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OPEN ACCESS **Original Article**

Phytochemical Screening, Antibacterial, Antioxidant, and *In Vitro* Anti-lithiatic Effects of *Ocimum basilicum* Leaves Extracts

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ABSTRACT

Background: *Ocimum basilicum* L., commonly referred to as "Reyhan". It has various pharmacological effects, such as anti-hypertensive, antioxidant, and anti-inflammatory activities.
Objective: In the present study, various phytochemical constituents, antioxidant potential, antibacterial activity, and *in vitro* anti-urolithiasis properties of *Ocimum basilicum* leaves were investigated.
Methods: Powdered basil leaves were extracted using water, ethanol, methanol, and chloroform by the maceration method. The well-diffusion assay was employed for testing antibacterial activity. Antioxidant capacity was assessed qualitatively by the TLC autography method using two mobile phases and quantitatively by the DPPH radical-scavenging assay using ascorbic acid as a standard. The turbidity method was utilized to assess the anti-urolithiasis efficacy of cystone *in vitro*.
Results: Phytochemical analysis indicated the existence of flavonoids, tannins, alkaloids, amino acids, lipids, fixed oils, and carbohydrates; however, anthraquinones were absent. All extracts demonstrated antibacterial action against Gram-positive and Gram-negative bacteria. An aqueous extract showed the widest diameter of inhibition zones, with *S. aureus* being 18.71 mm, *P. aeruginosa* 21.65 mm, and *E. coli* 20.65 mm. However, it showed very negligible action against *K. pneumoniae*. The qualitative antioxidant test indicated that all the extracts possessed antioxidant properties. Among these, the methanolic extract possessed the maximum quantitative antioxidant activity; vitamin C was used as a positive control. The *in vitro* anti-urolithiasis assay showed that the chloroform extract exhibited 61.86% inhibition of crystal formation.
Conclusion: Leaves of *Ocimum basilicum* exhibited marked antioxidant and antibacterial potentials. The plant also exhibited significant anti-urolithiasis property, which justifies its traditional use in herbal medicine.

Keywords: Phytochemical screening, *Ocimum basilicum*, antioxidant activity, qualitative and quantitative analysis, anti-urolithiasis activity.

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INTRODUCTION

Medicinal plants represent a vital component of healthcare in every part of the world, providing very useful phytochemicals that play a significant role in the treatment and prevention of a wide variety of diseases. Among these, the Labiatae family is renowned for its aromatic and medicinal species, especially those belonging to the genus Ocimum, which comprises more than 60 species widely used in Asian and African traditional medicine [1-4]. *Ocimum basilicum* L., commonly referred to as "Reyhan," is one of the well-known species in this group. This plant's essential oils and extracts include a variety of bioactive chemicals, including linalool, eugenol, and estragole, which contribute to its well-known antibacterial and antioxidant properties (5–8). It is also said to have anti-inflammatory, anti-hypertensive, and oxidative stress-protective qualities (9–11). Antibacterial drugs are a broad category of substances that, either by producing bactericidal or growth-inhibiting effects, or killing or stopping the growth and multiplication of bacteria. The bacterial cell is destroyed or disrupted by bactericidal agents. Bacteriostatic medications prevent bacteria from growing or multiplying without actually killing them (12). Given its wide range of biological activities, the present study focuses on the exploration of the phytochemical profile of *Ocimum basilicum* for assessing its antibacterial, antioxidant, and anti-urolithiasis potentials.

Figure 1: *Ocimum basilicum*

METHODOLOGY

Collection and Extraction of *Ocimum Basilicum* Leaves

Ocimum basilicum leaves were obtained from a farm in Al-Hamra, Tuban, Lahj, Yemen, and then were authenticated at the Department of Biology, University of Aden. After a week of air drying, the leaves were processed into a fine powder using an electric grinder. After that, the powder was dried at 37°C for a whole day. The powdered leaves were macerated 25 g of in 250 ml of distilled water, ethanol, methanol, and chloroform. The mixtures were filtered with Whatman No. 1 filter paper; the filtrates were then concentrated using a rotary evaporator. Afterwards, the extracts were dried in an oven at 40°C (13).

Phytochemical Screening

Phytochemical screening of the dried plant extracts was done using standard qualitative methods for the identification of flavonoids, alkaloids, steroids, glycosides, carbohydrates, saponins, tannins, fats, fixed oils, amino acids, and proteins, according to the principles described in the reference (14).

Flavonoids

-Lead acetate test: One ml of the test solution supplemented with a few drops of 10% lead acetate. A heavy white precipitate revealed the presence of phenol compounds.

