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Assessment of The Antibacterial Susceptibility of *Ocimum basilicum*

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ABSTRACT

The current study was designed to assess the antibacterial activities of an ethanol extract of *Ocimum basilicum* (*O. basilicum)*. Using disc diffusion and direct contact methods, the extracts were tested *in vitro* against three bacterial strains. The direct contact test was used and compared with the agar diffusion test. The optical density was measured using a spectrophotometer to collect data. The results showed that methanol extracts of plant parts of *O. basilcum* leaves contained tannins, flavonoids, glycosides, and steroids, whereas alkaloids, saponins, and terpenoids. In contrast, *O. basilcum* seeds contained saponins, flavonoids, and steroids. The *O. basilicum* stems contained saponins and flavonoids, *O. basilucum* had antibacterial activity against the identified bacteria. The plant extracts inhibited *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (*E. coli*). The result revealed that the *Ocimum basilicum* leaves were more potent than seeds and stems. *Ocimum basilicum* ethanol extract combined with established conventional antibiotics may enhance their antimicrobial properties, giving rise to synergistic effects against clinically important bacterial species.

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Introduction

The *Ocimum basilicum* is a tropical aromatic plant called holy basil, as the Orthodox churches use it to prepare holy water in Egypt. It was found around the tomb of Christ. Peace be upon him after his ascension to heaven. In Europe, they placed it in the hands of the dead when they were buried; in the hope of they go on a safe journey. In India, they put it in the mouths of the dead to ensure they reach the Creator. In Africa, they used to expel scorpion poisons (1). In ancient Egypt and Greece, it is believed that it opened the door of heaven for a person to pass through. Its English name, Basil, is derived from the ancient Greek word “Basilikohn”, which means "noble herbs" or "holy herbs", and basil represents an icon of hospitality in India and a symbol of love in Italy**.**

O. basilicum is a flowering plant known for its ornamental and medicinal properties. Among the chemical compositions isolated from the plant are terpenoids, alkaloids, flavonoids, tannins, saponins, glycosides, and ascorbic acid. There have been reports of hepatoprotective, immunomodulatory, antihyperglycemic, hypolipidemic, antitoxic, anti-inflammatory, antimicrobial activity, and antifungal properties (2).

More than 60 varieties of basil were found that differ in appearance and taste. It is still reputed to be the king of herbs and is added to cooked food at the last minute so as not to lose the essential oils because of boiling. It is also used in many dishes, salads, and jams, especially strawberry jam. It is also used when keeping fresh plants inside the refrigerator in plastic bags for a short period or in the freezer for long periods. It is noted that dried basil loses a large proportion of its volatile oils, and when basil seeds are soaked in water, they become gelatin and can be used in desserts and drinks (3).

Antimicrobial resistance is a life-threatening challenge to the world. Most of the well-known antibiotics are currently ineffective for several microbial diseases. Ampicillin, metronidazole, amoxicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, nalidixic acid, gentamicin, and ceftazidime are common antibiotics whose resistance pattern has been elevated in recent years (4).

Antimicrobial susceptibility testing can be utilized for drug discovery, epidemiology, and prediction of therapeutic outcomes. Antibiotics are known to be used in the treatment of many bacterial diseases. Moreover, repeated use of these vital materials may lose their effect by increasing the resistance of microbes. At present, failure to treat these substances is largely related to drug-resistant bacteria, and it has become a major problem for human health (5, 6). Rahimi and colleagues (7) used the Colorimetric technique for antibacterial properties for profit, especially in clinical situations of determined or intermittent disease quantitative assay. We used simple Colorimetric and disc diffusion methods to assess the in vitro antimicrobial susceptibility of *Ocimum basilicum* ethanol extract.

Materials and Methods

Plant material

We collected *O. basilicum* plants grown in the garden of the Department of Biology Faculty of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Saudi Arabia. Identification by Botanist member at the Department. The plant samples were dried in shadow, then ground, powdered and keep until used.

