

## EVALUATION OF THE ANTIBACTERIAL EFFICACY OF *Ocimum gratissimum* EXTRACT ON *Klebsiella* SPECIES ISOLATES OF CLINICAL AND FOOD ORIGIN

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### ABSTRACT

*This study evaluated the antibacterial efficacy of Ocimum gratissimum leaf extracts against Klebsiella species isolates of food and clinical origin. The isolates were identified using cultural, morphological, and biochemical characteristics. Aqueous and ethanolic extracts of Ocimum gratissimum were prepared. Thereafter, extract was screened for the presence of bioactive constituents such as tannin, phenol and phlobatannin. Antibiotic susceptibility test was carried out using the Kirby - Bauer disc diffusion method. Antibacterial activity was determined by agar well diffusion technique, while at concentrations ranging from 125mg/ml to 2000mg/ml, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was also established. Six (6) Klebsiella isolates were identified, with Klebsiella sp.<sup>1</sup>, Klebsiella sp.<sup>2</sup>, Klebsiella sp.<sup>3</sup> and Klebsiella sp.<sup>4</sup> as clinical isolates and Klebsiella sp.<sup>5</sup> and Klebsiella sp.<sup>6</sup> as food isolates. Phytochemical screening indicated the presence of phenol, tannin and phlobatannin in the leaf extract. Most isolates recorded high resistance index ranging from 0.6 - 1.0. Antibacterial assay revealed that ethanol extract exhibited higher antibacterial activity, with inhibition zones ranging from 5.33 ± 5.03 mm (Klebsiella sp.<sup>3</sup>) to 67.33 ± 18.58 mm (Klebsiella sp.<sup>6</sup>) at 250mg/ml and 2000mg/ml, respectively. The MIC of the ethanolic and aqueous extracts ranged from 60 - 800 mg/ml and 100 - 900 mg/ml respectively. An MBC of 2000mg/ml was observed. The findings suggest that O. gratissimum extracts, particularly ethanol-based formulations, may serve as potential alternatives in combating infections caused by multidrug-resistant Klebsiella species isolates.*

**KEYWORDS:** Antibacterial activity, Phytochemical, Leaf extract, Antibiotic resistance

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### INTRODUCTION

Antimicrobial resistance (AMR) is a growing public health concern globally, with *Klebsiella* sp., being a major contributor to multidrug-resistant infections. These opportunistic pathogens are responsible for a range of clinical conditions, including pneumonia, urinary

tract infections, and septicemia, as well as contaminating food and environmental sources. With the increasing incidence of diseases caused by bacteria and other microorganisms, as well as the development of drug resistance, there is an urgent need to search for alternatives from plant sources to combat these pathogens

(Levy, 2002). *Ocimum gratissimum* L is widely distributed in tropical and warm temperate regions. It has an English name of “Tea Bush”. It is commonly called “scent leaf” because it usually gives out sweet scent and pleasant aroma. The leaves of the plant are thought to contain both thymol and eugenol as well as volatile oil which has been shown to contain some antibacterial properties. *Ocimum gratissimum* leaves or the whole herbs are popular treatment for diarrhea (Batool et al., 2018). It is an aromatic herbaceous plant also known as basil, basil-clove, or *alfavaca*. It belongs to the family *Lamiaceae*. (Nweze and Eze, 2009). The antimicrobial activity of the water-saturated oil has been shown to be proportional to the thymol content in preparations where *O. gratissimum* is used as cold infusion (Batool et al., 2018). It has also been reported to be active against several species of bacteria and fungi (Nakamura et al., 1999). Medicinal plants have long been utilized in traditional medicine for their therapeutic properties. Among these, *O. gratissimum* have garnered significant attention for its antimicrobial efficacy. Previous studies have highlighted the antimicrobial potential of *O. gratissimum* against diverse bacterial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Prabhu and Patel, 2020; Udochukwu et al., 2015). The aim of this study was to evaluate the antibacterial efficacy of *Ocimum gratissimum* against *Klebsiella* species isolates of clinical and food origin.