Alkaloids

- Hager test: Several drops of Hager's reagent (saturated aq. solution of picric acid) applied to the test sample. The test was positive, as indicated by a strong yellow precipitate.
- Wagner's test: A few drops of Wagner's reagent were added to the side of the test tube, to a few ml of filtrate. A red-brown precipitate indicated the experiment's success. Color in the bottom layer of the test tube betrays triterpenoid.

Anthraquinone Glycosides

-Hydroxyanthraquinone Test: A few drops of 10% potassium hydroxide solution were added to 5 ml of the plant extract. In a test tube, 10 ml of dilute sulfuric acid was added to 5 ml of the plant extract, which was then boiled for 15 minutes, cooled, and neutralized with 10% NaOH before adding 5 ml of Fehling solution. The presence of a glycoside was indicated by the pink color.

Cardiac Glycosides

-Keller-Killian test: 0.4 ml of glacial acetic acid and a few drops of 5% ferric chloride solution were added to a plant extract. Further, 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully. The presence of a glycoside was indicated by the green color.

Carbohydrates

-**Moloch's Test:** The test solution was combined with 2-3 mL of concentrated sulfuric acid, which was added to the side of the test tube. The violet ring formation revealed the presence of carbohydrates.

– **Benedict Test:** To 0.5 ml of the extracts, 0.5 ml of the Benedict reagent was added. The solution was then heated in the boiling water bath for 2 minutes. A colored precipitate indicated the presence of sugar.

- **Fehling's Test:** 1 ml of Fehling's solution A+B was mixed with 1 ml of the plant extracts. The solution was then heated in the water bath for 10 minutes, shaking it occasionally. The formation of red precipitation revealed the presence of sugar. A few drops of a 1% alpha-naphthol solution were added to 1 ml of the test solution.

Proteins

-**Biuret test:** 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH were added to 2 ml of the test solution. They were mixed thoroughly. If a violet or pink color is formed, it indicates the presence of proteins.

Fats and Fixed Oils

Five drops of the sample were added to 1 ml of 1% copper sulfate solution and a few drops of 10% sodium hydroxide. Formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Amino Acids

-**Millon's test:** 5 drops of Millon's reagent were added to 1 ml of test solution and heated on a water bath for 10 min, then cooled, and 1% sodium nitrite solution was added. A white precipitate indicated the presence of proteins.

Antibacterial Activity Test

The antibacterial activity of *Ocimum basilicum* was evaluated using the well diffusion method on agar. The medium used was the Mueller-Hinton Agar. The microorganisms (*E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*) were activated by pouring one entire loopful of each organism into the sterile last water, then incubated at room temperature. A loop-full inoculum with an inoculum size of 10^8 cells/ml according to the McFarland standard was taken from the activated microorganisms and then inoculated into each of the four bottles containing 100 ml of molten Mueller Hinton agar medium before homogenizing and then poured into petri dishes until dry. Wells were created on each seeded plate with the help of the sterile pipettes, with each well receiving 50 µL of the extracts. The positive controls consisted of ciprofloxacin 5 mg/mL, erythromycin 10 mg/mL, gentamicin 10 mg/mL, and lincomycin 10 mg/mL, while the blank was DMS for all extracts. The test extracts were placed into the wells with different concentration levels of 300, 400, and 500 mg/ml. The growth of the microorganisms was indicated by the size of the inhibition zones created by the extracts, with measurements taken according to the method described by (15).

Antioxidant Test

Qualitative Antioxidant Activity

The qualitative antioxidant activity was assessed using the TLC autography method. Determining the antioxidant activity of plant extracts with the DPPH method is a simple, quick approach that requires few chemicals. The DPPH sample measurements indicated that antioxidants, which are generally not dependent on inhibition, were able to produce measurable results. Using a 0.1% DPPH solution, the location of each plant that removed the radical spray indicated a positive antioxidant through the presence of pale-yellow spots after 30 minutes. Two mobile phases were utilized: toluene-acetone-formic acid (8:2:0.15) (16) and butanol-acetic acid-water (4:1:5) (17).

Quantitative Antioxidant Activity via DPPH Radical Scavenging

The quantitative estimation of the scavenging effect was done in a universal bottle. The reaction mixture was composed of 1 ml of extracts (test samples) or 80%

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MeOH (blank) and 1 ml of 0.004% solution of DPPH in methanol. The comparison was made with ascorbic acid. The discoloration was assessed at 517 nm after incubation for 30 min. The inhibition of DPPH radicals by the samples was calculated based on the following equation: DPPH-scavenging activity (% = (absorbance of control - absorbance of sample)/absorbance of the control × 100 (18).

