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Phytochemical, Antioxidant and Antibacterial Properties of Leaves of *Psidium guajava* and *Gongronema latifolium* extracts on wound infective Bacteria

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Abstract

Antibiotic resistance amongst pathogenic bacteria resulting from drug abuse and mutation has been widely discussed. Plant extracts in combination withnanotechnology has improved disease therapy tremendously. This study reports on the phytochemicals and antioxidants inherent in two medicinal plants, namely,*Psidium guajava* (guava) and *Gongronema latifolium* (*utazi*).Phytochemicals and antioxidant properties were determined using standard methods. Antibacterial potentials of plant - mediatenanoparticles synthesized from zinc oxide were assessed against five bacteria isolated from diabetic and non-diabetic patients.Antibacterial activities was determined by measuring the sensitivity on Mueller Hinton Agar, minimum inhibitory concentration and minimum bactericidal concentration of the plant extracts against *Stahylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus haemolyticus*. The activities of some commercial antibiotics (oxoid) were also determined against the wound isolates. Both plants are rich in eight active components known for antibacterial activities. The scavenging abilities of the plants showed high free radicals antioxidants relevant in disease control and plant based food protection. Antibacterial activities of the green plants synthesized from ethanol and ethyl acetate showed tremendous inhibition against the test isolates. *Klebsiella pneumoniae* and *Staphylococcus haemolyticus* were highly susceptible compared *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* on ethanol extracts. The activities of the commercial antibiotics was significant compared to the plant extracts. The antibacterial potentials of the zinc nano synthesized extracts of guava and *utazi* has added to the pool of knowledge in plant therapy on wound isolates.

Keywords: antioxidants, phytochemicals, antimicrobials, wound bacteria

Introduction

Globally, microbial infections are a major cause of public health issues, and as a result of the widespread use of commercial antibiotics, numerous antibiotic resistances in human and animal pathogens are not only widespread but increasing rapidly (Oyama *et al*., 2016). The ability of microorganisms like bacteria and fungi to thrive despite exposure to antimicrobial

(antibacterial or antifungal) treatments intended to restrict their growth is known as antimicrobial resistance (AMR) (Schwarz *et al*., 2016). A genetic mutation that dramatically modifies a pathogenic microbe's structure or physiology enables it to avoid or resist the effects of an antimicrobial treatment (Purssell, 2019). The selection pressure brought about by the appropriate and inappropriate use of antimicrobial drugs in people and animals further hastens this naturally occurring process.

According to Schwartz *et al*. (2016), AMR genes (ARGs) acquired through horizontal gene transfer (HGT) (transformation, transduction, and conjugation) from other microorganisms confer innate and/or acquired mechanisms on microorganisms, such as the absence of a drug target site and/or enzymatic drug degradation. Microbial resistance to antibiotics has been predicted to the point of certainty, and its inevitable appearance has been noted since the earliest stages of antibiotic research and clinical practice. However, the emergence of the most well-known resistant microbial strains in the last 25 years has made the problem one that poses a threat to life (Chokshi *et al.,* 2019). AMR is a significant health problem that affects everyone in the world, and as a result, there is growing concern about the damage it poses to human life (Urbaniak *et al.*, 2018; McCann *et al.*, 2019)**.**

Phytochemicals are recognized as bioactive components in traditional herbal medicines used in herbal formulations (Tanaka and Kashiwada 2022). The use of medicinal plants (phytobiotics) for disease treatment has been explored over a period of time, and it is now getting more interest, because plants are believed to possess phytoconstituents with antimicrobial activities (Akharaiyi and Oyama, 2019). Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases (Chaudhary *et al.,* 2023). *Gongronema latifolium*, commonly called '*utazi*' used as spice and vegetable in traditional folk medicine has been explores for its numerous in Nigeria medicinal properties of all parts of *G. latifolium* have been exploited by different ethnic groups in Nigeria (Amrelia 2022; Mosango *et al*., 2022).

Guava (*Psidium guajava* L.) is a very unique and traditional plant which is grown due to its diverse medicinal and nutritive properties (Kumar *et al*., 2021). Guava leaves are also widely used for their antispasmodic, cough sedative, anti-inflammatory, antidiarrheic, antihypertension, anti-obesity, and antidiabetic properties. The presence of a unique variety of bioactive polyphenolic compounds, like quercetin and other flavonoids, and ferulic, caffeic, and gallic acids, present in guava leaves primarily determine their bioactive and therapeutic properties.

