Inhibition effect of rosemary and black cumin essential oils on some micro-organisms

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ABSTRACT

The present study was conducted to investigate the antimicrobial activity of rosemary and black cumin essential oils against different isolates of microorganisms including two bacteria strains: gram-positive bacteria (*Staphylococcus aureus)* and gram-negative bacteria (*Escherichia coli)* as well as two fungus strains (*Aspergillus niger* and *Aspergillus flavus*) and one yeast strain (*Saccharomyces cerevisiae*). Four concentrations of rosemary and black cumin essential oils (5, 10, 15, or 20 µl /ml) were examined against all tested strains of microorganisms by the agar diffusion method. The results showed that all various concentrations of rosemary and black cumin essential oils possess a significant inhibitory effect on the microorganisms strains used in this study. Rosemary is significantly the most effective essential oil against all tested strains of microorganisms compared to black cumin essential oil. The higher concentration of the essential oil has the greatest effect on growth inhibition compared to other concentrations. Depending on the findings of this study, it can be said that the essential oils of rosemary and black cumin have a greater and wider range of antimicrobial activity against several food-borne bacteria, and the crude extract can be used to find biological active items that may act as a starting point for the creation of new antimicrobial compounds. So, it can be recommended to use these essential oils as a source of potential of the active components in food preservatives.

Keywords: antimicrobial activity; rosemary; black cumin; essential oils; bacteria; fungus; yeast.

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a woody perennial herb belonging to the family Lamiaceae. Due to its essential oils and extracts, use as a spice, and the various biological functions, this plant, which is originally from the Mediterranean region, is today grown all over the world (Bozin *et al.*, 2007).

Black cumin (*Nigella sativa* L.) is an annual herbaceous plant belonging to the family Ranunculaceae. It has been associated with numerous medical benefits, including antioxidant, anticancer, anti-inflammatory, anti-allergic, antibacterial, and antifungal activities (Adegbeye *et al.*, 2020).

Numerous essential oils have been demonstrated to have potent antibacterial properties (Orhan *et al.*, 2012). Essential oils have shown antimicrobial properties against a wide variety of bacteria including antibiotic-resistant species and fungi (Soni and Soni, 2014). The capacity of essential oils to permeate cell membranes, damage cell membranes, impede cell functional characteristics, and finally cause cell contents leakage leading to cell death may be the cause of essential oils' antibacterial activity (Cai *et al.*, 2019).

The essential oil concentration affects the microbial population reduction where high concentrations of essential oil effectively inhibit the growth of microorganisms (Kalemba and Kunicka, 2003).

The investigation aims to evaluate the potentiality of rosemary and black cumin essential oils against different isolates of microorganisms.

MATERIAL AND METHODS

Plant materials

This investigation was carried out in the central laboratory of Horticulture Research Institute, Agricultural Research center to study the antimicrobial activity of rosemary *(Rosmarinus officinalis* L.) and black cumin (*Nigella sativa* L.) essential oils, which were obtained from the experimental station of Medicinal and Aromatic Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt and they were isolated by hydrodistillation.

Antimicrobial activity assay

Micro-organisms isolates

Five isolates of microorganisms, including two bacteria strains: gram-positive bacteria (*Staphylococcus aureus)* and gram-negative bacteria (*Escherichia coli)* as well as two fungus strains, (*Aspergillus niger* and *Aspergillus flavus*) and one yeast strain, (*Saccharomyces cerevisiae*) were obtained from Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt.

Media agar medium

All media used were obtained from (Difco).

Bacteria agar: includes l0.0g peptone, l0.0g meat exacts, 5.0g sodium chloride, 15.0 g agar, 1 liter distilled water, and 6.8±0.1 pH.

Yeast and fungi agar: includes 200.0g peeled potatoes, 20.0g D-glucose, 15.0 g agar, 1 liter distilled water, and 5.6±0.2 pH.

Muller Hinton Liquid and solid media were used for testing the antibacterial activity of all bacterial strains (Dhanalakshmi and Manimegalai, 2013) but for fungal strains were sub-cultured in nutrient yeast extract sucrose media (YES) peptone water used for diluting culture and nutrient agar media were used in plate count method.

