Research Article

In Vitro Investigating the Efficacy of Clove Extract Against *Proteus* spp

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Abstract | The study aimed to evaluate the antibacterial efficacy of clove extract against *Proteus* spp isolated from chicken meat and human urine samples. A total of 320 samples were collected including120 urine samples from patients with urinary tract infections and 200 chicken meat samples collected from local markets. PCR results confirmed the isolation of *Proteus mirabilis* from human samples (25%) and chicken meat samples (13%), as well as the isolation of *Proteus vulgaris* (6.6%) and chicken meat samples (4%). Gas chromatography-mass spectrometry (GC-MS) analysis confirmed the presence of several biologically active compounds in cloves, including eugenol, phenol, and saponins after alcoholic extraction at a rate of 22.5%. The antibacterial activity of clove extract at different concentrations (1.25, 2.5, 5, and 10 mg/ml) against isolates was evaluated in vitro, where the well diffusion test revealed an inhibitory activity against the growth of *P. mirabilis* where the diameters of inhibition zone increased with increasing extract dose. The diameters of inhibition zones were 14.54±0.13 mm,17.52±0.18 mm, 20.70±0.16 mm, and 24.70±0.20 mm, respectively. whereas the diameter of the inhibition zone for *P. vulgaris* was 14.04±0.11 mm, 16.05±0.18 mm,18.20±0.26 mm and 23.00±0.18 mm, respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for P. mirabilis were 0.31±0.03 mg/ml and 0.60±0.11 mg/ml, respectively, while the MIC for P. vulgaris was 0.60±0.11 mg/ml and 1.22±0.22 mg/ml, respectively. Taken together, these findings suggest that the alcoholic extract of cloves possesses significant antibacterial activity and could be a potential natural alternative for managing infections, particularly those related to chicken meat and urinary tract pathogens.

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Introduction

Foodborne pathogens pose a significant threat to global public health, contributing to substantial illness and mortality worldwide (Tropea, 2022). Chicken meat, one of the most widely consumed animal proteins globally, accounts for approximately 36% of total meat production and is valued for its high protein and low-fat content (Abbasi *et al.*, 2020; Avi, 2024). However, contamination of chicken meat with pathogenic microorganisms remains a critical concern, particularly under inadequate

handling and processing conditions (Hashem and Abdaljaleel, 2024; Yulistian and Praseptiangga, 2019). Among these pathogens, *Proteus* spp especially *Proteus mirabilis* and *Proteus vulgaris* have emerged as important opportunistic and foodborne bacteria capable of causing gastrointestinal and urinary tract infections (Faiq and Ahmed, 2024 and Li *et al*., 2022). These gram-negative bacilli are widely distributed in the environment and can colonize both animals and humans, posing risks of cross-species transmission. Contamination often occurs through poultry droppings, feathers, and processing surfaces, which can facilitate the spread to consumers and slaughterhouse workers (Ram *et al*., 2019). *Proteus* contains virulence factors and enzymes, including biofilm formation, protease, hemolysin, and urease, where these factors increase the resistance of bacteria and thus their survival in the host tissues (Abd Sharad *et al*., 2020).

Antimicrobial resistance (AMR) in humans and animals is seen as a global concern since it has implications for human public health as well as a rise in animal morbidity and death (Beikzadeh, 2023). Multi-drug resistance (MDR) bacteria endanger human and animal health and food safety, and antibiotic resistance remains a major concern, particularly in developing countries where sanitation is poor and antibiotic use is often indiscriminate and poorly monitored (Dougnon *et al*., 2020). *Proteus spp*. is the multidrug-resistant *Enterobacteriaceae* that produces the most extended-spectrum beta-lactamases (ESBLs) and carbapenemases, both of which are linked to UTIs (Alattar *et al*., 2024). Bacteria that cause urinary tract infections (UTIs) form biofilms, which in turn inhibit the effectiveness of antibiotics through the development of resistance genes (virulence genes) (Kadhim *et al*., 2014). Therefore, internal medical devices (IMDs) are the most susceptible to microbial infection and biofilm-producing pathogens (Hasan *et al*., 2021). Due to the presence and emergence of antibiotic-resistant bacteria, scientific research, and researchers continue to discover new, alternative, and effective therapeutic methods and agents (Molan and Hussien, 2024). Because medicinal plants are often used to treat illnesses, scientists have been encouraged to research plants that have pharmacological value and can be used therapeutically to regulate human health (Hashim and Ibrahim, 2024; Saeed and Abdulwahid, 2022). Such as cloves are considered a broad-spectrum herb rich in natural chemical compounds, which have broad biological effects, such as antifungal, antiviral, antioxidant, and antibacterial properties (Al-Juburi and Al-Sammarraae, 2022; Dakheel *et al*., 2023; Yassin *et al*., 2020). Given the positive efficacy of medicinal herbs used in traditional medicine and the lack of side effects, there has been an increase in the exploration of many medical alternatives with biological efficacy as antibiotics, such as the *aromatic syzygium* plant, which is widely used in folk medicine, locally available, and generally considered safe (Hasan *et al*., 2022; Seriana *et al*., 2021).