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Preparation of plant extract

For ethanol extraction, 20 g of each air-dried powder was added to 100 ml of ethanol 70% and incubated for 24 hours on a shaker. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. Then concentrated by repeated filtering steps and again use the rotary evaporator boils off the ethanol, leaving a pure extract, to make the final volume one-fourth of the original volume (stock solution)

Phytochemical screening of extracts

The chemical constituents of *O. basilcum* were identified through preliminary phytochemical screening of selected parts of the plant by the methods described by Sulieman et al. (8), with minor modifications. The constituents determined included: alkaloids, saponins, tannins, flavonoids, glycosides, steroids, and terpenoids.

Bacterial Test

In the current study, we used the following bacterial strains: *Staphylococcus aureus* (ATCC 29253), *Escherichia coli* 10536*, and Pseudomonas aeruginosa* 22893. At 37 ˚C, these strains were grown aerobically on brain heart infusion (BHI) broth. Centrifugation was used to remove the cells, which were then re-suspended in a new medium. We made the inoculum by re-suspending washed cells to predetermined optical densities corresponding to known concentrations. The tested extracts were categorized as follows.

Group I: *O. basilicum* leaves ethanol extract
Group II: *O. basilicum* seeds ethanol extract
Group III: *O. basilicum* stems ethanol extract
Group IV: control-bacterial suspension in each test extract.

The direct contact test (DCT)

The DCT (Figure 1) was used according to Weiss et al. (9) for the estimation of the antibacterial activity of the plant extract. The method was based on the bacteria counting method in 96-well microliter plates (96-well, TKA Teknolabo, 313). Tested materials were added for each 10 µL bacterial suspension (1x10^6 CFU/ml). The plate was held vertically, and wells were inspected for evaporation of the suspension's liquid, which occurred within 1 hr at 37°C. The wall of uncoated wells was contaminated with bacterial suspensions and used as a control. During incubation, evaporation of suspension liquid ensured direct contact between bacteria and the tested material (plant extracts). The bacterial suspension of each well was transferred, and brain heart infusion BHI broth (245 μL) was added to each A-wells and gently mixed for 2 minutes with a Vortex shaker. Take 15 μL of broth was transferred from A wells to an adjacent set of B wells containing fresh medium (215 μL) by automatic pipette. This resulted in two sets of 4 wells for each tested material containing an equal volume of the liquid medium so that bacterial outgrowth could be monitored both in the presence and absence of the tested material to detect surviving bacteria. After 12 hours of incubation at 37°C, colonies were counted, and cfu/ml was calculated. For group (IV), after the polymerization of the adhesives, 10 µL of bacterial suspension and (245 μL) of BHI were added to each of the wells. The antibacterial activity of plant parts extract was tested simultaneously, as described above. All experiments were performed in triplicate.

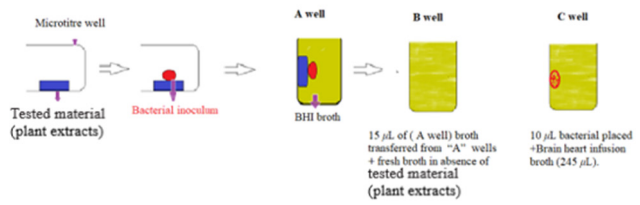

Figure 1. The direct contact test performed.

Antibacterial test by Disc-diffusion assay

Antibacterial activities of *O. basilicum* ethanolic extracts were assessed using filter disc diffusion assay on nutrient agar media, diffusing from its reservoir through the agar medium seeded with the test microorganisms. The disc diffusion method on Mueller-Hinton Agar (Merck Co., Germany). A plate of Mueller Hinton agar was inoculated with the suspension using a sterile cotton swab. After 24 hours at 37°C of incubation, antibacterial results were recorded by measuring inhibition zone diameters.

Statistical Analysis

Data were recorded, then plotted and statistically analyzed by One-Way ANOVA to compare the mean± standard division of the tested sample.

