



## Phytochemical and Antibacterial Activities of *Ocimum Gratissimum L.* on Isolates from Kunu-Zaki

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### ABSTRACT

This study involved a comprehensive analysis of phytochemical components of the aqueous extract of *Ocimum gratissimum* which revealed ascorbic acid to be the most abundant component, followed by tannins and flavonoids, while alkaloids, phytate, and cardiac glycosides were found in minimal amounts, while the ethanolic extract revealed the high components of ascorbic acid followed by flavonoid then tannin while other components were recorded in minimal amount. The study also evaluated the antibacterial properties of *Ocimum gratissimum L.* (scent leaf) against bacteria isolated from Kunu-zaki. Gram-negative organisms exhibited sensitivity to Gentamicin, Augmentin, and Ciprofloxacin, but demonstrated resistance to erythromycin, tetracycline, amoxicillin, ampicillin, and chloramphenicol. In contrast, gram-positive organism displayed sensitivity to Rocephin, Streptomycin, Pefloxacin, Ciprofloxacin, Gentamicin, and Zinnacef. Furthermore, the study examined the antimicrobial susceptibility of *Ocimum gratissimum* aqueous and ethanolic extracts on the isolated bacteria, the ethanolic extract yielded more favorable results, while ciprofloxacin was used as a control.

### Introduction

Kunu is an important non-alcoholic beverage mostly found in Northern Nigeria as opposed to wine or traditional palm wine and burukutu, which is alcoholic (Mbachu *et al.*, 2014; Okeke *et al.*, 2015; Anaukwu *et al.*, 2015). *Ocimum gratissimum L.*, belonging to the Lamiaceae family, is an aromatic perennial plant originating from Africa, Madagascar, and Southern Asia. Its leaves are elliptic-lanceolate, and it produces long flower spikes that range from pale white to pinkish. Related plants in the same genus include *Ocimum basilicum* (commonly known as sweet basil), *Ocimum canum* (African mint), *Ocimum campechianum* (Amazonian basil), and *Ocimum tenuiflorum* or *Ocimum sanctum* (known as holy basil). Traditionally, this plant has been utilized in various African countries for specific purposes. These include inducing abortion, assisting in childbirth and alleviating associated pain, as well as managing diabetes (Prabhu *et al.*, 2009; Mohammed *et al.*, 2007). Additionally, it has been reported to have properties such as preventing hair loss, protecting the central nervous system, cardiovascular benefits, renal function protection, anti-carcinogenic effects, and acting as a scavenger of free radicals (Anigbogu and Uzoaga, 2008; Effraim *et al.*, 2003; Prabhu *et al.*, 2009). These findings have been documented in various studies by researchers (Ugbogu *et al.*, 2021; Casanova *et al.*, 2014; Orafidya *et al.*, 2004; Sarraf *et al.*, 2013; Njoku *et al.*, 2011).

The antimicrobial and therapeutic attributes of *Ocimum gratissimum* are ascribed to its rich content of various phytochemicals, which encompass alkaloids, tannins, glycosides, phytate, saponins, phenolics, flavonoids, resins, steroids, terpenes, aromatic and volatile oils. Furthermore, it contains essential fatty acids such as linolenic acid and oleic acid, along with additional vital oils like eugenol, methyleugenol, ocimene, germaerne, cineole, and selinene (Talabi and Makarnjuola, 2017; Ujong *et al.*, 2021; Silva *et al.*, 2016; Brada *et al.*, 2011). In addition to these constituents, *Ocimum gratissimum* also houses vitamins and minerals (Alexander, 2016; Olumide *et al.*, 2019).

Extensive research has been conducted on *Ocimum gratissimum*, unveiling its numerous properties. It has been documented to possess antimicrobial capabilities, as reported by Nakamura *et al.*, (1999). Eugenol, found within the plant, contributes to its ovicidal activity, as noted by Pessoa *et al.*, (2002). Notably, it exhibits leishmanicidal properties, particularly effective against *Plasmodium berghei*, as reported by Tchoumboungang *et al.*, (2005). In experiments involving rats, the plant's potential as an anti-diarrheal agent was demonstrated by administering varying doses of its extract orally (Ezekwesili *et al.*, 2004). This resulted in a reduction in transit point and an increase in transit time, suggesting its promise in managing diarrhea, as observed by Amengialue *et al.*, (2013).

