

Evaluation of the Antibacterial Activity of Various Spice Extracts Commonly Consumed in the Libyan Diet

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تقييم النشاط المضاد للبكتيريا لمستخلصات التوابل المختلفة المستهلكة بشكل شائع في النظام الغذائي الليبي

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Abstract:

Spices have long been used in cooking to improve the taste of food. This study investigated the antibacterial properties of six different spices, using both water-based and alcohol-based extracts. The spices examined were black pepper, red pepper, cardamom, turmeric, caraway, and coriander seeds. The current study tested these extracts against three types of bacteria: *Staphylococcus aureus* (a Gram-positive bacterium), as well as *Escherichia coli* and *Pseudomonas aeruginosa* (both Gram-negative). The results showed that ethanol (alcohol) extracts of the spices significantly reduced the growth of all three bacteria tested ($p < 0.05$). When ranking the antibacterial effectiveness of the six spices, coriander was the most effective, followed by turmeric, caraway, black pepper, cardamom, and finally red pepper. Among the water-based extracts, caraway stood out for its particularly strong antibacterial action. Interestingly, red pepper extract was especially potent against *P. aeruginosa*, while extracts of cardamom, black pepper, and turmeric did not inhibit bacterial growth in some cases. Generally, the spices showing the best antibacterial effects were coriander, caraway, red pepper, black pepper, turmeric, and cardamom. Notably, there was no significant difference in antibacterial effectiveness between the two types of extracts ($p > 0.05$). Overall, the findings suggest that these six spices have the potential to combat bacteria effectively. Of the bacteria tested, *P. aeruginosa* was the most sensitive to the spice extracts, followed by *E. coli*, whereas *S. aureus* was the most resistant. These results indicate that these common spices could offer a safe and natural alternative for treating bacterial infections in the future.

Keywords: Antibacterial activity, Ethanol extract, Aqueous extract, Spices.

الملخص

لطالما استخدمت التوابل في الطهي لتحسين مذاق الطعام. بحثت هذه الدراسة في الخصائص المضادة للبكتيريا لستة توابل مختلفة، باستخدام مستخلصات مائية وكحولية. التوابل التي تم فحصها هي الفلفل الأسود والفلفل الأحمر والهيل والكرم والكرامة وبذور الكزبرة. اختبرت الدراسة الحالية هذه المستخلصات ضد ثلاثة أنواع من البكتيريا: المكورات العنقودية الذهبية (بكتيريا موجبة الجرام)، بالإضافة إلى الإشريكية القولونية والزائفة الزنجارية (كلاهما سالبة الجرام). أظهرت النتائج أن مستخلصات الإيثانول (الكحول) من التوابل قللت بشكل كبير من نمو جميع البكتيريا الثلاثة المختبرة ($p < 0.05$). عند تصنيف الفعالية المضادة للبكتيريا للتوابل الستة، كانت الكزبرة الأكثر فعالية، تليها الكرم والكرامة والفلفل الأسود والهيل وأخيرًا الفلفل الأحمر. من بين المستخلصات المائية، برزت الكزبرة بشكل خاص. وبشكل مثير للاهتمام، كان مستخلص الفلفل الأحمر فعالاً بشكل خاص ضد الزائفة الزنجارية (*P. aeruginosa*)، بينما لم تثبط مستخلصات الهيل والفلفل الأسود والكرامة نمو البكتيريا في بعض الحالات. وبشكل عام، كانت التوابل التي أظهرت أفضل التأثيرات المضادة للبكتيريا هي الكزبرة والكرامة والفلفل

الأحمر والفلفل الأسود والكرم والهيل. والجدير بالذكر أنه لم يكن هناك فرق كبير في الفعالية المضادة للبكتيريا بين نوعي المستخلصات ($p > 0.05$). وبشكل عام، تشير النتائج إلى أن هذه التوابل الستة لديها القدرة على مكافحة البكتيريا بفعالية. ومن بين البكتيريا المختبرة، كانت الزائفة الزنجارية (*P. aeruginosa*) الأكثر حساسية لمستخلصات التوابل، تليها الإشريكية القولونية (*E. coli*)، بينما كانت المكورات العنقودية الذهبية (*S. aureus*) الأكثر مقاومة. وتشير هذه النتائج إلى أن هذه التوابل الشائعة يمكن أن تُقدم بديلاً آمناً وطبيعياً لعلاج الأمراض البكتيرية في المستقبل.

