

## Antibacterial Activity of *Ocimum basilicum* (Rehan) Leaf Extract against Bacterial Pathogens in Sudan

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### Abstract

**Background:** *Ocimum basilicum* (Rehan) is a well-known medicinal plant which has received a great deal of attention over the past few decades around the world. Extracts of *Ocimum basilicum* having strong antibacterial and antioxidant properties are widely used for medicinal purposes.

**Objective:** To assess the antimicrobial activity of *Ocimum basilicum* (Rehan) leaf extract against human pathogenic bacterial pathogens; and to compare that with the antimicrobial activity of synthetic antibiotics.

**Material and methods:** 100 bacterial test strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* were enrolled in the study. Ethanolic extracts of *Ocimum basilicum* leaves were prepared at varying concentrations and soaked on Whatmann filter paper discs, which were applied on inoculated plates of Muller Hinton agar. Standardized discs of the synthetic antibiotics: ciprofloxacin, erythromycin, norfloxacin, co-trimoxazole, ceftriaxone, and gentamicin were also applied on inoculated plates of Muller Hinton agar. The disc diffusion method was used to screen the antibacterial activity of both *Ocimum basilicum* leaf extract and synthetic antibiotics.

**Results:** Leaf extract *Ocimum basilicum* exhibited a potent antibacterial activity against various strains of bacterial pathogens. Against *Klebsiella pneumoniae* there was significant difference of antimicrobial activity of leaf extract at concentration of 50µg/disc when compared with the antibiotics ciprofloxacin, erythromycin and gentamicin (p=0.001, 0.006 and 0.009) respectively.

**Conclusion:** *Ocimum basilicum* leaf extract showed strong antimicrobial activity against all bacterial species studied at all the concentrations tested.

**Keywords:** *Ocimum basilicum*; antibiotics; pathogenic bacteria; disc diffusion method.

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## Introduction

*Ocimum basilicum* is widely distributed in tropical and warm temperate regions of the world. It is also named as Rehan and it is a plant with extraordinary medicinal properties and contains several antioxidant compounds. In traditional medicine, *Ocimum basilicum* has been used as an antiseptic, preservative, sedative, digestive regulator and diuretic<sup>1</sup>. It also has been recommended for the treatment of headache, cough, infections of upper respiratory tract, kidney malfunction, and to eliminate toxins<sup>2</sup>. Both *Ocimum* oil and its extracts were shown to exhibit antibacterial activities against gram positive and gram negative bacteria by various researchers<sup>3</sup>. Rehan oil has also found a wide application in perfumery, as well as in dental and oral antimicrobial products. In addition, because the public nowadays prefers natural food additives, naturally derived antimicrobial agents such as Rehan, are becoming more important in antimicrobial packaging as they present a perceived lower risk to consumers<sup>4</sup>. Leaves and flowering parts of *O. basilicum* are traditionally used externally, when applied for the treatment of acne, disguising of smell, insect stings, snake bites, and skin infections<sup>5</sup>. The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria*, *Shigella*, *Salmonella*, *Proteus*, *Trichophyton*, *Cryptococcus*, *Penicillium*, and *Candida*<sup>6</sup>. The aim of the present study was to verify the registered benefit of leaves of *Ocimum basilicum* and to determine its antimicrobial activity against the test microorganisms.

## Materials and methods

Fresh leaves of *Ocimum basilicum* (Rehan) were collected locally and were air dried in shade. The *Ocimum basilicum* leaf extract was then prepared by grounding 50 g of leaves using mortar and pestle, and the yield was successively soaked in 80 % ethanol for about 72 hours, with daily filtration and evaporation. Solvents were evaporated under reduced pressure to dryness using rotary evaporator apparatus. Filtration and extraction were carried out in the Center of Medicinal and Aromatic Plants, Khartoum (Sudan). Extracts were exposed to air till complete dryness.

The bacterial test strains used were 100 bacterial pathogens, isolated from various clinical specimens: urine, blood, sputum, and wound infections. The clinical specimens were collected for microbiological testing at Soba University Hospital (Khartoum). Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques. The bacterial test strains used were *Escherichia coli* (21 strains), *Pseudomonas aeruginosa* (12 strains), *Proteus mirabilis* (21 strains), *Klebsiella pneumoniae* (21 strains), *Staphylococcus aureus* (17 strains) and *Enterococcus faecalis* (8 strains).