Based on this, the present study aims to investigate the prevalence of *Proteus* spp. in human urine and raw chicken meat samples collected from Wasit Governorate, Iraq, and to evaluate the potential antibacterial activity of a natural antimicrobial agent—clove extract.

Materials and Methods

Sample collection and bacterial isolation

A total of 320 samples were collected between March and September 2023 from regions in Wasit Governorate, Iraq. These included 120 urine samples obtained from patients diagnosed with urinary tract infections. In parallel, 200 raw chicken meat samples were randomly collected under sterile conditions from local superstores and retail markets. All samples were immediately transported to the microbiology laboratory in sterile cooler boxes. *Proteus* species were isolated and identified from chicken meat samples according to ISO 21528-2 (International Organization for Standardization, 2017). For processing, 25 grams of each sample were homogenized in 225 ml of buffered peptone water and incubated at 37 ˚C for 24 hours. Subsequently, 1 ml of this pre-enrichment was transferred to 10 ml of tetrathionate broth and incubated at 42 ˚C for 18–24 hours. The enriched cultures were streaked on selective media for isolation and were later subjected to biochemical, serological, and molecular identification procedures.

Cultural media

Proteus species were isolated and identified from samples of chicken flesh. Following weighing, 25 grams of each sample were mixed with 225 milliliters of buffered peptone water in a sterile flask and incubated for 18 to 24 hours at 37˚C. Nine milliliters of tetrathionate broth were combined with one milliliter of the suspension, and the mixture was incubated for 18 to 24 hours at 42˚C. Both regular MacConkey

agar and differential agar (Hichrome UTI agar) were used to culture the samples. Bacterial species were identified by standard methods after *Proteus* colonies were purified. Conventional biochemical methods were used to identify all probable colonies, and PCR was used for additional verification.

Detection of molecular

The detection of *Proteus* species in clinical and environmental samples was done using the conventional polymerase chain reaction (PCR). A particular 383-base pair (bp) fragment of the *Proteus* 16S ribosomal RNA (rRNA) gene was amplified in this process using reference sequences supplied from the GenBank database of the National Center for Biotechnology Information (NCBI). The target chromosomal DNA area was targeted and amplified using oligonucleotide primers (forward and reverse) made by Alpha DNA (Montreal, Canada). Following PCR, DNA sequencing was performed on the *Proteus* isolates that tested positive for additional characterization and confirmation (Figure 1).

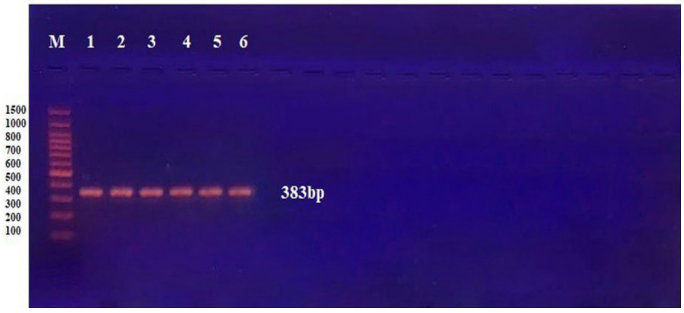

Figure 1: *DNA product analysis of Proteus Spp.*

Plant sample collection and extraction

Dried flower buds (seeds) of *Syzygium aromaticum* (clove) were purchased in 2023 from a local market in Wasit Province, Iraq. The plant material was identified and authenticated by the Ministry of Agriculture/ State Board for Seed Certification and Testing in Abu Ghraib, Baghdad, under certification number 1967 dated 4/6/2023. The clove samples were cleaned thoroughly and ground into a fine powder using an electric grinder. The powdered material was stored in airtight containers at 4 °C until further use.

Plant extraction

Clove extract was prepared using the Soxhlet extraction method. A total of 20 grams of powdered clove was placed in a cellulose extraction thimble and extracted with 100 ml of 75% Ethanol (Ethanol: distilled water, 3:1) at 50 °C for 8 hours. The extract was concentrated under reduced pressure using a rotary evaporator at 45 °C. The concentrated extract was further dried in an oven at 37 °C for 10 hours to obtain a semi-solid residue. The final dried extract was stored in a sealed container at 4 °C until analysis and use (Deshmukh and Borle, 1975).

