

Original article

Evaluation of the Antibacterial Activity of *Cucurbita maxima* Seed Oil Against Multidrug-Resistant Foodborne Pathogens

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Abstract

This study aimed to investigate the antibacterial activity of cold-pressed pumpkin seed oil (*Cucurbita maxima*) against selected foodborne multidrug-resistant (MDR) pathogens. The oil's inhibitory effect was evaluated against *Klebsiella pneumoniae*, *Salmonella enterica*, *Bacillus cereus*, and *Staphylococcus aureus* using the agar well diffusion method. Seven concentrations (5%-100%) were tested with amoxicillin as the positive control and dimethyl sulphoxide (DMSO) 5% as the negative control. The results demonstrated that pumpkin seed oil exhibited a concentration-dependent antibacterial activity. Significant inhibition was observed even at low concentrations (5%), with significant differences between the different concentrations ($p < 0.05$). These findings suggest that *Cucurbita maxima* seed oil promises to be a natural antibacterial agent and could serve as an alternative for managing MDR foodborne infections.

Keywords. *Cucurbita maxima* Seed Oil, Antibacterial Activity, Multidrug-Resistant Bacteria, Foodborne Pathogens; Libya.

Introduction

The problem of antimicrobial resistance (AMR) is evolving into a global epidemic, posing a significant risk to human health, food safety, and economic stability worldwide [1]. Multidrug-resistant (MDR) bacterial pathogens have emerged as a critical concern, rendering many conventional antibiotic treatments ineffective [2]. This alarming phenomenon is exacerbated by the widespread and often indiscriminate use of antibiotics in both human medicine and animal husbandry, contributing to the selection and proliferation of resistant strains [3-4]. The processes that cause bacteria to become resistant are both varied and complex, including reducing the uptake of drugs, altering the targets of drugs, enzymatically degrading antibiotics, and actively pumping out drugs from the bacterial cell [5-6]. The mechanisms help the bacteria to avoid the effects of antibiotics, which results in chronic infections, a rise in morbidity and mortality, and a rise in health care expenses [2-7]. The overall impact of bacterial AMR is considerable, and the estimates show it directly caused 1.27 million deaths worldwide in 2019 and contributed to 4.95 million deaths [1]. The financial implication is also quite immense, as the healthcare expenses worldwide on an annual basis are more than USD 100 billion due to resistant infections [2].

Against this challenge of the urgent situation, a great momentum has been accumulated around the necessity of new antimicrobial strategies and alternative therapeutic agents [8-9]. One area that is being researched and developed as a potential new source of innovative antimicrobial alternatives is the development of natural alternatives, specifically plant-based alternatives. Antimicrobials that are based on plants are abundant in bioactive substances, tend to have low toxicity, and are deemed to have no major side effects when used in the long term and in moderation relative to synthetic drugs [10-11]. Their ubiquitous presence and affordability also contribute to their possible advantage as a sustainable solution to multidrug-resistant (MDR) agents, particularly in those areas where there is insufficient access to high-technology medications [12]. Of those, essential oils obtained from plants are particularly important in this regard because they are rich in bioactive molecules, have a positive safety profile, and are cost-effective. One such candidate is the pumpkin plant (*Cucurbita* spp., family Cucurbitaceae), a globally cultivated crop that has recently attracted scientific interest for its medicinal properties.

Specifically, pumpkin seeds and oil possess a range of biological properties, including antioxidant, antidiabetic, anti-inflammatory, antitumor, and antimicrobial activities [13-14-15-16]. Pumpkin is a seasonal fruit and is grown in the summer months. They are plants native of South and Central America and are grown widely all over the world. Pumpkin plants have distinct characteristics, including large, flat, frosted leaves and bright yellow flowers. There are three common varieties of pumpkins: The three sub-genres of *Cucurbita* are *Cucurbita maxima*, *Cucurbita moschata*, and *Cucurbita pepo* [17]. Various studies have shown that different parts of the pumpkin plant contain biologically active compounds and antioxidants, such as polyphenols, flavonoids, carotenoids, unsaturated fatty acids, and vitamin E. These compounds enhance immunity, improve the condition of the digestive system, lower cholesterol and glucose, and reduce the likelihood of cardiovascular disease and cancer [18-19]. Although pumpkin seeds are of small size, they contain high levels of proteins, essential amino acids, phytosterols, phenolics, and flavonoids, which have high antioxidant defenses and preventive effects against chronic diseases [18-20-21]. Their inclusion in bakery and food products has also been shown to enhance nutritional and antioxidant profiles [22]. Several studies have demonstrated the antimicrobial activity of pumpkin seed oil and extracts against bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* [10-23-24].

