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Original Article

Study on the effectiveness of *Ocimum basilicum* seed extract against various kinds of bacteria that cause urinary tract infections

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Abstract

The present study was conducted to determine the inhibitory activity of *Ocimum basilicum* extracts on the bacteria causing urinary tract infections. Plant samples were collected from the Nasiriyah region in southern Iraq. It was dried and ground, then extracted from the sample seeds. Ethanol, hexane, and qualitative chemical tests were conducted to determine the active components of the extracts. The efficacy of these extracts was tested at concentrations (100%–75%–50%–25%) on bacteria isolated from urinary tract patients at Al-Hussein Teaching Hospital in Thi Qar. The results showed a significant effectiveness of the ethanol extract and less than that of the hexane extract. The extracts with concentrations (100% and 75%) recorded a higher inhibition rate than (50% and 25%) and the minimum inhibitory concentration (MIC) was 25%. The ethanolic extract of *O. basilicum* seeds was the most effective against the tested gram-positive and gram-negative bacteria strains, whereas the hexane extracts were less effective against all tested pathogenic bacteria. This suppression may result from the chemical composition of extracts containing secondary metabolites.

Keywords: *Ocimum basilicum*, medicinal plants, antimicrobial, pathogenic bacteria.

Introduction

The use of plant extracts to treat many diseases has increased at present, especially after increasing the resistance of pathogenic bacteria that cause a major problem for an individual's health, whether in developed or developing countries; studies have shown that S. *aureus* is one of the most pathogenic staph bacteria that can cause infection urinary tract infections in addition to E. coli causes pneumonia, and it also causes meningitis, mastitis and respiratory system [1]. Many antibacterial agents are used to treat a variety of ailments. Nonetheless, the rising use of these drugs, frequently sporadic and long-term, has resulted in adverse effects that harm human health and antibiotic strains [2]. Nowadays, people are being bombarded with a thousand unhealthy products, and the problem of antibiotic resistance is a worldwide public health problem that continues to grow [3]. Oc*imum basilicum*, a Labiatae herb, is peppery and annual. Basil, from the Greek "Basileus", meaning "Royal" or "King", is known as the "King of the Herbs" because of its many uses in health, cosmetics, pharmacology, and food [4]. This tropical shrub may be found in Southern Asia, Africa, and India. It has been raised in Pakistan and India for 5,000 years. In France, Greece, southern Europe, North and South America, and other warm and temperate locations, O. *basilicum* is produced commercially [5].

Its stem is straight and branched. It can reach 50 to 100 cm in height. Its leaves vary in color, in shades of green or purple; they can be smooth or wavy. The tiny flowers, which can be white, lilac, or red, are grouped vertically in branches and often come in groups of three. The basil of green leaves is the most famous and most cultivated; the rarest and the most are the red leaves with black seeds and oval in shape with mean dimensions of 3.11±0.29 mm (length), 1.82±0.26 mm (width), and 1.34±0.79 (height). O. *basilicum* seeds are

traditionally used as a natural remedy for treating indigestion, ulcers, diarrhea, sore throats, and kidney disorders [6]. In this context, Gajendiran *et al.* [7] demonstrated its effectiveness against nine clinical pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Enterococcus spp.*, *Proteus mirabilis*, *Shigella dysenteriae*, *Salmonella spp.*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*), showing that it was most effective against *Pseudomonas aeruginosa* at a concentration of 100 mg oil/ml. In addition, in a study by [8]. Therefore, this study aimed to examine the inhibitory effectiveness of O. *basilicum* seed extract in ethanolic and hexane against certain gram-positive and gram-negative bacteria.

Material and methods

Plant samples for this study were gathered in november 2022 in Nasiriyah, Dhi Qar Governorate, southern Iraq. Dr. Haider Radi, a science professor at Thi-Qar University, diagnosed and identified the plants. They were completely cleansed of impurities before being ground into a fine powder with an electric mill and kept in sterile glass vials until usage.

Preparation ethanol extract

The plant powder ethanol extract utilized in the study was created by Soxhlet equipment and was used to combine 50 g of plant powder with 250 ml of pure ethanol for 8 hours at 40–60°C. In a rotating evaporator, the solution was dried [9].