Results

Phytochemical analysis

Qualitative screening of phytochemical constituents of various parts of *O. basilcum* was investigated, and the results shown in Table 1 indicate variations in the various constituents of the different plant parts. *O. basilcum* leaves contained tannins, flavonoids, glycosides, and steroids, whereas alkaloids, saponins, and terpenoids were devoid. In contrast, *O. basilcum* seeds contained saponins, flavonoids, and steroids, whereas alkaloids, glycosides, tannins, and terpenoids were absent. The *O. basilicum* stems contained saponins, and flavonoids, whereas the glycosides, steroids, alkaloids, tannins, and terpenoids were absent.

The direct contact test (DCT)

The data in Tables 2-4 present the comparison of the mean OD across different plant extract parts against the various tested bacteria. The mean OD across different plant extract parts (leaf, seed and stem) against *Staphylo-*

Table 1. Phytochemical constituents of the *O. basilicum* plant parts used in this study.

Plant parts	Alkaloids	Saponins	Tannins	Flavonoids	Glycosides	Steroids	Terpenoids
Leaves	-	-	+	+	+	+	-
Seeds	++	-	++	-	+	+	-
Stems	+	-	+	-	-	-	+

+ (Present); − (Absent)

Table 2. Comparison of mean OD across different plant extracts parts against *Staphylococcus aureus* using ANOVA (p> 0.05).

Plant parts	A-Well Mean ± SD	B-Well Mean ± SD
Leaves	0.15±0.008	0.22±0.010
Seeds	0.24±0.008	0.20±0.007
Stems	0.22±0.005	0.24±0.034

Table 3. Comparison of mean OD across *O. basilicum* parts extract against *E. coli* 10536 using ANOVA.

Plant parts	A-Well Mean ± SD	B-Well Mean ± SD
Leaves	0.24±0.008	0.20±0.012
Seeds	0.36±0.015	0.27±0.007
Stems	0.42±0.020	0.28±0.033

Table 4. Comparison of mean OD across *Ocimum basilicum* parts extract against *Pseudomonas aeruginosa 22893* using ANOVA.

Plant parts	A-Well Mean ± SD	B-Well Mean ± SD
Leaves	0.38±0.043	0.21±0.015
Seeds	0.39±0.015	0.22±0.005
Stems	0.42±0.047	0.25±0.034

coccus aureus using ANOVA in A-well were 0.15±0.008, 0.24±0.008, and 0.22±0.005, respectively, compared with that B-well, which was 0.22±0.010, 0.20±0.0007, and 0.24±0.043, respectively (Table 2). However, tests of ethanol extracts of these plant parts against *E. coli* ATCC 10536 were 0.24±0.008, 0.36±0.015, and 0.42±0.020, respectively, in A-well compared with those of B-well, which were 0.20±0.012, 0.27±0.007, and 0.28±0.033, respectively as indicated in Table 3. Whereas bacterial kinetics of the same *O. basilicum* parts against *Pseudomonas aeruginosa* 22893 were 0.38±0.043, 0.39±0.015, and 0.42±0.047, respectively, compared with that of B-well, which was 0.21±0.015, 0.22±0.005, and 0.25±0.034, respectively as shown in Table 4. The result revealed that the *O. basilicum* leaf was more potent than seeds and stems.

Data in Figure 2 shows the sensitivity of tested organisms to plant extracts compared with that of the control. The results revealed that *Staphylococcus aureus* 29253 is more sensitive to plants extracted understudy than *E. coli* 10536 and *Pseudomonas aeruginosa* 22893

Figure 3 shows the plant extract parts on the tested organisms. The *O. basilicum* leaf has high efficacy than others, followed by *O. basilicum* seeds showed, and finally, the *O. basilicum* stem.

