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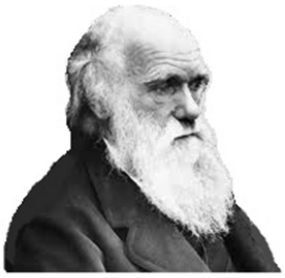

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Determination of antibacterial activity of ethanolic and aqueous stem extracts of *Ocimum gratissimum* (African basil) against common bacteria

Faidatullahi Oyiza Mohammadbasheer*, Hajara Kabir Ahmad

Department of Plant Biology, Faculty of Science, Bayero University Kano, Kano State, Nigeria

*E-mail address: faidatmbasheer@gmail.com

ABSTRACT

The Lamiaceae plant species *Ocimum gratissimum* is the subject of this experiment. Three common pathogenic bacteria were used to test the antibacterial activity of the aqueous and ethanolic extracts of the stem of Ocimum gratissimum. They underwent tests against Shigella species, Salmonella enterica, and Pseudomonas aeruginosa. Each extract was evaluated for its antibacterial properties, minimum bactericidal concentrations (MBC), and minimum inhibitory concentration (MIC). The plant extracts were given at various concentrations of 250 mg/mL, 125 mg/mL, 61.5 mg/mL, and 31.2 mg/mL in order to assess their antibacterial activities against the chosen bacteria using the agar well diffusion method. The outcomes demonstrated that while both plant stem extracts inhibited the organisms, the ethanolic stem extract was superior to the aqueous counterpart in terms of its effectiveness. The range of the minimum bactericidal concentrations (MBCs) and minimum inhibitory concentrations (MICs) was 3.96 mg/mL to 31.25 mg/mL. The results of this investigation demonstrate that the stems of Ocimum gratissimum possess antibacterial properties on par with those of the plant's leaves. The low minimum inhibitory concentrations (MICs) further suggest a potent antibacterial action against the pathogens, especially when it comes to the ethanol extracts. As a result, *Ocimum gratissimum* extracts may be utilized to treat infections linked to the test organisms, establishing the plant's status as a natural antibiotic and a necessary ingredient in pharmaceutical manufacturing.

***Keywords*:** Antibacterial, Bactericidal, Inhibitory, *Ocimum gratissimum*

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1. INTRODUCTION

Plants have traditionally been used to treat a wide range of illnesses. It is well known that medicinal plants generate a wide range of compounds to defend themselves against various pathogens. In an effort to find more effective medications against strains of resistant microorganisms, researchers are focusing more and more on herbal products as sources of lead compounds.

The effectiveness of the active ingredients in herbal medicine as natural healing agents, as well as their availability, accessibility, affordability, and well-regarded less-or non-toxic effects, may be the reason behind the growing preference for using herbal medicines these days over conventional ones (Ikpeazu *et al*., 2018; Ijioma *et al*., 2021). Medicinal plants are considered the best source of various drugs, according to the World Health Organisation. In developed nations, traditional medicine makes use of about 80% of plants. Consequently, research has been done on these plants to learn more about their potential medical benefits. Many researchers from all over the world have looked into the antimicrobial qualities of various plants (Adamu *et al*., 2005). Furthermore, due to their potential to treat and prevent chronic diseases like cancer, diabetes, stroke, and arthritis (Bernell and Howard, 2016), as well as serve as an alternative therapy for the treatment of psychiatric disorders (Venuprasad *et al.*, 2014) and meet the health needs of the elderly (WHO, 2019), medicinal plants and their bioactive compounds have drawn the attention of several researchers during the past ten years. These days, these medicinal plants are used to treat a wide range of illnesses and are also a source of new drugs for conventional or traditional medicine. According to (Mbanaso *et al.*, 2020), medicinal plants like *Cinchona officinalis, Digitalis purpurea, Saix alba,* and *Papaver somniferum* were used to make drugs like quinine, digoxin, aspirin, and morphine. Because pathogenic bacteria are becoming more resistant to conventional antibiotics, attention must be paid to alternative antibiotic sources (Tarun *et al.*, 2012). There are several culinary and medicinal uses for medicinal plants, including *Ocimum gratissimum*, *Vernonia amygdalina, and Aframomum melegueta*. Certain bacteria are subject to the bacteriostatic and bactericidal effects of these medicinal properties (Okigbo *et al.*, 2006; Okeke *et al*., 2008; Funmilayo *et al.*, 2010).