Furthermore, the ethanolic extracts of its essential oil have exhibited antifungal activity against specific aerial pathogens, as reported by Sessou *et al.*, (2013) and Terezinha *et al.*, (2006). In terms of antibacterial activity, its steam-distilled extract displayed inhibitory effects on selected bacteria, as documented by Prabhu *et al.*, (2009) and Adebolu and Salau, (2005). The plant's positive influence on wound healing, attributed to an increase in vascular

permeability, and was reported by Orafidiya *et al.*, (2005) and Prabhu *et al.*, (2009). Additionally, Sahouo *et al.*, (2003) demonstrated its anti-inflammatory properties.

In the realm of analgesic effects, Aziba *et al.*, (1999) conducted research that revealed the extract's ability to significantly extend reaction times by 85% during a 20-minute observation period in experimental subjects, all without eliciting any overt signs of toxicity. Meanwhile, on leukemia cells, the essential oil demonstrated cytotoxic effects, as evidenced by Dubey *et al.*, (1997).

Turning to its impact on the cardiovascular system, studies conducted by Anigbogu and Uzoaga, 2008; Lahlou *et al.*, (2004) provided indications of antihypertensive effects. In an ethanolic extract study involving albino rats, Effraim *et al.*, (2003) observed immunostimulatory effects through the examination of hematological indices. In streptozocin-induced diabetic rats, Mohammed *et al.*, (2007) conducted research that showcased antidiabetic effects, with the extract leading to an 81.3% reduction in blood glucose levels after 24 hours of administration. Okoli *et al.*, 2010, delved into the plant's anticonvulsant effects. The extensive body of research on *Ocimum gratissimum* underscores its considerable potential for a wide range of therapeutic applications.

The increasing problem of antibiotic resistance in various organisms poses a significant challenge to the treatment of diseases, and it has escalated into a global issue as bacteria continue to develop reduced responsiveness to a wide range of antibiotics. This situation has created an urgent need to constantly search for natural alternatives. Furthermore, there is concern about the presence of microorganisms in locally produced beverages sold by vendors and there is a pressing need to impart knowledge to these vendors about maintaining aseptic conditions during the production of their goods.

The Aim of the study is to assess the phytochemical components of *Ocimum gratissimum* and Antibacterial activities of *Ocimum gratissimum* on isolates from kunu-zaki.

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## Materials and Methods

### (1) Study Area

The specimens were acquired from various market locations through local vendors, after which they were conveyed to the laboratories of the Department of Applied Microbiology and Brewing, as well as the Department of Biochemistry, situated in Awka, Anambra State, Nigeria, for further research and analysis. Awka is situated in southeastern Nigeria, and it serves as the political and administrative center of Anambra State.

### Sample Collection

#### (a) Plant material

Fresh leaves of *Ocimum gratissimum* was procured from Eke-Awka market in Anambra state, Nigeria. A plant scientist from the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria, confirmed their identity. Subsequently, the leaves were air-dried in the laboratory for a duration of 10 days. Afterward, they were crushed using a laboratory mechanical grinder, and the resulting fine powders were carefully stored for future analysis.

### Sample Preparations and Extraction

The air-dried plant sample was ground into a powdered form, sieved and stored in plastic container. A 20g of the plant sample was added into a 200ml of distilled water and ethanol each at room temperature to obtain the crude extract. Muslin cloth was used to filter the plant leaves residues and the filtrate obtained was further purified by filtration through Whatman No 1 filter paper under aseptic condition. The filtrate collected was then concentrated by using rotary evaporator. The extract was then collected in fresh sterile universal bottles and stored in the refrigerator at 4°C until when required for use (Ladipo *et al.*, 2010).

