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Effect of black pepper on various microorganism, Basra, Iraq

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Abstract

The antifungal and antibacterial properties of black pepper (Piper nigrum) were examined in this study. Against a number of isolated bacteria and fungi. 58 oral swabs in all were collected from 40 women and 18 men with diabetes and urinary tract infections at Al-Moani and Al-Fayhaa Hospitals in Al-Basra province during the period from September to November 2023. Disc diffusion was used in antimicrobial experiments to identify inhibitory zones against various bacterial isolates. According to the findings, black pepper revealed strong antifungal efficacy against Candida albicans (80%), Candida dubliniensis (80%), and Candida tropicalis (100%). Furthermore, the extract showed strong antibacterial activity, particularly against Staphylococcus aureus (100%) and Staphylococcus epidermidis (60%), and moderate activity against Escherichia coli (80%) and Klebsiella pneumoniae (70%). The findings confirm the broad-spectrum antibacterial properties of black pepper, which are likely caused by its bioactive components, such as piperine and essential oils. These results suggest that black pepper could be a natural alternative for controlling dangerous bacteria and fungi in the food preservation and pharmaceutical industries.

Keywords: *Basra, antimicrobial properties, antifungal, antibacterial, black pepper*

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Introduction

The rise of the Roman Empire coincided with the global popularity of black pepper. Because of its special qualities, Piper nigrum is referred to as the "lord of spices." According to archaeologists, humanity utilized spices 50,000 years ago for their unique fragrant qualities in a variety of culinary preparations. Since then, black pepper has been used in Indian cuisine and later became widely traded throughout Asia [1]. Piper nigrum, sometimes known as black pepper, was the first plant in the Piperaceae family to be shown to have medicinal properties by science [2]. Since they have been demonstrated to be an excellent variety of bioactive chemicals used in conventional medical therapy and exhibiting exceptional benefits, they are recommended in clinical therapies [3]. Maintaining health and preventing disease depend on the human gut microbiota, which is composed of many bacteria that reside in the gastrointestinal tract [4]. Pepper may include harmful molds and yeasts in addition to human pathogens that produce spores, such as Salmonella, Clostridium perfringens, and Bacillus cereus [5].

Black pepper includes a number of potent antibacterial substances, including caffeic, piperine, and other alkaloids. Specialty cheeses containing herbs, spices, and vegetables have gained popularity [6]. An analysis of these compounds' effects on bacteria and fungi showed that they prevented these microbes from growing and procreating [7]. The many antibacterial qualities of traditional herbal plants, such as black pepper (Piper nigrum L.), are currently the subject of several studies. Black pepper can be used to treat stomach problems, diarrhea, sinusitis, fever, asthma, and obesity. The active components of black pepper extract include alkaloids, phenols, tannins, coumarin, saponins, flavonoids, glycosides, and essential oils. Compared to white pepper, black pepper frequently has higher levels of oleoresin (6–13%) and essential oil (2.0–2.6%) [8]. Volatile oils and pepper ethanol extracts prevent food deterioration. Plants of the Piperaceae family, such as Piper nigrum L., commonly known as black pepper, naturally contain piperine [9].

Black pepper, or Piper nigrum, was the first member of the Piperaceae family to be identified as a therapeutic agent with scientific validation [2].They are advised in clinical treatments since they have been shown to be an exceptional variety of bioactive chemicals employed in conventional medicinal therapy that yield remarkable effects. Numerous studies have found that black pepper includes a wide range of phytochemicals with important health benefits, such as alkaloids, flavonoids, steroids, phenols, terpenes, tannins, and amides [10,11].Vitamins, minerals, carbohydrates, proteins, and secondary metabolites are all found in black pepper [2]. Piperine (2.1-8.9%), oleoresin (2.31-12%), essential oil (0.4-6.9%), starch (28-51%), and fatty acids (1.8-15%) are the biochemical components of black pepper. β-caryophyllene (30%), limonene (13.4%), β-pinene (8%), α-pinene (4.6%), sabinene (6%), caryophyllene oxide (9%), 3-carene (30%), and camphene (11%) are some of the bioactive compounds found in black pepper. Hydroxytyrosol 4-O-glucoside, 6-hydroxyluteolin 7-O-rhamnoside, 2-hydroxybenzoic acid, apigenin 6,8-di-C-glucoside, scopoletin, rhoifolin, sesamin, and hydroxytyrosol were among the phytochemicals discovered [1].