الكلمات المفتاحية: النشاط المضاد للبكتيريا، مستخلص الإيثانول، المستخلص المائي، التوابل.

Introduction

Countless plant-based compounds have shown therapeutic potential [1]. Nature has been responsible for the vast diversity seen in natural products over its long history of evolution, providing an inspirational source to scientists discovering new compounds useful for humanity [2]. Spices are acknowledged natural antioxidants and provide a major role with respect to the chemoprevention of disease and aging processes. Spicy food is a prominent aspect of many culinary food ways around the globe [3]. Spices have many uses to add to the flavour, colour, aroma and preservation of foods or beverages. Spices have been consumed in many cultures for many centuries and were traditionally consumed for their flavouring and aroma qualities. However, scientific studies are now confirming their biological activities beyond just their flavour and aroma [4].

In general, spices can be made from many parts of the plant, such as bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the complete plant tops [5]. With regard to spices, in Arabic, Baharat means "spices". Baharat is also a spice mixture or blend that is used throughout Libya. The general population of Fezzan region (southwest of Libya) is commonly recognized for its widespread consumption of a spice mixture known as "Baharat", particularly in local traditional dishes. The herbalists all recognize Baharat as having caraway, coriander, cardamom, cumin, cinnamon, ginger, black pepper, turmeric, fennel, red pepper, nutmeg, and cloves. For the purposes of this investigation, we chose to investigate six spices.

Worldwide, infectious diseases account for the majority of morbidity and mortality. The World Health Organization has recently reported that of the approximately 55 million people that died worldwide in 2011, one third was attributed to infectious diseases [6]. Morality can be increased by antibiotic resistant microorganisms due to their ability to withhold and recover transmission of their antibiotic resistance from antibiotic drugs, which are one of the treatments for infectious diseases [7]. Antibiotic resistant bacteria are putting people at risk and is jeopardizing the overall effectiveness of antibiotics by even limiting the treatment pathway for common infections [8]. The decrease of new antibacterial agents being tested and on the market relevant to antibiotic resistance pathogens like *S. aureus*, is only worsening the issue of antibiotic resistance [9]. Therefore, there is a lot of space in the literature to examine natural products that can be used as an effective antidote against human diseases [10], and act on pathogen with high efficacy and less side effects. The primary aim of this study is to identify and compare the *in vitro* antibacterial activity of six spices extracts black pepper (*Piper nigrum*), red pepper (*Capsicum frutescens* L.), cardamom (*Elettaria cardamomum*), turmeric (*Curcuma longa*), caraway (*Carum carvi*) and coriander (*Coriandrum sativum* L.).

Material and methods

Aqueous extract preparation

In March 2021, spices obtained from the local market of Sebha. The dried spices were ground to fine powders with an electric grinder. Regularly, a total of five (5g) powdered spices were added to the same conical flask containing 20mL of distilled water. The flasks were capped with a cooked wooden cork, and the flasks were shaken by hand for a few minutes, at those same times the mixtures in the conical flasks were placed at a shaker adjusted to 100 rpm overnight. The mixtures in the flask were then filtered using muslin cloth, and the mixture was centrifuged at 2000rpm for 5 min and the supernatant was transferred to sterile falcon tube after filtration and stored at 4°C in fridge [11].