## MATERIALS AND METHODS

### *Collection of Plant Materials and Test Organisms*

Leaves of *O. gratissimum* were collected from the demonstration garden

at the Faculty of Agriculture, University of Benin, Benin city, Edo State. The plant was authenticated at the Department of Plant Biology and Biotechnology (PBB). The leaves were thoroughly washed with distilled water to remove any surface debris. They were then air-dried in a shaded area for 7-10 days and grinded into a fine powder. *Klebsiella* species isolates of clinical origin were obtained from the department of Medical Microbiology of the University of Benin Teaching Hospital, Benin city, while isolates of food origin, previously isolated from ready-to-eat foods were obtained from a commercial research laboratory in Benin City, Edo State. To ensure accuracy, identification of the isolates was re-confirmed using standardized procedures, guaranteeing that the test organisms were properly characterized.

### *Preparation of Plant Extracts*

Aqueous and ethanolic extracts of *O. gratissimum* leaves were prepared. For the aqueous extraction, 500g of powdered plant material was soaked in 2000ml of distilled water, over a period of 24hours. While for the ethanolic extraction, the same amount of powdered plant material was soaked in 3000mL of 95% ethanol, left to stand at room temperature for 72 hours with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain the crude extracts. The concentrated extracts were then stored in a refrigerator at 4°C to preserve their integrity until they were required for further testing (Kubmarawa et al., 2008).

### *Phytochemical Screening of Plant Extracts*

The phytochemical screening (qualitative analysis) of the extracts was

performed to detect the presence of bioactive compounds which include; saponins, tannins, flavonoids, alkaloids, phenolics and phlobatannins (Kebede *et al.*, 2021).

#### **Identification of Test Organisms**

The identities of test organisms obtained were reconfirmed. Following subculturing, isolates were identified based on cultural, morphological and biochemical characteristics (Cheesebrough, 2006).

#### **Antibiotic Susceptibility Testing**

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. The following antibiotic discs were used: amoxicillin (30 µg), ciprofloxacin (10 µg), tarivid (10 µg), streptomycin (30 µg), augmentin (20 µg), sparfloxacin (30 µg), gentamicin (10 µg), septrin (30 µg), chloramphenicol (25 µg) and perfloxacin (10 µg). Antibiotic resistance was evaluated based on the diameters of inhibition zones around the antibiotic discs. The results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines, allowing for the determination of resistance profiles (CLSI, 2017).

#### **Antibacterial Assay**

To prepare the inoculum, test organisms were cultured in nutrient broth and incubated for 24 hours at 37°C. After incubation, the cultures were adjusted to a 0.5 McFarland turbidity standard to ensure a standardized bacterial concentration. A 0.2 ml aliquot of bacterial culture was diluted in normal saline and then evenly spread onto solidified Mueller-Hinton agar plates using a glass rod. The antibacterial activity of both extracts was assessed using the agar well diffusion method. Following inoculation, the agar plates were allowed to dry. Wells, 4 mm in diameter, were created on the agar

surface using a cork borer. A 0.2 ml volume of each extract, prepared at different concentrations (125mg/ml-2000mg/ml), was introduced into the wells using a Pasteur pipette. The wells were spaced adequately to prevent overlap of inhibition zones. The plates were then incubated at 37°C for 24 hours to allow the development of inhibition zones. Each test was performed in triplicate to ensure reproducibility. The diameters of the inhibition zones were measured using a ruler (Ibekwe *et al.*, 2001).

#### **Test for Minimum Inhibitory Concentration**

The minimum inhibitory concentration (MIC) of the extracts was determined to identify the lowest concentration needed to effectively inhibit the growth of the test organisms. The MIC was assessed using standardized microbiological techniques (Nakamura *et al.*, 2021).