Preparation hexane extract

Twenty gm of dried seeds powder was combined with 200 ml of hexane by Soxhlet continuous extraction, and the solution was filtered using Whatman No.13 filter paper. The filtrate was concentrated under reduced pressure on a rotary evaporator at 50°C and dried at 25°C. The extract was collected in sterilized glass tubes until use [10].

Stock solution

Prepare the concentrated solution by dissolving 1 g of extracted in 10 mL of Dimethyl sulfoxide to obtain a solution with a concentration of 100 mg/mL, sterilizing the concentrated solution for each extract using microfiltration filters Millipore filter with a diameter of 0.22 mm, and then repeating the concentration of 3 mg/ml [11].

Qualitative tests of some active compounds in plant extract

Detection of tannin compounds in plant extract

Hydrous acetate mercury chloride was employed at a concentration of 5%, and 1 mL of the reagent was added to 1 mL of the Extract if a white precipitate indicated a positive reaction [12].

Detection of peptides and amine-free groups

These groups were detected using 1% Ninhydrin reagent according to the method obtained by Romay *et al.* [13], where 1 ml of the reagent was added to 1 ml of the Extract with heating for 10 minutes using a boiling water bath. The violet color indicates the presence of amino acids and peptides.

Detection of alkaloids

Was used to detect Drakendorff's reagent was used to detect the alkaloid, where a few drops of picric acid ($C_6 H_3 N_3 O_7$) 0.1% were added to 5 ml of the plant extract in the test tube; the appearance of a yellow color indicates the presence of alkaloids [14].

Detection of glycosides

Five ml of plant extract was added to two ml of acetic acid, then a drop of ferric chloride $fecl_3$ solution and one ml of concentrated sulfuric acid H_2SO_4 were added. The appearance of a brown ring on the inner surface indicates the presence of glycosides [14].

Detection of flavonoids

One ml of potassium hydroxide ethanol with a concentration of 5% was added to 1 ml of the Extract, and the yellow precipitate's appearance indicates a positive reaction [15].

Detection of phenols

0.5 ml of plant extract was put in a test tube. A few drops of Ferric chloride solution at a concentration of 0.5% indicates the appearance of dark green and the presence of phenolic compounds [9].

Sample culture

Diagnosed and ready bacterial samples were obtained from consultancy patients in Imam Hussein Teaching

Hospital for patients with urinary tract infections. Some biochemical tests were conducted on them to confirm the diagnosis of bacteria in the laboratories of the Faculty of Science, and from these tests: (the Catalase test), (the Oxidase test), (the API 20E) and (the IMVC test).

Antibiotic sensitivity test

The Disk diffusion method mentioned is used to conduct an antibiotic sensitivity test. Mueller Hinton agar medium included Nitrofurantoin (300 µg), Norfloxacin (30 µg), Gentamicin (10 µg), Amikacin (10 ug), Tetracycline (25ug) and Ciprofloxacin (10 µg) (Bioanalysis, Turkey) were compared results with the standard tables mentioned in to determine the diameter of the inhibition zone—0.22 Micrometer. Concentrations of 1000 ppm were attended [16].

Results

Qualitative tests of some active compounds in plant extract

The tests have shown that the ethanol and hexane extract for O. *basilicum* seed contain flavonoid, alkaloid glycoside, and amino acid; it also does not contain phenol compounds (Table 1).

Activity of antibiotics on growth of isolated bacteria

The current results showed a significant statistical difference between antibiotics used in this study against all isolated bacteria, except that Amikacin was not a significant difference. The Gentamicin antibiotic scored high activity against E. coli. In contrast, with low activity against P. *aeruginosa* and P. *mirabilis*, the ceftriaxone antibiotics scored high activity against P. *mirabilis*, low activity against both E. coli and S. *aureus*, the Norfloxacin antibiotic scored high activity against all isolated bacteria, while not scored antibacterial activity against P. *aeruginosa*, the Nitrofurantoin antibiotic scored high activity against S. *aureus*, while the low activity against E. coli, also, have not activity against both P.mirabllis and P. *aeruginosa*, the Ciprofloxacin antibiotic show the high activity all against isolated bacteria, while not scored antibacterial activity against P. *aeruginosa*, finally the Tetracycline antibiotic scored high activity against E. coli. In contrast, the low activity against S. *aureus* also means no activity against both P.mirabllis and P. *aeruginosa* (Table 2 and Figure 1).