Ethanol extract preparation

To prepare ethanolic extract, 5 gm of powdered herbs were stirred in 20 mL of 95% ethanol in a flask and sealed with a cork. Similar method was applied to mix and shake for 24 hours and filter with muslin cloth. Centrifugation at 2000 rpm for 5 min was done and the supernatant was decanted. The pellet was thrown away, the supernatant was filtered and concentrated in a rotary evaporator. The extract was kept at 4°C in a sterile falcon tube in refrigerator until further use [11].

Preliminary phytochemical analysis

The extract would then be filtered and qualitatively examined for the detection of different phytochemical components. Alkaloids would be assessed from the Dragendorff test [12], and carbohydrates and proteins would be determined from the Molisch and Biuret tests, respectively [13]. Cardiac glycosides would be determined by a concentrated H_2SO_4 test [14], coumarin would be identified using alcoholic sodium hydroxide and flavonoids

would be determined from Pew's test [15]. Saponin would be detected using a foam test, tannins would be estimated using ferric chloride test, and terpenes would be determined using Salkowski's test [12].

Sterility test of extracts

Sterility of all the extracts was examined on nutrient agar. 1ml of each extract were inoculated in nutrient agar plates and incubated for 24 hours at 37°C. Bacterial growth was observed to check the contamination. No growth in the plates indicated that the extracts were sterile.

Preparation of the bacterial cultures

The test organisms, *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Escherichia coli* (*E. coli*), were obtained from stock culture in the Microbiology laboratory, Botany department, Faculty of Science, Sebha University. The inoculum for each of the three microorganisms in the experiment was prepared to a final concentration of 10⁵ CFU/mL, confirmed using the pour plate method. For each trial, 10 mL of freshly made sterile nutrient broth was inoculated directly from the slant cultures.

Assessment of Antibacterial Activity

The bacteria used in this study include one species that is gram-positive (*S. aureus*) and two species that are gram-negative (*E. coli* and *P. aeruginosa*). The antibacterial properties of the spice extracts were evaluated using the agar disk diffusion technique, as outlined by Banjara (2012) [16]. Sterile nutrient agar, melted and cooled to 45°C, was poured (20 mL each) into standard sterile petri dishes to form a base layer. Once set, 0.1 mL of each bacterial strain was spread evenly over the surface using the spread plate method. Sterile filter paper discs (Whatman No. 1.5 mm in diameter) were soaked with spice extracts. These discs, measuring 8 mm, were carefully placed onto the inoculated agar plates and allowed to dry under a laminar flow hood to maintain sterility. The plates were then inverted and incubated at 37°C for 24 hours. After incubation, the clear zones around the discs, indicating bacterial inhibition, were measured. For controls, discs soaked only in the pure solvents that used to prepare the extracts (without any spice extract) were used. Each experiment was performed in triplicate to ensure accuracy.

Statistical analysis

The gathered information was examined with the help of the Statistical Package for Social Science (SPSS) for Windows version 25.0. The evaluation included both one-way, two-way ANOVA methods and t- test. One-way ANOVA was employed to compare numerical data among multiple groups, while two-way ANOVA looked at how two independent factors influenced the dependent variable. t- test was used to compare between aqueous and ethanol extracts. A p-value of under 0.05 was identified as statistically important.

Results and discussion

Determination of phytochemical active agents in spices

In the current investigation, we looked at spices first for identifying prominent constituents. The study investigated the extracts of chosen spices and found the presence of phytochemical active agents (Table 1). The extracts contained compounds such as alkaloids; carbohydrates; amino acids; glycoside; triterpenoid; tannins; and flavonoids.

Table 1: Phytochemical active components in spices.