The liquid medium was sterilized by auto-cleaving at 121°C for 20 min and then used for subculture and optical density assay while solid media was used for agar – well diffusion assay.

Extraction of essential oils

Thirty grams of dried medical plants along with 600 ml distilled water were subjected to HD for four hours using two Clevenger – type apparatus. The essential oils obtained this way were separated from water (due to their immiscibility with water and also as a result of a difference in its density level) and then dried over anhydrous sodium sulfate and stared in violas at 4°C. The extraction was repeated three times (Tepe *et al.,* 2005).

Disc diffusion method

The disc diffusion method was carried out to measure the antimicrobial activity according to Sleigh and Timburg (1981). Base agar was overlaid with agar (agar, 5ml) with inoculums of bacteria to yield a low growth. After the solidification of agar, each of the various crude essential oils of rosemary and black cumin was added at an amount of (5, 10, 15, or 20 µl /ml) on sterile paper discs (5mm diameter, Whatman No. 1 filter paper) in triplicates, then placed on agar plates previously inoculated and incubated at 35°C for 24- 48h. The inhibition zone diameter of the microbial growth produced by different essential oils was measured in mm (Orak *et al.,* 2011).

Experimental design and statistical analysis

A completely randomized design was performed for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Cochran (1980). Averages were compared using the L.S.D. values at 5% level (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of various concentrations of rosemary and black cumin essential oils on antimicrobial activity

As shown in Tables (1, 2, 3, 4 & 5) and Figures (1, 2, 3, 4 & 5), data revealed that the positive effect of various concentrations of rosemary and black cumin essential oils against all tested strains microorganisms including two bacteria: gram-positive bacteria (*Staphylococcus aureus)* and gram-negative bacteria (*Escherichia coli)* as well as two fungi (*Aspergillus niger* and *Aspergillus flavus*) and one yeast, (*Saccharomyces cerevisiae*) by the agar diffusion method.

Staphylococcus aureus

As regards the type of oil, it is obvious that rosemary oil had the highest inhibition zone diameter (41.5mm) against *Staphylococcus aureus.* On the other hand, black cumin oil resulted in the lowest one (15.3mm).

With respect to oil concentration, it is shown that the high concentration (20µl/ml) exhibited the maximum value of inhibition zone diameter (42.3mm) against *Staphylococcus aureus* followed by the moderate concentration (15 or 10µl/ml) produced zone diameter (30.8 and 24.7mm) respectively, while the low concentration (5µl/ml) gave the minimum value of inhibition zone diameter (15.9mm).

Concerning the interaction between type and concentration of oil, it is found that rosemary oil with a high concentration (20µl/ml) had the maximum value of inhibition zone diameter (61.2mm) against *Staphylococcus aureus*, whereas, black cumin oil at a low concentration (5µl/ml) resulted in the lowest inhibition zone diameter (7.4mm).

Escherichia coli

As regards the type of oil, it is obvious that rosemary oil had the highest inhibition zone diameter (38.5mm) against *Escherichia coli.* On the other hand, black cumin oil resulted in the lowest one (14.1mm).

Concerning oil concentration, it is shown that a high concentration (20µl/ml) exhibited the maximum value of inhibition zone diameter (40.3mm) against *Escherichia coli* followed by the moderate concentration (15 or 10µl/ml) produced zone diameter (33.8 and 20.3mm) respectively, while a low concentration (5µl/ml) gave the minimum value of inhibition zone diameter (10.7mm).

Concerning the interaction between type and concentration of oil, it is found that rosemary oil with the high concentration (20µl/ml) had the maximum value of inhibition zone diameter (58.2mm) against *Escherichia coli*, whereas black cumin oil at the low concentration (5µl/ml) resulted in the lowest inhibition zone diameter (7.3mm).

Aspergillus niger

As regards the type of oil, it is obvious that rosemary oil had the highest inhibition zone diameter (23.1mm) against *Aspergillus niger.* On the other hand, black cumin oil resulted in the lowest one (13.2mm).

With respect to oil concentration, it is shown that a high concentration (20µl/ml) exhibited the maximum value of inhibition zone diameter (32.3mm) against *Aspergillus niger* followed by the moderate concentration (15 or 10µl/ml) produced zone diameter (19.3 and 13.3mm) respectively, while a low concentration (5µl/ml) gave the minimum value of inhibition zone diameter (7.7mm).