The activity of different concentrations of *O. basilicum* ethanol extract against isolated bacteria

In this study, the effect of O.gbasilicum seed extract on the expansion of all isolated bacteria was statistically significant at the 0.05 level. Furthermore, the results demonstrated that the activity of the extract increased with increasing concentration against all isolated bacteria, except for S. *aureus*, for which a non-significant difference was noted between 100 and 75 concentrations; furthermore, both P. *mirabilis* and P. *aeruginosa* exhibited no activity at 25 concentrations. High activity was found against *Staphylococcus aureus*, followed by *Proteus mirabilis*, *Escherichia* coli, and *Pseudomonas aeruginosa*, with activity against *Staphylococcus aureus* and E. coli being the weakest (Table 3 and Figure 2).

The activity of different concentrations of *O. basilicum* hexane extract against isolated bacteria

In this study, the effect of an O. *basilicum* seed extract on the expansion of all isolated bacteria was

Table 1: Qualitative disclosures for ethanol and hexane extract.

Ethanol and hexane chemical compound	O. *basilicum*	
	Ethanol	Hexane
Alkaloids	+	+
Amino acid	+	+
Glycoside	+	+
Tannins	+	+
Phenols	-	-
Flavonoid	+	+

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Table 2: Antibiotic sensitive test against isolated bacteria (mm).

Bacteria	*P. mirabilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Antibiotics	**Inhibition zone of antibiotics Mean±SD**			
Gentamicin	**I** 12.0±1.00^{c}	**S** 19.0±1.00^{b}	**R** 11.6±1.52^{b}	**S** 15.6±1.15^{cd}
Ceftriaxone	**R** 18.3±2.51^{b}	**R** 10.6±0.57^{e}	**R** 14.3±1.52^{a}	**R** 10.6±1.52^{e}
Amikacin	**I** 15.3±1.52^{b}	**R** 14.3±0.57^{d}	**R** 13.3±1.52^{ab}	**I** 15.6±2.30^{cd}
Norfloxacin	**S** 23.0±3.00^{a}	**S** 24.0±2.00^{a}	**R** 0.00±0.00^{c}	**S** 26.0±2.00^{a}
Nitrofurantoin	**R** 0.00±0.00^{d}	**R** 11.0±1.00^{e}	**R** 0.00±0.00^{c}	**S** 18.0±1.73^{c}
Ciprofloxacin	**I** 22.3±1.52^{a}	**I** 24.0±1.00^{a}	**R** 0.00±0.00^{c}	**I** 22.0±2.00^{b}
Tetracycline	**R** 0.00±0.00^{d}	**I** 16.7±1.15^{c}	**R** 0.00±0.00^{c}	**R** 14.6±1.15^{d}
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LSD	**3.03**	**1.98**	**1.75**	**3.05**

Note: S – Sensitive; I – Intermediate; R – Resistance.