One of the newly found medicinal plants, Ocimum gratissimum L., also referred to as scent leaf, has the potential to be used as a new drug source or as an alternative therapy for treating a variety of illnesses. It is a perennial herbaceous plant that is widely distributed, highly aromatic, and profitable to grow. Africa, Asia, and South America are home to this member of the Lamiaceae family (Tanko *et al*., 2008; Akara *et al.*, 2021). It can be added to fish, meat, soups, and stews as a natural flavouring agent, condiment, or vegetable. According to (Akara *et al.*, 2021) it is also used in traditional medicine to treat a number of illnesses, including fever, diarrhoea, anaemia, cough, pneumonia, inflammation, and bacterial and fungal infections. Ocimum gratissimum is being used to treat even the most severe medical conditions; additional research on the plant even suggests that it is an effective treatment for patients with HIV and AIDs. (Elujoba, 2000).

Bacteria are commonplace, largely free-living organisms that frequently only have one cell. In other words, they are organisms with only one cell. Due to their ability to form pairs, chains, or clusters, they are typically found in large quantities. While certain bacteria are harmful to humans and can cause fatal or deadly events in large quantities, other bacteria are helpful and beneficial to humans. *Salmonella, Pseudomonas*, and *Shigella* species are a few of

these harmful bacteria (Dekker *et al.*, 2015, Wilson and Pandey, 2023). Based on the two distinct forms of cell walls that they possess, bacteria can be divided into two main categories: gram-positive and gram-negative. The established method for differentiating between bacteria, gramme staining, is used to determine how bacterial cells react, leading to this classification.

Gramme negative bacteria with thin cell walls, often known as pathogens such as *Shigella, Pseudomonas,* and *Salmonella*, exhibit pink or red staining when stained with gramme stain (WHO, 2019). These are lethal bacteria that can cause a wide range of illnesses, including intestinal, bloodstream, and pneumonia infections.

Antibiotics are used to treat diseases brought on by organisms that cause disease. Antibiotics have been successful, but infectious diseases continue to be the worlds leading cause of death (Harriet *et al.*, 2020). Given that the pathogenic microbes that cause these diseases are becoming more resistant to conventional antibiotics, attention must be paid to alternative antibiotic sources (Tarun *et al.*, 2012). Furthermore, plants with a high concentration of phytochemicals and medicinal properties are being investigated as potential therapies for neglected tropical diseases (NTDs) affecting humans and animals in Nigeria and throughout Africa (Harriet, 2020).

This study aims to investigate the antibacterial efficacy of *O. gratissimum* stems, which have been shown in earlier research to possess medicinal properties (Effriam *et al.*, 2000). To find out how effective the plant extracts are against bacteria and how much of them can be used, *Ocimum gratissimum* stem extracts will be tested on microorganisms.

2. MATERIALS AND METHODS

2. 1. Experimental site

The experiment was conducted at the old Bayero University Kano campus in Kano, Nigeria, in the Plant Pathology Laboratory of the Department of Plant Biology. The location of the lab was 11.9794° latitude and 8.47838° longitude.

2. 2. Sample collection

In the month of March, between the hours of 4-6 pm, the *Ocimum gratissimum* plant was purchased from vendors at the Yankura market in Yankura, Kano state, Nigeria. Then, Mallam Bahahudeen, the curator of the herbarium at the Department of Plant Biology, Bayero University, Kano, recognised and verified them as *Ocimum gratissimum* leaves and stems. Bayero University's Department of Microbiology provided the bacterial samples, which were verified by chief technologist Mallam Idris. All chemicals used were of analytical grade.

2. 3. Preparation and extraction of plant materials

The method of (Gidado *et al.*, 2005) as described by (Ekeleme *et al.*, 2017) was slightly modified for the preparation of the extract. The plants were cleaned, and the leaves were removed from the stems. After gathering the stems, they were allowed to air dry for six days. They were then roughly crushed and then put through an electronic blender to get even smaller pieces. For later use, the powdered sample was kept in airtight containers and kept at room temperature. A precise 40g portion of the powdered stem samples was added to 400 mL of each solvent. The extract was prepared using two solvents: 90% ethanol and distilled water. The extracts that were obtained were the ethanolic and aqueous stem extracts. For a duration of three

days, these were kept at room temperature and were regularly shaken. After being filtered through a lab cheesecloth, they were placed in the water bath to be extracted. To obtain the crude extracts, which were kept in labeled airtight containers for later use, the filtrates were evaporated to dryness at 50 °C. To achieve the required concentration, 0.5 grammes of each of these extracts were subsequently dissolved in 1 milliliter of dimethyl sulfoxide (DMSO).