It is a popular spice due to its enticing perfume, strong flavor, and tingling sensation. Terpenoids, the principal chemicals found in black pepper essential oil (BPEO) [12]. In contrast to chemical antibiotics, an essential characteristic of EO components is their hydrophobicity, which enables them to partition into the lipids of the bacterial cell membrane, upsetting cell structures, increasing their permeability, and causing intracellular compound lysis and leakage[13].To the best of our knowledge, however, nothing is known about the antibacterial activity and method of action of BPEO against microorganisms.

Methodology

Study Population and Sample Collection

A total of 58 clinical samples were collected through oral swabs from diabetic patients and those suffering from urinary tract infections. The study population consisted of 40 females and 18 males, and samples were obtained from Al-Moani and Al-Fayhaa Hospitals in Basra Governorate during the period from September to November 2023.

Culture Media Preparation

Two main types of culture media were used in this study:

Fungal Media: Sabouraud Dextrose Agar (SDA) was used for fungal isolation. A total of 65 g of SDA was mixed with 250 mg of chloramphenicol and dissolved in 1000 ml of distilled water. Then the medium was heated with continuous stirring, sterilized by autoclaving, poured into Petri dishes, and allowed to solidify. All fungal specimens were cultured on SDA containing cycloheximide to inhibit bacterial growth and were incubated at 37°C for 24–48 hours under aerobic conditions. After incubation, 50 µL of black pepper extract was applied to the medium, and the inhibition zones were measured after three days to assess antifungal activity.

Bacterial Media: MacConkey Agar was prepared by suspending 38 g of the powder in 1 L of distilled water, heating, and sterilizing at 121°C for 15 minutes, while Mueller-Hinton Agar was prepared by suspending 50 g of the powder in 1 L of distilled water, autoclaving at 121°C for 15 minutes, cooling to 45–50°C, and pouring into sterile Petri dishes. A bacterial inoculum of 1×10^6 CFU/ml was prepared in sterile saline, and 10 µL of each bacterial suspension was inoculated onto the agar surface, with control plates containing saline only. All plates were incubated at 37°C for 72 hours, and the diameters of the inhibition zones were measured in millimeters to evaluate the antibacterial activity of the black pepper extract.

Identification of Bacterial and Fungal Isolates

Colony morphology, Gram staining, routine biochemical assays (such as catalase, coagulase, oxidase, and indole tests), and lactose fermentation on MacConkey agar were used to identify the bacterial isolates. The macroscopic appearance of the fungal isolates on Sabouraud Dextrose Agar (SDA), the microscopic characteristics using Lactophenol Cotton Blue (LPCB) staining, and the germ tube test for *Candida* albicans and other differential tests for *Candida* species were used to identify them.

Extracting and analyzing active compounds from black pepper

Using a Soxhlet system, the active components of black pepper were extracted using the Guenther (1972) technique. A thimble made of thick filter paper was filled with ground black pepper seeds and put into the Soxhlet extractor's main chamber. The extraction solvent was 200 milliliters of 70% ethanol. Continuous solvent circulation over the solid sample was made possible by heating the solvent with a hot plate and condensing the vapor in the cooling unit. Until the solvent in the flask turned black, signifying that the extraction process was finished, this extraction cycle was repeated. After filtering the resultant extract through filter paper to get rid of contaminants, it was centrifuged for 30 minutes and then concentrated in a water bath with constant stirring. The concentrated extract was placed in tightly sealed glass vials and stored at 4°C prior to further analysis.

GC–MS Analysis

The chemical composition of the black pepper extract was assessed using Gas Chromatography–Mass Spectrometry (GC–MS) [14]. The extract was made with one milliliter of extract and one milliliter of hexane, a dilution solvent. A 0.5 µL aliquot of the diluted sample was introduced into the GC-MS instrument under the specified separation conditions. The compounds were identified by comparing their mass spectra and retention times to those of established reference compounds. The relative abundance of each peak was automatically calculated by dividing the peak area by the total chromatogram area. Every peak on the chromatogram represented a distinct material.