In Vitro Anti-Lithiatic Activity Test

The *in vitro* anti-lithiatic activity test was conducted using the turbidity method. The *in vitro* anti-lithiatic activity of the extracts was evaluated based on the calcium oxalate inhibition of formation by the extracts with/without inhibitors (standard drugs & extracts). The precipitation of calcium oxalate at 37°C, pH 6.8, was evaluated based on the turbidity units/measurements at 620 nm. A spectrophotometer UV/Vis was used to evaluate the turbidity units caused by the formation of calcium oxalate in the treatment groups (19). The growth of the stone nucleus *in vitro* without the presence of any inhibitor was carried out initially. For the experiment, the following procedure was followed: A quantity of 1.0 ml of 0.025 M $CaCl_2$ solution, along with 2 ml of Tris buffer solution of pH 7.4, was poured into the test tube. Immediately, 1.0 ml of 0.025 M sodium oxalate solution was also added to the same test tube. Then, the growth of the turbidity was followed immediately, taking the measurements of the formed turbidity levels in terms of the absorption value pronounced at 620 nm inside the UV/Vis spectrophotometer immediately until the period of 10 minutes or 600 seconds elapsed from the time the chemicals were mixed inside the spectrophotometer. The above process was repeated three times to obtain the growth of the turbidity levels without the presence of the inhibitor inside the human body. The process was continued to observe the effect of the plant extracts on the formation of the stone nuclei inside the human body. In this experiment, four sets of test tubes with 1 ml of 0.025 M calcium chloride, 2 ml of Tris buffer, and 1 ml (50 mg/ml solution) of chloroform extract were taken. Another set of test tubes was prepared with the polyherbal formulation (Cystone®) synthetic drug. Then 1 mL of 0.025 M sodium oxalate was added to each test tube. Each set was replicated three times. Immediately after mixing sodium oxalate, we measured the change in turbidity of the solution for up to 10 minutes.
Inhibition in stone nucleus formation was calculated by the graphical method using the following mathematical formula:

$$\text{Inhibition \%} = [\ Ac - Ai \backslash Ac\ ] \times 100$$

Where;
Ac: absorbance for the Control, Ai: absorbance in the presence of inhibitor (drugs/extracts). The method used was similar to that described by (20).

Statistical Analysis

SPSS version (26), was used for analyzing the data. One-way ANOVA was used for the antibacterial assay and Pearson correlation for the antioxidant activity. The P-value was considered significant at 0.05.

RESULTS

Phytochemical Screening

The phytochemical analysis of the different extracts (aqueous, ethanol, methanol, and chloroform) from the leaves of *Ocimum basilicum* revealed the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, triterpenes, tannins, cardiac glycosides, carbohydrates, fat, and fixed oils. Meanwhile, the phytochemical constituents like anthraquinone glycosides were not present, as shown in Table 1.

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Table 1: Phytochemical screening test of water, ethanol, methanol, and chloroform extracts of *Ocimum basilicum*

Phytochemicals	Test performed	Water extract	Ethanol extract	Methanol extract	Chloroform extract
Alkaloids	Hager test Wagener test	+ +	_ _	+ _	+ +
Flavonoids	Lead acetate test	+	+	+	+
Saponins	Frothing test	+	_	_	_
Triterpenoids	Salkowski test	+	_	_	+
Tannins	Ferric chloride	+	+	+	+
Anthraquinone glycosides	Hydroxyanthraquinone Test	_	_	_	_
Cardiac glycosides	Keller-killiani test	_	+	_	+
Carbohydrates	1- molosch test 2- Benedict test 3- fehling test	+ + _	+ + _	+ + _	+ _ _
Amino acids	Millon test	_	+	_	+
Proteins	Biuret test	+	_	_	_
Fat and fixed oils	Fat and fixed oils test	_	+	+	+

Antibacterial Effects

Table 2 presents the antibacterial activity of water, ethanol, methanol, and chloroform extracts of *Ocimum basilicum* leaves with 300, 400, and 500 mg/ml against chosen Gram-positive and Gram-negative bacteria. All the extracts exhibited different degrees of antibacterial efficacy on the tested germs. Except for *Klebsiella pneumonia*, where it demonstrated no influence, the Water extract displayed the greatest inhibition region against all other bacteria. Inhibition zones of positive controls were shown in Table 3.

Table 2: Results of antibacterial activities of *Ocimum basilicum* extracts

Species	Concentration mg/ml	Inhibition zones in mm				
		Aqueous	Ethanol	Methanol	Chloroform	
P. aeruginosa	300	18.00	14.87	13.00	10.23	P= 0.009 F= 7.912
	400	19.45	16.43	15.69	11.10	
	500	21.65	17.15	17.38	14.67	
	Mean ± SD	19.70 ± 1.84	16.15 ± 1.17	15.36 ± 2.21	12.00 ± 2.35	
E. coli	300	16.85	13.93	13.35	13.77	P= 0.048 F= 4.146
	400	17.36	14.52	16.86	13.87	
	500	20.65	15.86	17.77	13.90	
	Mean ± SD	18.29 ± 2.06	14.77 ± 0.99	15.99 ± 2.33	13.85 ± 0.07	
K. pneumonia	300	-	-	11.52	11.22	P= 0.209 F= 2.244
	400	-	-	13.49	11.80	
	500	-	-	15.80	12.11	
	Mean ± SD	-	-	13.60 ± 2.14	12.00 ± 2.35	
S. aureus	300	14.16	12.89	13.84	10.78	P= 0.018 F= 6.104
	400	17.34	13.57	14.40	12.18	
	500	18.71	13.80	16.22	12.79	
	Mean ± SD	16.74 ± 2.33	13.42 ± 0.47	14.82 ± 1.24	11.92 ± 1.03	