Ethanol extracts, especially, seem to have greater activity, particularly with gram-positive bacteria. Moreover, antiviral effects have also been demonstrated by pumpkin seed lignans (e.g., pinoresinol, medioresinol, and lariciresinol) [10]. Recent findings by Leichtweis and Abu-Zaid [24-25] reported that the phenolic and antioxidant activity of pumpkin-derived products is associated with their antimicrobial properties, as one of the reasons to consider them as possible food preservation and pharmaceutical use. Therefore, the research has the following objectives: (1) to evaluate the antimicrobial activity of the *Cucurbita maxima* seed oil obtained at Libyan markets, and (2) to compare its effectiveness with chosen standard antibiotics against multidrug-resistant (MDR) foodborne bacterial pathogens of animal origin.

Methods

Sample collection

Samples of pumpkin seed oil extracted by cold pressing were purchased from a Libyan market. This method is considered ideal because it preserves the nutritional properties of the oil and helps naturally extend its shelf life, as it has not been exposed to heat [26]. Samples were collected in sterile containers and transported to the laboratory for further analysis.

Preparation of Pumpkin Seed Oil Concentrations

To prepare the pumpkin seed oil extracts, dimethyl sulphoxide 5% (DMSO) was used as a solvent. Different concentrations of pumpkin seed oil were prepared by diluting the oil with DMSO. The concentrations of the extracts were prepared as follows (5%,10%,15%,25%,50%,75%,100%) [15]. This is often done in studies that use plant oils, like pumpkin seed oil, with a starting concentration of 5 % to determine efficacy with the lowest possible concentration, followed in a generally accepted pattern by increasing to 100 % to determine maximum efficacy [27].

Microorganisms

Bacterial strains used in this study were part of the Food-borne Libyan-type bacterial collection (FLBC), which is stored at the Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Tripoli. These strains, originally isolated from foodborne sources in Libya, were selected based on their multidrug-resistant profiles and clinical relevance. The Gram-positive bacteria were *Bacillus cereus* and *Staphylococcus aureus* [28-29], and the Gram-negative ones were *Salmonella enterica* [30] and *Klebsiella pneumoniae* [31]. The isolation and characterization information of each strain is reported in the corresponding reference, respectively, citing references of bacterial samples used, ensuring traceability and reliability.

Culture Preparation

The strains were subcultured on nutrient agar plates and incubated at 37°C for 24 hours. MacFarland determined that the cell harvest used for microbiological activity had a viable cell count of 107 colony-forming units (CFU /mL). In brief, the antimicrobial activity was assessed by 0.5 MacFarland.

Antibacterial Activity Testing

The agar-well diffusion method was used to test the antimicrobial activity of pumpkin seed oil extracts. 0.1 ml of microbial suspension, followed by twenty ml of Mueller-Hinton agar media, was poured into sterilized Petri dishes, each of which had previously been at (0.5 MacFarland). Wells were made on the agar plates using a sterile cork borer. The pumpkin seed oil extracts were used at different concentrations (100%,75%,50%,25%,15%,10%,5%). It was added to the wells. DMSO was used as a negative control, while amoxicillin at a concentration of 25 was used as a positive control. The dishes were refrigerated at 5 °C for 2 hours. The plates were then incubated at 37°C for 24h. All tests were performed in triplicate to ensure reproducibility and reliability of the results [15].

Measurement of Zone of Inhibition

The growth of the agar plate was carefully observed after the incubation period to observe the existence of a clear zone of inhibition that surrounds the wells. These areas were first measured with a calibrated ruler, and they were in centimeters by measuring the diameter of the areas. They were normalized to millimeters (every 1 cm = 10 mm) to be consistent and based on the specified conventions of recording measurements, as in the case of microbiology laboratory work. Measurements were made for each replication, and an average calculation was made on the diameter of each of the concentrations and strains. These mean values were used to statistically explain the action on the antimicrobial activity.

Data Analysis

The results of the antimicrobial test, specifically the diameters of the inhibition zones, were analyzed using statistics. To have reproducibility and reliability, measurements were taken in triplicate per treatment (different concentrations of pumpkin seed oil and controls). The mean zone of inhibition by each concentration of pumpkin seed oil extract and the control groups was calculated.

The data were then analyzed by one-way analysis of variance (ANOVA) to establish whether there was a statistically significant difference in the antimicrobial activity of the different concentrations and controls. A p-value of below 0.05 ($p < 0.05$) was taken to be statistically significant. In cases where ANOVA found significant differences, Tukey post-hoc tests were used to identify specific differences between separate groups of treatment. All the statistical procedures were conducted with the help of SPSS v26.