Results and Discussion

Antifungal Activity of Black Pepper Extract

The effects of black pepper extract on a number of yeast isolates (*Candida* spp.) are displayed in Table 1. With 80% (16 of 20 isolates) exhibiting positive inhibition and 20% (4 isolates) being resistant, the results reveal that the majority of *Candida albicans* isolates were susceptible to the extract. Similarly, 20% (1 isolate) of *C. dubliniensis* showed resistance, whereas 80% (4 out of 5 isolates) were sensitive. On the other hand, all three isolates of *C.tropicalis* (100%) showed complete suppression, indicating complete extract sensitivity. These results validate its usage in therapeutic applications as a natural antifungal medication. The bioactive components of black pepper (Piper nigrum L.), such as piperine and volatile oils, have a strong antifungal effect by rupturing fungal cell membranes and preventing proliferation [15].

Table (1): Black pepper's effects on yeast

Yeast isolates	Negative		Positive	
	N	%	N	%
C . Albicans (20)	4	20%	16	80%
C . dubliniesis (5)	1	20%	4	80%
C . tropicals (3)	0	0%	3	100%

**Note: The number given in parentheses next to each microbial species shows the total number of isolates acquired from clinical samples.*

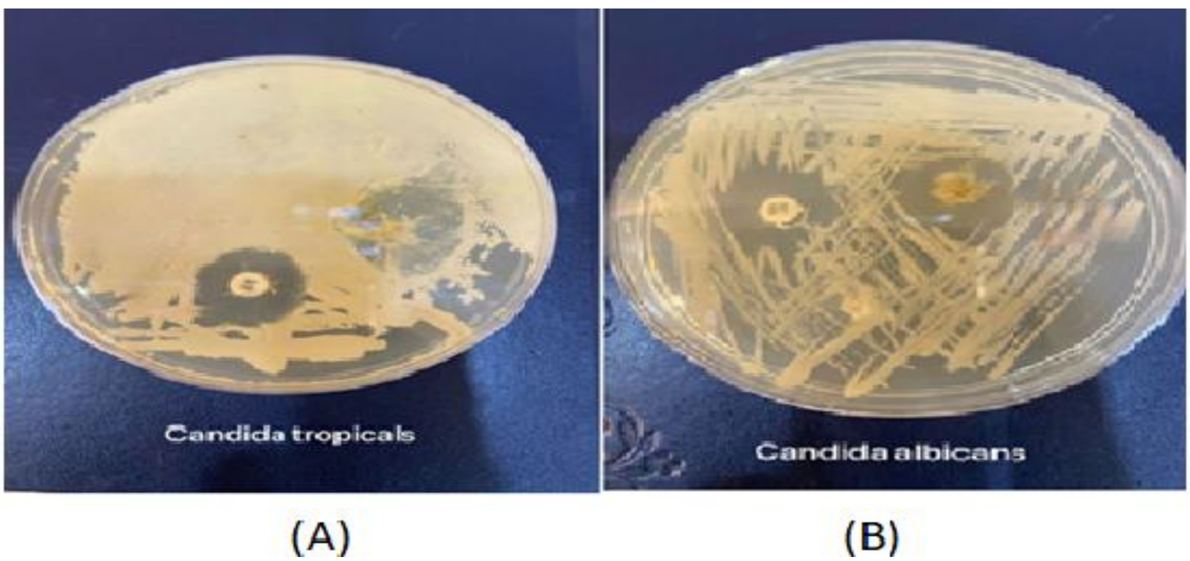

Figure (1): *Candida albicans* growth on SDA **(A)** and *Candida tropicalis* growth on SDA **(B)**