#### **Test for Minimum Bactericidal Concentration**

The minimum bactericidal concentration (MBC) was done to determine the minimum concentration of the extract that is bactericidal. It was determined by re-using extract concentrations that inhibited the growth of the test organism (i.e., those at the MIC). Broth dilutions were streaked onto solidified extract agar and incubated for 24 to 48 hours. The MBC is the lowest dilution of antimicrobial that prevents growth of the organism on the agar plate (Nweze and Eze, 2020).

## **RESULTS**

The isolates used in this study, includes, *Klebsiella* sp.<sup>1</sup>, *Klebsiella* sp.<sup>2</sup>, *Klebsiella* sp.<sup>3</sup>, *Klebsiella* sp.<sup>4</sup> (clinical isolates) and *Klebsiella* sp.<sup>5</sup>, *Klebsiella* sp.<sup>6</sup> (food isolates). Phytochemical

screening of the leaf extracts (Table 1) revealed that *Ocimum gratissimum* extract showed the presence of tannin, phenol and phlobatannin.

The antibiotic susceptibility pattern of the isolates (Table 2) revealed that most of the isolates were resistant to the antibiotics. The isolates recorded a resistance index range from 0.6 (*Klebsiella* sp.<sup>5</sup>) to 1.0 (*Klebsiella* sp.<sup>2</sup> and *Klebsiella* sp.<sup>3</sup>). Table 3a and 3b shows the antibacterial activity of aqueous and ethanolic extract of *Ocimum gratissimum* against *Klebsiella* species isolates respectively. The highest zone recorded for ethanolic extract was 67.33±18.58(mm) at 2000mg/ml for *Klebsiella* sp.<sup>6</sup>, the lowest zone was

5.33±5.03(mm) at 250mg/ml for *Klebsiella* sp.<sup>3</sup>. For aqueous extract, the highest zone was 68.66±4.16(mm) at 2000mg/ml for *Klebsiella* sp.<sup>6</sup> and the lowest zone was 2.66±1.15(mm) at 125mg/ml for *Klebsiella* sp.<sup>2</sup>

The Minimum Inhibitory Concentration (MIC) and the Minimum bactericidal concentration (MBC) of *Ocimum gratissimum* extracts on bacterial isolate is shown in Table 4. The results revealed that the MIC for ethanolic extract ranges from 60-800mg/ml while results for aqueous extracts ranges from 100-900mg/ml. The Minimum bactericidal concentration (MBC) of the aqueous extract recorded for some of the isolates was 2000 mg/ml.

Table 1: Phytochemical composition of *Ocimum gratissimum* Extract

Phytochemical constituent	<i>Ocimum gratissimum</i>
Alkaloid	-
Flavonoid	-
Saponin	-
Tannin	+
Phenol	+
Phlobatannin	+

Key: = Absent; + = Present

Table 2: Antibiotic Susceptibility Pattern of *Klebsiella* species

ISOLATES	AU	CN	PEF	OFX	S	SXT	CH	SP	CPX	AM	RI
<i>Klebsiella</i> sp. <sup>1</sup>	0(R)	0(R)	8(R)	10(R)	6(R)	0(R)	6(R)	10(R)	20(S)	0(R)	0.9
<i>Klebsiella</i> sp. <sup>2</sup>	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	4(R)	0(R)	0(R)	0(R)	1.0
<i>Klebsiella</i> sp. <sup>3</sup>	0(R)	0(R)	0(R)	6(R)	0(R)	8(R)	8(R)	0(R)	0(R)	0(R)	1.0
<i>Klebsiella</i> sp. <sup>4</sup>	0(R)	0(R)	8(R)	10(R)	0(R)	0(R)	0(R)	6(R)	14(I)	0(R)	0.9
<i>Klebsiella</i> sp. <sup>5</sup>	10(R)	0(R)	22(S)	12(I)	0(R)	8(R)	14(I)	0(R)	20(S)	0(R)	0.6
<i>Klebsiella</i> sp. <sup>6</sup>	0(R)	0(R)	8(R)	12(I)	0(R)	0(R)	8(R)	0(R)	20(S)	0(R)	0.8