Concerning the interaction between type and concentration of oil, it is found that rosemary oil with the high concentration (20µl/ml) had the maximum value of inhibition zone diameter (39.4mm) against *Aspergillus niger*, whereas black cumin oil at the low concentration (5µl/ml) resulted in the lowest inhibition zone diameter (6.2mm).

Aspergillus flavus

As regards the type of oil, it is obvious that rosemary oil had the highest inhibition zone diameter (20.3mm) against *Aspergillus flavus.* On the other hand, black cumin oil resulted in the lowest one (15.8mm).

With respect to oil concentration, it is shown that a high concentration (20µl/ml) exhibited the maximum value of inhibition zone diameter (29.8mm) against *Aspergillus flavus* followed by the moderate concentration (15 or 10µl/ml) produced zone diameter (20.2 and 14.8mm) respectively, while a low concentration (5µl/ml) gave the minimum value of inhibition zone diameter (7.2mm).

Concerning the interaction between type and concentration of oil, it is found that rosemary oil with the high concentration (20µl/ml) had the maximum value of inhibition zone diameter (31.4mm) against *Aspergillus flavus*, whereas, black cumin oil at the low concentration (5µl/ml) resulted in the lowest inhibition zone diameter (6.1mm).

Saccharomyces cerevisiae

As regards the type of oil, it is obvious that rosemary oil had the highest inhibition zone diameter (29.4mm) against *Saccharomyces cerevisiae.* On the other hand, black cumin oil resulted in the lowest one (17.2mm).

With respect to oil concentration, it is shown that a high concentration (20µl/ml) exhibited the maximum value of inhibition zone diameter (34.4mm) against *Saccharomyces cerevisiae* followed by the moderate concentration (15 or 10µl/ml) produced zone diameter (25.3 and 20.2mm) respectively, while a low concentration (5µl/ml) gave the minimum value of inhibition zone diameter (13.3mm).

Concerning the interaction between type and concentration of oil, it is found that rosemary oil with the high concentration (20µl/ml) had the maximum value of inhibition zone diameter (41.7mm) against *Saccharomyces cerevisiae*, whereas, black cumin oil at the low concentration (5µl/ml) resulted in the lowest inhibition zone diameter (9.1mm).

Depending on the type and concentration of essential oil and the type of examined microorganisms, all of the examined essential oils inhibited the growth of the bacterial, fungal, and yeast strains employed in this experiment at varying rates. On the other hand, raising the content of essential oils reduced the activity of bacterial strain growth.

Antimicrobial activity may include intricate mechanisms, such as cell membrane permeabilization, membrane obliteration, suppression of functional cell characteristics, and, eventually, leakage of cell contents leading to cell death. (Cai *et al.,* (2019).

A good to moderate antimicrobial activity of rosemary and black cumin essential oils has

been reported by Jiang *et al.* (2011) who stared that the essential oil of rosemary showed antibacterial and antifungal activity. On the other hand, El-Nour *et al.* (2015) found that methanolic extracts of *Nigella sativa* seeds had antimicrobial activity against *Escherichia coli*.

CONCLUSION

It can be concluded that all of the evaluated essential oils have a major inhibition activity on the microorganism strains used in this study. Compared to black cumin essential oil, rosemary is noticeably more effective than all evaluated strains of microorganisms. Additionally, there are very substantial statistical disparities between the concentrations of the essential oils that were examined. The essential oil's higher concentration has the strongest impact on growth inhibition as compared to the lower one. Therefore, it is suggested that these essential oils be used as a potential source of active components for food preservatives.