#### (b) Kunu-zaki Samples Collection

A total of 70 kunu-zaki samples were bought from five distinct local vendors operating in various market locations, specifically Eke-Awka, Amaenyi, Okpuno, Tempsite, and Ifite markets.

### Isolation of *E. coli* from kunu-zaki

One millilitre of each kunu sample was aseptically transferred into 9 ml of sterile distilled water to achieve an initial dilution. The mixture was then vortexed to ensure homogeneity, and further serial dilutions ranging from  $10^{-1}$  to  $10^{-7}$  were performed to facilitate bacterial counts, following the methodology outlined by Falegan *et al.*, in 2017. Subsequently, 1 ml from the fifth test tube was inoculated into Eosin Methylene Blue (EMB) and nutrient agar using the pour plate method and incubated at 37°C for 24 hours. The morphological and biochemical characteristics of the observed bacterial growth on the media were subsequently determined.

### Phytochemical Components of the leaves of *Ocimum gratissimum* was determined using the following methods;

**Total Phenol Content:** Phenol content was determined using Barros *et al.*, (2007) method. Sample extract (1ml) was mixed with Folin-Ciocalteu's reagent and left for 3 minutes. Sodium carbonate was added (1ml) and was adjusted to the volume of 10ml using distilled water. Absorbance at 725nm was measured after 90 minutes in the dark. Results expressed as mg gallic acid equivalent per ml extract.

### Alkaloid determination

The alkaloid content was determined following Harbone's (1998) method. Five milliliters of plant extract was mixed with 200ml 20% acetic acid in ethanol. After 4 hours at 25°C, the mixture was filtered, and the filtrate concentrated using a water bath. Ammonium hydroxide was added dropwise until a precipitate formed. The precipitate was collected, washed, and dried at 80°C. Alkaloid content was calculated as a percentage of the sample's weight.

**Flavonoid determination:** A 0.5ml plant extract was mixed with 2ml water, then with 0.15ml of 5% NaNO<sub>2</sub>. After 6 minutes, 0.15ml of 10% AlCl<sub>3</sub> was added to stand for 6 minutes, and then 2ml of 4% NaOH was added to the mixture. Water was added to bring the volume to 5ml, allowed to stand for 15 minutes. Absorbance measured at 510nm with water as blank. Catechin used as reference standard. Result expressed as mg catechin equivalent per gram of sample (mg CE/g). (Barros *et al.*, 2007).

### Ascorbic Acid Determination

Klein and Perry (1982) method was adopted, extracted 20mg dried leaf powder with 10ml 1% metaphosphoric acid. Filtrate (1ml) reacted with 9ml 50µm 2, 6-dichlorophenolindophenol sodium salt hydrate, measuring absorbance at 515nm after 30 minutes. Ascorbic acid calculated from calibration curve, expressed as mg/g dried sample.

### Saponin Content

Obadoni and Ochuko (2002) method was employed, 5ml of sample extract was mixed with 200ml of 20% aqueous ethanol, the sample was left for 3 hours, filtered, concentrated to 10ml using water bath at 90°C. Diethyl ether (5ml) was added and aqueous layer recovered while the ether layer was discarded. Purification process was repeated with 15 ml of n-butanol added and washed twice with aqueous sodium chloride. Remaining solution was dried and saponin content calculated.

$$\text{Percentage saponin} = \frac{W_2 - W_1}{W_0} \times 100$$

Where W<sub>0</sub> = weight of sample

W<sub>1</sub> = weight of evaporating dish

W<sub>2</sub> = weight of evaporating dish + dried extract

### Tannin

A 0.5ml sample extract was mixed with 4.5ml water, FeCl<sub>3</sub> (0.1M, 0.5ml), and potassium ferrocyanate (0.1M, 0.3ml) (AOAC, 1990). Absorbance measured at 720nm. Tannic acid used as standard, results reported as mg TAE/ml.