Data in Tables 2-4 and Figures 4-6 show that *O. basilicum* leaves, seeds and stems varied in their antibacterial activity against the tested organisms (mean of inhibition zone diameter mm). The diameter zone of inhibition of *O. basilicum* leaves, seeds, and stems were 11.3±0.071, 8.0±0.212, and 6.8±0.021, respectively, against *Staphylococcus aureus* 29253. Whereas the zones of inhibition against *E. coli* 10536 were 9.87±2.99, 7.50±4.87, and 5.93±1.93, respectively. Moreover, the zones of inhibition against *Pseudomonas aeruginosa* 22893 were 7.75±1.28, 7.00±1.51, and 5.67±0.83, respectively (Table 5).

The current result supported the result collected by coulometric direct contact. The inhibition occurs by *Ocimum basilicum* parts ethanol extracts were significantly different at level p ≥ 0.0001.

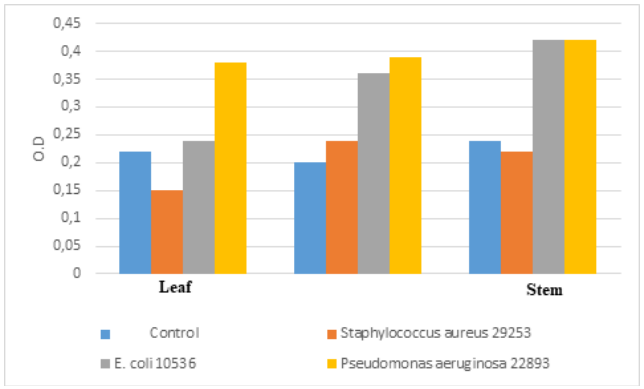

Figure 2. The sensitivity of tested organisms to plant extracts compared with the control.

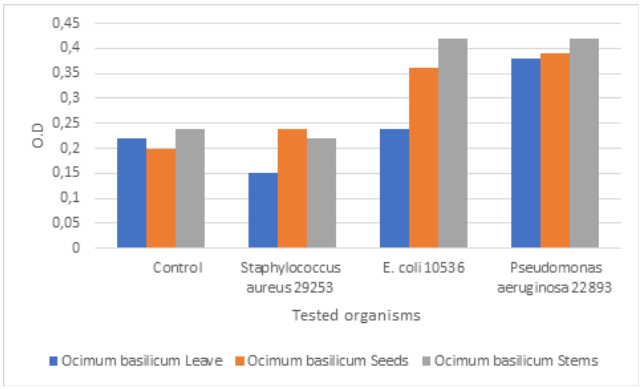

Figure 3. The efficacy of the plant extract parts on the tested organisms .

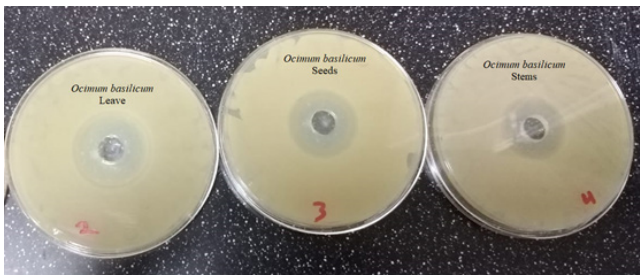

Figure 4. The inhibition of *Staphylococcus aureus* 29253 growth in agar by tested plants parts.

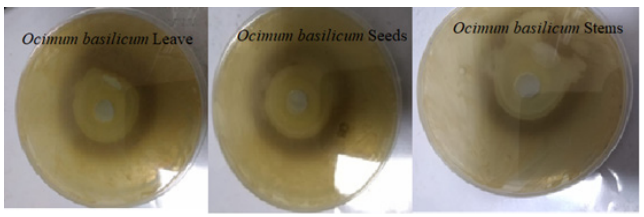

Figure 5. The inhibition of *E. coli 10536* growth in agar by tested plants parts.