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Table 1 : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Staphylococcus aureus*

Essential oil Type (A)	Essential oil concentration (µl /ml) (B)				MEANS (A)
	5	10	15	20	
Rosemary	24.3	37.1	43.4	61.2	41.5
Black Cumin	7.4	12.3	18.1	23.3	15.3
MEANS (B)	15.9	24.7	30.8	42.3	
LSD (A) =	3.7				
LSD (B) =	5.2				
LSD (AXB) =	7.4				

Table 2 : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Escherichia coli*

Essential oil Type (A)	Essential oil concentration (µl /ml) (B)				MEANS (A)
	5	10	15	20	
Rosemary	14.1	29.4	52.1	58.2	38.5
Black Cumin	7.3	11.2	15.4	22.3	14.1
MEANS (B)	10.7	20.3	33.8	40.3	
LSD (A) =	2.9				
LSD (B) =	4.1				
LSD (AXB) =	5.8				

Table 3: Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Aspergillus niger*

Essential oil Type (A)	Essential oil concentration (µl /ml) (B)				MEANS (A)
	5	10	15	20	
Rosemary	9.2	17.4	26.2	39.4	23.1
Black Cumin	6.2	9.1	12.3	25.1	13.2
MEANS (B)	7.7	13.3	19.3	32.3	
LSD (A) =	4.6				
LSD (B) =	6.5				
LSD (AXB) =	9.2				

Table 4 : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Aspergillus flavus*

Essential oil Type (A)	Essential oil concentration (µl /ml) (B)				MEANS (A)
	5	10	15	20	
Rosemary	8.3	18.2	23.1	31.4	20.3
Black Cumin	6.1	11.4	17.3	28.2	15.8
MEANS (B)	7.2	14.8	20.2	29.8	
LSD (A) =	4.1				
LSD (B) =	5.8				
LSD (AXB) =	8.2				

Table 5: Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Saccharomyces cerevisiae*

Essential oil Type (A)	Essential oil concentration (µl /ml) (B)				MEANS (A)
	5	10	15	20	
Rosemary	17.4	26.1	32.4	41.7	29.4
Black Cumin	9.1	14.3	18.2	27.1	17.2
MEANS (B)	13.3	20.2	25.3	34.4	
LSD (A) =	3.3				
LSD (B) =	4.7				
LSD (AXB) =	6.6				

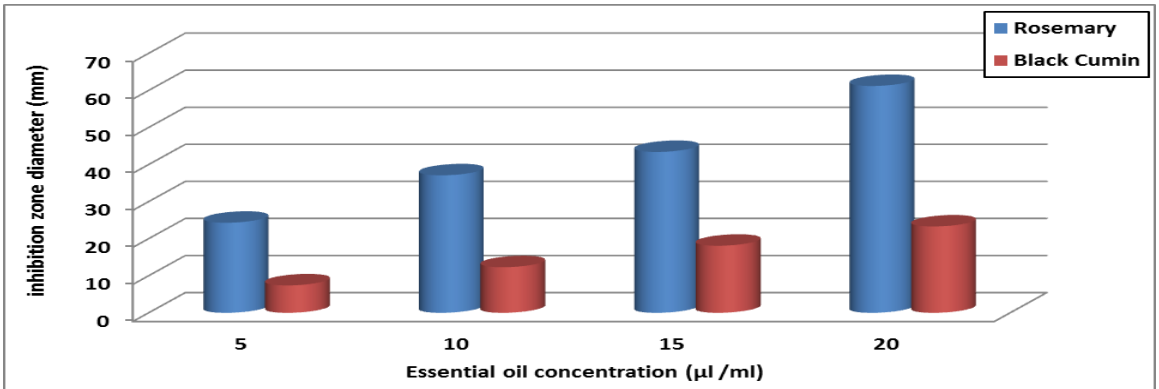

Figure (1) : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Staphylococcus aureus*

