Research Article ISSN 2641-4295

Food Science & Nutrition Research

Antibacterial Effect of Turkish Black Cumin Seed Oil Against Pathogen Bacteria's

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Received: 19 August 2021; **Accepted:** 21 September 2021

Citation: Guldal I, Karabiyikli S. Antibacterial Effect of Turkish Black Cumin Seed Oil Against Pathogen Bacteria's. Food Sci Nutr Res. 2021; 4(2): 1-3.

ABSTRACT

Black cumin seeds are used for medicinal and aromatic purposes in Turkey. Black cumin seed oil (BSCO) was provided from a local producer. BCSO was extracted by cold-pressing method. Antibacterial effect of BCSO was investigated against B. cereus (ATCC 10876), L. monocytogenes (ATCC 19115), E. coli (ATCC 25922), Salmonella Thypimurium (ATCC 14028) and S. aureus (ATCC 25923) by minimum inhibitory concentration (MIC) method.

MIC values were determined according to micro tube dilution method. BCSO had antibacterial effect against B. cereus and S. aureus and didn't have antibacterial effect against other bacterias. B. cereus and S. aureus are Gram (+) and others are Gram (-). In various studies, it had been found that Gram (+) bacterias are less resistance to aromatic oils.

Fatty acid composition of BCSO was investigated for additional information of material and major fatty acids of BCSO are linoleic acid, oleic acid and palmitic acid.

As a results, study suggests that cold-pressed BCSO can be used for its functional property.

Practical Applications
Black seed oil is widely used in ethnopharmacology. It is marketed as a treatment agent for many mild diseases in the shops called "Aktar" in Turkish culture and it finds a response by the public. However, its use in industry is limited due to its pungent odor and taste. In this study, the functional properties of black cumin oil were investigated.

Keywords
Antibacterial effect, Black cumin seed oil, Functional food.

Introduction
In early 1900's, food-borne deaths were mostly caused by infectious diseases by pathogenic microorganisms. It is also known that elderly people and children are less resistant to diseases by pathogenic microorganisms [1].

Black cumin seed is used widely in ethno-cultural pharmacological disciplines in treatment of diseases such as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and flu. Major component of black cumin oil is thymoquinone. In addition, it contains terpens such as thymohydroquinone and dithymoquinone [2]. Black cumin seed contains 36-38% fixed oil and 0.4-2.5% essential oil [3]. Chemical composition may vary according to pressing method and raw material.

In this study, antibacterial effect of BCSO was investigated against pathogen bacterias to find out role for BCSO as preservative.

Study was funded by Gaziosmanpasa University BAP (Scientific Research Project Commission), Tokat, Turkey.

Materials and Methods

Bacterial Strains

Test cultures, *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923), *Salmonella Thypimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 10876), were obtained from the Ministry of Food, Agriculture and Husbandry, Tokat Food Control Laboratory Directorate, Turkey. Stock cultures of bacteria were stored at -80°C in Brain Hearth Infusion Broth (BHIB, Lab M, LAB 049, UK) containing 20% glycerol (Merck, 1.04092.2500, Germany). Before the analyzes, *L. monocytogenes*, *S. aureus*, *Salmonella Thypimurium*, and *E. coli* were enriched and activated twice in BHIB at 37 ± 2°C and *B. cereus* at 30 ± 2°C for 18 - 24 hours.

BCSO Samples

In this study, BCSO samples were purchased from local producers (Tokat, Turkey) and kept away from light and heat in room temperature.

Preparation of BCSO samples

In every solution which contains oil sample, Tween 80 (Merck, 8.22187.0500, Germany) was used as an emulsifying agent for the dilution liquid, pre-enrichment, enrichment and broth in 0.1% ratio.

Determination of antibacterial activity

Growth rate of test cultures was determined by inoculating on PCA agar and target microorganisms was arranged to be 10^6–10^7 cfu to inoculate into the plate-wells.

96-well plates were used in study. 200 µl of sample was placed in the first well. There are 8 x 12 wells in plates. First well was used as witness for only oil sample. It shouldn't have microbial growth. Also last well was used as witness for only test cultures. It should have microbial growth. With this design, we had control points of test cultures' and oil samples' efficiency. 100 µl of BHI Broth was added to other wells. 100 µl of sample was taken from the first well; homogenized and then transferred to the second well. Likewise, 100 µl of sample was taken from the second well; after homogenization; transferred to the third well. In this way, the dilution processes up to the eleventh well was completed. The final concentration of the samples in the wells at the end of dilution is respectively: 1:1 (100%), 1:2 (50%), 1:4 (25%), 1:8 (12.5%), 1:16 (6.25%), 1:32 (3.13%), 1:64 (1.56%), 1:128 (0.78%), 1:256 (0.39%), 1:512 (0.20%), 1:1024 (0.10%) and witness of test culture in last well (0.0%).