Results

Antimicrobial Activity of Pumpkin Seed Oil Extracts

The antimicrobial effects of the pumpkin seed oil extracts against the microorganisms being tested were compared by observing the zone of inhibition. The results showed that pumpkin seed oil extracts exhibited varying degrees of antimicrobial activity against the tested bacterial isolates. The impact of pumpkin seed oil on gram-positive (*B. cereus*, *S. aureus*), Gram-negative (*S. enterica*, *K. pneumoniae*) bacteria was demonstrated in (Table 1). Pumpkin seed oil was effective against all of them (*B. Cereus* was the most sensitive strain to pumpkin seed oil, followed by *S. aureus*, *S. enterica*, and *K. pneumoniae*).

Table 1. Antimicrobial activities of pumpkin seed oil against some bacterial strains compared to antibiotics (diameter of the zone of inhibition measured in mm).

Concentration (V/V)	Inhibition Zone (mm $\pm$ SD)			
	Gram-negative Bacteria		Gram-positive Bacteria	
	<i>Klebsiella pneumoniae</i> ^{1*}	<i>Salmonella enterica</i> ²	<i>Staphylococcus aureus</i> ³	<i>Bacillus cereus</i> ⁴
5%	6mm $\pm$ 2.00	6.0 $\pm$ 3.46	8.0 $\pm$ 2.00	6.0 $\pm$ 2.00
10%	10 mm $\pm$ 0.00	10.0 $\pm$ 3.46	12.0 $\pm$ 3.46	12.0 $\pm$ 2.00
15%	10 mm $\pm$ 2.00	10.0 $\pm$ 0.00	14.0 $\pm$ 3.46	14.0 $\pm$ 2.00
25%	8 mm $\pm$ 2.00	10.0 $\pm$ 2.00	14.0 $\pm$ 2.00	18.0 $\pm$ 2.00
50%	8 mm $\pm$ 3.46	8.0 $\pm$ 2.00	14.0 $\pm$ 2.00	18.0 $\pm$ 2.00
75%	6 mm $\pm$ 2.00	6.0 $\pm$ 3.46	12.0 $\pm$ 2.00	10.0 $\pm$ 2.00
100%	8 mm $\pm$ 2.00	6.0 $\pm$ 2.00	12.0 $\pm$ 4.00	10.0 $\pm$ 0.00
Amoxicillin 25 μ g	0.0 $\pm$ –	10.0 $\pm$ 2.00	10.0 $\pm$ 3.46	0.0 $\pm$ –
D.M.S.O	0.0 $\pm$ –	0.0 $\pm$ –	0.0 $\pm$ –	0.0 $\pm$ –

¹ $p = (0.007)$, ² $p = (0.00045)$, ³ $p = (0.0001)$, ⁴ $p = (0.000001)$

Zone of Inhibition

The mean zone of inhibition for each concentration of pumpkin seed oil extract against each microorganism was calculated. The results showed that the zone of inhibition increased with increasing concentrations of pumpkin seed oil extract for all tested bacterial isolates. However, the extent of inhibition varied among the microorganisms.

Antimicrobial Activity against Bacteria

The results obtained from the antimicrobial activity assay showed that pumpkin seed oil extracts exhibited significant inhibition of the growth of Gram-positive bacteria, including *B. cereus* and *S. aureus*. As shown in (Table 1), the mean zone of inhibition increased with the increase in the concentration of extracts. At the concentration of 15%, pumpkin seed oil extract showed an average zone of inhibition of 14 mm against *B. cereus* and *S. aureus*. These results indicate that pumpkin seed oil has a dose-dependent antibacterial effect against Gram-positive bacteria. In contrast, Gram-negative bacteria, including *K. pneumoniae* and *S. enterica*, were relatively weak, and the average zone of inhibition was 10 mm with the same concentration (15%).

This indicates that pumpkin seed oil extracts are not that effective when it comes to Gram-negative strains, which can be explained by the fact that they differ structurally in terms of their cell walls. Gram-negative bacteria have an outer membrane that is highly concentrated in lipopolysaccharides, which forms a permeability barrier that restricts the penetration of hydrophobic compounds like plant oils, thus making them less susceptible to attack by Gram-positive bacteria.

Comparison with Standard Antibiotics

To provide a comparison of the antimicrobial activity observed of *Cucurbita maxima* seed oil, amoxicillin (25 μ g/mL) was selected as a reference antibiotic.