### Terpenoids

Terpenoids were analyzed following Ghorai *et al.*, (2012). Sample extract (0.5ml) and 3.5ml iced-cold 95% methanol was left for 48 hours. Centrifuged at (400g for 15 mins), 200µl of supernatant and 1.5ml chloroform was allowed to stand for 3 minutes, 100µl conc. H<sub>2</sub>SO<sub>4</sub> was added and allowed to stand in dark for 90 minutes. Decanted supernatant, and the red precipitate were dissolved with 1.5ml of 95% methanol. Absorbance measured at 538nm against 95% methanol (blank). Linalool used as standard.

### Phytate

Young and Greaves' (1999) method was used for phytate determination. Exactly, 2ml sample extract was mixed with 100ml 2% concentrated HCl for 3 hours. Filtrate (25ml) was placed in 250ml beaker and, 50ml distilled water added together with 5ml of 0.3% ammonium thiocyanate which served as an indicator, and titrated with standard iron (III) chloride solution containing 0.00195g iron per ml

$$\text{Phytic acid (\%)} = \text{Titre value} \times 0.00195 \times 1.1952 \times 100$$

### Cardiac Glycosides Determination

Cardiac glycosides were determined using Osagie's (1998) method. Exactly, 1ml extract plus 1ml of 2% 3,5-DNS in methanol and 1ml of 5% aqueous NaOH was boiled for 2 minutes. The (brick red precipitate formed), was then filtered.

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## Antibiotic Sensitivity Testing

### Standardization of Inoculum

A 100 ml volume of 0.5 McFarland standard solution was prepared by combining 99.5 ml of concentrated sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and 0.5 ml of barium chloride dihydrate (BaCl<sub>2</sub>·2H<sub>2</sub>O). This solution was stored in a brown bottle in a dark environment, and a small quantity was withdrawn from it whenever needed for the standardization of the inoculum. Inoculum standardization involved mixing overnight bacterial culture with sterile normal saline (0.85% NaCl) in a test tube and comparing its turbidity to that of the McFarland standards, as described by Cheesbrough *et al.*, (2015).

The bacterial isolates were then plated on Muller Hinton agar plates and incubated for 24 hours. Subsequently, 0.1 ml of the standardized isolates was spread evenly on the agar plates using sterile glass spreaders. For susceptibility testing, a variety of antibiotics were applied to the surface of the agar plates. For gram-negative organisms, this included Gentamycin, Amoxicillin, Erythromycin, Ampicillin, Tetracycline, Augmentin, Chloramphenicol, and Ciprofloxacin. For gram-positive organisms, the antibiotics used were Rocephine, Ampiclox, Septrin, Streptomycin, Erythromycin, Pefloxacin, Ciprofloxacin, Amoxicillin, Gentamicin, and Zinnacef. These plates were then incubated overnight at 37°C.

#### Antimicrobial Susceptibility Testing of Crude Extracts

Antimicrobial activity screening was conducted using the modified agar well diffusion method, following the procedure outlined by (Agu *et al.*, 2013; Agu *et al.*, 2014; Awah *et al.*, 2016; Adindu *et al.*, 2017; Awah *et al.*, 2017; Saeed and Tariq, 2017; Ubaoji *et al.*, 2020). Duplicate plates of Mueller-Hinton Agar (MHA) were prepared, with 20 ml poured into each sterile Petri dish and allowed to solidify. Subsequently, each plate was inoculated with 0.1 ml of the suspension of isolates obtained from an overnight broth culture. The turbidity of the suspension was adjusted to 0.5 of the McFarland scale, equivalent to approximately  $1 \times 10^8$  cfu/ml. The inoculum was evenly spread across the agar surface and allowed to air-dry for 30 minutes. Three holes were aseptically made approximately 1 cm away from the edges of each plate using a sterile cork borer with a diameter of 6 mm. The extracts were reconstituted and the aqueous extracts were dissolved in distilled water, while the ethanol extracts were dissolved in 6% dimethyl sulfoxide (DMSO). A stock solution with a concentration of 100 mg/ml was prepared by dissolving 1 g of each plant extract in 10 ml of the respective diluent. Next, the agar wells were filled with 0.01  $\mu$ l of the extract solution at different concentrations, including 50 mg/ml, 25 mg/ml, and the last well with only the diluent to serve as the control. This entire process was performed in duplicates. The Petri dishes were then incubated at 37°C for a duration of 16 to 18 hours. After incubation, the zones of inhibition around each well were measured using a transparent meter rule and recorded, to the nearest millimeter (mm).