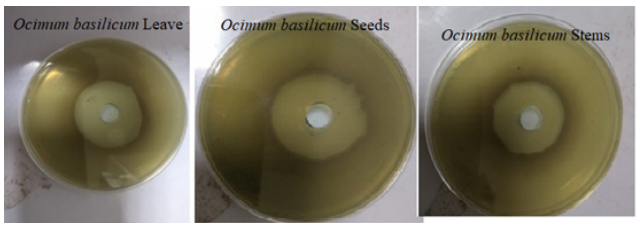

Figure 6. The inhibition of *Pseudomonas aeruginosa 22893* growth in agar by tested plants.

Table 5. Antibacterial activity of *Ocimum basilicum* water extracts against bacteria by disc diffusion.

Tested plants parts	*Staphylococcus aureus* 29253	*E. coli 10536*	*Pseudomonas aeruginosa 22893*
Leaves	11.3±0.071	9.87±2.99	**7.75±1.28**
Seeds	8.0±0.212	7.50±4.87	**7.00±1.51**
Stems	6.8±0.021	5.93±1.93	**5.67±0.83**
p-value	0.0001		

Discussion

The current research aimed to assess the antimicrobial activities of *Ocimum basilicum (O. basilicum)*. Aqueous extract phytochemical screening and chemical composition of *O. basilicum* revealed the presence of saponins, tannins, and cardiac glycosides (*O. basilicum*). The extracts were tested *in vitro* against three bacterial strains using the method of disc diffusion. Basil has antioxidant properties that help the body protect against free radical damage, thereby combating most types of cancer. Basil also contains flavonoids, which protect cell structures from damage caused by radiation and oxygen. Basil is high in beta-carotene, which protects cells from free radical damage. According to an Australian study, basil serves to protect against toxic chemical damage by increasing the body's levels of antioxidant molecules like glutathione and increasing the activities of antioxidant enzymes like superoxide dismutase and catalase, which avenge cell components and membranes. Harmful free radicals are reached because of a lack of oxygen and other toxic factors. Basil also aids in the prevention of cancers caused by toxic compounds by reducing DNA damage and inducing cancer cell death, which slows the growth of interesting new tumors and promotes survival.

Our findings are consistent with previous research, which has shown that basil contains significant amounts of phenolic acids, which make a significant contribution to its increased antioxidant capacity (10-12). The antibacterial activity of *O. basilicum* parts water extract was investigated against gram-negative and gram-positive bacteria, including *Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus*, respectively. Agar disk diffusion tests revealed the highest inhibition zones. In the inhibition zones of leave extracts, S. aureus measured 11.3mm, E. coli 9.87mm, and Pseudomonas aeruginosa 7.75mm. These findings were significant compared to those of Moghaddam et al (13), who reported a range of 36-18 and 18-9 mm for *S. aureus* and *E. coli*, respectively.