The effects of black pepper on microorganisms are a fascinating subject that has been extensively investigated in research. Black pepper is often used as a condiment in many different recipes due to its distinct flavor. But it also contains a wealth of bioactive compounds that have the ability to influence microbial activity. Black pepper includes piperine and other compounds that may have antifungal effects against dangerous bacteria and fungi, according to many studies. These bioactive substances, however, could also have an impact on helpful gut microbes. The antifungal action of black pepper extract against *Candida albicans* is probably due to its volatile oil, which possesses antibacterial qualities. Black pepper (Piper nigrum L.) extract was found to have antifungal properties against a number of *Candida* species, including *Candida albicans*, *Candida dubliniensis*, and *C. tropicalis*. These results are consistent with previous research by[15–17].The extract includes active components that can prevent fungal development, as evidenced by the observed inhibitory zones. Because they may damage fungal cell membranes and interfere with essential metabolic functions, piperine and the volatile oil components of black pepper are principally responsible for the antifungal action of black pepper. The impact of black pepper on microorganisms generally seems to rely on dose. It is important to stress that further research is needed to identify the precise effects and any negative outcomes. The disc diffusion method was used to investigate how black pepper affected the growth of fungi. For every sample, the diameter of the inhibitory zone (black pepper) was measured. Many kinds of *Candida* fungus are susceptible to the antifungal actions of black pepper. A few species of *Candida* have demonstrated resistance to black pepper.

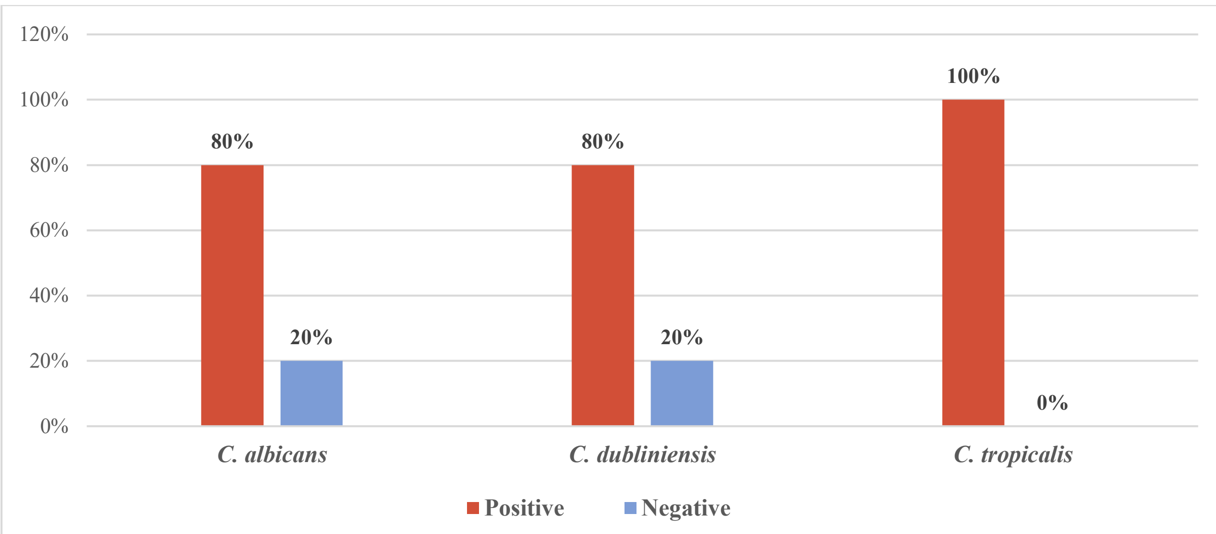

Figure 2: The results of effect of the black pepper on yeast

The ethanolic extract of black pepper (Piper nigrum L.) showed strong antifungal activity against many *Candida* species, suggesting its potential as a natural therapeutic agent, according to the current study. Twenty isolates of *Candida albicans* were collected from diabetic individuals, as shown in Table [1]. Of these, four isolates (20%) showed no discernible inhibition, whereas sixteen isolates (80%) were sensitive to the black pepper extract, with inhibition zone sizes ranging from 10 to 20 mm. Comparably, four isolates (80%) of *Candida dubliniensis* (n = 5) showed positive inhibition zones of 10–20 mm, whereas one strain (20%) showed resistance. Complete fungicidal efficacy was not always seen, despite the extract's excellent inhibition of fungal growth. On the other hand, isolates of *Candida tropicalis* (n = 3) showed 100% total inhibition with 20 mm inhibition zones, indicating that this species is more susceptible to the extract (Figure 2). The current study's findings are in line with other studies that showed Piper nigrum's antifungal qualities. According to Dorman and Deans (2000), black pepper volatile oils have strong inhibitory effects on fungal infections [18].