Key: Resistant (R) = 0-10mm, Intermediate (I) = 11-16mm, Sensitive(S) =17mm and above, SXT = Septrin (30 µg), CH = Chloramphenicol (30 µg), SP = Sparifloxacin (10 µg), CPX = Ciprofloxacin (30 µg), AM = Amoxicillin (30 µg), AU = Augmentin (10 µg), CN = Gentamycin (30 µg), PEF= Pefloxacin( 30 µg), OFX = Tarivid (10 µg), S = Streptomycin (30 µg).

Table 3a: Antibacterial Activity of Ethanolic Extract of *Ocimum gratissimum* against *Klebsiella* species

Isolates	Zones of Inhibition				
	2000mg/ml	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
<i>Klebsiella</i> sp. <sup>1</sup>	41.33 ± 16.04	10.66 ± 3.04	12.00 ± 10.58	8.00 ± 7.21	-
<i>Klebsiella</i> sp. <sup>2</sup>	11.33 ± 1.15	10.00 ± 2.00	8.66 ± 1.15	4.33 ± 3.78	2.66 ± 1.15
<i>Klebsiella</i> sp. <sup>3</sup>	30.00 ± 14.00	28.00 ± 10.58	14.00 ± 5.28	6.33 ± 4.50	-
<i>Klebsiella</i> sp. <sup>4</sup>	24.00 ± 2.00	7.66 ± 2.51	-	-	-
<i>Klebsiella</i> sp. <sup>5</sup>	58.66 ± 29.95	56.66 ± 16.65	31.33 ± 10.06	-	-
<i>Klebsiella</i> sp. <sup>6</sup>	68.66 ± 4.16	56.66 ± 11.05	29.33 ± 6.42	-	-

  

Isolates	Zones of Inhibition (mm)				
	2000mg/ml	1000mg/ml	500mg/ml	250mg/ml	125mg/ml
<i>Klebsiella</i> sp. <sup>1</sup>	29.33 ± 6.429	11.333 ± 2.30	50.00 ± 12.16	8.66 ± 9.018	-
<i>Klebsiella</i> sp. <sup>2</sup>	12.00 ± 2.00	8.00 ± 8.00	-	-	-
<i>Klebsiella</i> sp. <sup>3</sup>	32.00 ± 2.00	23.33 ± 5.78	12.33 ± 4.51	5.33 ± 5.03	-
<i>Klebsiella</i> sp. <sup>4</sup>	51.33 ± 8.08	36.66 ± 8.32	-	-	-
<i>Klebsiella</i> sp. <sup>5</sup>	44.66 ± 11.05	43.33 ± 3.05	34.66 ± 8.08	23.00 ± 8.54	11.66 ± 2.08
<i>Klebsiella</i> sp. <sup>6</sup>	67.33 ± 18.58	60.66 ± 9.86	47.33 ± 11.01	36.00 ± 4.00	15.33 ± 1.15

Values expressed as mean±SD ;KEY:- = no zone of inhibition observed; Clinical isolates = *Klebsiella* sp.<sup>1-4</sup>; Food isolates = *Klebsiella* sp.<sup>5-6</sup>

**Key:** = no zone of inhibition observed; Clinical isolates = *Klebsiella* sp.<sup>1-4</sup>; Food isolates = *Klebsiella* sp.<sup>5-6</sup>

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *O. gratissimum* Extracts against *Klebsiella* Isolates