After oil sample dilution process, 100 µl of test culture was inoculated into each well. *E. coli*, *L. monocytogenes*, *Salmonella Thypimurium* and *S. aureus* were incubated at 37 ± 2°C in BHI broth for 18 - 24 hours and *B. cereus* at 30 ± 2°C for 18 - 24 hours. As a result of incubation, single drop colony and drawing method was performed from each well onto BHI agar. Results were recorded as negative and positive.

Process was repeated four times for each microorganism and results were obtained as positive (+) and negative (-). Results were evaluated as minimum inhibitory concertation.

Fatty acid composition

Fatty acid composition analysis was performed by Gaziosmanpaşa University Chemistry Department, Tokat, Turkey. 50 mg of oil sample was weighed and dissolved in 3 ml of hexane. 3 ml, 2 molar KOH (prepared in methanol) was added into solution and vortexed for 1 minute. After keeping at room temperature for 30 minutes to separate the phases, 1 ml of GC was taken from the upper phase and analyzed. Analysis of fatty acids was performed on "Perkin Elmer Clarus 500" brand gas chromatography device. Flame ionization detector and capillary column (RTX-2330; 30 m x 0.25 mm x 0.20 µm) were used. Detector temperature of GC device was determined as 250°C, injector temperature 250°C, injection split 50/1, carrier gas (helium) flow rate 1 ml/min. The oven temperature program was set at 120°C for 2 minutes, it was increased to 180°C within 2 minutes, the temperature was increased 4°C per minute from 180°C to 200°C and the furnace program was completed by keeping it at 200°C for 3 minutes.

Results and Discussion

Antibacterial effect of BCSO is shown in Table 1. BCSO has shown antibacterial effect against *B. cereus* and *S. aureus* at the most diluted concentration (0.10%). Inhibition wasn't observed against other bacterias. In aspect of Gram positive and negative classification; It has been found that Gram (+) bacterias are less resistant to aromatic oils than Gram (-) bacterias in various studies. This may cause according to different cell wall structure.

Table 1: Results of fatty acid composition analyses.

Peak #	Time (Minute)	Component	Result (%)
1	3.127	C8:0 Caprylic Acid ME	0.00
3	5.757	C11:0 Undecanoic Acid ME	0.01
4	7.189	C12:0 Lauric Acid	0.00
5	9.201	C13:0 Tridecanoic Acid	0.00
6	11.402	C14:0 Myristic Acid	0.18
7	13.003	C14:1 Myristoleic Acid	0.00
8	14.024	C15:0 Pentadecanoic Acid	0.05
9	16.963	C16:0 Palmitic Acid	12.46
10	18.371	C16:1 Palmitoleic Acid	0.22
11	19.856	C17:0 Heptadecanoic Acid	0.05
12	21.215	C17:1 cis-10-Heptadecanoic Acid	0.03
13	23.244	C18:0 Stearic Acid	2.86
14	24.403	C18:1n9c Oleic Acid	23.33
16	26.690	C18:2n6c Linoleic Acid	57.41
17	29.197	C18:3n3 Alpha Linoleic Acid	0.43
18	30.244	C20:1 cis-11-Eicosenoic Acid	0.33
19	32.435	C20:2 cis-11-14- Eicosenoic Acid	2.40
20	34.712	C22:1n9 Erucic Acid	0.07
22	36.413	C23:0 Tricosanoic Acid	0.09
23	36.748	C20:5n3	0.04
24	36.985	C24:0 Lingoceric Acid	0.00
25	38.801	C24:1 Nervonic Acid	0.01
26	39.609	C22:6n3	0.00
		Total	100

Fatty acid composition results are shown in Table 1. Primary fatty acid composition of BSCO was linoleic acid (18:2n-6c) at a level of 57.41 g / 100 g total fatty acid and followed by oleic acid

(18:1n9c) at a level of 23.33 g / 100 g; palmitic acid (16:0) at a level of 12.46 g / 100 g.

As a result of the study, it can be suggested that BCSO can be used as an antibacterial additive in food production process.

In addition, it is difficult to determine the inhibition effects of aromatic oils. The reason for this is many variables such as harvest conditions of the plant, method of extraction, geography, activity of the emulsifying agent used in broth and dilutions, and the differences in the method used in antibacterial examination may cause differences in the results. Therefore, in the literature, inhibition diameter, MIC and MBC values of aromatic oils can vary.

Conclusion

With the trend of consumers to natural products in past years, manufacturers prefer natural additives in regard. Pathogenic microorganisms threaten human health and can lead to foodborne diseases which can result in death. Black cumin seed oil can be herbal additives candidates for use as preservatives in food production with their functional properties.

Acknowledgments

Financial support for this research (scientific research project) was provided by Gaziosmanpaşa University (Tokat-Turkey).

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