Table 1.

When the agar diffusion method was used, the black cumin oils caused the different inhibition zones on the tested bacteria. Antibacterial effects of the tested black cumin oil concentrations showed variations against the bacterial strains. Additionally, the control treatment (absolute methanol) was inactive against all the bacteria.

The antibacterial effects of the oils were similar to each other. The most active oil was sample A at 1.0% and 2.0% concentrations completely inhibiting the growth of all bacteria. Also, other black cumin oils had some inhibitive effects against all bacteria at 1.0% and 2.0%, while samples of D and E had the lowest activity (Table 1).

The lowest active concentration was 0.5% in all of the cumin samples. In general, this concentration was ineffective against *E. coli*, *E. coli* O157:H7, *K. pneumoniae*, *P. aeruginosa*, *Y. enterocolitica*, *Lb. casei* ssp. *casei*, *Leu. pseudomesenteroides* and *W. paramesenteroides*. In 0.5% concentrations of B, C, D and E samples also had no inhibitory effect against *E. aerogenes*, *S. typhimurium*, *S. salivarius* ssp. *thermophilus* and *Lb. paracasei* ssp. *paracasei* (Table 1).

Of all the bacteria, *A. hydrophila* was the most sensitive bacteria against all of the concentrations of black cumin oils, while *Y. enterocolitica* was the most resistant bacteria. Generally, the fixed oils of the black cumin samples had higher antibacterial activity against spoilage and pathogenic bacteria than LAB.

The inhibitory effects of black cumin were previously determined against bacteria, yeasts and moulds by some researchers. Akgul (1989) reported that 0.05% and 1% concentrations of black cumin essential oil had antibacterial and antifungal effects. Hanafy and Hatem (1991) announced that the diethyl ether extract of *Nigella sativa* seeds (25-400 µg extract/disc) caused concentration dependent inhibition (but not *S. thyhimurium*) of *E. coli*, *P. aeruginosa*, *S. aureus* and *Candida albicans*. Farrag et al. (2000) found that the fixed oil of black cumin had an inhibitory effect against gram positive such as *S. aureus* and *B. cereus* and Gram negative bacteria. Ozcan (1998), De et al. (1999) and Khan et al. (2003) reported that the extract from *Nigella sativa* seeds had antifungal activity against *Aspergillus parasiticus*, *Candida albicans* and *Saccharomyces cerevisiae*, respectively.

Many components of black cumin were characterised by Burits and Bucar (2000) using GC-MS, but the major ones were thymoquinone, *p*-cymene and carvacrol. All of these compounds had antibacterial effect (Ali and Blunden, 2003). Hence, the antibacterial effects of our samples may be closely related to their high percentage of these compounds.

Table 1

Inhibition zones (measured as mm) of the essential oils from various black cumin at different concentrations against bacteria*

	Different concentrations of the black cumin samples (%)														
	A			B			C			D			E		
Bacteria	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2	0.5	1	2
Spoilage and/or pathogenic															
A. hydrophila	22	27	37.5	21	26	36.5	19.5	25	35.5	20	25.5	35	21	24.5	34.5
B. cereus	12.5	19.5	29.5	11.6	19.2	28.5	12.1	18.5	28.2	11.2	18.2	27.8	11.3	18.1	27.5
B. subtilis	14	21	27	13.8	20.1	27	13.5	20.8	26.8	13.5	20.2	26.5	12.8	19.5	26
C. xerosis	17.5	22.5	32.5	17.5	22.1	32.4	17.2	21.8	32	17.2	21.5	31.8	17.4	21.5	31.6
E. aerogenes	7	16.5	23.5	-	16.2	23.6	-	16.5	22.8	-	15.8	23.2	-	15.8	23
E. coli	-	16	19.5	-	15.8	19.5	-	15.5	19.5	-	15.5	19.2	-	14.8	19.4
E. coli O157:H7	-	15	19	-	15.2	19.3	-	14.8	18.8	-	14.2	18.8	-	14.4	18.4
E. faecalis	8.5	17.5	24	8.5	16.8	23.8	8.6	17	23.5	8.4	16.4	23.5	8.6	16.4	23.8
K. pneumoniae	-	13.5	18.5	-	12.8	18.8	-	13	18.2	-	12.8	18.5	-	12.2	18.2
L. monocytogenes	7	15.5	21.5	-	14.6	21	-	14.8	20.6	-	14.2	21	-	14.2	20.4
M. smegmatis	17	22	32	16.6	21.5	32.2	16.5	22	32	16.5	21.8	32.2	16.6	21.5	31.6
P. vulgaris	18.5	24.5	35	18.5	23.8	34.8	18.2	24.2	35	18	23.6	34.6	18.2	23.8	34.6
P. aeruginosa	-	16	21	-	16.4	20.6	-	16	21.2	-	15.8	20.4	-	15.8	20.6
P. fluorescens	7.5	13.5	21.5	8	13	21.4	7.6	13.4	21.2	7.2	12.8	20.8	7.2	13	20.6
S. aureus	16.5	21.5	29.5	16.5	21.5	29.2	16.2	21.5	28.8	15.8	21	29	15.8	21.2	29
S. typhimurium	7	16.5	24.5	-	16.8	24.4	-	16.2	24	-	16.2	23.8	-	16	23.5
Y. enterocolitica	-	11	16.5	-	11	16.4	-	10.6	16.2	-	10.8	16	-	10.6	16
LAB															
S. salivarius ssp. *thermophilus*	10.5	16.5	24	-	13.5	19	-	13	18.8	-	13.2	19.2	-	13	19
Lb. casei ssp. *casei*	-	13.5	19.5	-	14.3	20.8	-	14	20.5	-	14.2	20.2	-	13.8	20.5
Lb. paracasei ssp. *paracasei*	7	14.5	21	-	12.2	18.8	-	12.5	19	-	12	18.6	-	12	18.4
W. paramesenteroides	-	12.5	19	10.4	16.2	23.3	10	16.5	23	10.2	16	22.8	10.2	15.8	22.8
Lb. delbrueckii ssp. *bulgaricus*	11	18.5	24.5	11	18.3	23.8	10.8	18.1	24.4	11	18.1	24	10.6	17.6	23.6
Leu. pseudomesenteroides	-	14	19	-	14.5	19.3	-	14	19.1	-	14.2	18.6	-	13.8	19
Leu. gelidum	8.5	14	19.5	8	14.4	19.6	8.4	13.5	19.2	8.1	13.8	19	8.3	13.5	19

-: inactivity
*: The diameter of the well (4 mm) is included in the final zone measure.
A: Antalya region in Turkey; B: Erzurum region in Turkey; C: Kayseri region in Turkey; D: Konya region in Turkey; E: Tekirdag region in Turkey

As a result, antibacterial activities of the black cumin fixed oils against food spoilage and/or pathogenic, and lactic acid bacteria are an important finding. Therefore, the oil of black cumin may be used in food as a preservative.

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