



## **Comparative Study of *Ocimum gratissimum* Mediated Nanoparticles and Conventional Antibiotics against Endophytic Leguminous Bacteria and Selected Clinical Isolates**

**Oludare Temitope Osuntokun<sup>1\*</sup>, Olayemi Stephen Bakare<sup>1</sup>,  
Owolabi Mutolib Bankole<sup>2</sup> and O. Ajayi Ayodele<sup>3</sup>**

<sup>1</sup>*Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.*

<sup>2</sup>*Centre for Bio-Computing and Drug Development (CBDD), Adekunle Ajasin University, Nigeria.*

<sup>3</sup>*Department of Microbiology, Federal University Oye Ekiti, Oye Ekiti, Nigeria.*

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors OTO, OSB, OMB and OAA. Author OTO designed the materials and methods used in the course of the research work. Authors OTO and OSB designed the antimicrobial assay procedure. Author OAA designed the materials and methods used for the isolation and characterization of endophytic leguminous bacteria. Author OMB performed the preparation and synthesis of *Ocimum gratissimum* mediated nanoparticles. Author OTO wrote the final draft of the manuscript. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/AJARR/2020/v13i130301

#### Editor(s):

(1) Dr. Longan Shang, Zhejiang University, China.

(2) Dr. Maria Luisa Kennedy Rolon, National University of Asuncion, Paraguay.

(3) Dr. Nadia Sabry El-Sayed El-Gohary, Mansoura University, Egypt.

#### Reviewers:

(1) Camila Martins de Oliveira, Universidad Federal de Rondonópolis, Brazil.

(2) Dinesh Singh, ICAR - Indian Agricultural Research Institute, India.

(3) Benmati Mahboubba, National School Superieure De Biotechnologie, Algeria.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59147>

**Received 24 May 2020**

**Accepted 30 July 2020**

**Published 07 August 2020**

**Original Research Article**

### **ABSTRACT**

The purpose of this research work is to compare the antimicrobial activities of conventional antibiotics and *Ocimum gratissimum* mediated nanoparticle against microorganisms isolated from endosphere of leguminous plants and selected clinical organisms. The microorganisms are

\*Corresponding author: Email: [osuntokun4m@gmail.com](mailto:osuntokun4m@gmail.com), [oludare.osuntokun@aaau.edu.ng](mailto:oludare.osuntokun@aaau.edu.ng);

becoming a resistant reservoir to conventional antibiotics, there is a great need, to research into plant mediated nanoparticles, to combat this great menace of resistant organisms, this therefore was the basic hypothesis of this research work. The Endophytic Leguminous plants used for this research work were *Mucuna pruriens*, *Calopogonium mucunoides* and *Vigna unguiculata*. The plants were randomly collected from Federal College of Agriculture, Akure (7.2704° N, 5.2241° E). Endophytic bacteria were isolated, identified and characterized from the endospheres of the leguminous plants using biochemical test, sugar fermentation and Bergey's manual. The antimicrobial susceptibility test for endophytic isolates and clinical isolates were performed using Kirby-Bauer disc diffusion technique. The same procedure was repeated for the two sulfur nanoparticles. The sulfur nanoparticles were synthesized using Sodium thiosulfate pentahydrate, citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>,AR) with leaf extract of *Ocimum gratissimum*, SNP1 and SNP2 (Solid reflectance spectrum); SNP1/SNP2 was synthesized in the presence/absence of *O. gratissimum* and characterized on shimadzu UV-VIS-NIR spectrophotometer UV-3100 with a MPCF-3100 sample compartment. The probable number of organisms isolates from the leguminous plants ranges between 10<sup>9</sup>-10<sup>12</sup> cfu and all the endophytic isolates were found to be Gram positive rods. The probable organisms isolated from the leguminous plants were *Microbacterium lacticum*, *Cellulomonas flavigena* and *Bacillus* spp including; *B. pumilus*, *B. subtilis* and *B. amylolyticus*. *Bacillus subtilis*. All endophytic bacteria were resistant to antimicrobial susceptibility test using Cephalosporins (oxid) and multiple susceptibility disc with a diameter between 11mm and 20mm. Endophytic bacteria were susceptible to the SNP1 sulfur nano particles with a diameter between 9mm and 14mm diameter zones of inhibition. The clinical isolates were susceptible to multiple susceptibility disc with of diameter of 11mm and 20mm. Antimicrobial susceptible to SNP1 ranges between 18-20mm zones of inhibition to SNP2. This study discovered that nanoparticles has better antimicrobial efficacy against both endophytic isolated bacteria and clinical isolates. The present of *Ocimum gratissimum* drastically increases the efficacy of sulphur mediated nanoparticles. it can be deduced that the *Ocimum gratissimum* mediated nanoparticle as a potential alternative to antibiotics in this widespread antibiotics resistance era.

**Keywords:** *Conventional antibiotics; Ocimum gratissimum; biosynthetic sulfur nanoparticles; endophytic leguminous bacteria.*

## 1. INTRODUCTION

The microbiome of plants is complex and dynamic, this plant are increasingly being considered as holobionts commonly inhabited by endophytic microbial communities [1,2]. Indeed, endophytes are ubiquitous and have been found in all species of plants studied to date [1]. The term "endophyte" is derived from the Greek words "endon" meaning within, and "phyton" meaning plant. The microorganisms that can behave as endophytes are easy to describe taxonomically, they can be classified as archaea, bacteria, fungi, protozoans and viruses. However, in the absence of a really clear-cut accepted definition, it is more difficult to describe what features of these microorganisms qualify them as endophytes [3,4,5]. More recently, Haridoim et al. [1] defined endophytes as microbes including bacteria, archaea, fungi, and protists that colonize the plant interior regardless of the outcome of the association, In recent years, many endophytes have been identified through culture-independent approaches such as sequencing of the 16S rRNA gene, the internal

transcribed spacer regions, ITS1 and ITS2, or through whole genome sequencing of endophyte communities [6]. During the plant-endophyte interaction, ROS-detoxification occurs early on, after entry of the endophyte into the plant [7,8,9].