Components	Spices					
	Red pepper	Black pepper	Turmeric	Caraway	Cardamom	Coriander
Dragendorff's (Alkaloids)	+	+	-	-	-	-
Tannins	+	+	+	+	+	-
Flavonoids	-	+	-	-	-	-
Ninhydrin (amino acids)	+	-	+	+	-	+
Triterpenoid	-	+	+	+	+	+
Glycoside	+	+	-	+	-	+
Reducing sugar Fehling test	+	+	+	+	+	-

Evaluation of antibacterial activity of extracts

As illustrated in Figure 1, the antibacterial effectiveness of ethanolic extracts of selected spices against *S. aureus*, *P. aeruginosa*, and *E. coli* is demonstrated. The findings demonstrated that turmeric and red pepper exhibited minimal activity towards *E. coli*. The inhibitory zones against *E. coli* were as follows: black pepper (14.67 ± 3.06 mm), coriander (17.00 ± 2.00 mm), cardamom (11.33 ± 2.52 mm), caraway (12.33 ± 2.52 mm), turmeric (10.0 ± 1.00 mm), and red pepper (9.33 ± 0.58 mm). The inhibition zones against *S. aureus* were as follows: black pepper (13.33 ± 2.52 mm), coriander (12.33 ± 2.52 mm), cardamom (13.67 ± 1.53 mm), caraway (13.0 ± 3.00 mm), turmeric (18.33 ± 2.08 mm), and red pepper (12.00 ± 1.73 mm). It was observed that turmeric extract exhibited a comparatively high inhibitory effect against *S. aureus*. The inhibition zones of black pepper, coriander, cardamom, caraway, turmeric, and red pepper against *P. aeruginosa* were measured to be 11.33 ± 1.15 mm, 16.33 ± 1.53 mm, 13.00 ± 2.65 mm, 14.33 ± 2.52 mm, 13.00 ± 2.65 mm, and 12.33 ± 2.52 mm, respectively.

The results in Figure 1 and Table 2 indicate that all tested bacterial strains were inhibited by the ethanolic extracts of black pepper, coriander, cardamom, caraway, turmeric, and red pepper. The two-way ANOVA experiment results showed that the ethanolic extracts of black pepper, coriander, cardamom, caraway, turmeric and red pepper inhibited *S. aureus*, *P. aeruginosa*, and *E. coli*; $p < 0.05$.

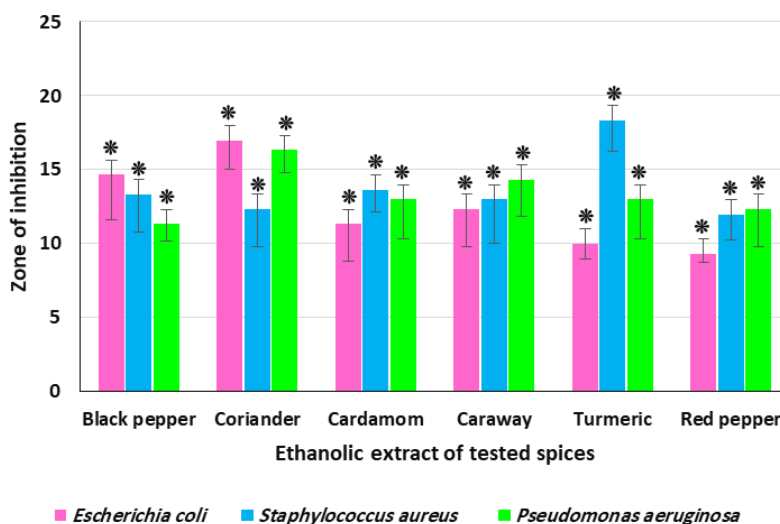


Figure 1: Antibacterial activity of ethanolic extract of selected spices .