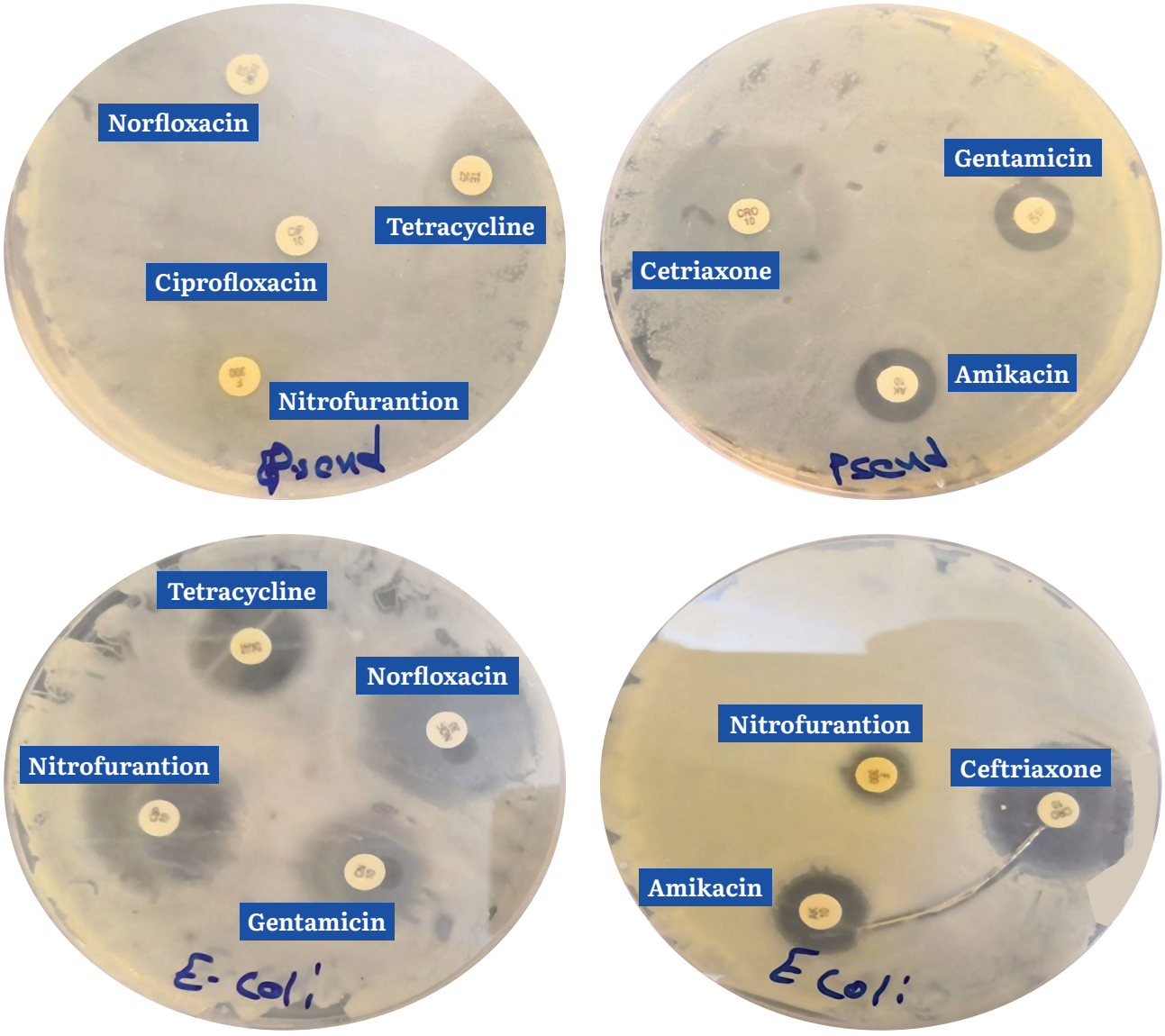

Figure 1: Antibiotic sensitive test against isolated bacteria (mm).

Table 3: Activity of O. *basilicum* seeds ethanol extract on the growth of isolated bacteria.

Bacteria	*P. mirabilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Mg/ml concentration	**Inhibition zone of O. *basilicum* ethanolic extract Mean±SD**			
100	34.3±1.15[a]	33.0±1.00[a]	32.6±2.08[a]	36.0±1.73[a]
75	29.6±0.57[b]	28.3±1.52[b]	28.0±1.00[b]	33.6±1.52[a]
50	24.3±0.57[c]	19.6±2.08[c]	20.0±2.64[c]	17.7±2.51[b]
25	0.00±0.00[d]	11.0±2.00[d]	0.00±0.00[d]	9.00±2.64[c]
Control	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[d]
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LSD	**1.33**	**3.21**	**3.30**	**4.06**

Note: The DMSO that was used as the control group in this table does not have a biological action. Every two averages in this table have the same small letter; there is no significant difference between them, and every two different letters have a significant difference between them. The effectiveness of the extract increases with the introduction of letters such as (a, b, c etc).