Gas chromatography-mass spectrometry (GC–MS) Analysis

Gas chromatography-mass spectrometry (GC–MS) analysis of the clove ethanolic extract was conducted by the Department of Environment and Water, Ministry of Science and Technology, Iraq. The analysis was performed using an automated pyrolysis gas chromatograph coupled to a Shimadzu mass spectrometer (Japan). The conditions are listed in Table 1. In a sealed glass tube, 1 mg of dried extract was combined with 50 µL of pyridine and 100 µL of N-methyl-N-trimethylsilyl-trifluoroacetamide for derivatization. The mixture was then heated at 80°C for 30 minutes for the shock-stop reaction. To analyze the derivative sample, 1 µL was injected into the quartz chamber of the pyrolysis unit using a 10 µL syringe. The following steps were taken: The syringe was rinsed before and after sample injection, and the capillary column was 35% phenyl-coated fused silica with a length of 60 meters, a film thickness of 0.25 mm I.D., and a film thickness of 0.25 m (Shashanka *et al.*, 2016).

Table 1: *GC–MS Operating conditions*

System Component	Condition
Injection mode	Split/splitless
Injector temperature	280 °C
Split ratio	10:1
Column	Capillary (60 m × 0.25 mm I.D., film thickness 0.25 µm)
Oven temperature program	40 °C for 5 min, ramped to 280 °C and held for 15 min
Carrier gas	Helium (99.99%)
Flow rate	1 mL/min
Mass Spectrometer	
Ionization mode	Electron impact (EI)
Ionization energy	70 eV
Scan range	40–600 m/z
Ion source temperature	200 °C
Detector temperature	230 °C
Minimum scan rate	0.5 scan/sec
Scan speed	5000 u/sec

Antibacterial activity test of clove extract (In Vitro)

The agar well diffusion method employed in this study followed the procedures described by (Daoud *et al*., 2019) to evaluate the antibacterial activity of clove extract. Briefly, a stock solution was prepared by dissolving the clove-extracted powder in distilled water to achieve a final concentration of 10 mg/mL. The solution was then filtered using a 0.22 µm Millipore membrane filter. Distilled water served as the negative control. A fresh, pure culture of a previously identified bacterial isolate was used for the assay. To prepare the inoculum, at least 3 to 5 well-isolated colonies from a fresh bacterial culture were transferred into test tubes containing 5 mL of sterile normal saline and incubated at 37 ˚C for 2 hours. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, equivalent to 1.5×10^8 CFU/mL. Using sterile disposable swabs, the standardized inoculum was spread uniformly onto the surface of Mueller-Hinton agar plates (with a medium depth of approximately 4 mm). The plates were rotated three times at 60˚ angles to ensure even distribution. After allowing the plates to dry, wells with a 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Then, 100 µL of the clove extract solution at concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL were added to the respective wells. Plates were incubated at 37 ˚C for 18–24 hours. Following incubation, the diameter of the inhibition zones was measured in millimeters using a digital vernier caliper.

Minimum inhibitory concentration (MIC)

The MIC was defined as the lowest concentration of the clove extract capable of inhibiting visible bacterial growth, as per the guidelines outlined by the Clinical and Laboratory Standards Institute (CLSI) and described by (Al-Malkey *et al*., 2020; Eloff, 1998), with slight modifications. The broth microdilution method using a 96-well polystyrene microtiter plate was employed.

Preparation of antimicrobial agent

A stock solution of ethanolic clove extract was prepared by dissolving the dry extract in 10% distal water to a final concentration of 10 mg/mL, followed by filtration through a 0.22 µm Millipore filter. Two-fold serial dilutions were prepared using Mueller-Hinton Broth (MHB) to obtain concentrations ranging from 5 mg/mL to 0.01 µg/mL.

Preparation of inoculum

Fresh bacterial colonies were transferred into tubes containing 4–5 mL of MHB and incubated at 35 ˚C for 2 hours. The suspension turbidity was adjusted to 0.5 McFarland standard and then diluted 1:100 to obtain approximately 1×10^6–10^8 CFU/mL for use in the MIC assay.

MIC Assay procedure

In each well of the 96-well microtiter plate, 100 µL of diluted clove extract and 100 µL of standardized bacterial suspension were combined, making a final volume of 200 µL per well. Column 11 served as the positive control (broth + bacteria + solvent), and column 12 served as the negative control (broth + solvent only, without bacteria). Plates were incubated at 37 ˚C for 18–24 hours. After incubation, 30 µL of Alamar Blue reagent was added to each well and incubated again for 1 hour at 37 ˚C. Alamar Blue is a resazurin-based, cell-permeable, non-toxic indicator dye. In the presence of metabolically active bacteria, resazurin is reduced to resorufin, leading to a color change from blue (no growth) to pink/red (growth). The MIC was recorded as the lowest concentration that prevented a visible color change. All tests were performed in duplicate to confirm reproducibility.