Likewise, [19]emphasized the significance of phenolic compounds in causing damage to fungal cell membranes and changing permeability, while [15]found that methanolic extracts of black pepper considerably decreased *Candida albicans* growth and oil contents. When taken as a whole, these results support the current findings, demonstrating that black pepper extract has broad-spectrum antifungal efficacy that differs among Candida species and might be a viable natural antifungal substitute.

Table 2: The effect of black pepper on bacteria.

Yeast isolates	**Negative**		**Positive**	
	N	**%**	**N**	**%**
E . coli (10)	2	20%	8	80%
Klebsiella pneumonia(10)	3	30%	7	70%
Staph.aureus (5)	0	0%	5	100%
Staph.epidermidis (5)	2	%40	3	60%

****Note:*** *The total number of isolates from clinical samples is indicated by the number in parenthesis next to each microbial species.*

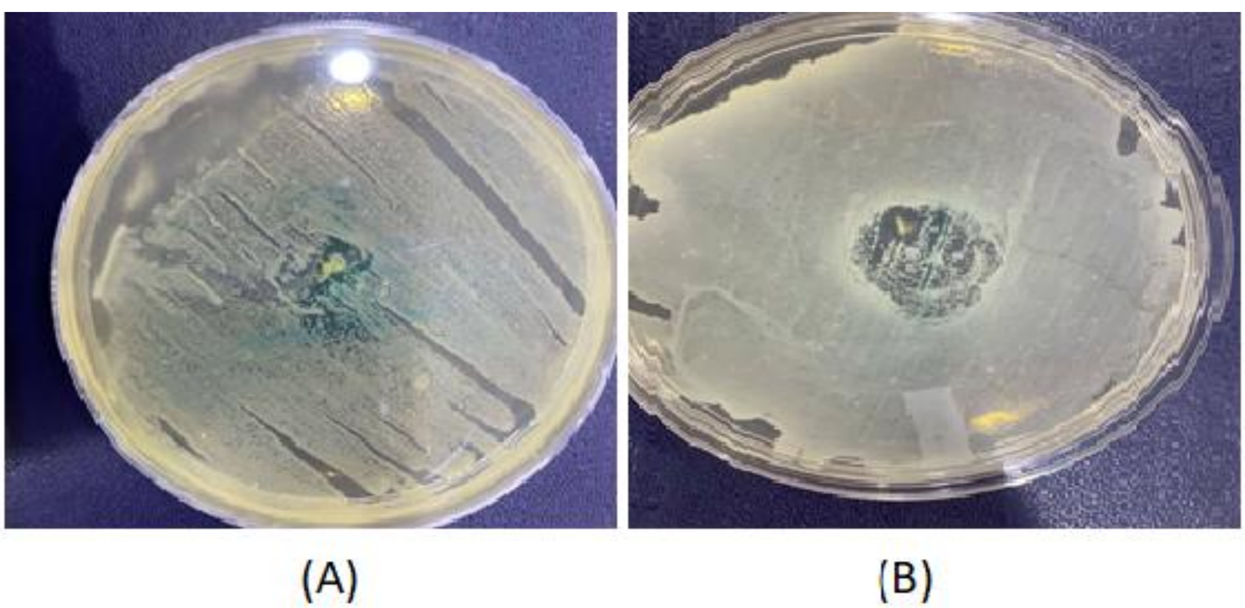

Figure (3): (A*)* *E.Coli* **, (B)** *Staph orus* Growth on Muller Hinton Agar

Antibacterial Activity of Black Pepper Extract

Because of its active ingredients, particularly piperine and essential oils, which weaken bacterial cell walls and obstruct enzyme activity, black pepper (Piper nigrum L.) possesses potent antibacterial properties. Studies have shown that black pepper significantly inhibits both Gram-positive and Gram-negative microorganisms, such as Escherichia coli and Staphylococcus aureus. Black pepper may be a natural alternative to manufactured antibiotics due to its antibacterial properties. Its bioactive components cause cell lysis and growth inhibition by interfering with microbial metabolism [20]