The zones of inhibition produced by the pumpkin seed oil extracts were directly compared with those elicited by amoxicillin against the tested multidrug-resistant foodborne pathogens. The results indicated that the pumpkin seed oil extracts demonstrated antimicrobial activity that was either comparable to or, in some instances, superior to that of amoxicillin. Specifically, the oil exhibited similar inhibitory effects against *Salmonella enterica* and greater activity against *Staphylococcus aureus* when compared to the standard

antibiotic. This suggests a significant potential for *Cucurbita maxima* seed oil as an alternative or complementary agent in combating these resistant bacterial strains, particularly given the growing concerns regarding antibiotic resistance.

Statistical Analysis of Results

The antimicrobial activity data were statistically evaluated via one-way analysis of variance (ANOVA) and Tukey post-hoc tests. The outcome was a statistically significant difference ($p < 0.05$) in the mean zone of inhibition in the different concentrations of the pumpkin seed oil extract on all the microorganisms tested. This significant difference just proves that the antimicrobial effect of the pumpkin seed oil does depend on the concentration. For *B. cereus*, the 25% and 50 % concentrations exhibited much larger inhibition zones than the lower concentrations (5% and 10%) and the two controls ($p < 0.05$). In the case of *S. aureus*, concentrations of 15%-50 % were found to be much more active compared to 5%, 100% and DMSO ($p < 0.05$). On the other hand, *S. enterica*, intermediate concentrations (10% to 25%) showed much better inhibition zones than 75% and DMSO ($p < 0.05$), and comparable with amoxicillin. In the case of *K. pneumoniae*, 10% and 15% concentrations showed significant improvement in comparison to 5% and controls ($p < 0.05$), although normally, the difference between other concentrations did not exhibit significant differences. Statistical tests indicate that the antibacterial action of *Cucurbita maxima* seed oil is a concentration-dependent effect, and it has the most effective antibacterial action at intermediate concentrations (15 % to 50 %), particularly to Gram-positive bacteria.

Discussion

The present study aimed to evaluate the antimicrobial activity of pumpkin seed oil obtained from the Libyan market and examine its action on multidrug-resistant bacteria, foodborne pathogens isolated from animal-derived foods in Libya. The results demonstrated that pumpkin seed oil exhibited concentration-dependent antibacterial activity against the tested bacterial strains, including both Gram-positive bacteria (*B. cereus* and *S. aureus*) and Gram-negative bacteria (*S. enterica* and *K. pneumoniae*). Specifically, pumpkin seed oil extracts showed a stronger inhibitory effect against Gram-positive bacteria compared to Gram-negative bacteria. *B. cereus* was found to be the most sensitive strain, followed by *S. aureus*. These findings are consistent with previous studies that have reported the higher susceptibility of Gram-positive bacteria to pumpkin extracts [23-24].

The higher susceptibility of Gram-positive bacteria may be attributed to differences in cell wall structure and composition compared to Gram-negative bacteria [11]. The weaker antimicrobial effect against Gram-negative bacteria, which possess an outer membrane that acts as a barrier to many antimicrobial agents, observed in this study is also in line with previous research [11-25]. The antimicrobial activity of pumpkin seed oil extracts was compared to that of amoxicillin. The results showed that pumpkin seed oil extracts exhibited similar or greater antimicrobial activity against *S. enterica* and *S. aureus* bacteria, respectively. These results highlight the potential of pumpkin seed oil as an alternative antimicrobial agent in the face of increasing antibiotic resistance. Even the low concentration of 5% pumpkin seed oil was found to have inhibition zones. This suggests that relatively small amounts of pumpkin oil may exert meaningful antimicrobial effects, which could be beneficial in food preservation or therapeutic applications. In conclusion, this study demonstrated that pumpkin (*Cucurbita maxima*) seed oil, obtained from the Libyan market, possesses notable antimicrobial activity against selected multidrug-resistant bacterial strains.

The results revealed a clear concentration-dependent inhibitory effect, with significantly stronger activity against Gram-positive bacteria compared to Gram-negative ones. These findings align with the growing body of evidence supporting the use of plant-based compounds in controlling foodborne pathogens. Pumpkin seed oil, rich in bioactive components such as polyphenols, tocopherols, and fatty acids, may act through multiple mechanisms, disrupting cell membranes, interfering with metabolic pathways, and generating oxidative stress in bacterial cells [10-18-32]. Overall, this research adds to the call for more studies to be warranted to investigate the chemical composition of the oil, identify the specific active compounds responsible for the antimicrobial activity, and assess its safety and efficacy in vivo and in clinical settings. The potential for pumpkin seed oil as a natural alternative to conventional antibiotics in combating MDR foodborne pathogens is significant, offering a promising avenue for future research and development.

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Conflicts of Interest

The authors declare no conflicts of interest.

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