## Results

The result of the phytochemical analysis conducted on *Ocimum gratissimum* leaves indicated the presence of several bioactive compounds. These included ascorbic, saponin, tannin, flavonoids, phenol, alkaloid, terpenoids, glycosides and phytate as detailed in Table 1. The qualitative analysis of ethanolic and aqueous extracts of *Ocimum gratissimum* involves identifying the presence of specific chemical compounds, while quantitative analysis aims to determine the concentrations of these compounds. Both types of analysis are crucial for understanding the chemical composition and potential therapeutic properties of the extracts, which can be valuable in traditional and modern medicine, as well as in research and development for various applications.

Out of the seventy Kunu-zaki samples that were procured, thirty-eight isolates were successfully obtained, the samples were from five different market locations as shown in Table 2. These isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus species* and *Salmonella species*. It is noteworthy that *Escherichia coli* was the most prevalent bacteria as highlighted in Table 3.

Biochemical test which includes gram staining, methyl red, voges proskauer, oxidase, citrate, catalase, motility and sugar fermentation test was further employed to identify the organisms of interest as shown in Table 4. Antibiotic susceptibility test carried out indicated that *Escherichia coli* displayed a notable level of resistance to Ampicillin, chloramphenicol, and erythromycin, followed by *Pseudomonas species* and *Salmonella species*. In contrast, *Staphylococcus species* exhibited the least resistance. However, most of the isolates exhibited relatively lower resistance levels to ciprofloxacin, gentamycin, and Augmentin. Consequently, ciprofloxacin was employed as the antibiotic control, as detailed in Tables 5 and 6.

Antimicrobial susceptibility testing was conducted using crude extracts of *Ocimum gratissimum* ethanolic extract on the isolates. At a concentration of 25 mg/ml, all isolates were inhibited except for *Salmonella species*. When the concentration was increased to 50 mg/ml, only *E. coli* was inhibited, and all the isolates demonstrated sensitivity or susceptibility to the antibiotics employed in the analysis.

Similarly, antimicrobial susceptibility testing was performed using crude extracts of *O. gratissimum* aqueous extract on the isolates. At a concentration of 25 mg/ml, all isolates displayed resistance. However, at the higher concentration of 50 mg/ml, *E. coli* and *Salmonella species* were inhibited. Furthermore, all the isolates exhibited varying degrees of susceptibility to the antibiotics used in the study as control, as detailed in Tables 7 and 8

**Table 1: Phytochemical Analysis of the *Ocimum gratissimum* (Scent leaf)**

Parameter	Aqueous Extract of Scent leaf	Ethanol Extract of Scent leaf
Ascorbic acid (mgAAE/G)	26.30 $\pm$ 1.31	17.61 $\pm$ 1.96
Saponin (mg/g)	1.02 $\pm$ 0.21	0.27 $\pm$ 0.05
Phenol (mgGAE/g)	4.46 $\pm$ 0.29	4.97 $\pm$ 0.22
Flavonoid (mgCE/g)	6.88 $\pm$ 1.25	14.38 $\pm$ 1.25
Tanin (mgTAE/g)	8.32 $\pm$ 0.27	7.59 $\pm$ 0.15
Alkaloids (mg/g)	0.02 $\pm$ 0.03	0.03 $\pm$ 0.01
Phytate	0.20 $\pm$ 0.05	0.24 $\pm$ 0.02
Cardiac Glycosides (mg/g)	0.08 $\pm$ 0.01	0.06 $\pm$ 0.00
Terpenoids (mgL/g)	0.00 $\pm$ 0.00	0.11 $\pm$ 0.02

MgGAE: Milligram Gallic equivalent

MgCE: Milligram Catechin equivalent

MgAAE: Milligram Ascorbic Acid Equivalent

MgL: Milligram Linalool equivalent

Table 2: Location and Number of Samples Collected.