2. 4. Maintenance of bacteria isolates

The bacteria were kept in nutrient agar slants and refrigerated at 4 °C until they were needed.

2. 5. Standardisation of test isolates

For each of the three bacteria, 10 millilitres of normal saline was added to a test tube. Then, using a sterilised wire loop, specific amounts of each bacteria were added to the test tubes and mixed until the Mcfarland standard was reached.

2. 6. Antibacterial testing

The agar well diffusion technique, as outlined by (Cheesbrough, 2006), was utilised to conduct the antibacterial testing. The antibacterial activity of the extracts was ascertained using it. Hinton Mueller A dish of sterile Petri was filled with agar. After the Petri dish was slowly rotated and the bacterial isolate had been distributed uniformly, the seeded plates were left to set. On the agar surface, uniform wells were cut using a sterile cork borer with a 6 mm diameter. Using a sterile syringe, add 3–4 drops of varying concentrations of each extract to the wells.

The plates were then left to stand at room temperature for an hour in order to give the extract time to properly diffuse. Zones of inhibition were noted after every plate was incubated for 24 hours at 37 °C. Each well has a clearance zone surrounding it, which represents inhibition.

The diameter of these zones was measured in millimetres (mm). In separate plates, ampicillin was used as the positive control and DMSO as the negative control. The plates were equally incubated at 37 °C for 24 hours in an incubator, and a ruler was used to measure the diameter of the zones of inhibition in millimetres. The mean of the duplicate determination was recorded for each value.

2. 7. Determination of minimum inhibitory concentration (MIC)

The tube dilution method (Wagenlehner *et al*., 2006) was slightly modified in order to determine the minimum inhibitory concentration. 1 millilitre of the extract solution (62.25 mg/mL) was combined with 1 millilitre of nutritional broth, and then 1 millilitre was moved from test tube 1 to test tube 2, and so on, up to test tube 6, with the exception of test tube 5. Next, 1 millilitre of the test organisms was introduced into every test tube, with the exception of the sixth one.

The fifth served as the positive control since it contained only the test samples, and the sixth served as the negative control because it contained only the nutrient broth. Following a thorough shaking, each test tube was incubated for 24 hours at 37 °C. The minimal inhibitory concentration was determined by looking at the tube with the lowest dilution in which there was no discernible growth of the test organisms. Table 6 below shows the results of this process, which was repeated for each of the two extracts.

2. 8. Determination of minimum bactericidal concentration (MBC)

First, tubes that did not grow during the MIC determination process were chosen in order to determine the minimum inhibitory concentrations. Each tube was then filled with a loopful, which was then subcultured onto extract-free agar plates and incubated for an additional 24 hours at 37 °C. The MBC was identified as the lowest concentration at which no growth was seen. The bactericidal effect of the concentration of the extracts used was indicated by the absence of visible growth on all of the Nutrient Agar plates. (Jones *et al*, 2014).

3. RESULTS

The antibacterial properties of the ethanolic and aqueous stem extracts on the test organisms are displayed in Table 1's results.

Table 1 below shows the stem extracts' performance against the test organisms. The stem extract exhibited the highest activity against *Salmonella* (16.60 mm) in the ethanolic extract, with inhibition values of 16.10 mm and 16.00 mm for *Shigella* and *Pseudomonas*, respectively, according to the results. The aqueous stem extracts showed the least activity against *Pseudomonas*, measuring 13.60 mm.

As shown in Table 1 below, *Salmonella* was the organism on which the extracts had the greatest effects at 250 mg/mL, followed by *Shigella* and *Pseudomonas*. With regard to *Pseudomonas* and *Salmonella*, but not *Shigella*, the concentrations of 125 mg/mL and 62.5 mg/mL had the same effects. The lowest effect of the extracts was seen at 31.25 mg/mL, and the extracts had the same effects on all three test samples. As indicated in Table 1.1, there was only an interaction for *Shigella* between the concentration and extracts used.