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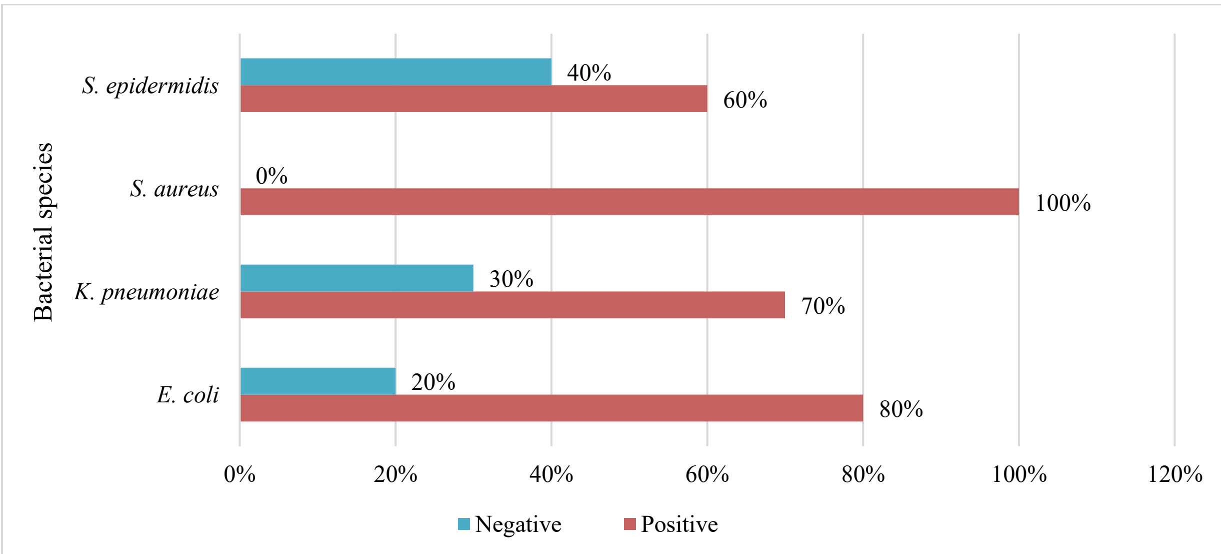

Figure 4: The results of the effect of black pepper on bacterial growth

The antibacterial activity of black pepper extract was assessed against four bacterial species: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. As shown in Table (2), the extract inhibited the bacterial strains to varying degrees. Eight (80%) of the isolates displayed positive inhibition with inhibition zone widths ranging from 13 to 14 mm, while two (20%) of the *E. Coli* isolates (n = 10) recovered from patients with UTIs were resistant. Many investigations have shown that black pepper (Piper nigrum L.) has broad-spectrum antibacterial action against both Gram-positive and Gram-negative bacteria. According to Karsha and Lakshmi (2010), black pepper's bioactive components, such as piperine and essential oils, which compromise the integrity of bacterial cell walls and enzyme systems, give it strong antibacterial qualities, particularly against *Staphylococcus aureus* [20]. Similarly, 70% of isolates (n = 10) displayed inhibition zones of about 10 mm, while 30% of Klebsiella pneumoniae isolates remained resistant to the extract. Conversely, gram-positive bacteria were more susceptible. All five *Staphylococcus aureus* isolates from oral samples demonstrated total inhibition (100%) with inhibition zones ranging from 18 to 20 mm, suggesting that black pepper extract has potent antibacterial qualities against this pathogen. Three (60%) of the five Staphylococcus epidermidis isolates had inhibition zones between 16 and 18 mm, while two (40%) showed no inhibition (Figure 4). These results suggest that black pepper extract is more effective against Gram-positive bacteria than Gram-negative ones. The outer membrane of Gram-negative bacteria acts as a barrier to lipophilic substances like piperine, whereas the thick peptidoglycan layer of Gram-positive bacteria facilitates their diffusion and activity. Their cell walls' structural variations can account for this discrepancy. Our results are in line with previous studies [20], which showed that black pepper extracts were effective against Gram-positive bacteria by considerably inhibiting the growth of S. aureus. Similarly, [18] found that volatile oils from Piper nigrum exhibited significant antibacterial activity against both Gram-positive and Gram-negative pathogens. Similar results were found by [16,21], who demonstrated that black pepper extracts induce cell lysis by interfering with metabolic activities and compromising the integrity of bacterial membranes. The available data largely support the notion that black pepper has strong antibacterial properties, particularly against Gram-positive infections, and could be used as a natural alternative to conventional antibiotics.