Statistical analysis

The Chi-squared test was used to compare isolation proportions. Additionally, the odds ratio was estimated. $P < 0.05$ indicates significance (Cary, 2010)

Results and Discussion

Based on PCR and cultural characteristics, two *Proteus* species *P. mirabilis* and *P. vulgaris*, were successfully isolated and identified, as shown in Table 2 and Table 3. As outlined in Table 2, *P. mirabilis* was detected in 13% (26/200) of chicken meat samples and 25% (30/120) of human urine samples, yielding a total prevalence of 17.5% (56/320). A statistically significant difference ($\chi^2 = 7.48$, $P = 0.006$) was observed between the two sources, with human samples showing a higher isolation rate. While the isolation rate was 4% (8/200) of chicken meat samples, it was 6.6% (8/120) in human urine samples (Table 3).

Based on the chemical composition results from this investigation, the ethanol extract of clove (*Syzygium aromaticum*) was found to contain a

higher concentration of active chemical components (Table 4). The use of medicinal plant extracts is increasingly important in the search for novel antibacterial biomolecules to combat antibiotic resistance (Pavesi *et al*., 2018). Clove extract contains a range of potentially bioactive compounds, including eugenol, phenol, 2-methoxy-4-(2-propenyl)-acetate, essential oils such as oleic acid, *n*-hexadecanoic acid, 2,3,4-trimethoxyacetophenone, caryophyllene, flavonoids, saponins, and tannins (Gowri and Manimegalai, 2019; Ibrahim and Mohammed, 2025). These constituents are associated with various biological activities, including antipyretic, antispasmodic, anticarcinogenic, inhibition of 5-LOX enzyme activity in human polymorphonuclear leukocytes, antioxidant effects, protection against peroxynitrite-mediated tyrosine nitration and lipid peroxidation, antifungal, antimicrobial, and antibacterial activities (Rosarior *et al*., 2021).

The antimicrobial activity of clove was due to the presence of eugenol, 2-heptanone, methyl salicylate, kaempferol, gallic acid, isoeugenol, and oleanolic acid (Faujdar *et al*., 2020). These chemicals typically denatured proteins that reacted with the cell membrane and altered their permeability (Kaur and Kaushal, 2019). The relationship between anti-nociceptive and anti-inflammatory actions is widely recognized for a variety of nonsteroidal anti-inflammatory drugs. As with NSAIDs, the extract may reduce vascular permeability and hence decrease edema production. Thus, the plant could be employed as a possible source of anti-inflammatory and anti-nociceptive chemicals, supporting the local claim of its usage in painful and inflammatory disorders (Tanko *et al*., 2008). The peaks were detected using GC-MS data (Figure 3). The primary peaks were recognized as eugenol, phenol,2-methoxy-4-(2-propenyl)-acetate, oleic acid, n-hexadecanoic acid, 2,3,4, Trimethoxyacetophenone, and caryophyllene. Similar ethanol extract components were observed in GC-mass (Gowri and Manimegalai, 2019; Rasmey and Mahran,2018). Similar to Oleic Acid components were recorded in GC-mass (Eltak *et al*.,2023) and

Table 2: *Isolation percentage of P. mirabilis from different sources*

Source	No. of Samples	*P. mirabilis*	Yates' χ^2	P-value
Chicken meat	200	26 (13%)	7.48	0.006
Human urine	120	30 (25%)		
Total	320	56 (17.5%)		

Table 3: *Isolation percentage of P. vulgaris from different sources*

Source	No. of Samples	P. vulgaris	Yates' χ^2	P-value
Chicken meat	200	8 (4%)	1.12	0.28 NS
Human urine	120	8 (6.6%)		
Total	320	16 (5%)		