Isolates	Ethanol		Aqueous	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
<i>Klebsiella</i> sp. <sup>1</sup>	225	-	200	2000
<i>Klebsiella</i> sp. <sup>2</sup>	700	-	100	-
<i>Klebsiella</i> sp. <sup>3</sup>	225	-	200	-
<i>Klebsiella</i> sp. <sup>4</sup>	800	-	900	2000
<i>Klebsiella</i> sp. <sup>5</sup>	100	-	400	2000
<i>Klebsiella</i> sp. <sup>6</sup>	60	-	300	-

**Key:** = > 2000mg/ml Clinical isolates = *Klebsiella* sp.<sup>1-4</sup>; Food isolates = *Klebsiella* sp.<sup>5-6</sup>

## DISCUSSION

*Klebsiella* species are Gram-negative, rod-shaped bacteria commonly found in clinical and environmental samples. All the isolates were Gram-negative rods, fermented lactose, sucrose, glucose, fructose, maltose, and sorbitol, but not starch. This pattern is characteristic of *Klebsiella* species, which are known to ferment a wide range of carbohydrates, aiding their adaptation to different

environments (Madigan *et al.*, 2021). The inability to hydrolyze starch suggests a lack of amylase production, which differentiates them from some other Enterobacteriaceae (Forbes *et al.*, 2018). The phytochemical screening of *Ocimum gratissimum* leaf extract revealed the presence of tannins and phenols, but an absence of alkaloids, flavonoids, saponins, and phlobatannins (Table 1). Tannins and phenols are known to exhibit

antimicrobial properties by interfering with bacterial cell membranes and enzyme systems (Cowan, 1999).

The antibiotic susceptibility profile of *Klebsiella* species in this study indicates a high level of resistance to most tested antibiotics, with only a few showing susceptibility. The antibiotic resistance index (RI) values ranged from 0.6 to 1.0, indicating significant resistance patterns.

Among the six isolates, *Klebsiella* sp.2 and *Klebsiella* sp.3, both clinical isolates had the highest resistance index (RI = 1.0), meaning they were resistant to all tested antibiotics. This suggests exposure to high levels of antibiotics in the environment or previous antibiotic misuse, which has selected for multidrug-resistant strains (Davies and Davies, 2010). Most isolates were resistant to Augmentin, Gentamycin, Amoxicillin, Septrin, Streptomycin, and Sparfloxacin, which are commonly used antibiotics (Livermore, 2012). Ciprofloxacin was one of the few antibiotics with notable sensitivity, showing effectiveness against *Klebsiella* sp.1, sp.5, and sp.6. Pefloxacin also exhibited sensitivity to *Klebsiella* sp.5, while Tarivid had an intermediate effect on *Klebsiella* sp.5 and sp.6. The limited effectiveness of fluoroquinolones suggests that resistance to this class of antibiotics is emerging but not yet widespread. The resistance to multiple antibiotics, especially beta-lactams such as Augmentin and Amoxicillin, may indicate the presence of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella* species (Pitout and Laupland, 2008). This poses a serious challenge in clinical settings, where *Klebsiella* species are a major cause of hospital-acquired infections. The high levels of antibiotic resistance observed in *Klebsiella* spp. isolates in this study

highlight the urgent need for antimicrobial stewardship programs to control the overuse and misuse of antibiotics. There is also a need for alternative antimicrobial strategies, including the use of medicinal plants such as *Ocimum gratissimum*, whose phytochemical constituents may contribute to antibacterial effects (Igbinosa et al., 2009).

The antimicrobial activity of *Ocimum gratissimum* extracts against *Klebsiella* species at various concentrations reveals a dose-dependent inhibition pattern. The ethanol and aqueous extracts displayed varying degrees of antibacterial activity, with higher concentrations generally producing larger zones of inhibition (Table 3a and 3b). These findings align with previous studies that have reported the antibacterial properties of *O. gratissimum*, which are attributed to its rich phytochemical content, including eugenol, thymol, and other bioactive compounds (Nakamura et al., 2021).