This study evaluates the efficacy of two medicinal plants for the control of antibiotic resistant bacteria associated with wound infection.

Materials and Methods

Collection of plants material:

Fresh plant leaves of *Psidium guajava* (Guava) and *Gongronema latifolium* (*Utazi*) were obtained within the premises of Owerri west Local Government Secretariat at about 7:45 am on the 12th of July 2023 and identified by Mr. Iroka Finian a botanist in the Department of Botany, Nnamdi Azikiwe University Awka. Voucher specimens for *Psidium guajava* (NAUH – 03^{A}) and *Gongronema latifolium* (NAUH 034^{C}) were deposited at Nnamdi Azikiwe University Herbarium in the Department of Botany. The leaves were brought to the laboratory, stripped from their stems and place on a clean area of the laboratory floor for air drying. This was done according to methods described by Aneta *et al*. (2022).

Extraction of Plants material

The active components of the plants part were extracted using sterile distilled water for phytochemical and antioxidant analysis as well as ethanol and ethyl acetate for antibacterial analysis. Adopting nanotechnology, Zinc nitrate was used for the extraction of active antimicrobial

components from the ethanol and ethyl acetate extracts.

The aqueous and organic solvents extracts were filtered using Ashless No. 42 filter paper and the filtrate air-dried and used for phytochemical and antibacterial analysis. The nano extract was used to determine the antimicrobial potentials of the leaf extracts against clinical bacterial species such as Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus haemolyticus and Klebsiella sp isolated from wounds adopting the methods described by (Elvino et al., 2022).

Preparation ZnO Nanoparticles

Zinc oxide (ZnO) nanoparticles was synthesized by mixing plant extract with clear solution with 0.5 Mm solution of hydrated zinc sulfate/zinc oxide/zinc nitrate and boiling the above mixture at desired time and temperature to get effective mixing. The reaction showing the change in colour revealed confirmation of ZnO nanoparticles (Pranjali *et al.*, 2019).

Phytochemical Screening

Phytochemicals such as alkaloids, flavonoids, tannins, saponin, steroids, glycosides, anthraquinone, and terpenoids were determined according to the methods described by Maria *et al*. (2018), Edori *et al*. (2019), Thilagavathi *et al*. (2015) and Smyslova*et al*. (2019).

Preparation of Plant extracts

Aqueous extract

Fifty grams (50 g) of the leaves (powdered material) was soaked in 400 ml of distilled water. This was heated to boil in a water bath. The mixture was stirred at regular intervals (3-5 minutes) and left to stand for 24 h and filtered with *Ashless No. 42 filter paper*. The filtrate was concentrated in hot water bath at 80^{o}C for 5 h. The filtrate was then refrigerated at 4^{o}C (Smyslova *et al*., 2019).

Antioxidant Preparation and Analysis

This was prepared by dissolving 0.004 g of DPPH in 100 ml of ethanol and mix properly in a shaker and kept in cool dark place until used. 2 ml of DPPH solution was added into 2 ml of plant extract and incubated in the dark for 20 mins at room temperature (Baliyan et al., 2022).

The result of the reaction was read in a spectrophotometer calibrated at 517 nm. The absorbance of the extract was read and recorded in triplicates. The percentage (%) radical scavenging activity of the plant extract was calculated from the absorbance reading using the formula below:

$$\% \text{ RSA} = \frac{\text{Abs of control - Abs of sample}}{\text{Abs of control}} \times 100$$

Where: RSA is the radical scavenging activity
Abs control is the absorbance of DPPH radical + ethanol
Abs sample is the absorbance of plant samples

Preparation of Test Organisms

Pure cultures of test organisms were sub-cultured on nutrient broth at 37^{o}C for 24 h (*Naqqash* et al., 2022). Test isolates were standardized by McFarland 0.5 turbidity equivalent to 1.5 x 10^{8} Cfu/ml (Cheesbrough, 2003).

Antibacterial Susceptibility of bacterial isolates using Plant Extracts

Susceptibility of the test isolates to the extract was done by well-in-agar diffusion assay. Four wells of 6.25 mm deep were made with a sterile cork borer on Mueller Hinton Agar previously seeded with the 24 h old standardized broth cultures. The wells were filled with different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml) of the ethanol and ethyl acetate extracts separately. The plates were incubated for 24 hours at 37^{0}C. Zones of inhibition around the wells were measured and

recorded in millimeters (mm) in duplicates (Cheesbrough 2003).