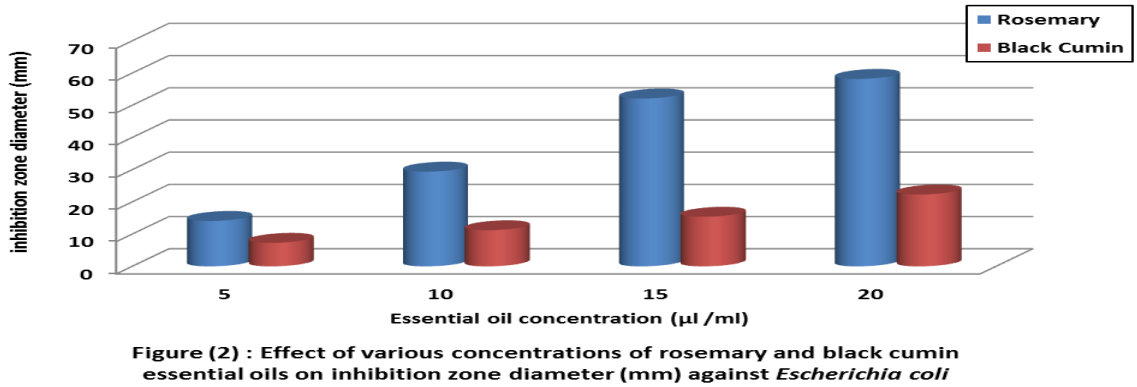

Figure (2) : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Escherichia coli*

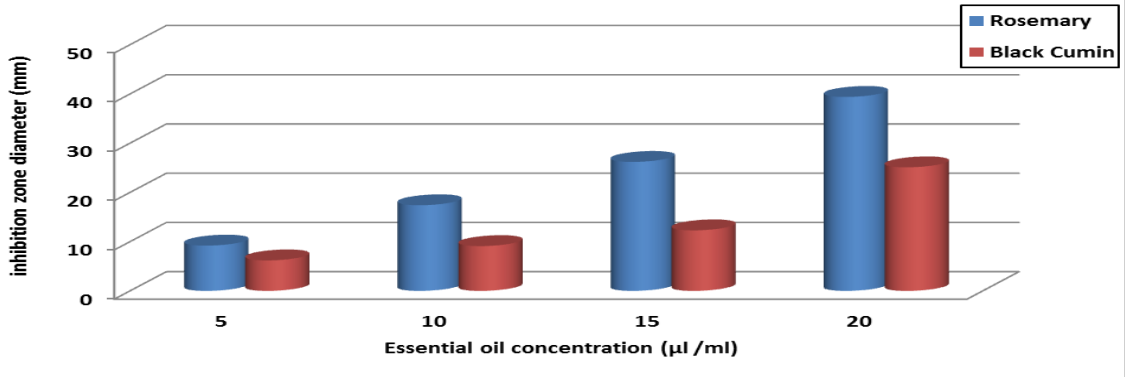

Figure (3) : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Aspergillus niger*

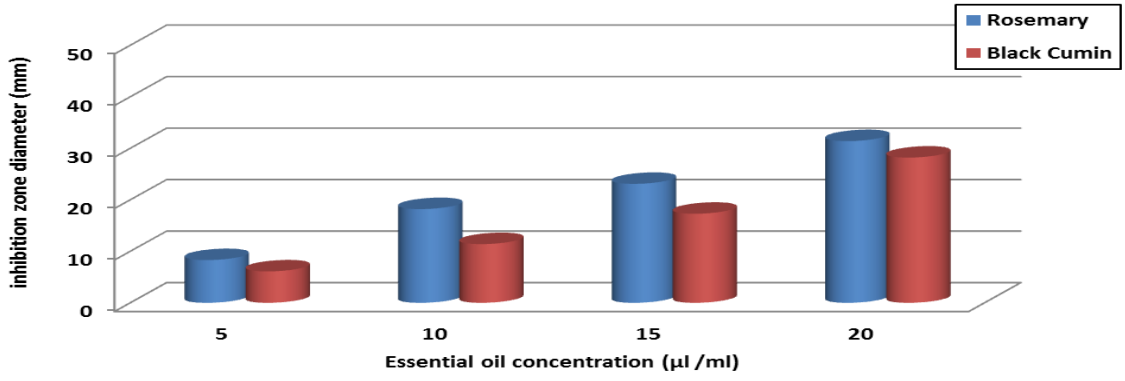

Figure (4) : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Aspergillus flavus*