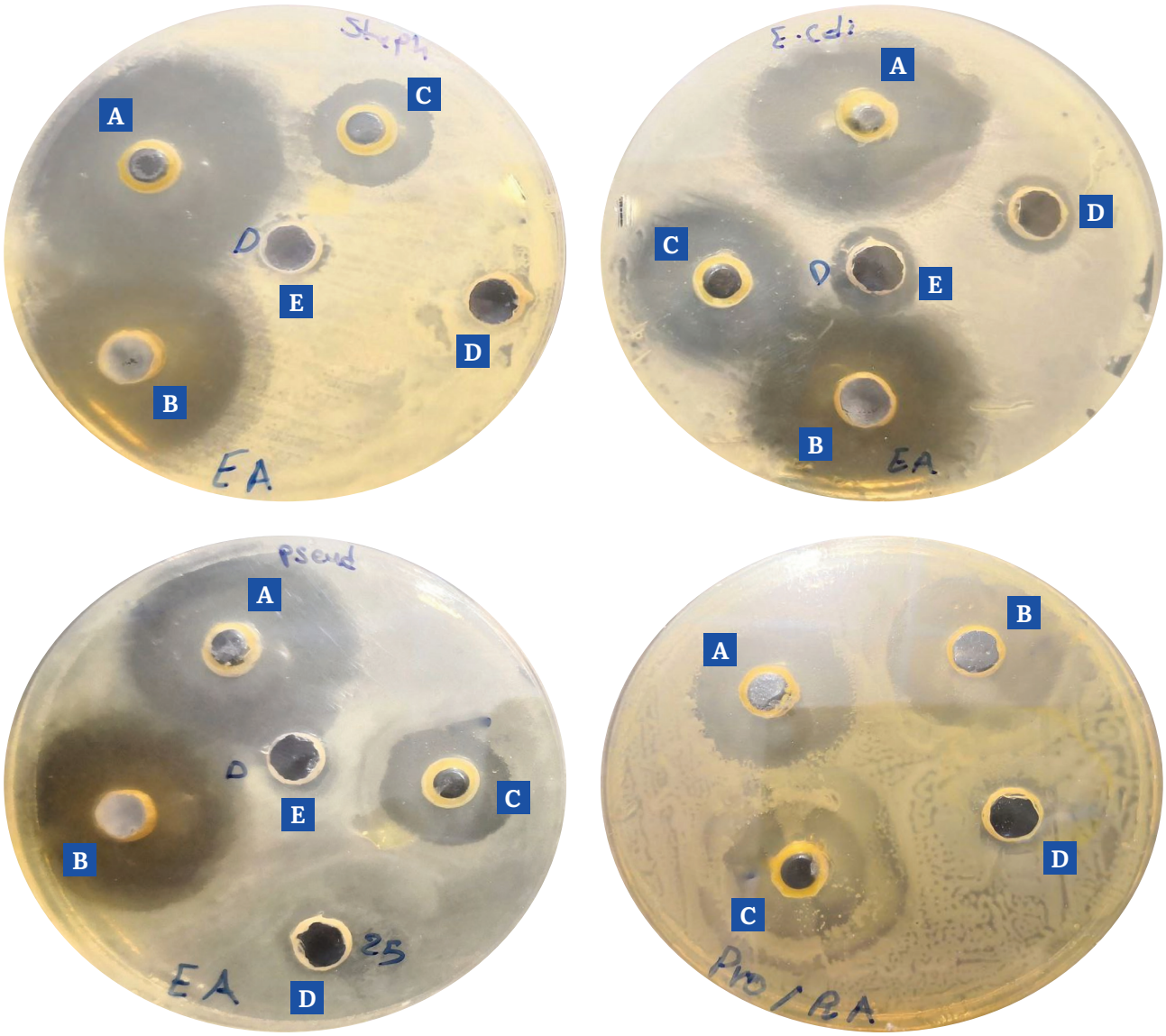

Figure 2: Activity of O. *basilicum* L. extract ethanol against isolated bacteria: Well A concentration 100%; Well B concentration 75%; Well C concentration 50%; Well D concentration 25%; Well E concentration control.

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found to be statistically significant (0.05). The study also revealed a statistically significant difference in activity across concentrations for all of the isolated bacteria, and it was also noted that none of the 25 concentrations of the O. *basilicum* extract showed any activity against the microorganisms P. *mirabilis*, P. *aeruginosa*, or S. *aureus*. High activity was found against P. *aeruginosa*, then against E. coli, then against S. *aureus*, and finally against P. *mirabilis*; activity against E. coli was the lowest (Table 4 and Figure 3).

Discussion

Medicinal plants may be able to provide antibiotic alternatives for antibiotic-resistant microorganisms. This study found that active chemicals in the same seeds can affect various pathogenic species in different quantities. The active chemicals extract affected gram-positive bacteria more than gram-negative bacteria. Gram-negative organisms may have a protective outer membrane [17].