O. basilicum, *Satureja hortensis*, and *Anethum graveolens* extracts were tested against pathogenic microorganisms such as *E. coli*, *Staphylococcus aureus*, *Streptococcus cricetus,* and *Candida albicans*, and the inhibitory zone diameter was used as an indicator of antimicrobial activity (14, 15). When *O. basilicum* aqueous extract was evaporated at 80°C, the largest inhibition zone diameter was observed for *E. coli* and *Candida albicans* in the case of alcoholic extracts. Other extracts demonstrated an average inhibitory zone diameter for pathogenic microorganisms tested (16). Furthermore, Ahonkhai et al. (17) used agar diffusion and agar dilution methods to investigate the antimicrobial activities of *O. basilicum* and *O. gratissimum* volatile oils. At a concentration of 0.51% in agar, the volatile oils of both plants slowed the growth of *Streptococcus viridian, Staphylococcus albus*, and *Klebsiella pneumonia, Pseudomonas aeruginosa. O. basilicum* inhibited Proteus vulgaris at 0.67% and *O. gratissimum* at 0.53%). *Ocimum basilicum* also had a concentration-dependent effect on *Escherichia coli*. Surprisingly, the results of *Ocimum basilicum* extract had lower activity against *E. coli*. Kahya (18) and Muhannad et al. (19) evaluated the well-diffusion method for testing the susceptibility of Staphylococcus aureus. He discovered that applying *O. basilicum* at concentrations ranging from 0.34 to 10.96 mg/ml inhibited the growth of S. aureus. At *O. basilicum* concentrations of 20 and 100 mg/ml, the inhibition zones were larger (12.2 0.3 to 20.0 0.2 mm). Previous findings corroborated our findings. The antibacterial activity of *O. basilicum* leaf extract against all bacteria tested at all concentration levels. In the minimum concentration (6.25 mg/ml) of the leaf extract, the greater antimicrobial activity was detected against *E. coli* and *Pseudomonas aeruginosa* (7.8 mm inhibition zone), and the minimum antibacterial activity was detected against Staphylococcus aureus (4.4 mm inhibition zone) (20). These findings are consistent with those obtained in the current study. Disk-diffusion and minimal inhibition concentration (MIC) methods were used to assess the antimicrobial potential of *O. basilicum* extracts in ethanol, methanol, and hexane. The antimicrobial activity of the three extracts differed. The hexane extract exhibited the broadest spectrum of antimicrobial activity (21). The hexane extract inhibited 10, 9, and 6% of the 146 bacterial strains tested, respectively, while the methanol and ethanol extracts inhibited 10, 9, and 6%, respectively (22). This finding may be related to the current study's findings from water extracts, which showed greater inhibition than these solvents.

The current study found that *O. basilicum* leaf extract had potent antibacterial activity against a variety of bacterial pathogen strains (23). These findings support the idea that this plant could be useful in herbal medicine, and it is suggested that advanced scientific techniques be used to isolate and separate the bioactive compounds responsible for this antibacterial activity.

Drug resistance is a growing threat to health and development, necessitating immediate multipronged action to accelerate progress (24). Antimicrobial resistance has been named one of the top ten worldwide health challenges confronting humanity by the World Health Organization.

Antimicrobial misuse and overuse are the primary causes of the emergence of drug-resistant pathogens.

Lack of clean water and sanitation, as well as inadequate infection prevention and control, promote the spread of microbes, some of which are resistant to antimicrobial treatment. The costs of antimicrobial resistance are enormous. In addition to the deaths and disabilities caused by long-term illnesses, they result in prolonged hospital stays the need for more expensive medicines, and financial difficulties (25).

The findings of this study support the use of basil oil

as an antibacterial agent to control bacteria (26). A direct contact test was developed by Weiss and Cropanzano (9). The kinetics of bacterial growth can be used to determine antibacterial activity. The volatile oils in basil have antibacterial and antifungal properties. They kill gram-positive and gram-negative bacteria, fungi, viruses, yeasts, and molds. It is resistant to a wide range of bacteria, the most important of which are Listeria, *Staphylococcus aureus*, *E. coli*, and Salmonella. Yersinia, and *Pseudomonas aeruginosa*. Antibiotic resistance has become widely used because this bacterium has a high ability to mutate and produce antibiotic-resistant strains. *O. basilicum* L. extracts were tested for antibacterial properties in an organic solvent and two different concentration levels of methanol. Using the disc diffusion method, these extracts were tested in vitro against pathogenic bacteria. *O. basilcum* methanol extracts were found to have antimicrobial activity against the microorganisms tested. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and two strains of *E. coli* were all inhibited by methanol extracts, whereas chloroform and acetone extracts had no effect. A scanner electron microscope was used to examine the cells of microbes treated and untreated with plant extracts. The treated cells were discovered to be damaged (27).

Conclusion

The *Ocimum basilicum* plant extras showed potent inhibition against tested pathogenic bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli (E. coli)*. The result revealed that the plant leaves were more potent than seeds and stems. *Ocimum basilicum* ethanol extract combined with established conventional antibiotics may enhance their antimicrobial properties.

Interest conflict

The authors report no conflict of interest.

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