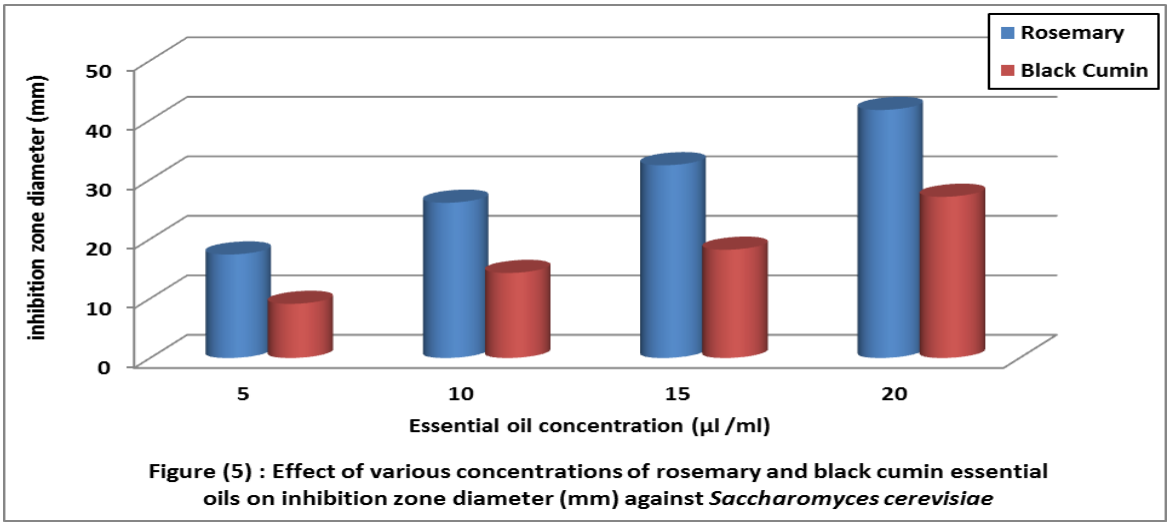

Figure (5) : Effect of various concentrations of rosemary and black cumin essential oils on inhibition zone diameter (mm) against *Saccharomyces cerevisiae*

التأثير التثبيطي للزيوت الطيارة للحصالبان وحبة البركة على بعض الكائنات الحية الدقيقة

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الملخص العربى

أجريت هذ التجربة لدراسة فعالية الزيوت الطيارة المستخرجة من الحصالبان وحبة البركة كمضادات الميكروبات ضد عزلات مختلفة من الكائنات الحية الدقيقة ويتضمن ذلك سلالتين من البكتيريا وهما: البكتيريا موجبة الجرام (*Staphylococcus aureus*) والبكتيريا سالبة الجرام (*Escherichia coli*) بالإضافة إلى سلالتين من الفطريات وهما: (*Aspergillus flavus* & *Aspergillus niger*) وسلالة واحدة من الخميرة وهى (*Saccharomyces cerevisiae*). تم اختبار أربعة تراكيز من الزيوت الطيارة للحصالبان وحبة البركة (5 ، 10 ، 15 أو 20 ميكرو لتر / مل) ضد جميع سلالات الكائنات الحية الدقيقة المختبرة بطريقة انتشار الآجار بطريقة الانتشار في الآجار. أظهرت النتائج أن جميع التركيزات المختلفة للزيوت الطيارة من الحصالبان وحبة البركة لها تأثير مثبط كبير لسلالات الكائنات الحية الدقيقة المستخدمة في هذه الدراسة. يعتبر الزيت الطيار للحصالبان الأكثر فعالية بشكل ملحوظ ضد جميع الكائنات الحية الدقيقة المختبرة مقارنة بالزيت الطيار لحبة البركة. كما أن التركيز الأعلى من الزيت الطيار له التأثير الأكبر على تثبيط النمو للميكروبات مقارنة بالتركيزات الأخرى. بناءً على النتائج التي تم الحصول عليها في هذه الدراسة ، يمكن الاستنتاج أن الزيوت الطيارة للحصالبان وحبة البركة لها طيف أقوى وأوسع من النشاط المضاد للميكروبات ضد عدد من البكتيريا التي تنقلها الأغذية ويمكن استخدام المستخلصات لاكتشاف المنتجات الطبيعية النشطة بيولوجيًا التي قد تخدم كمصدر أساسي لتطوير مركبات جديدة مضادة للميكروبات. لذلك، يمكن التوصية باستخدام هذه الزيوت الطيارة كمصدر أساسى للمكونات النشطة في حفظ المواد الغذائية.

الكلمات الاسترشادية: النشاط المضاد للميكروبات، الحصالبان، حبة البركة، الزيوت الطيارة، البكتيريا، الفطريات، الخميرة.