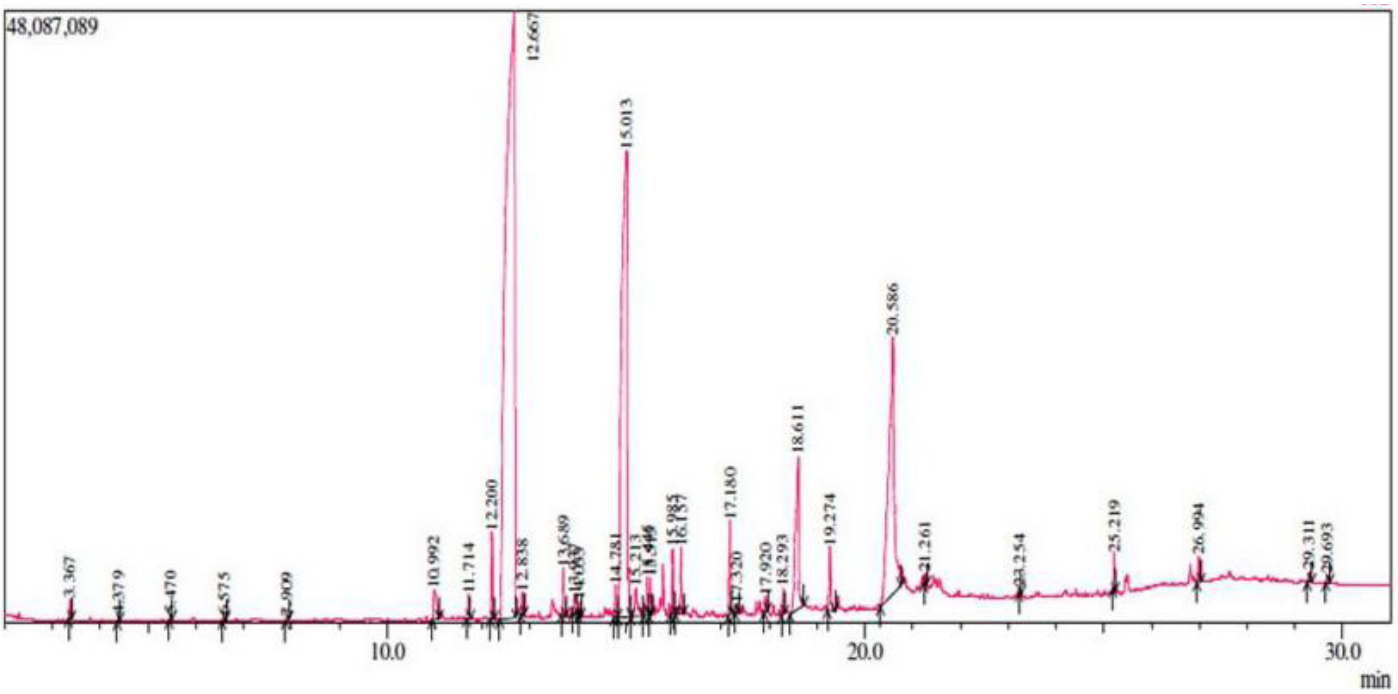

Figure 2: *Chromatogram of GC-MS analysis of the ethanolic extract of Cloves.*

Table 4: *Major volatile compounds of the ethanolic extract of Cloves and its metabolites area percentage*

Peak No.	Compound	Retention time	Chemical formula	Area %
1	Phenol, 4-(2-propenyl)	10.992	C9H10O	0.83
2	12,15-Octadecadiynoic acid, methyl ester	11.105	C19H30O2	0.85
3	Caryophyllene	12.200	C15H24	0.98
4	Eugenol	12.667	C10H12O2	48.15
5	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	15.013	C12H14O3	22.61
6	2,3,4, Trimethoxyacetophenone	17.180	$C_{11}H_{14}O_4$	1.02
7	n-Hexadecanoic acid	18.611	$C_{16}H_{32}O_2$	4.70
8	(3S,3aS,6R,7R,9aS)-1,1,7-Trimethyldecahydro-3a,7-methano-cyclopenta [8]annulene-3,6-diol	19.274	C15H26O2	0.91
9	Oleic Acid	20.586	C18H34O2	13.01