Table 3: Inhibition zones of positive controls

	Ciprofloxacin 5 mg/ml	Erythromycin 10 mg/ml	Gentamycin 10 mg/ml	Lincomycin 10 mg/ml
P. aeruginosa	17.07 mm	-	22.39 mm	-
E. coli	44.55 mm	-	20.00 mm	-
K. pneumonia	16.48 mm	-	14.59 mm	-
S. aureus	12.50 mm	29.73 mm	24.68 mm	29.88 mm

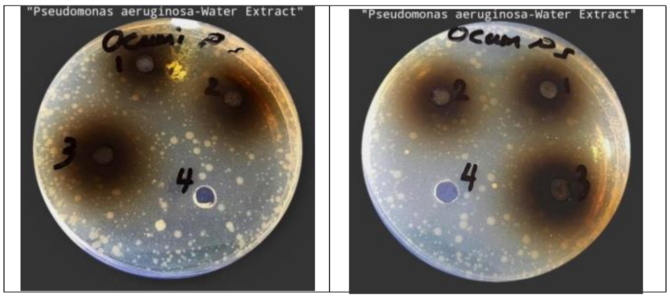

Figure 2: Inhibition zones of water extract on *P. aeruginosa*, 1=300 mg/ml. 2=400 mg/ml, 3=500 mg/ml, 4= Blank.

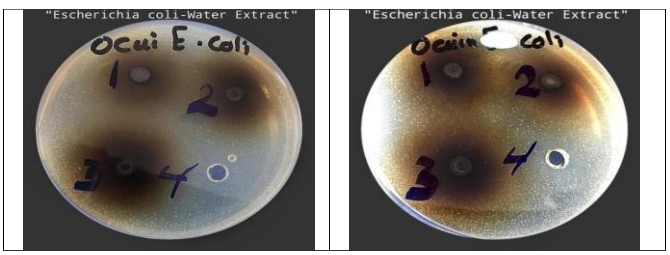

Figure 3: Inhibition zones of water extract on *E. coli*, 1=300 mg/ml. 2=400 mg/ml, 3=500 mg/ml, 4= Blank.

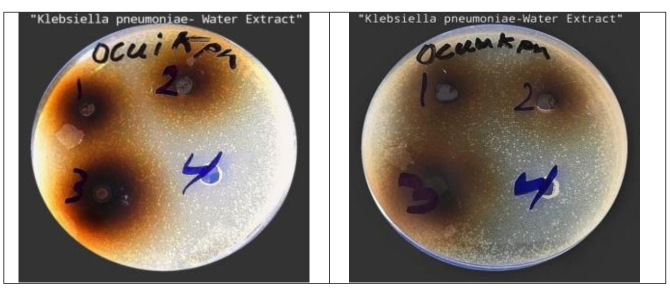

Figure 4: Inhibition zones of water extract on *K. pneumonia*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

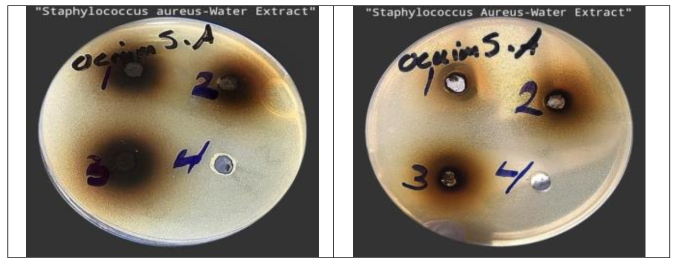

Figure 5: Inhibition zones of water extract on *S. aureus*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

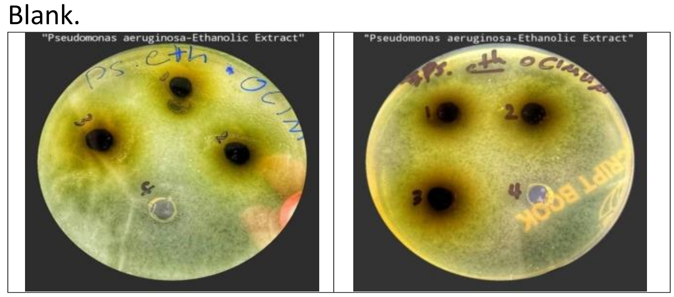

Figure 6: Inhibition zones of ethanolic extract on *P. aeruginosa*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