The antimicrobial sensitivity testing was conducted by the agar disc diffusion method. The sensitivity medium (Muller-Hinton agar) was prepared by adding 3.8g of Muller-Hinton agar powder to 100 ml distilled water and autoclaved at 121°C for 15 minutes at 15 lbs., and poured in sterile Petri plates up to a uniform thickness of approximately 4 mm and the agar was allowed to set at ambient temperature before use. The bacterial isolates were suspended in peptone broth and incubated at 37° C for 3-4 hours before

used as inocula. The turbidity of the broth culture was adjusted to 0.5 McFarland units. This gives a suspension containing approximately  $1-2 \times 10^6$  colony forming units (CFU)/ml. A sterile cotton swab was inserted into the bacterial suspension, rotated, and then compressed against the wall of the test tube to express any excess fluid. The swab was then streaked on the surface of the Muller-Hinton agar plate. To ensure a uniform, confluent growth, the swab was streaked three times over the entire plate surface. To test antibacterial activity of Rehan leaf extract, it was first dissolved in a methanol solvent, and then varying concentrations of the extracts (100 $\mu$ g, 50 $\mu$ g, 25 $\mu$ g, 12.5 $\mu$ g, and 6.25 $\mu$ g) were soaked on autoclaved discs of Whatmann filter paper. These filter paper discs were placed on a streaked Muller-Hinton agar plate surface. The plates were incubated overnight at 37° C for 18-24 hours. The antimicrobial activity was detected by measuring zones of inhibition. To test antibacterial activity of the synthetic antibiotics, standardized discs of ciprofloxacin (5 $\mu$ g), erythromycin (15 $\mu$ g), norfloxacin (10 $\mu$ g), co-trimoxazole (25 $\mu$ g), ceftriaxone (30 $\mu$ g), and gentamycin (10 $\mu$ g) were tested by the agar disc diffusion method by placing on a streaked Muller-Hinton agar plate surface. The antimicrobial activity was also detected by measuring zones of inhibition.

## Results

Table I exhibits the antibacterial activity of *Ocimum basilicum* leaf extract against all tested bacteria at all concentrations. As regard the lowest concentration (6.25 mg/ml) of the leaf extract, its highest antibacterial activity was detected against *Escherichia coli* and *Pseudomonas aeruginosa* (7.8 mm inhibition zone); and its lowest antibacterial activity was detected against *Staphylococcus aureus* (4.4 mm inhibition zone).

**Table I. Mean zones of inhibition (in mm) for different concentrations of *Ocimum basilicum* leaf extract**

Bacterial test strains (No. tested)	Concentrations of leaf extract (in $\mu$ g/disc)				
	<b>100</b>	<b>50</b>	<b>25</b>	<b>12.5</b>	<b>6.25</b>
<i>Escherichia coli</i> (21)	13.6	12	11	8.9	7.8
<i>Klebsiella pneumoniae</i> (21)	13	10.8	10.3	7.6	5.4
<i>Proteus mirabilis</i> (21)	14	12.2	10.7	6.7	5.4
<i>Staphylococcus aureus</i> (17)	13.9	11	10	9	4.4
<i>Pseudomonas aeruginosa</i> (12)	13.9	11.8	11.2	9.2	7.8
<i>Enterococcus faecalis</i> (8)	13.6	11	9.5	8.5	5.5

Table II exhibits the mean zones of inhibition (in mm) for the different synthetic antibiotics used. Regarding the antibacterial activity of the antibiotics tested, the highest activity was due to the action of ciprofloxacin against *Pseudomonas aeruginosa* (28 mm inhibition zone); and the lowest activity was due to the action of erythromycin against *Proteus mirabilis* (4.4 mm inhibition zone).