The MIC results for each test sample are displayed in the Tables 2, 3, and 4 below. The MBC results are displayed in Table 5 below. For both the aqueous and ethanolic extracts, the stem extracts' MICs were found to be 31.25 mg/mL and 15.62 mg/mL, respectively.

The extracts' minimum inhibitory concentration (MIC) against *Shigella* is displayed in Table 3. MIC was found to be 7.81 mg/mL for ethanolic stem extracts and 15.62 mg/mL for aqueous stem extracts.

Table 4 indicates that the minimum inhibitory concentration (MIC) for *Pseudomonas* was determined to be 15.62 mg/mL for both the ethanolic and aqueous stem extracts.

Table 1. Antibacterial activity (Diameter zones of inhibition) of plant extracts on test organisms.

Sources of variation	***Pseudomonas* spp (mm)**	***Salmonella* spp (mm)**	***Shigella* spp (mm)**
Extracts			
ASE	13.60[a]	15.60[a]	15.70a[a]
ESE	16.00[a]	16.60[a]	16.10[a]
LSD (0.05)	3.09	3.64	0.92

Concentrations (mg/mL**)**			
250	14.63^{b}	16.37^{b}	14.75^{b}
125	11.38^{bc}	14.87^{bc}	14.38^{b}
62.5	12.38^{bc}	13.00^{bc}	12.50^{c}
31.25	10.63^{c}	11.75^{c}	10.37^{d}
Control (+)	28.00^{a}	24.50^{a}	35.21^{a}
LSD (0.05)	3.45	4.07	1.03
Interaction			
Extracts x Concentrations			***
LSD (0.05)	NS	NS	0.45

Keys: ASE = aqueous stem extracts, ESE = ethanolic stem extracts, NS = No significant difference and LSD is least significant difference.

Table 1.1. Interaction effect between extracts and concentrations in *Shigella* spp zones of inhibition.

	250 mg/mL	**125 mg/mL**	**62.5 mg/mL**	**31.25 mg/mL**	**Control (+)**
ASE	14.00^{c}	12.00^{e}	9.50^{g}	8.50^{i}	34.50^{b}
ESE	12.50^{d}	12.00^{e}	11.00^{f}	9.00^{h}	36.00^{a}

Keys: ASE = aqueous stem extracts, ESE = ethanolic stem extracts.

Table 2. Minimum inhibitory concentration (MIC) on *Salmonella enterica.*

Concentration (mg/mL)					**MIC (mg/mL)**
Extracts	31.25	15.62	7.81	3.96	
ASE	+	-	-	-	31.25
ESE	+	+	-	-	15.62

+ = presence of growth, - = absence of growth
Keys: ASE = aqueous stem extracts, ESE = ethanolic stem extracts.

Table 3. Minimum inhibitory concentration (MIC) on *Pseudomonas aeruginosa*

Concentration (mg/mL)					MIC (mg/mL)
Extracts	31.25	15.62	7.81	3.96	
ASE	+	+	-	-	15.62
ESE	+	+	-	-	15.62

+= presence of growth, -= absence of growth
Keys: ASE=aqueous stem extracts, ESE=ethanolic stem extracts.

Table 4. Minimum inhibitory concentration (MIC) on *Shigella* spp

Concentration (mg/mL)					MIC (mg/mL)
Extracts	31.25	15.62	7.81	3.96	
ASE	+	+	-	-	15.62
ESE	+	+	+	-	7.81

+ = presence of growth, - = absence of growth
Keys: ASE = aqueous stem extracts, ESE = ethanolic stem extracts.

Table 5. Minimum bactericidal concentration (MBC) RESULTS

Extracts	***Salmonella***	***Pseudomonas***	***Salmonella***
ASE	31.25	31.25	31.25
ESE	31.25	31.25	15.625

Keys: ALE = aqueous leaves extracts, ASE = aqueous stem extracts, ELE = ethanolic leaves extracts, ESE = ethanolic stem extracts.

4. DISCUSSION

In this study, *Salmonella*, *Pseudomonas*, and *Shigella* species were used as test samples. Following the process of extracting *O. gratissimum* stems with ethanol and distilled water, the

antibacterial activities of the various extractions were ascertained and were discovered to have antibacterial effects against the experimental samples. This is consistent with research by (Udochukwu *et al*., 2015), who found that extracts from *O. gratissimum* have both antifungal and antibacterial properties against a variety of bacterial species.