Conclusions and Recommendations

Since its ethanolic extract has shown robust antifungal and antibacterial action against a variety of pathogenic microorganisms, including *C.tropicalis* and *Staph. aureus*, black pepper (Piper nigrum L.) has the potential to be a natural antimicrobial agent. The findings demonstrate that its bioactive components, particularly piperine and volatile oils, have a major impact on altering microbial cell membranes and metabolic activities. The extract's antifungal efficacy varied according to the species of *Candida*, and it was more effective against Gram-positive bacteria than Gram-negative infections. These findings show the potential of black pepper as a natural medicinal agent. Black pepper is a natural food preservative that can help inhibit the growth of hazardous germs. Further research is needed to isolate the active chemicals, define the minimal inhibitory doses, assess toxicity and safety, and investigate synergistic effects with current antimicrobial medications for possible clinical and pharmaceutical uses.

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Conflict of Interest: Authors declare there is no conflict of interest.

References

1. Garg AP. Biomedical applications of black pepper, the king of spices-A review. Biomed J Sci Tech Res. 2023;53(1):44364–71.

2. Ramakrishnan Nair R, Dutta Gupta S. Somatic embryogenesis and plant regeneration in black pepper (Piper nigrum L.): I. Direct somatic embryogenesis from tissues of germinating seeds and ontogeny of somatic embryos. J Hortic Sci Biotechnol. 2003;78(3):416–21.

3. Ali A, Wu H, Ponnampalam EN, Cottrell JJ, Dunshea FR, Suleria HAR. Comprehensive profiling of most widely used spices for their phenolic compounds through LC-ESI-QTOF-MS2 and their antioxidant potential.

Antioxidants. 2021;10(5):721.

4. Ayeni FA, Biagi E, Rampelli S, Fiori J, Soverini M, Audu HJ, et al. Infant and adult gut microbiome and metabolome in rural Bassa and urban settlers from Nigeria. Cell Rep. 2018;23(10):3056–67.

5. Djordjević M, Sterniša M, Možina SS, Beszédes S, Simović DŠ, Hodúr C, et al. Black pepper (Piper nigrum L.) bacterial decontamination by sterilization and microwave treatments. Analecta Tech Szeged. 2019;13(2):1–5.

6. Himabindu D, Arunkumar H. Effect of black pepper (Piper nigrum L.) on the keeping quality of spiced cottage cheese. Res Rev J Food Dairy Technol. 2017;5:30–6.

7. Ali NH, Faizi S, Kazmi SU. Antibacterial activity in spices and local medicinal plants against clinical isolates of Karachi, Pakistan. Pharm Biol. 2011;49(8):833–9.

8. Hakim RF, Alwi F. Effect of Black Pepper Extract (Piper nigrum L) towards Streptococcus Mutans Growth: Pengaruh Ekstrak Lada Hitam (Piper nigrum L) terhadap Pertumbuhan Streptococcus Mutans. Dentika Dent J. 2020;23(1):13–7.

9. Hikal DM. Antibacterial activity of piperine and black pepper oil. Biosci Biotechnol Res Asia. 2018;15(4):877.

10. Siddiqui BS, Gulzar T, Mahmood A, Begum S, Khan B, Rasheed M, et al. Phytochemical studies on the seed extract of Piper nigrum Linn. Nat Prod Res. 2005;19(7):703–12.

11. Garg AP. Health Benefits of Cumin in Foods: A. J ISSN. 2023;2766:2276.

12. Zhang J, Ye K-P, Zhang X, Pan D-D, Sun Y-Y, Cao J-X. Antibacterial activity and mechanism of action of black pepper essential oil on meat-borne Escherichia coli. Front Microbiol. 2017;7:2094.

13. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. Food Res Int. 2011;44(9):3057–64.

14. Baser KHC, Ozek T. Analysis of Essential Oils and Fragrances by Gas Chromatography. Gas Chromatogr. 2012 Dec 31;519–27.

15. Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (Zingiber officinale Roscoe). Int J Curr Pharm Res. 2010;2(4):40–3.

16. Takooree H, Aumeeruddy MZ, Rengasamy KRR, Venugopala KN, Jeewon R, Zengin G, et al. A systematic review on black pepper (Piper nigrum L.): from folk uses to pharmacological applications. Crit Rev Food Sci Nutr. 2019;59(sup1):S210–43.

17. Busatta C, Mossi AJ, Rodrigues MRA, Cansian RL, Oliveira JV de. Evaluation of Origanum vulgare essential oil as antimicrobial agent in sausage. Brazilian J Microbiol.

2007;38:610–6.

18. Dorman H, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 2000;88(2):308–16.

19. Santos VR, Pereira EMR. Antifungal activity of Brazilian medicinal plants against Candida species. Candida albicans. 2018;

20. Karsha PV, Lakshmi OB. Antibacterial activity of black pepper (Piper nigrum Linn.) with special reference to its mode of action on bacteria. Indian J Nat Prod Resour. 2010;1(2):213–5.

21. Busatta C, Vidal RS, Popiolski AS, Mossi AJ, Dariva C, Rodrigues MRA, et al. Application of Origanum majorana L. essential oil as an antimicrobial agent in sausage. Food Microbiol. 2008;25(1):207–11.

تأثير الفلفل الأسود على مختلف الكائنات الدقيقة، البصرة، العراق

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الملخص

اختبرت هذه الدراسة التأثيرات المضادة للفطريات والبكتيريا للفلفل الأسود (Piper nigrum) ضد العديد من العزلات البكتيريا والفطرية. تم الحصول على 58 مسحة فموية من مرضى السكري والتهابات المسالك البولية، من بينهم 40 أنثى و18 رجلاً، من مستشفى الموانئ العام ومستشفى الفيحاء العام في محافظة البصرة خلال فترة من سبتمبر الى نوفمبر 2023. تم إنتاج مستخلصات الفلفل الأسود وتقييمها في المختبر لتحديد فعاليتها ضد بعض الميكروبات المختلفة . تم تحضير المستخلصات الإيثانولية من الفلفل الأسود باستخدام طريقة Soxhlet باستخدام جهاز سوكسليت، وتم تحليل مكوناتها النشطة بيولوجيًا باستخدام تقنية كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) أُجريت اختبارات مضادة للميكروبات باستخدام طريقة انتشار القرص لتحديد مناطق التثبيط ضد العزلات الميكروبية المختلفة.

أظهرت النتائج أن الفلفل الأسود أظهر نشاطًا مضادًا للفطريات ملحوظًا ضد *Candida albicans* (%80) ، *Candida dubliniensis* (%80)، وتثبيطًا كاملًا *Candida tropicalis* (%100). علاوة على ذلك، أظهر المستخلص تأثيرات قوية مضادة للبكتيريا، وخاصة ضد *Staph. aureus* (%100) و*Staph epidermidis* (%60)، مع نشاط معتدل ضد *E.coli*(%80) و *Klebsiella pneumonia* (%70). تؤكد النتائج أن الفلفل الأسود يمتلك خصائص مضادة للميكروبات واسعة الطيف، ويعزى ذلك على الأرجح إلى مركباته النشطة بيولوجيًا مثل Piperine والزيوت العطرية. تشير هذه النتائج إلى أن الفلفل الأسود يمكن أن يكون بديلًا طبيعيًا لمكافحة البكتيريا والفطريات المسببة للأمراض، وقد تكون له تطبيقات محتملة في الصناعات الدوائية وحفظ الأغذية.

الكلمات المفتاحية: *الفلفل الأسود، النشاط المضاد للميكروبات، مضاد للفطريات، مضاد للبكتيريا، البصرة*