Minimum Inhibitory Concentration (MIC) Assay

The Minimum Inhibitory Concentration Assay determines the lowest concentration of a particular antibiotic needed to kill an organism. This procedure was done according to Tomasz-Swebocki *et al*. (2023).

Serial dilution of the extracts (at different concentrations of 500 $mgml^{-1}$, 250 $mgml^{-1}$, 1.25 $mgml^{-1}$ and 62.5 $mgml^{-1}$) were added to a growth medium (nutrient broth) in separate test tubes. These tubes were then inoculated with24 h standardized test isolates and incubated overnight. The MIC of the toxicant (plant extract) is the lowest concentration that does NOT show growth. This was confirmed using the spectrophotometer at 340 nm.

Minimum Bactericidal Concentration (MBC) Assay

Minimum Bactericidal Concentration is the lowest number of bacteria recorded on the plate after 24 h incubation on nutrient agar. A loop full of the different concentrations (after spectrophotometric reading) were streaked on a freshly prepared surface driednutrient agar and incubated overnight. Concentrations of growth after incubation wasused to determine the MBC **(**Tomasz-Swebocki *et al*., 2023).

Antibiotic Susceptibility/Sensitivity Test using Commercial (Oxoid) Antibiotics

Commercial antibiotics (oxoid) of known concentrations were placed at equidistant on freshly prepared and surfaced dried Mueller Hinton Agarpreviously seeded with standardized pure cultures of test organisms and incubated at 37^0C for 24 h. Zone of inhibition (ZOI) was measured and recorded after incubation. The resistance, sensitivity and intermediate activities of the organisms were compared with the Clinical and Laboratory Standards Institute (CLSI, 2018).

Results

The leaves of *Psidium guajava and Gongronema latifolium* contains active phytochemicals. Saponis, tannin, terppenoid and glycosides are present in significant amount (Table 1). This result was confirmed in the quantitative analysis shown in Table 2. The % RSA values of the two plants is high suggesting the presence of free radical scavengers in the plants (Table 3).

Table 1 Qualitative Phytochemical Composition of Plant Extracts

Phytochemical parameters	Extract of *Psidium guajava*	Extract of *Gongronema latifolium*
Saponin	+++	+++
Flavonoid	++	+
Tannin	+++	+++
Alkaloid	++	+
Anthraquinone	+	+
Terpenoid	+++	++
Glycosides	+++	+++
Steroids	+++	+

+, Low; ++, Moderate; +++, High

Table 2 Quantitative Phytochemical Composition of Plant Extracts (n=5)

Phytochemical parameters	Extract of *Psidium guajava*	Extract of *Gongronema latifolium*
Saponin	1.021±0.01	0.912±0.01
Flavonoid	0.623±0.05	0.328±0.01
Tannin	0.991±0.01	1.112±0.05
Alkaloid	0.451±0.01	0.213±0.01
Anthraquinone	0.122±0.02	0.100±0.01
Terpenoid	1.006±0.01	0.567±0.02
Glycosides	0.851±0.05	0.925±0.01
Steroids	1.002±0.01	0.122±0.01

Table 3 Antioxidant Assay of Plant Extracts (n=5)

Plant Extract	Absorbance	% RSA Values
Guava	2.590±0.5	13.67±0.5
Utazi	2.498±0.5	16.73±0.5

RSA, radical scavenging activity; Ads control; 3.000; SD±; Standard Deviation from mean values

The four isolates are resistant to rifampicin, ceftriaxone and tetracycline, but sensitive to levofloxacin and ciprofloxacin. *Escherichia coli* and *Staphylococcus haemolyticus* are resistanceto seven antibiotics used, whereas *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* are sensitive to five antibiotics. *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* aresensitive to cefotaxime,ceftazidine and Amikacin respectively, whereas *Staphylococcus aureus* and *Staphylococcus haemolyticus* showed resistance (Table 4).

Table 5 shows the sensitivity profile of the different concentrations of the zinc nono synthesized ethanol extract of guava leaf extract (toxicant) compared to the control (CIP). High activities of the plant extract was expressed in the 500 mg/ml and 250 mg/ml concentrations. 500 mg/ml concentration was significant in activity for the zinc nono synthesized ethyl alcohol extract of guava leaf extract against the four bacterial isolates (Table 6). *Staphylococcus aureus* was resistant to all the concentrations in Table 6.