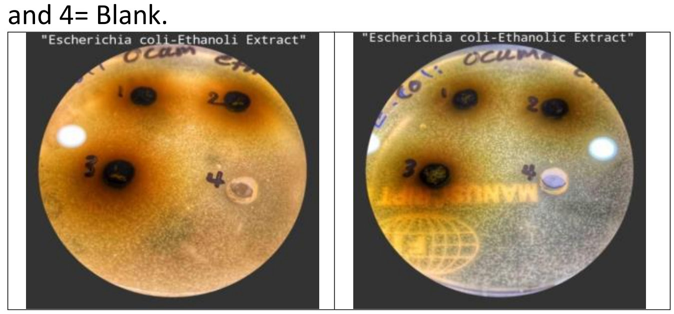

Figure 7: Inhibition zones of ethanolic extract on *E. coli*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

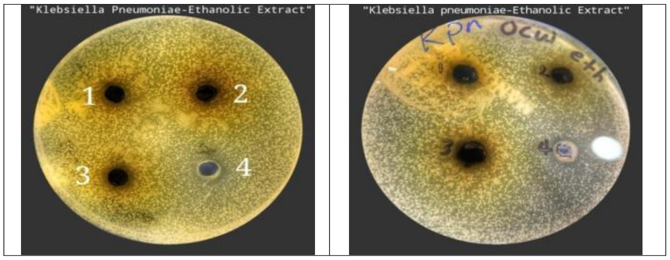

Figure 8: Inhibition zones of ethanolic extract on *K. pneumonia*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

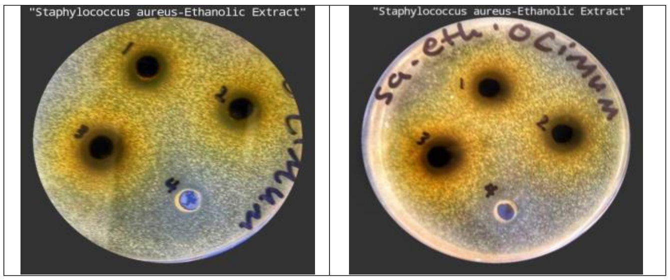

Figure 9: Inhibition zones of ethanolic extract on *S. aureus*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

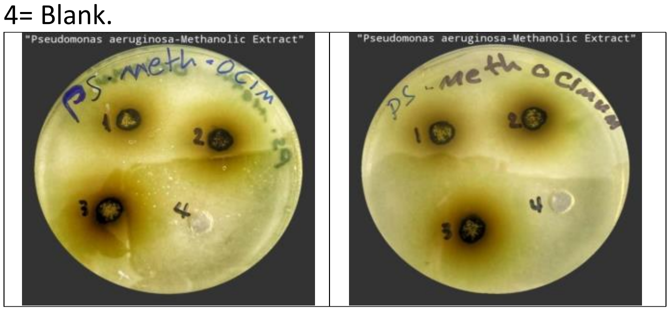

Figure 10: Inhibition zones of methanolic extract on *P. aeruginosa*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

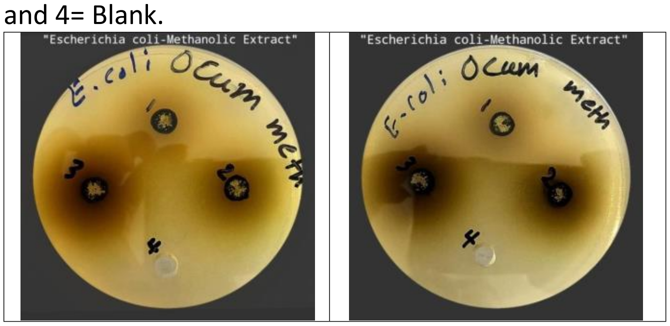

Figure 11: Inhibition zones of methanolic extract on *E. coli*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

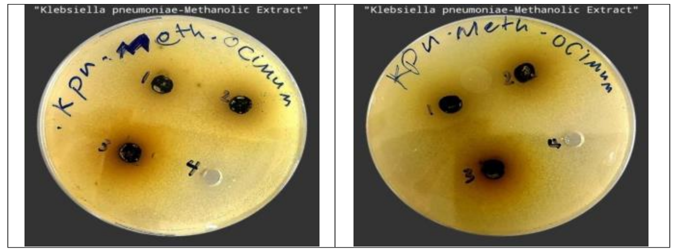

Figure 12: Inhibition zones of methanolic extract on *K. pneumonia*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