The ethanol extract demonstrated notable antibacterial activity, with inhibition zones ranging from  $8.66 \pm 9.02$  mm at 125 mg/mL to  $67.33 \pm 18.58$  mm at 2000 mg/mL, particularly in *Klebsiella* sp.6. The largest inhibition zones were observed at higher concentrations across most isolates, confirming that the ethanol extract is highly effective in inhibiting *Klebsiella* species as seen in Table 3a. Ethanol is known to efficiently extract phenolic compounds and essential oils, which exhibit strong antibacterial activity by disrupting bacterial membranes and interfering with metabolic processes (Ogunlana et al., 2020).

Among the isolates, *Klebsiella* sp.6 (food isolate) showed the highest susceptibility, with a mean inhibition zone of  $67.33 \pm 18.58$  mm at 2000 mg/mL. In contrast, *Klebsiella* sp.2 (clinical isolate)

exhibited the lowest susceptibility, with a zone of  $12.00 \pm 2.00$  mm at the highest concentration, suggesting strain-specific variations in resistance mechanisms. This variability could be due to differences in membrane composition, efflux pump activity, or biofilm formation (Akinjogunla *et al.*, 2019).

The aqueous extract also exhibited antibacterial effects, but the inhibition zones were generally smaller than those of the ethanol extract. The highest inhibition zone was  $68.66 \pm 4.16$  mm in *Klebsiella* sp.6 at 2000 mg/mL, whereas the lowest inhibition zone was  $2.66 \pm 1.15$  mm in *Klebsiella* sp.2 at 125 mg/ml. This difference in activity may be attributed to the lower solubility of essential oils and phenolic compounds in water compared to ethanol, resulting in reduced bioavailability of antimicrobial compounds (Aboh *et al.*, 2022).

Despite the lower efficacy of the aqueous extract compared to ethanol, significant inhibition was still observed, particularly at higher concentrations. The presence of bioactive compounds, such as flavonoids and tannins, in the aqueous extract may contribute to its antibacterial effects, although in lower concentrations than in ethanol extracts (Ekezie *et al.*, 2021).

The ethanol extract demonstrated superior antibacterial activity across all isolates and concentrations compared to the aqueous extract. This suggests that ethanol extracts a broader range of antimicrobial compounds more efficiently than water. Previous studies have also reported higher antimicrobial efficacy of ethanol extracts of medicinal plants due to their ability to dissolve non-polar phytochemicals, which contribute to bacterial cell membrane disruption (Okoh *et al.*, 2018).

The dose-dependent increase in inhibition zones observed in both extracts supports the concept that higher concentrations of *O. gratissimum* result in greater bacterial inhibition. However, the variability in inhibition zones among *Klebsiella* species isolates suggests potential differences in susceptibility, which could be due to strain-specific resistance mechanisms or variations in metabolic activity (Oyeleke and Dauda, 2020). The minimum inhibitory concentration (MIC) of *Ocimum gratissimum* extracts against *Klebsiella* species varied across different isolates and extraction solvents. The ethanol extract exhibited lower MIC values for most isolates compared to the aqueous extract, indicating a higher antimicrobial potency. This aligns with previous research suggesting that ethanol extracts of medicinal plants contain a higher concentration of bioactive compounds with antimicrobial properties (Ogunlana *et al.*, 2020).

The ethanol extract exhibited MIC values ranging from 60 mg/mL (*Klebsiella* sp.6) to 800 mg/mL (*Klebsiella* sp.4). The lowest MIC (60 mg/mL) was observed in *Klebsiella* sp.6, suggesting high susceptibility to the ethanol extract. Conversely, *Klebsiella* sp.4 had the highest MIC (800 mg/mL), indicating reduced susceptibility or potential tolerance mechanisms. (Table 4) The variations in MIC values among isolates may be attributed to differences in bacterial membrane composition, efflux pump activity, or biofilm formation, which can influence bacterial resistance to plant extracts (Akinjogunla *et al.*, 2019).