Table 2: Mean values of antibacterial activity of selected spices against *S. aureus*, *P. aeruginosa* and *E. coli*

Spices	Extract	Bacteria		
		<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>
Black pepper	Aqueous	0.00±0.00 ^b	11.0±1.0 ^b	12.33±1.53 ^b
	Ethanolic	13.33±2.52 ^{a,b,c}	11.33±1.15 ^{a,b,c}	14.67±3.06 ^{a,b,c}
Coriander	Aqueous	8.00±1.00 ^a	12.33±3.06 ^a	9.67±2.08 ^a
	Ethanolic	12.33±2.52 ^a	16.33±1.53 ^a	17.00±2.00 ^a
Cardamom	Aqueous	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d
	Ethanolic	13.67±1.53 ^{b,c}	13.00±2.65 ^{b,c}	11.33±2.52 ^{b,c}
Caraway	Aqueous	10.0±1.00 ^a	12.33±2.08 ^a	11.33±1.53 ^a
	Ethanolic	13.0±3.00 ^{a,b,c}	14.33±2.52 ^{a,b,c}	12.33±2.52 ^{a,b,c}
Turmeric	Aqueous	0.00±0.00 ^c	6.33±0.58 ^c	9.33±2.52 ^c
	Ethanolic	18.33±2.08 ^{a,b}	13.00±2.65 ^{a,b}	10.0±1.00 ^{a,b}
Red pepper	Aqueous	9.0±2.65 ^a	14.0±1.73 ^a	9.67±1.53 ^a
	Ethanolic	12.00±1.73 ^c	12.33±2.52 ^c	9.33±0.58 ^c

Mean values in each column for each spice followed by the same letters are not significantly different ($p < 0.05$) (Duncan's Multiple Range Test).

As indicated in Table 2 and Figure 2, it appears that the water (aqueous) extract of caraway produced the greatest antibacterial effects. *S. aureus* showed the greatest level of resistance to black pepper. The turmeric water extract, on the other hand, had fairly low antibacterial activity and did not inhibit *S. aureus* at all. On the other hand, the water extract from cardamom showed no antibacterial action against any tested bacteria. The spice extracts in water demonstrated strong antibacterial effects against *P. aeruginosa* and *E. coli* ($p < 0.05$), as illustrated in Figure 2. The order of how effective the spice extracts are at fighting bacteria is: caraway, red pepper, coriander, black pepper, turmeric, and cardamom.

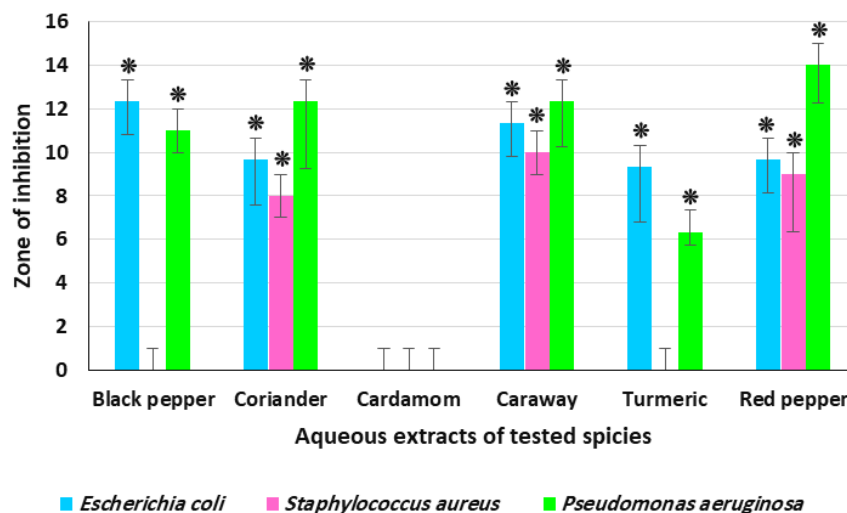


Figure 2: Antibacterial activity of aqueous extract of selected spices

The analysis of variance (ANOVA) findings revealed very significant impacts ($p < 0.001$) from Extract, Bacteria, and Spices on the dependent variable, with high F-statistics. Notably, all interactions, both two-way and three-way, involving Extract, Bacteria, and Spices were also significantly notable ($p < 0.001$) (see Table 3). This suggests that the effect of these factors does not occur uniformly across levels, implying that the influence of Extract is affected by the levels of Bacteria and Spices. For instance, the effects of Extract vary according to the levels of both Bacteria and Spices. The bacteria studied in this experiment were chosen based on their association with food spoilage and disease. *S. aureus* has been identified as the principal causative agent of disease affiliated with food. The genera *P. aeruginosa* and *E. coli* could, however, create dangerous toxins and other materials that create problems in the stomach of the consumer.