Table 4 Sensitivity Profile of Commercial Antibiotics against Bacteria Isolated from Wounds (n=5)

Bacterial Isolates	C	GEN	RD	TE	CTX	CAZ	AMK	CRO	CIP	LEV
Pseudomonas aeruginosa	S	S	R	R	R	R	S	R	S	S
E. coli	R	R	R	R	S	R	R	R	S	S
Staphylococcus aureus	R	S	R	R	R	R	R	R	S	S
Klebsiella pneumoniae	S	S	R	R	R	S	R	R	S	S
Staphylococcus heamolyticus	R	S	R	R	R	R	R	R	S	S

C, Chloramphenicol; GEN, Gentamicin; RD, Rifampicin; TE, Tetracycline; CTX, cefotaxime; CAZ, ceftazidine; AMK, Amoxyillin Clavulanic Acid; CRO, ceftriaxone; CIP, Ciprofloxacin; LEV, Levofloxacin, CLSI Standard.

Table 5 Sensitivity and zone of inhibition of Zinc Nano synthesized Ethanol Extract of Guava Leaf on Wound Bacterial Isolates (n=5)

Test isolates	CIP	500 mg/ml	250 mg/l	125 mg/l	62.5 mg/l
Staphylococcus aureus	43±0.01	25±0.01	15±0.05	0±0.00	0±0.00
Pseudomonas aeruginosa	35±0.01	20±0.01	20±0.01	0±0.00	0±0.00
Escherichia coli	40±0.05	20±0.05	15±0.01	0±0.00	0±0.00
Staphylococcus epidermidis	42±0.01	15±0.01	10±0.02	10±0.05	0±0.00
Klebsiella sp	30±0.02	13±0.05	10±0.01	0±0.00	0±0.00

SD±; Standard Deviation from mean values; Measurement in mm diameter; CIP, Ciprofloxacin; mg/ml; milligram per milliliter

Table 6 Sensitivity and zone of inhibition of Zinc Nano synthesized Ethyl Acetate Extract of Guava Leaf on Wound Bacterial Isolates (n=5)

Test isolates	CIP	500 mg/ml	250 mg/l	125 mg/l	62.5 mg/l
Staphylococcus aureus	43±0.01	0±0.00	0±0.00	0±0.00	0±0.00
Pseudomonas aeruginosa	35±0.02	12±0.01	0±0.00	0±0.00	0±0.00
Escherichia coli	40±0.05	10±0.01	0±0.00	0±0.00	0±0.00
Staphylococcus epidermidis	42±0.05	15±0.01	0±0.00	0±0.00	0±0.00
Klebsiella pneumoniae	30±0.01	15±0.01	0±0.00	0±0.00	0±0.00

Tables 7 and 8 shows the antibacterial potentials of zinc nano synthesized ethanol and ethyl acetate leaf extracts of *Utazi* respectively against wound isolates. Concentrations at 500 mg/ml and 250 mg/ml are significant. *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* are highly susceptible compared to the other isolates. *Staphylococcus aureus* showed high resistance (Table 7) and *Escherichia coli* (Table 8).

Table 7 Sensitivity and zone of inhibition of Zinc Nano synthesized Ethanol Extract of *Utazi* Leaf on Wound Bacterial Isolates (n=5)

Test isolates	CIP	500 mg/ml	250 mg/l	125 mg/l	62.5 mg/l
Staphylococcus aureus	43±0.01	10±0.01	0±0.00	0±0.00	0±0.00
Pseudomonas aeruginosa	35±0.01	15±0.01	0±0.00	0±0.00	0±0.00
Escherichia coli	40±0.01	15±0.01	10±0.01	0±0.00	0±0.00
Staphylococcus haemolyticus	42±0.01	35±0.01	20±0.01	18±0.01	15±0.01
Klebsiella pneumoniae	30±0.01	20±0.01	12±0.01	10±0.01	0±0.00

SD±; Standard Deviation from mean values; Measurement in mm diameter; CIP, Ciprofloxacin; mg/ml; milligram per milliliter

Table 8 Sensitivity and zone of inhibition of Zinc Nano synthesized Ethyl Acetate Extract of *Utazi* Leaf on Wound Bacterial Isolates (n=5)