Plant bioactive phytochemicals may combat resistant microorganisms better than chemical or manufactured antimicrobial medicines [18]. In the current study, O. *basilicum* seeds possessed substantially varying levels of bioactive phytochemicals, which may have contributed to their bactericidal effect. Secondary products, including flavonoid chemicals, alkaloids, steroids, and tannins, provide plant materials with medicinal characteristics [19]. Tannins from plants have been shown to have antimicrobial activity [20]; the hypothesized action is to interact with cell proteins via a variety of bioactive forces, such as hydrogen bonding, covalent bond formation, or hydrophobic interactions [21]. As a result, tannins may exert antibacterial activity by inhibiting microbial essential processes such as extracellular enzyme activity, microbial adhesions, oxidative phosphorylation inhibition, or transport proteins in the cell membrane [22].

Furthermore, tannins have been hypothesized to interact with proline-rich peptides, limiting protein synthesis [23]. The seeds of this plant are widely utilized in the treatment of numerous ailments, earning it the moniker "miracle herb" [24].

The presence of thymoquinone, a key component of essential oil, is linked to the medicinal benefits of these seeds [24]. According to Table 1 in the results, the extracts of the seeds plant obtained revealed the presence of alkaloids and Tannins; on the other hand, phenol was absent in the extracts; these results are similar to Behidj-Benyouness *et al.* [25] who state that the results obtained from the phytochemical study of the P. *harmala*, show that the main major compounds present in large quantities are the alkaloids, followed by coumarins and saponins. In contrast, Maitham *et al.* [26], through their phytochemical screening studies of P. *harmala* seeds, showed the absence of flavonoids, coumarin and resins and the presence of alkaloids, tannins glycosides, anthraquinones, terpenoids and steroids.

Some isolates sensitive to both extracts were found, as shown in the results. This finding demonstrated the potent effect of plant seed extracts against clinical isolates used in the present study, which were resistant to seven commonly used antibiotics (Nitrofurantoin, Norfloxacin, Gentamicin, Amikacin, Ciprofloxacin, Tetracycline and Ciprofloxacin) that may lead this extracts to be an alternative treatment to infectious diseases caused by this organism. Similarly, many different studies reported the antimicrobial activity of plant seed extracts against different microorganisms, including clinical bacteria; these two studies were done by Shama *et al.* [27], Awdalla *et al.* [28], and another study conducted by Hero and Jwan [29]. Therefore, plant seeds' antibacterial activity may be related to their ability to inactivate cell envelope transport proteins, enzymes, and microbial adhesions and may be complex with polysaccharides [30].

The antimicrobial methods could also affect MIC and MBC values. The disc diffusion test used by Teixeira *et al.* [31] is simple, convenient, and well-standardized but presents qualitative results and approximate MIC values [32]. Our study used a microdilution test, a quantitative test that allows for higher accuracy and reproducibility, to determine MIC and MBC values [32].

The seed plant extracts in our study showed high bactericidal activity, mostly against P. *aeruginosa* and S. *aureus*. Increasing the number of multi-drug resistance pathogenic microbes in humans and animals, as well as unwanted side effects of certain antibiotics, has encouraged enormous interest in searching for new antimicrobial drugs of plant origin [33].

All of the four tested bacteria in this study responded to water extract distillation and hydroalcoholic extracts with greater results for hydroalcoholic extract. However, it has been reported by many researchers that hydroalcoholic extract, compared to the aqueous extract, is more effective and has a superior inhibitory influence [34]. Seeds of O. *basilicum* are recognized for their biological activities, especially antidiabetic, anti-inflammatory and antioxidant activities [35]. Azzi *et al.* [36], Ouelbani *et al.* [34] and Telli *et al.* [37] reported

Table 4: Activity of O. *basilicum* hexane compound on the growth of isolated bacteria (mm).