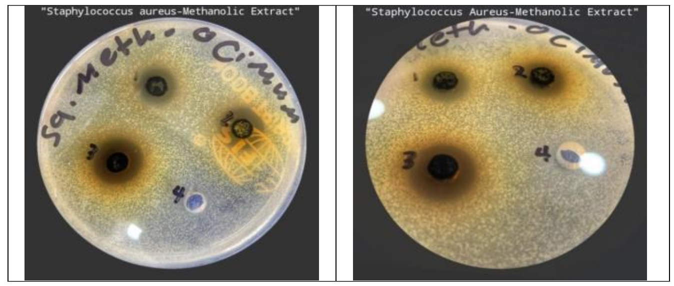

Figure 13: Inhibition zones of methanolic extract on *S. aureus*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

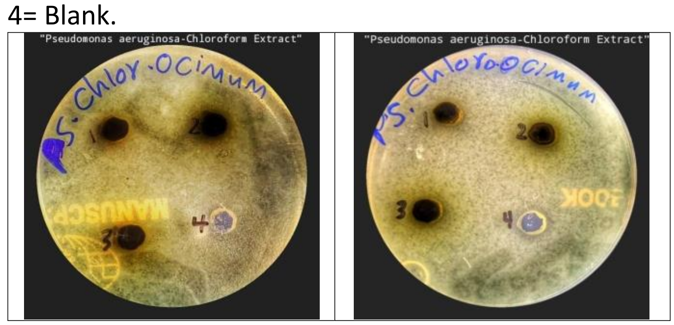

Figure 14: Inhibition zones of chloroform extract on *P. aeruginosa*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

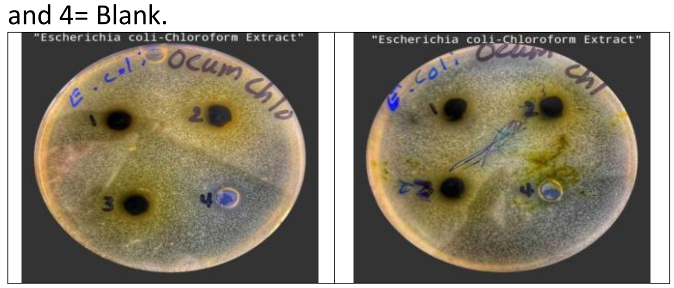

Figure 15: Inhibition zones of chloroform extract on *E. coli*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

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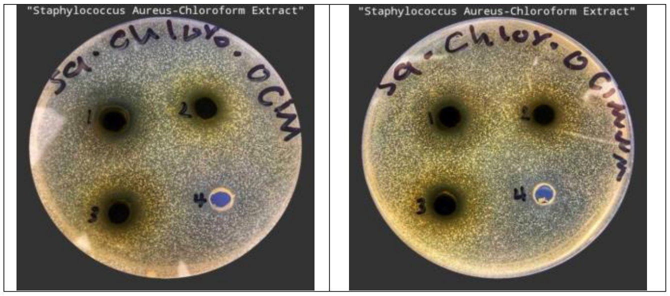

Figure 16: Inhibition zones of chloroform extract on *S. aureus*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

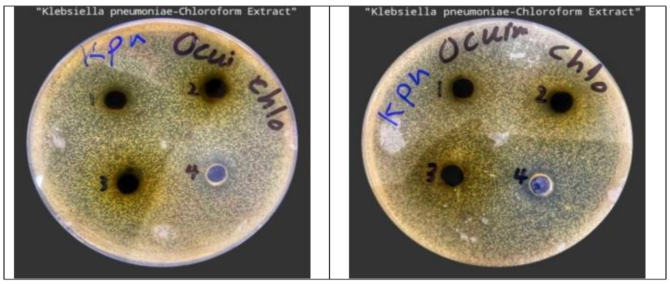

Figure 17: Inhibition zones of chloroform extract on *K. pneumonia*, 1=300 mg/ml, 2=400 mg/ml, 3=500 mg/ml, and 4= Blank.

Figure 18: Inhibition zones of positive controls on *E. coli*, 1=Ciprofloxacin, 2= Erythromycin, 3= Gentamycin, 4= Lincomycin.

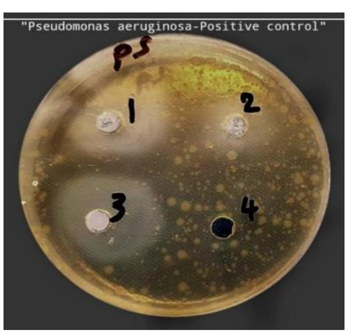

Figure 19: Inhibition zones of positive controls on *P. aeruginosa*, 1=Ciprofloxacin, 2= Erythromycin, 3= Gentamycin, 4= Lincomycin.