Test isolates	CIP	500 mg/ml	250 mg/l	125 mg/l	62.5 mg/l
Staphylococcus aureus	43±0.01	30±0.01	10±0.01	0±0.00	0±0.00
Pseudomonas aeruginosa	35±0.01	20±0.01	10±0.01	0±0.00	0±0.00
Escherichia coli	40±0.01	15±0.01	0±0.00	0±0.00	0±0.00
Staphylococcus haemolyticus	42±0.01	20±0.01	20±0.01	10±0.01	10±0.01
Klebsiella pneumoniae	30±0.01	20±0.01	15±0.01	10±0.01	0±0.00

Minimum inhibitory concentration of zinc nano-synthesized ethanol and ethyl acetate extracts of guava and *utazi* leaves on wound bacterial isolates are shown in Tables 9 and 10 respectively. Concentrations at 500 mg/ml and 250 mg/ml are significant in activity for *E. coli* and *K. pneumoniae.* Other organisms showed varied activities in the concentrations of 125 mg/ml and 62.5 mg/ml. Minimum bactericidal concentrations of ethanol and ethyl acetate extracts of guava and *utazi* leaves are shown in Tables 11 and 12. Lower concentrations of 125 mg/ml and 62.5 mg/ml exhibited higher activities compared to the higher concentrations of 500 mg/ml and 250 mg/ml.

Table 9 Minimum Inhibitory Concentration (MIC) of Zinc Nano-synthesized Ethanol and Ethyl Acetate Extracts of Guava Leaf on Wound Bacterial Isolates (ƛ=340 nm)

Zinc Nano-synthesized Ethanol Extract of Guava Leaf	Wound Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
	Escherichia coli	1.261±0.01	1.185±0.01	1.320±0.01	1.082±0.01
	Pseudomonas aeruginosa	1.786±0.02	1.745±0.01	1.649±0.05	1.448±0.02
	Staphylococcus aureus	1.462±0.01	1.710±0.02	1.295±0.01	2.097±0.01
	Staphylococcus haemolyticus	1.124±0.01	1.449±0.01	1.881±0.05	1.509±0.05
	Klebsiella pnuemoniae	1.423±0.05	1.664±0.05	1.745±0.01	1.787±0.05
Zinc Nano-synthesized Ethyl Acetate Extract of Guava Leaf					
	Escherichia coli	0.458±0.01	1.407±0.05	1.129±0.01	1.574±0.01
	Pseudomonas aeruginosa	1.477±0.05	1.263±0.01	1.850±0.02	1.575±0.02
	Staphylococcus aureus	1.605±0.01	1.611±0.01	1.472±0.02	1.917±0.03
	Staphylococcus haemolyticus	1.516±0.02	1.406±0.05	1.361±0.01	1.182±0.01
	Klebsiella pneumoniae	1.654±0.01	1.386±0.05	1.504±0.01	1.480±0.01

Table 10 Minimum Inhibitory Concentration (MIC) of Zinc Nano-synthesized Ethanol and Ethyl Acetate Extract of*Utazi* Leaf on Wound Bacterial Isolates (ƛ=340 nm)

Zinc Nano-synthesized Ethanol Extract of *Utazi* Leaf (Z)	Wound Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
	Escherichia coli	1.351±0.01	1.206±0.01	1.157±0.02	1.194±0.02
	Pseudomonas aeruginosa	1.041±0.01	1.134±0.02	1.162±0.01	1.201±0.05
	Staphylococcus aureus	1.376±0.05	1.541±0.01	1.527±0.05	1.906±0.01
	Staphylococcus epidermidis	1.759±0.01	1.158±0.02	1.124±0.05	1.247±0.01
	Klebsiella pneumoniae	1.601±0.05	1.185±0.01	1.247±0.01	1.129±0.05
Zinc Nano-synthesized Ethyl Acetate Extract of *Utazi* Leaf (V)					
	Escherichia coli	1.124±0.01	1.599±0.02	1.662±0.01	1.541±0.05
	Pseudomonas aeruginosa	1.873±0.05	1.843±0.01	2.401±0.01	2.891±0.01
	Staphylococcus aureus	1.581±0.05	1.500±0.05	1.613±0.01	1.750±0.01
	Staphylococcus epidermidis	1.082±0.01	1.592±0.01	1.131±0.05	1.489±0.02
	Klebsiella pneumoniae	1.214±0.01	1.662±0.05	1.124±0.01	1.231±0.05

Table 11 Minimum Bactericidal Concentration (MBC) of Guava Plants Extracton Wound Bacterial Isolates (n=5)