Ethanol is known to efficiently extract a broad range of antimicrobial compounds, including phenolics, flavonoids, and essential oils such as

eugenol, which have strong bactericidal effects by disrupting bacterial cell walls and interfering with enzymatic processes (Okoh *et al.*, 2018). The observed trend supports the findings of Aboh *et al.* (2022), who reported that ethanol extracts of *Ocimum gratissimum* were more effective against Gram-negative bacteria than aqueous extracts due to the higher solubility of hydrophobic antimicrobial compounds.

The MIC values of the aqueous extract ranged from 100 mg/mL (*Klebsiella* sp.2) to 900 mg/mL (*Klebsiella* sp.4) as shown in table 4. Compared to the ethanol extract, the aqueous extract generally exhibited higher MIC values, suggesting lower antimicrobial efficacy. The lower potency of the aqueous extract could be due to the limited solubility of essential oils and certain phenolic compounds in water. Although aqueous extracts still contain bioactive compounds such as tannins and alkaloids, they may be present in lower concentrations or lack certain hydrophobic antimicrobial agents that are more effectively extracted with ethanol (Ekezie *et al.*, 2021).

A comparison of MIC values shows that ethanol extracts exhibited stronger antibacterial activity than aqueous extracts in most cases. Similar findings have been reported in previous research. *Ocimum gratissimum* extracts exhibited significant antibacterial activity against *Klebsiella* species with ethanol extracts showing more pronounced inhibition than aqueous extracts. Additionally, *Klebsiella* sp.6 (food isolate) exhibited the lowest MIC values for both ethanol (60 mg/mL) and aqueous (300 mg/mL) extracts, indicating high susceptibility to *O. gratissimum*. In contrast, *Klebsiella* sp.4 (clinical isolate) had the highest MIC values for both ethanol (800 mg/mL) and

aqueous (900 mg/mL) extracts, suggesting greater resistance. These findings suggest that while *O. gratissimum* extracts are effective against *Klebsiella* species, susceptibility varies among isolates, likely due to intrinsic resistance mechanisms or strain-specific factors (Nakamura *et al.*, 2021).

The Minimum Bactericidal Concentration (MBC) is a crucial parameter in antimicrobial studies as it indicates the lowest concentration of an antimicrobial agent required to kill a given bacterial isolate. The results presented for the ethanol and aqueous extracts of *Ocimum gratissimum* against *Klebsiella* isolates show variations in bactericidal efficacy. The results revealed that the MBC for the ethanolic extracts was greater than the highest concentration of MIC used (2000mg/ml) for all isolates, whereas the MBC for the aqueous extract was 2000mg/ml for some isolates. This suggests that the phytochemical constituents of the plant exert differential antibacterial effects depending on the extraction solvent used. The MBC values for *Ocimum gratissimum* indicate that the plant extracts are bactericidal at relatively high concentrations, ranging from 2000 mg/ml for most isolates. The bactericidal activity of these extracts suggests that they are not only effective in inhibiting bacterial growth but may also be capable of killing the bacteria at higher concentrations.

## CONCLUSION

This study highlights the antibacterial potential of *Ocimum gratissimum* against *Klebsiella* species isolates of clinical and food origin. All isolates exhibited significant resistance to conventional antibiotics, however highest resistance index was exhibited by clinical isolates,

underscoring the need for alternative antimicrobial strategies. The phytochemical analysis of *O. gratissimum* revealed the presence of tannins and phenols, which are known for their antibacterial properties. The ethanol extract demonstrated greater antibacterial activity than the aqueous extract, with a dose-dependent inhibition pattern. Lowest MIC values were recorded for both extracts against food isolates, however *O. gratissimum* extracts can be bactericidal at higher concentrations. These findings support the potential use of *O. gratissimum* as a natural antibacterial agent against multidrug-resistant *Klebsiella* strains.

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