Figure 20: Inhibition zones of positive controls, 1=Ciprofloxacin, 2= Erythromycin, 3= Gentamycin, 4= Lincomycin, on *S. aureus*.

Figure 21: Inhibition zones of positive controls, 1=Ciprofloxacin, 2= Erythromycin, 3= Gentamycin, 4= Lincomycin, on *K. pneumonia*

Antioxidant Tests

The qualitative antioxidant activity results from the TLC autography method showed positive antioxidant activity in all extracts, using both mobile phases, represented by yellow spots on the TLC plate.

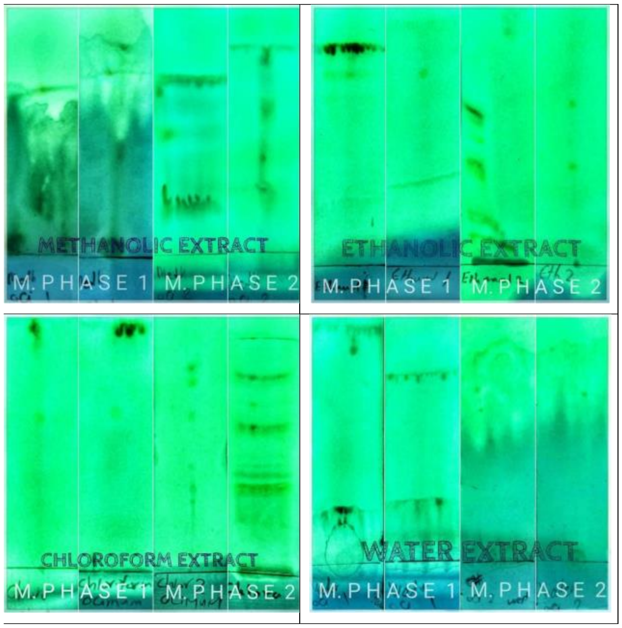

Figure 22: TLC qualitative antioxidant test of *Ocimum basilicum* extracts

Antioxidant Results

Table 4 summarizes the free radical scavenging capacity of *Ocimum basilicum* extracts as well as the real antioxidant activity of ascorbic acid. Results from this test clearly showed that all extracts exhibited high antioxidant activity toward DPPH radicals. Methanolic extract revealed (96.21 ± 0.57), which was the maximum effect among all extracts, along with the positive control, following the positive control (95.23 ± 1.00). Where the ethanolic extract represented (94.54 ± 1.91). Water extract was revealed (90.38 ± 1.49). The minimum effect was observed with chloroform, which appeared to be (64.60 ± 12.73).

Table 4: Antioxidant activities of *Ocimum basilicum* as measured by DPPH radical scavenging

			Radical scavenging %		
Extract Conc.	**Ascorbic Acid**	**Water**	**Ethanol**	**Methanol**	**Chloroform**
100 µL	95.84%	91.2%	95.91%	95.56%	83.13%
150 µL	94.8%	89.9%	95.10%	95.9%	62.69%
200 µL	96.25%	88.53%	91.10%	96.7%	57.2%
300 µL	94.04%	91.9%	95.34%	96.69%	55.4%
Mean±SD	95.23±1.00	90.38±1.49	94.51±1.91	96.21±0.57	64.60±12.73
P- value	<0.05	<0.05	<0.05	<0.05	<0.05
Pearson Correlation	-0.999952**	-0.999886**	-0.999999**	-0.999379**	-1.000000**

In Vitro Anti-lithiatic Activity Test

Calcium oxalate crystals were produced when the metastable solutions of sodium oxalate and calcium chloride were incubated. Comparing the induction time in the presence of the extract to that of the control (Cystone®) allowed one to determine the rate of stone creation. In comparison to the control, the turbidity of the solution in the presence of chloroform extract was lower, demonstrating less oxalate crystallization in the presence of chloroform extract.

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Table 5: Results of anti-lithiatic activity

Samples	UV absorptions in nm (After 60 minutes)	%	Mean of inhibition%
Cystone	1.767	26.18%	
	1.693	29.27%	**29.71%**
	1.6012	33.69%	
Chloroform	0.486	79.70%	
extract	1.160	51.52%	**61.86%**
	1.102	54.36%	
Negative	(At 10		
control	minutes)	0%	
	2.3935		**0%**
	2.3925		
	2.4146		

DISCUSSION

Over the duration of this century, the indiscriminate use of antibiotics has increased the number of bacteria that are resistant to such drugs. As a result, depending on the individual, the majority of these antibiotics have undesirable side effects. As a result, researchers have to look for alternative materials for microorganisms. People can purchase a wide range of botanical-herbal combinations from numerous local markets, which are used to treat a variety of illnesses [21].