Bacteria	*P. mirabilis*	*E. coli*	*P. aeruginosa*	*S. aureus*
Concentration	**Inhibition zone of O. *basilicum* seeds hexane extract Mean±SD**			
100	23.6±1.52[a]	28.7±0.57[a]	28.3±1.52[a]	26.7±0.57[a]
75	20.6±1.52[b]	23.0±0.00[b]	22.3±2.08[b]	22.0±1.00[b]
50	11.3±1.52[c]	13.6±0.57[c]	11.3±0.57[c]	9.33±0.57[c]
25	0.00±0.00[d]	7.66±1.15[d]	0.00±0.00[d]	0.00±0.00[d]
Control	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[e]	0.00±0.00[e]
P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LSD	**2.49**	**1.33**	**2.49**	**1.21**

Note: The DMSO that was used as the control group in this table does not have a biological action. Every two averages in the table have the same small letter; there is no significant difference between them, and every two different letters have a significant difference between them. The effectiveness of the extract increases with the introduction of letters such as (a, b, c etc).

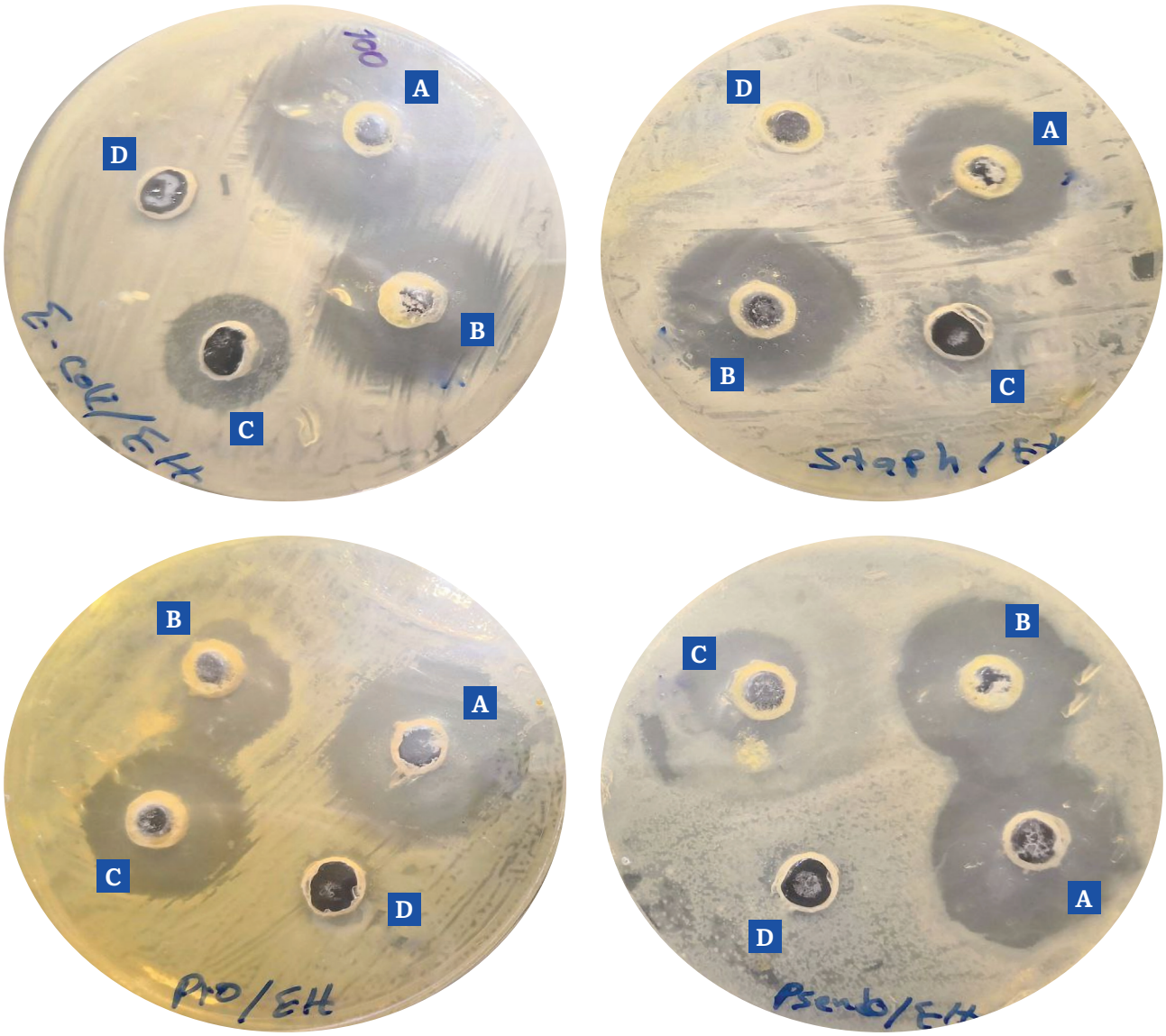

Figure 3: Activity of O. *basilicum* L. extract Hexane against isolated bacteria: Well A concentration 100%; Well B concentration 75%; Well C concentration 50%; Well D concentration 25%.