S/N	Location	Number of Samples
1	Eke-Awka	14
2	Amaenyi	14
3	Okpuno	14
4	Tempsite	14
5	Ifite	14

Table 3: Percentage of Most Probable Isolate from Kunu sample

Organism	No. of Isolates	% of Isolates
<i>E. coli</i>	17	45
<i>Pseudomonas specie</i>	9	24
<i>Staphylococcus specie</i>	7	8
<i>Salmonella specie</i>	5	13
Total	38	100

Table 4: Biochemical characteristics of the kunu isolates

Sample no	methylred	V.P	Indole	oxidase	citrate	catalase	motility	glucose	lactose	sucrose	H <sub>2</sub> S	Gas prod	shape	probable organism
1	+	-	+	-	-	+	+	+	+	-	-	+	Rod	<i>E. coli</i>
15	-	-	-	+	+	+	+	+	-	-	-	-	Rod	<i>Pseudomonas specie</i>
40	+	-	-	-	+	+	-	+	+	-	-	-	Cocci	<i>Staphylococcus specie</i>
65	+	-	-	-	-	+	+	+	-	-	+	-	Rod	<i>Salmonella sp</i>

+ ..... positive, - ..... negative .

Table 5: Antibiotic Susceptibility of Gram negative isolates

Antibiotics	<i>E. coli</i>	<i>Pseudomonas</i>	<i>Salmonella</i>
Ampicillin	+	+/-	+
Tetracycline	+	+	+
Erythromycin	+	+	+
Amoxicillin	+	+	-
Gentamicin	-	-	-
Augmentin	-	-	-
Chloramphenicol	+	+	+/-
Ciprofloxacin	-	-	-

Keys: +  $\rightarrow$  Resistant to Antibiotic, -  $\rightarrow$  Sensitive

Table 6: Antibiotic Susceptibility of Gram positive isolates

Antibiotics	Results
Rocephin	-
Ampiclox	+
Seprin	+
Streptomycin	-
Erythromycin	+
Pefloxacin	-
Ciproflaxcin	-
Amoxicillin	+
Gentamicin	-
Zinnacef	-

Keys: +  $\rightarrow$  Resistant to Antibiotics, -  $\rightarrow$  Sensitive

**Table 7:** Antimicrobial Susceptibility Testing of *Ocimum gratissimum* ethanolic extract on the Kunu isoates using ciprofloxacin as control recorded in mm.

	25mg/ml	50mg/ml	Antibiotics control
<i>E. coli</i>	0	1	10
<i>Salmonella sp</i>	2	3	22
<i>Pseudomonas sp</i>	0	1	13
<i>Staphylococcus sp</i>	0	1	14

**Table 8:** Antimicrobial Susceptibility Testing of *Ocimum gratissimum* aqueous extract on the Kunu isolates using ciprofloxacin as control in mm.

	25mg/ml	50mg/ml	Antibiotics control
<i>E. coil</i>	0	1	9
<i>Pseudomonas</i>	0	0	12
<i>Staphylococcus sp</i>	0	0	14
<i>Salmonella sp</i>	0	2	23

## Discussion

The result of the phytochemical analysis conducted on *Ocimum gratissimum* leaves indicated the presence of several bioactive compounds. These included ascorbic, saponin, tannin and flavonoids, while alkaloid, terpenoids, cardiac glycosides and phytate were negligible in amount and it's in accordance with the findings of Alexander, 2016 and Adeniyi *et al.*, 2012.

Umar *et al.*, (2019), also showed the phytochemical components such as anthraquinone, saponins, tannins, terpenoids and alkaloids, were detected in methanol extracts. But, flavonoids, glycosides, phlobatannins and steroids were not in the methanol extracts analysed. While flavonoid was detected in chloroform extract and this is in contrast to the work because aqueous and ethanolic extract were utilized, while Umar *et al.*, (2019) used methanol and chloroform.