The presence of such secondary metabolites in plants produces some biological activity in humans and animals, and it is responsible for their use as herbs in primary health care (22). The medical value of the plants is derived from specific chemical substances that produce a definite physiological effect on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Presently, many scientists and organizations are in search of traditional remedies as alternative medicines. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (23).

In this study, at 500 mg/ml, the water extract showed the maximum inhibition zone against *P. aeruginosa*, which was 21.65 mm. In contrast, the weakest inhibition zone against this bacterium was with chloroform extract at 300 mg/ml, which was 10.23 mm. *E. coli* culture showed the broadest zone of inhibition (20.65 mm) at 500 mg/ml of the water extract. Conversely, the narrowest zone of inhibition was observed around 13.35 mm at 300 mg/ml of methanolic extract. Aqueous and ethanolic extracts of *Ocimum basilicum* showed no effect against *K. pneumonia*. Meanwhile, methanolic and chloroform extracts showed an inhibition zone, which was considered a non-significant effect (P-value > 0.05). Against *S. aureus*, the largest inhibition zone was given by the aqueous extract at 500 mg/ml, whereas the chloroform extract at 300 mg/ml exhibited the smallest zone of inhibition shown in Table 2.

Overall, we observed that all extracts showed significant activity in all bacteria except with *K. pneumoniae*, which displayed a non-significant influence with all extracts. Additionally, we noted a sharp expansion of the zone of inhibition with growing extract concentrations. Many earlier studies (24) have found antibacterial.

Regarding antioxidant activity, the methanolic extract displayed the highest antioxidant activity as the removal of stable DPPH radicals, and the lowest activity was found in the chloroform extract. However, results of this experiment revealed strong antioxidant capability for every extract. This test's foundation is the theory that a hydrogen donor serves as an antioxidant.

Furthermore, DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants (18). From the result of the DPPH assay, it can be concluded that *Ocimum basilicum* is a rich source of antioxidant compounds (25).

In the calcium oxalate inhibition assay, the control inhibited 29.71% after 60 minutes. Notably, the chloroform extract showed maximum inhibition of calcium oxalate formation by 61.86%. Kidney stone development is influenced by several elements, including dehydration and insufficient water intake, slow urinary flow, and lower urine volume. Moreover, some compounds, like uric acid and calcium oxalate concentrations in the urine, may increase during stone formation (26). The extract's ability to decrease nucleation raises the metastable threshold of oxalate in urine, thereby preventing the formation of CaOx crystals (27).

Recommendations

The study recommends confirming these results by studying quantitative phytochemical evaluations and conducting *in vivo* studies on specific experimental animals. Additionally, the identification and isolation of the active chemical constituents of *Ocimum basilicum* should be done. Further pharmacological investigations should be conducted to assess its possible activities, such as its antifungal and anti-inflammatory effects, as well as its potential role in the management of diabetes mellitus, or to show its ability to reduce blood pressure and alleviate stress. Finally, it is recommended to conduct further biological and isolation of phytochemical studies on the *Ocimum basilicum* plant.

CONCLUSION

This study demonstrated that *Ocimum basilicum* contains a wide spectrum of bioactive phytochemical constituents, including alkaloids, tannins, carbohydrates, flavonoids, triterpenoids, saponins, cardiac glycosides, amino acids, fats, and fixed oils, while anthraquinones were absent in all extracts. The plant exhibited notable antibacterial activity, with the methanolic extract producing the largest inhibition zone against *Klebsiella pneumoniae*, and the aqueous extract showing the strongest inhibitory effects against all tested bacterial strains except *K. pneumoniae*. Antioxidant evaluation revealed that the methanolic extract possessed higher antioxidant capacity than ascorbic acid, the reference standard. In addition, *O. basilicum* demonstrated significant antil-ithiatic activity, particularly in the chloroform extract, which showed the greatest inhibition of calcium oxalate crystallization. Collectively, these findings provide strong evidence that *Ocimum basilicum* possesses potent antioxidant, antibacterial, and anti-lithiatic properties, supporting its potential application as a natural antioxidant in food systems and as a promising candidate for pharmaceutical and therapeutic development.

Author's Contributions

Mohammed Suhail Okba and Yasmeen Nasser Hasan Mohmmed conceptualized and designed the study, contributed to data interpretation, drafted the original manuscript, and critically revised the article. Haithem Nasser Mohsen, Fatima Nasser Hassun, Anwar Abdullah Al-Jahwari, Abdulkhaleq Abdulhadi, Hesham Abdulwahid Numan, Saleh Abdulqwi Saleh, Abdulrahman Ahmed Ba-abbad, Aiman Fares Faisal Almarfady, Ali Gunaid Bawazir, and Rami Omar Karama contributed to study design, data collection, data analysis, interpretation of results, and manuscript drafting. All authors reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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