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that these medicinal plants are used for the treatment of many diseases.

In the present study, the phytochemical screening of the various extracts of all plants showed the presence of a large range of secondary metabolites. Amessis-Ouchemoukh *et al.* [38] reported that several studies affirmed the presence of polyphenols, terpenoids, and coumarins contrary to saponins in some plants. Ksouri *et al.* [39] confirmed the presence of polyphenols, saponins and terpenoids in Z. album. Elgamal *et al.* [40] identified and isolated a saponin triterpenoid and a quinovic acid from the aerial parts of some medicinal plants, which are probably responsible for many biological activities.

Whereas Hero and Jwan [29] found ethanolic extract was more inhibitory to *P. aeruginosa* than aqueous extract, another study done by Ibrahim and Kebede [41] found ethanol extract of O. *basilicum* showed highest activity as compared to aqueous extract. These variations may be due to differences in geographical area, strains, and sample size as these studies were worked on only one *P. aeruginosa* compared with *Pseudomonas* strains in this study.

The results of other solvents varied, some of which had a weak effect, such as water extract at a concentration of 25%, which was less effective than the rest of the other solvents, and this may be attributed to the difference in the polarity of the organic solvents. Alternatively, the inhibitory activity of plant seeds is due to the fact that they contain most of the active compounds—especially tanning materials, including tannic acid, which may have an effect on microorganisms [42].

As for the results of testing some commercial antibiotics against the tested bacteria, the results showed that the effect of these antibiotics varies from one antibiotic to another and from one bacterium to another [43].

As for the lack of effect of some other antibiotics, this is expected because they affect the cell wall, especially of Gram-positive bacteria, which is consistent with the results of this study. Ethanolic extract showed the highest antibacterial activity against all the isolates tested, with zones of inhibition between 36 mm.

This is in contrast to aqueous extract, which shows the lowest or no antimicrobial activity in concentration 25% against all isolates tested. These results are close to the results obtained by Rathi *et al.* [44], who mention three extracts of M. *oleifera* plant leaves and seeds (aqueous, methanolic, ethanolic).

The other extract, hexan, shows moderate antibacterial activity. The results showed that the extracts have an antibacterial activity on the four strains of bacteria under study and in all the concentrations used (50%, 75% and 100%), which affirms results obtained by Ishnaiwer [45] confirmed that the fenugreek has an antioxidant and anti-infection activity. According to our study, it inhibited the growth of the bacteria. Although the main focus of the current study is to determine the antibacterial activity of seed plant extract, further essential studies need to be conducted to confirm the antimicrobial activity and evaluate the potential use of the active compound/s in the current extract as therapeutic product to treat MDR bacterial infections in humans. First, the suggested compounds' *in vitro* and *in vivo* antimicrobial activity should be evaluated against diverse bacterial isolates, including MDR, to confirm their potential. Enzyme biochemical testing can also verify docking results. In addition, the extract's possible active components should be tested for the mechanism of action, physicochemical characteristics, toxicity and resistance. Future studies would validate the extract's active compounds' *in vitro* and *in vivo* activity and evaluate its safety for therapeutic usage in humans.

Conclusion

Medicinal plants can be a source of antibacterial agents due to their phytochemical composition. The ethanolic extract of O. *basilicum* seeds was the most effective against the tested gram-positive and gram-negative bacteria strains, whereas the hexane extracts were less effective against all tested pathogenic bacteria. This suppression may be a result of the chemical composition of extracts containing secondary metabolites. However, these extracts could be a viable alternative to antibiotics and could be used as effective therapeutic agents against pathogenic bacteria without posing a health risk.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the University of Thi-Qar (approval ID: 6098).

Consent to participate

Written informed consent was obtained from all participants in this study.

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