The presence of these various metabolites in *Ocimum gratissimum* underscores its potential as a valuable source of phytomedicines. These metabolites are recognized for their diverse pharmacological effects in both humans and animals. They represent naturally occurring chemical constituents in plants that possess medicinal properties and play a role in protecting against diseases, as highlighted by Alexander (2016). These phytochemicals are non-nutritive plant compounds with protective and disease-preventive attributes. Quantitative analyses; aqueous extract of ascorbic acid, saponin, tannin and cardiac glycosides recorded higher parameter than the ethanolic extract of *Ocimum gratissimum*, while higher amount were recorded in other paramters employed.

The different isolates derived from Kunu-zaki consisted of *Escherichia coli* (accounting for 45% of the total), *Staphylococcus species* (18%), *Pseudomonas aeruginosa* (24%), and *Salmonella species* (13%). Among these, *Escherichia coli* emerged as the predominant isolate. This outcome aligns with the findings of Makwin *et al.*, (2013), who also reported *Escherichia coli* as the most prevalent bacterium, followed by *Enterobacter*, *Staphylococcus aureus*, and then *Streptococcus spp*.

It's important to note that these findings contrast with the research conducted by Ekanem *et al.*, 2018, which not only identified bacterial isolates but also fungal isolates. Their results included *Lactobacillus sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Salmonella sp.*, *Escherichia coli*, and *Pseudomonas sp.* among the bacterial isolates, while the fungal isolates encompassed species from *Fusarium*, *Aspergillus*, *Penicillium species* and *Saccharomyces sp*. The presence of such a wide array of isolates in the samples suggests potential issues with hygiene during the production process.

This discovery underscores the apparent lack of adequate knowledge regarding food processing and handling practices among most vendors. Additionally, the absence of access to clean and portable water, along with inadequate storage and waste disposal facilities at both preparation and service points, has resulted in unsanitary conditions. These conditions serve as potential sources of microbial contamination, posing an increased risk to public health, as noted by Sperber, (2003). It is imperative to ensure the safety and microbiological purity of the water used, especially in the post-heating processing of Kunu-zaki, as it may have been a source of contamination. Spices and additives could also have contributed to the contamination (Essien *et al.*, 2009; Lawal, 2012)

Concerning the antibiotic susceptibility patterns, *Escherichia coli* displayed sensitivity to gentamicin, Augmentin, and ciprofloxacin, with the highest sensitivity observed for ciprofloxacin. However, it exhibited resistance to erythromycin, ampicillin, tetracycline, amoxicillin, and chloramphenicol. On the other hand, *Salmonella species* demonstrated sensitivity to gentamicin, Augmentin, amoxicillin, and ciprofloxacin, while being resistant to ampicillin, tetracycline, and erythromycin. *Pseudomonas species* exhibited resistance to tetracycline, erythromycin, amoxicillin, and chloramphenicol, while *Staphylococcus specie* demonstrated sensitivity to Rocephin, Streptomycin, Pefloxacin, Ciprofloxacin, Gentamicin, and Zinnacef.

The resistance to chloramphenicol aligns with the findings of Makwin *et al.*, (2013), which reported high resistance of *E.coli* to chloramphenicol, while the isolates were sensitive to gentamicin, ciprofloxacin, Augmentin, and septrin. This highlights the critical issue of antibiotic resistance among microorganisms, which poses a significant challenge in disease prevention and control efforts.

Furthermore, it's worth noting that the ethanolic extract of *Ocimum gratissimum* demonstrated higher antimicrobial activity compared to the aqueous extract, is in accordance with the findings of Oshim *et al.*, 2019, where all isolates exhibited resistance to the ethanolic extract of *Ocimum gratissimum*.

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## Conclusion

Medicinal plants have held a crucial position in the history of disease treatment and the improvement of human health. The analyzed leaves revealed substantial quantities of phytochemical components with diverse health-promoting attributes. The outcomes of this investigation emphasize the considerable promise of *Ocimum gratissimum* as a natural substitute for addressing a variety of health conditions and antibiotics resistance. The examination of its phytochemical constituents implies the presence of bioactive compounds with potential therapeutic properties. Additionally, the exhibited antibacterial efficacy against isolates from kunu-zaki underscores its ability to combat microbial infections."

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