Table 3: Results of the two-way ANOVA (F-zones) used to test between subjects' effects (extract, spices, bacteria) and their interaction.

Independent variables	F	P value
Extract	235.035	.000
Bacteria	12.329	.000
Spices	25.190	.000
Extract * Bacteria	23.531	.000
Extract * Spices	24.128	.000
Bacteria * Spices	4.576	.000
Extract * Bacteria * Spices	6.710	.000

In their study to evaluate the antimicrobial properties of spice extracts (water-based) they observed that *P. aeruginosa* was affected the most, *E. coli* was affected next, and *S. aureus* had the greatest resistance. The aqueous extract of caraway was the strongest in antibacterial effects against the bacteria they tested. They also reported that the aqueous extract of red pepper was effective against *P. aeruginosa* with an inhibition zone of 14.0 mm; but the growth of *P. aeruginosa* was not affected by the black pepper, cardamom, and turmeric extracts. Potential reasons for the aqueous extracts (cardamom against *P. aeruginosa*, *E. coli*, and *S. aureus*; and turmeric and black pepper against *S. aureus*) to be inactive could be that the individual part of the spice plant is devoid of antimicrobial activity as a spice or could be in part, stem from the method of extraction [17] or time of collection of the herbal materials and environmental influences may have impacted levels of active constituents in the plant material [18]. In addition, the different responses of Gram-positive and Gram-negative bacteria to certain agents

are often due to the unique structural composition of their cell walls [19]. The difference in cell wall composition between these two groups significantly affects their susceptibility to antimicrobial drugs.

The statics analysis, the t- test shows that the ethanolic extract and watery (aqueous) extract are no significant different ($p>0.05$). The results, later not in agreement with those obtained by [20, 21], which was best for the ethanolic extract of the tested plant on relative to the watery extract of the same plant. It is still uncertain what the exact mechanism of antibacterial action of spices and derivatives are [22]. Having said that, these mechanisms have been hypothesized by previous study [23] as: hydrophobic and hydrogen bonding of phenolic compounds in anchoring to membrane proteins, leading to partitioning into the lipid bilayer; membrane perturbation of permeability through expansion and increase fluidity causing inhibition of adjustable enzymes that are embedded in the membrane; membrane disruption; damage to electrons transport systems and perturbation of the cell wall. On the contrary, recent work suggests that could the mechanisms, in part, relate to increase of reactive oxygen species (ROS) that disrupt the permeability and confirmation of the membrane, resulting in subsequent damage to bacterial cells [24].

Also, in this study the extracts from selected spices on the antibacterial activity showed different results. These different and conflicting results in the antimicrobial activity of these spices may have been attributed to the unreliable presence of different chemical components in spices. It has shown that samples imported from different geographic origins with different climates and vegetation show different antimicrobial activities [25, 26].

A possible reason the Gram-negative bacteria were more resistant compared to the Gram-positive, is that the Gram-negative bacteria have a thick peptidoglycan layer in their cell walls which may limit the passage of antimicrobial agents through their cell walls and maintain a rigid structure for their cells [27].

Conclusion

This research found that extracts from spices such as coriander, turmeric, and caraway, particularly in ethanol, can inhibit the growth of several harmful bacteria. Water extracts were generally less effective, except for caraway. Among the bacteria tested, *P. aeruginosa* was the most sensitive to spice extracts, while *S. aureus* was the least. These findings highlight the potential of common spices as sources for developing new antimicrobial treatments.

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