The stems' ethanolic extract showed the highest level of activity. This finding is consistent with the findings of the study by (Udochukwu *et al.*, 2015), which showed that the ethanolic extracts of *V. amygdalina* and *O. gratissimum* outperform the water extracts in their ability to combat *Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.*

This observation may be explained by the fact that some plant active ingredients are insoluble in water, the presence of inhibitors of antimicrobial components, or the high volatility of ethanol, which tends to extract more active ingredient from the sample than water (Amadioha *et al,* 1999, Okigbo *et al*, 2005).

(Akinyemi *et al.*, 2006) conducted a comprehensive investigation on stem bark portions and concluded that *O. gratissimum*'s aqueous and ethanolic extract possessed antimicrobial properties. The two extracts demonstrated efficacy against both MRSA and S. aureus. Similar to the findings of the current investigation, they exhibit bacteriostatic properties at lower concentrations.

As demonstrated by the results of this study, *Ocimum gratissimum* has antibacterial properties against *Shigella, Salmonella, and Pseudomonas* species. These findings are consistent with those of previous studies (Ilori *et al*., 1996; Nweze and Eze, 2009; Prakash *et al.*, 2011; Melo *et al.*, 2019) that have verified *Ocimum gratissimum*'s antimicrobial activities. Furthermore, independent studies conducted by (Silva, 2005) and (El-said, 1996) demonstrated the antibacterial activity of *Ocimum gratissimum* oil and extracts against *Shigella*, *Salmonella,* and *Proteus bacteria.*

The results of this study indicate that ethanolic stem extracts, as opposed to aqueous stem extracts, have the greatest impact on *Salmonella and Pseudomonas*. This is somewhat consistent with the findings of (Talabi and Makanjuola *et al.*, 2017), who reported that *O. gratissimum*'s aqueous extract strongly inhibited *Pseudomonas aeruginosa* and moderately inhibited *Staphylococcus aureus.* However, a variety of antimicrobial activities were shown by the ethanolic leaf powder extract, which showed significant inhibitory properties against *E. coli, Bacillus cereus, P. aeruginosa,* and *S. aureus.*

Furthermore, (Nwiyi *et al*., 2009) reported that *O. gratissimum* ethanol extracts exhibited greater antibacterial activity against bacteria, which is consistent with the current study's findings that the ethanolic extracts had a wider zone of inhibition on the test organisms.
Furthermore, it was discovered that *Ocimum gratissimum*'s ethanolic stem extracts were more successful on the test organisms than the leaf extracts in the research conducted by (Odundare *et al*., 2014). Also, (Ogundare *et al.*, 2014) suggested that the observation may be explained by the fact that the stems contain a higher concentration of bioactive compounds than the leaves. It might also be caused by the plant's age, the extraction solvent, the extraction technique, and the time of plant material harvesting (Okigbo, 2006).

The aforementioned evidence demonstrates that the plant's stem has an equal antibacterial effect on the test organisms as the plant's leaves do. The stems had low minimum inhibitory concentrations (MICs), ranging from 31.25 mg/mL to 3.96 mg/mL, according to the plant extracts' minimum inhibitory concentration (MIC) assay. Particularly for the ethanol stem extract of *Ocimum gratissimum*, these low MICs are indicative of a strong antibacterial effect on the test organisms. It also suggests that very low dosages of the extracts yield very good

results. This also suggests that the stems can be applied to clinical medicine and could be more successfully used to treat infections linked to the test organisms.

4. CONCLUSION

The stems and leaves of *O. gratissimum* were extracted using ethanol and water to get ethanolic and aqueous extracts. These extracts were tested against *Pseudomonas, Shigella* and *Salmonella* species and the results obtained from this study suggest that the aqueous extract and the ethanolic extract of *O. gratissimum* leaf and stems possess antibacterial activity on the test organisms. Following various methodologies, the antibacterial effects on the test organisms were determined and the minimum inhibitory concentration (MIC) as well as the minimum bactericidal concentrations (MBC) for each of the extracts on the bacteria was also ascertained which prove the potential of *Ocimum gratissimum* as a remedy in curing or combating diseases caused by *Shigella*, *Salmonella* and *Pseudomonas.* The results were interpreted and discussed as the findings of the current study was compared with that of previous studies.

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