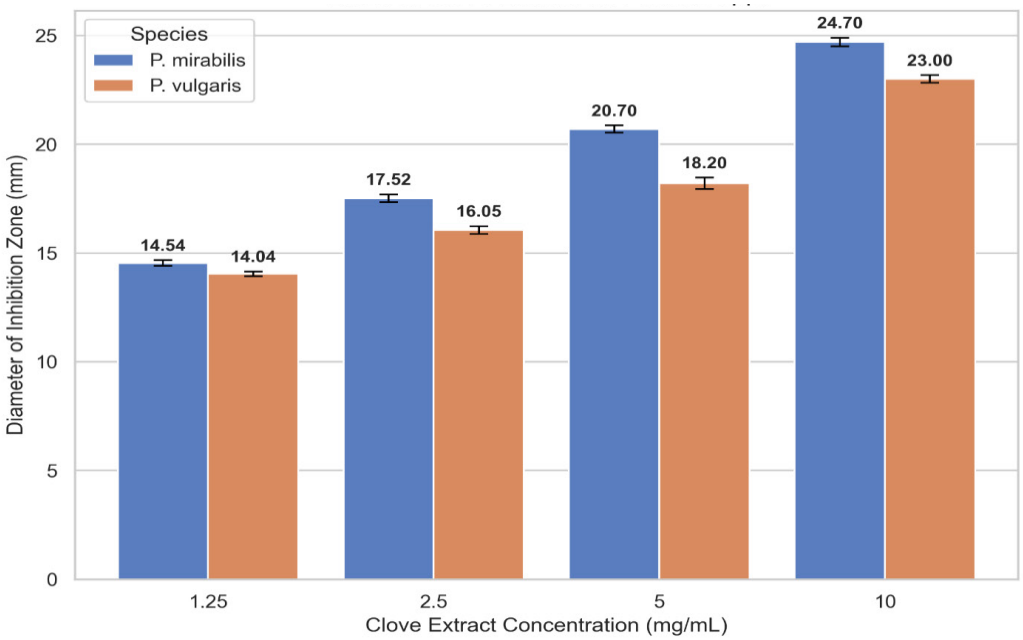

Figure 3: *The effect of clove extract on Proteus spp. at different concentrations.*

similar eugenol components were recorded in GC-mass (Mittal *et al.*, 2014; Wael *et al.*, 2018).

Several studies revealed that 80% ethanol was able to extract most of the bioactive phytochemical compounds, especially flavonoids effectively (Chon *et al.*, 2020). Previous research tested different solvent extraction methods on spices and found that 80% ethanolic extraction demonstrated the showed the highest inhibition against both gram-negative and gram-positive (Sakha *et al.*, 2018). The primary component of eugenol found in the ethanol extract of S. aromaticum has been linked to a variety of biological activities, including antimicrobial, antimutagenic, antiulcerogenic, antiviral, antioxidant, anti-inflammatory, antithrombotic, antifungal, and antiparasitic. Clove's antibacterial effect is attributable to its eugenol, which is extensively utilized in perfumery, dentistry, and food (Gowri and Manimegalai, 2019). Caryophyllene was able to affect bacterial membrane permeability and integrity, resulting in membrane degradation and intracellular content leakage, ultimately leading to cell death (Moo *et al.*, 2020). This result shows the primary region for eugenol, indicating that this active component was responsible for the plant greeting activity, which coincided with a study by some researchers who identified and examined the compounds in clove extract (Mandey *et al.*,2020). The results of this study encourage the use of natural sours like some plants or some parts of plants, and the present tests improve the bioactivity, including antibacterial action, thus related to active compounds in the Clove (*Syzygium aromaticum*), especially in the buds, this could be one of many promised to treat to solve some series problems caused by bacteria activities that cause food poisoning

and harmful to human health (Mejía-Argueta *et al.*, 2020). *S. aromaticum* is a powerful chemopreventive agent that has been utilized by traditional Ayurveda physicians in India from ancient times to treat respiratory and digestive illnesses (Banerjee *et al.*, 2006) S. aromaticum has several medicinal purposes; it controls nausea, vomiting, cough, diarrhea, flatulence, dyspepsia, stomach distension, and gastrointestinal spasm, relieves pain, causes uterine contractions, and stimulates the nerves (Tanko *et al.*,2008) . Previous research has examined the mechanisms of eugenol's antibacterial activity; the oil's hydrophobicity allows it to partition lipids and break the outer membranes of Gram-positive and Gram-negative bacteria (Pavesi *et al.*, 2018) . Eugenol was also observed to promote protein leakage from cell membranes in both species of bacteria (Oyedemi *et al.*, 2009).

Determination of antimicrobial activity of medicinal plant extracts

In this study, the clove (*Syzygium aromaticum*) plant was extracted by ethanol extract and the activity of this extract was evaluated in vitro against *Proteus spp*. using agar well diffusion and agar dilution methods. Natural plant-derived antibacterial agents are more cost-effective and exhibit fewer side effects, which has increased their popularity in recent years. Spices such as clove have been widely recognized for their diverse biological activities, including antioxidant, antiviral, anticancer, antifungal, and antibacterial effects (Cortés-Rojas *et al.*, 2014). In this study, significant differences were observed in the diameter of the inhibition zones among different extract concentrations and between the two *Proteus* species tested. The inhibition zone increased with extract concentration, indicating a dose-dependent effect. At a concentration of 10 mg/mL, the ethanolic extract exhibited the strongest activity. For *P. mirabilis*, the inhibition zones were recorded as 24.70 ± 0.20 mm (10 mg/mL), 20.70 ± 0.16 mm (5 mg/mL), 17.52 ± 0.18 mm (2.5 mg/mL), and 14.54 ± 0.13 mm (1.25 mg/mL). Similarly, *P. vulgaris* showed inhibition zones of 23.00 ± 0.18 mm, 18.20 ± 0.26 mm, 16.05 ± 0.18 mm, and 14.04 ± 0.11 mm at the respective concentrations. These results are summarized in Table 5. and visualized in Figure 3, and they agree with the findings (Sethi *et al.*, 2013).