**Table II. Mean zones of inhibition (in mm) for different antibiotics**

Bacterial test strains (No. tested)	Antibiotics concentration in ( $\mu\text{g}/\text{disc}$ )					
	CIP	E	NOR	CO	CEF	G
<i>Escherichia coli</i> (21)	16.9	7.5	9.4	10.7	12	13
<i>Klebsiella pneumoniae</i> (21)	15.8	10	9	6	6.8	12
<i>Proteus mirabilis</i> (21)	27	4.4	19	13	21	12
<i>Staphylococcus aureus</i> (17)	21.7	12.4	14	13.4	13	10
<i>Pseudomonas aeruginosa</i> (12)	28	8.6	18.9	14	15.5	10
<i>Enterococcus faecalis</i> (8)	16.7	11.7	10	9	13.8	10

CIP = Ciprofloxacin (5  $\mu\text{g}$ )

E = Erythromycin (15  $\mu\text{g}$ )

NOR = Norfloxacin (10  $\mu\text{g}$ )

CO = Cotrimoxazole (25  $\mu\text{g}$ )

CEF = Ceftriaxone (30 $\mu\text{g}$ )

G = Gentamycin (10  $\mu\text{g}$ )

There was an insignificant difference ( $p > 0.05$ ) between the antibacterial activity of the leaf extract and the synthetic antibiotics against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus* and *Enterococcus faecalis*.

Against *Klebsiella pneumoniae* there was significant difference of antimicrobial activity of leaf extract at concentration of 50 $\mu\text{g}/\text{disc}$  when compared with the antibiotics ciprofloxacin, erythromycin and gentamicin ( $p = 0.001$ , 0.006 and 0.009) respectively.

## Discussion

Due to the differences of methodologies evaluating the antimicrobial properties and the differences in herbal contents and compositions from different geographical regions, it becomes difficult to compare the various studies published on the antimicrobial activity of *Ocimum basilicum*. The results of our study showed that the activity of *Ocimum basilicum* extract was similar for both Gram-negative and Gram-positive pathogens. This result agrees with the study of Shafique, *et al*<sup>7</sup>; who also studied the antimicrobial activities of *Ocimum basilicum* leaf extract against eight bacterial strains using the disc diffusion method. Their results indicated that extracts of *Ocimum basilicum* exhibited higher antimicrobial activity against the tested Gram positive microorganisms.

Also Shweash *et al*<sup>8</sup> reported that there was an effect of ethanolic extract of Rehan leaves on *Enterobacteriaceae*. At a ratio of 100 mg/ml, *Ocimum basilicum* had a marked increased zone of inhibition associated with *Escherichia coli* growth. The inhibition zones were quite different and had substantially increased as per the concentration of *Ocimum basilicum* extract; and the growth was completely inhibited at the highest concentration. A similar outcome was observed after a 24 hours incubation period of bacterial growth.

Furthermore, *Ocimum basilicum* had a dependent concentration effect on *Escherichia coli*. Surprisingly, the results of *Ocimum basilicum* extract had lower action against

*E. coli*. Yahya<sup>9</sup> evaluated the well-diffusion method for testing the susceptibility of *Staphylococcus aureus*. He found the growth of *S. aureus* to be inhibited after application of *Ocimum basilicum* at concentrations of 0.34-10.96 mg/ml. The greater inhibition zones ( $12.2 \pm 0.3$  to  $20.0 \pm 0.2$  mm) were observed at *Ocimum basilicum* concentrations of 20 and 100 mg/ml respectively.

*Ocimum basilicum* extract enhanced wound healing and treated inflammation signs. Antibacterial activity of *Ocimum basilicum* extract could be attributed to some active ingredients having the ability to combine with extra cellular and soluble protein and to make a complex with bacterial cell wall disrupting microbial membranes. Unnithan, *et al*<sup>10</sup> reported that *Ocimum basilicum* extract had shown significant antibacterial activity against Gram positive bacteria (*Staphylococcus aureus*) as compared with Gram negative bacteria (*Escherichia coli*).

## Conclusion

The present study revealed that *Ocimum basilicum* leaf extract had potent antibacterial activity against various strain of bacterial pathogen. These findings support the fact that this plant could be useful in herbal healthcare and it is recommended to isolate and separate the bioactive compounds responsible for this antibacterial activity using advanced scientific techniques.

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