Zinc Nano-synthesized Ethanol Extract of Guava Leaf	Bacterial Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
	Escherichia coli	-	-	+++	+++
	Pseudomonas aeruginosa	+	++	+++	+++
	Staphylococcus aureus	-	++	+++	+++
	Staphylococcus haemolyticus	-	-	+	++
	Klebsiella pneumoniae	-	+	+++	+++

Zinc Nano-synthesized Ethyl Acetate Extract of Guava Leaf	*Bacterial Isolates*	500 mg/ml	250 mg/l	125 mg/mi	2.5 mg/ml
	Escherichia coli	-	-	-	-
	Pseudomonas aeruginosa	-	+++	+++	+++
	Staphylococcus aureus	+	+++	+++	+++
	Staphylococcus haemolyticus	-	-	+++	+++
	Klebsiella pneumoniae	-	-	+++	+++

Table 12 Minimum Bactericidal Concentration (MBC) of *Utazi* Plants Extract on Wound Bacterial Isolates (n=25)

Zinc Nano-synthesized Ethanol Extract of *Utazi* Leaf	Bacterial Isolates	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
	Escherichia coli	-	-	-	-
	Pseudomonas aeruginosa	-	+	+++	+++
	Staphylococcus aureus	+	+++	+++	+++
	Staphylococcus haemolyticus	-	+	+++	+++
	Klebsiella pneumoniae	-	+	+++	+++
Zinc Nano-synthesized Ethyl Acetate Extract of *Utazi* Leaf	**Bacterial Isolates**	**500 mg/ml**	**250 mg/l**	**125 mg/mi**	**62.5 mg/ml**
	Escherichia coli	-	-	-	-
	Pseudomonas aeruginosa	-	-	+++	+++
	Staphylococcus aureus	+	+	+++	+++
	Staphylococcus epidermidis	-	+++	+++	+++
	***Klebsiella* sp**	-	-	+++	+++

Discussion

Antimicrobial resistance (AMR) is a huge challenge to public health and directly impacts global economic growth negatively, with developing countries in Africa bearing the biggest burden of the adverse effects of AMR (Ayukekbong *et al.,* 2017; Morel *et al,* 2020; Meybodi *et al.*, 2021). AMR is also the ability of microorganisms to persist or grow in the presence of drugs designed to inhibit or kill them. This results in therapeutic failure, which negatively impacts the global control and management of infectious diseases (Meybodi *et al.*, 2021).

Antibiotics are important anti-infective agents which have been used since the 20th century for the treatment of human infections (Hutchings *et al*., 2019; Walesch *et al*., 2023). The β-lactam antibiotics are clinically important antimicrobial medicines and have remained the first-line chemotherapeutic intervention against Gram-positive and Gram-negative bacteria since the 1950s (Hutchings *et al*., 2019; Soliman *et al*., 2023). Bacterial resistance to β-lactams has increased substantially in past few decades (Jani *et al.,* 2021; Zaatout *et al*., 2021; Mutuku *et al.*, 2022; Tan *et al*., 2023). However, their irrational, injudicious, and excessive use is on steady rise which not only worsen the issue of antibiotic resistance but also results in their accumulation around the environment as micro-pollutant (Fazaludeen-Koya *et al*., 2022; Gitter *et al*., 2023). The emergence of antibiotic resistance has threatened the effective treatment of microbial infections (Sanz-García *et al*., 2023) hence the search for newer drugs of plant origin.

Guava and *utazi* leaves showed high concentration of phytochemicals and antioxidants capable of use as antimicrobial agents (Tanaka and Kashiwada 2022). Phytochemicals have been reported to exert strong antibacterial activity against several microbes associated with diseases (Akharaiyi and Oyama, 2019).Antioxidants have the ability to scavenge free radicals in the human body and have been suggested to contribute to the protective effect of plant-based foods on diseases (Chaudhary *et al.,* 2023).

The use of nanotechnology in the fortification of drugs had proven enormous advantages and improvement in medicine and antibacterial applications (Faryad *et al*., 2022; Sergio *et al*., 2023). Thus, green nanotechnology using plant extract has open up new possibilities for the synthesis of novel nanoparticles with desirable characteristics such as highly penetrable ability into the cells of microorganisms. This feat was demonstrated in the results obtained from this study. Extracts from ethanol exhibited greater potentials in the inhibition of microbial growth at higher concentrations compared to ethyl acetate extracts.

Staphylococcus haemolyticus and *Klebsiella pneumoniae* are highly susceptible compared to the other isolates. *Staphylococcus aureus* and *Escherichia coli* showed higher resistance.

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