Medicinal plants are widely recognized as a rich source of compounds suitable for drug development and synthesis. These plants contain a broad spectrum of chemical constituents, most of which fall into four main biochemical categories: alkaloids, glycosides, polyphenols, and terpenes. Some plants are valued for their nutritional benefits, while others are recommended for their therapeutic properties. In the present study, *Syzygium aromaticum* (clove) was investigated. Clove exhibited significant antibacterial activity across all tested concentrations, consistent with previous findings (Muhammad *et al.*, 2016). Eugenol, the primary active compound in clove, is particularly noted for its strong antimicrobial properties- findings that align with those reported earlier (Sakha *et al.*, 2018). The increasing prevalence of antibiotic-resistant microorganisms, especially in clinical settings, has made treatment increasingly difficult (Blatnik and Lešničar, 2006). Spread control measures have been developed, as well as treatments for MDR bacteria; an alternate treatment for these bacteria is to investigate natural substances (Lewis and Ausubel, 2006). Antibacterial activity was observed against MDR microorganisms. However, the clove plant derived from spices proved more potent against bacteria (Idowu *et al.*, 2021).

Table 5: *Diameter of inhibition zones (mm) of various concentrations of clove extract against Proteus spp.*

Concentration	*Proteus* mirabilis	*Proteus* vulgaris	LSD
1.25 mg/mL	14.54 ± 0.13[a]	14.04 ± 0.11[a]	0.57
2.5 mg/mL	17.52 ± 0.18[a]	16.05 ± 0.18[a]	
5 mg/mL	20.70 ± 0.16[a]	18.20 ± 0.26[a]	
10 mg/mL	24.70 ± 0.20[a]	23.00 ± 0.18[a]	

Means with different lowercase letters in the same row are significantly different at P < 0.05.

Table 6: *The clove extract against Proteus spp. (MBC and MIC) values (mg/mL)*

Proteus spp.	MIC (mg/mL)	MBC (mg/mL)
P. mirabilis	0.31 ± 0.03	0.63 ± 0.05
P. vulgaris	0.60 ± 0.11	1.22 ± 0.22
P-value	0.02 (NS)	0.02 (NS)

The diameter of the inhibitory zone varies with the concentration of the extract. If the effect of the extract is directly proportional to the concentration, this could be attributed to an increase in the concentration of the active compounds in the extract by increasing its concentration. The alcoholic extract of the clove plant affects the growth of these bacteria because it contains a variety of active ingredients. One of them could be

eugenol, which is known to be found in large quantities in this plant's flower buds. Aside from being a broad-spectrum antibacterial, it contains other chemicals that could be the explanation for its microbiological efficiency (Melander *et al.*, 2018). The effect of plant extracts on bacteria occurs through a mechanism similar to the action of antibacterial drugs, as they work to inhibit the synthesis of the bacterial cell wall, or to inhibit the synthesis of proteins and nucleic acids that the cell essentially needs, or to inhibit the synthesis of the plasma membrane (Chao *et al.*, 2000). According to Kurutas *et al.*, 2005, urinary tract infections can produce oxidative stress by depleting urine antioxidant enzymes. If clove ethanolic extract could provide substantial antioxidative benefits while also exerting antibacterial capabilities against UTI-causing microorganisms, it has the potential to be developed into an effective therapy for this infection.

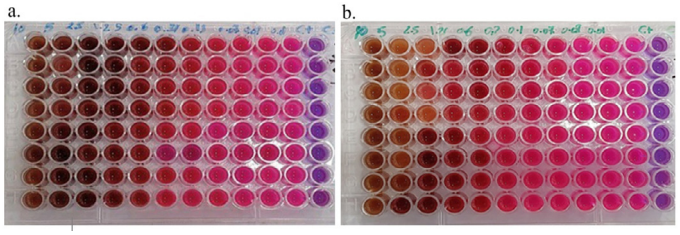

Figure 4: *The results of Broth microdilution assay (MIC) values of cloves extract for (a. P. mirabilis, b. P. vulgaris). (C-)" Negative control (MHB broth only), (C+)" Positive control (only bacteria, broth MHB) Blue color Wells had no or inhibited growth, red color Wells with growth*

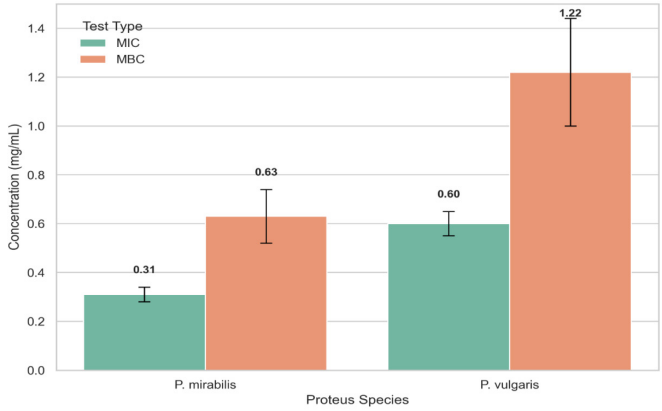

Figure 5: *The observed activity (MBC and MIC) of Antibacterial clove extract against (P. mirabilis and P. vulgaris).*

A correlation study between separated plant components and inhibition of microorganisms revealed those phenolic compounds had potent antibacterial properties (Dorman and Deans, 2000). Thus, the significant level of phenolic content observed in clove ethanolic extract could be one of the main elements contributing to the strong antibacterial capabilities in both Gram-positive and Gram-negative UTI-causing bacteria studied. The antibacterial activity of clove is linked to eugenol (2 methoxy-4 allyl-phenol) (Gupta *et al.*, 2008). The high tannin concentration (10-19%) in clove additionally provides additional antibacterial properties (Nanasombat and Lohasupthawee, 2005).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The result showed in (Agar dilution method) shown in *Proteus mirabilis* the MIC concentration mg/ml was (0.31±0.03) and in *Proteus vulgaris* was (0.60±0.11). While the MBC concentration mg/ml in *P. mirabilis* was (0.60±0.11) and in *p. vulgaris* was (1.22±0.22). This result showed no significant differences between the two types of bacteria and between MIC, and MBC concentration (Table 6), (Figures 4 and 5). This result matches the previous findings (Rosarior *et al.*, 2021). Eugenol, flavonoids, and tannins were among the bioactive components identified in the extract. This bioactivity could be due to eugenol, the primary clove component. Eugenol's hydrophobicity could break down the cellular lipid and disrupt the bacterial cell wall, resulting in cell lysis and intracellular fluid leakage (Pavesi *et al.*, 2018).

Considering this, we postulated that other bioactive molecules, particularly phenolic compounds, were functioning in tandem with eugenol to give the reported antibacterial properties of clove ethanolic extract. Eugenol has previously been shown to have a positive synergistic antibacterial effect on a variety of bacterial strains when combined with other antibiotics such as fluconazole, tetracycline, and colistin (Wang *et al.*, 2018).The findings of the current study may confirm previous recommendations for clove extract as a safe natural product with a variety of therapeutic effects for humans (Pavesi *et al.*,2018).

Conclusions

The research study demonstrated that the ethanol extract of clove (*Syzygium aromaticum*), has biological activity as a potential inhibitor for (*P. mirabilis,P. vulgaris*). GC-MS analysis confirmed the presence of several bioactive compounds, including eugenol, phenol, 2-methoxy-4-(2-propenyl)-acetate, oleic acid, n-hexadecanoic acid, 2,3,4-trimethoxyacetophenone,

caryophyllene, flavonoids, saponins, and tannins. (MBC and MIC) An in vitro antibacterial activity of clove extract against the identified *Proteus* strains. We can conclude that *P. vulgaris* is less sensitive than *P. mirabilis* to the effect of clove extract. This is attributed to the possibility of some differences in the structural composition of virulence factor proteins and the mutations they have undergone, which in turn causes a greater effect for some types of bacteria compared to other types, with regard to the effect of the compounds and their biological activity.

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Novelty Statement

This study provides the first molecular analysis of *Proteus mirabilis* and *Proteus vulgaris* isolates from chicken meat and human urine in Wasit, Iraq. It integrates antimicrobial susceptibility testing (AST), 16S rRNA gene sequencing to reveal high resistance rates and novel genetic mutations at multiple loci. The submission of new sequences to NCBI provides valuable insights into zoonotic transmission pathways and the public health implications of antimicrobial resistance in foodborne pathogens.

Author's Contribution

Mushtaq Talib Abdulwahid: Formulating the idea and general supervision, theoretically and practically. **Safa Jabbar Mudheher**: Help manage the article and conduct practical experiments.

Generative AI or AI assisted technology statement

The authors declare that no generative AI were used in the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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