A genomic survey using comparative genomics of endophyte strains hypothesized that many genes involved in biofilm production, adhesion, and motility contribute to plant colonization and the endophytic life style within the host plant [10-14].

Cowpea, *Vigna unguiculata*, is a climbing annual in the family Fabaceae grown for its edible seeds and pods. The cowpea plant is usually erect and possess ribbed stems and smooth trifoliate leaves which are arranged alternately on the stems. The plant produces clusters of flowers at the end of a peduncle (flower stalk) and 2-3 seed pods per peduncle. The seed pods are smooth, cylindrical and curved, reaching up to 35 cm (10 in) in length, with distinctive coloration, usually green, purple or yellow. Cowpea can

reach in excess of 80 cm (31.5 in) in height [14,15].

Velvet bean (*Mucuna pruriens* (L.) DC. var. *utilis*) is a leguminous vine. It is annual or sometimes short-lived perennial in the Family Fabaceae. Velvet bean is vigorous, trailing or climbing, up to 6-18 m in length [16]. It has a taproot with numerous, 7-10 m long, lateral roots. The stems are slender and slightly pubescent [17]. The leaves are generally slightly pubescent, alternate, trifoliolate with rhomboid ovate, 5-15 cm long x 3-12 cm broad, leaflets [16]. The inflorescence is a drooping axillary raceme that bears many white to dark purple flowers. The velvet bean seeds are variable in colour, ranging from glossy black to white or brownish with black mottling. Seeds are oblong ellipsoid, 1.2 to 1.5 cm long, 1 cm broad and 0.5 cm thick [16;17].

Calopo (*Calopogonium mucunoides*) is a vigorous, hairy annual or short-lived perennial trailing in the legume family, Fabaceae. It can reach several meters in length and form a dense, tangled mass of foliage, 30-50 cm deep. The root system is dense and shallow, at most 50 cm deep. The stems are succulent, covered with long, brown hairs. They are creeping in the lower parts, sometimes rooting at the nodes that come in contact with the soil. The upper part of the stem is twining. The leaves are up to 16 cm long and trifoliolate. The hairy leaflets are 4-10 cm long x 2-5 cm broad, ovate to elliptical. The inflorescence is a slender hairy raceme that may be up to 20 cm long and bears 2 to 12 blue or purple small flowers. The fruits are 3-8 seeded hairy pods, 2-4 cm long [18].

Nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics. Bacterial infections are a major cause of chronic infections and mortality. Antibiotics have been the preferred treatment method for bacterial infections because of their cost-effectiveness and powerful outcomes. The primary reason why NPs are being considered as an alternative to antibiotics is that NPs can effectively prevent microbial drug resistance in certain cases. The rampant use of antibiotics has led to the emergence of numerous hazards to public health, such as superbugs that do not respond to any existing drug and epidemics against which medicine has no defense [19].

The search for new, effective bactericidal materials is significant for combating drug resistance, and NPs have been established as a

promising approach to solve this problem. The most serious concern with antibiotic resistance is that some bacteria has becoming resistant to almost all of the easily and readily available antibiotics. These bacteria are able to cause serious disease thereby result to a major public health problem. Bacteria from the environment which were supposed to be neutral amidst the global resistance crisis were not left of the antimicrobial resistant war. Therefore, the need to finding effective alternative means to combat antibiotics resistant becomes important; hence resulted to this project study. The aim of this study is to use leguminous endophytic bacteria and selected clinical isolate to explore sulfur mediated nanoparticles of medicinal plant origin as an alternative antimicrobial agents [20].

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Samples

Mature plant specimen of three leguminous plants; *Mucuna pruriens*, *Calopogonium mucunoides* and *Vigna unguiculata* were randomly collected from the school farm of Federal College of Agriculture, Akure (7.2704° N, 5.2241° E) in triplicates.

### 2.2 Collection of Clinical Isolates

Pure cultures of Five [5] selected clinical isolates; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella pullorum* were collected from the Microbiology laboratory of Adekunle Ajasin University for this study. These isolates were pathogenic and have been known to cause human and poultry diseases. All the culture were grown and maintained in nutrient broth at 37°C and used for further studies.

### 2.3 Bacteria Isolation from Leguminous Plants

The samples collected were separately washed with tap water, followed by surface sterilization using 70% ethanol for 30 seconds, 2% Sodium hypochlorite (NaOCl) for 5 minutes, 3% Hydrogen peroxide for 30 seconds and then rinsed five times with distilled water, to remove epiphytic microbes. Ten grams of these samples were cut to 2-3 cm pieces and macerated using sterilized mortar and pestle with 12.5 mM potassium phosphate buffer (pH 7.1), followed by a 10-fold serial dilution where 0.5ml of the 10<sup>-3</sup> dilution was plated using the pour plate method on Nutrient Agar supplemented with

cycloheximide (100 µg/mL) to inhibit fungal growth. Inoculated Petri plates were incubated at 37°C for 24 hours. After the incubation time, the colony forming units (CFU) for each plate was estimated [21]. Isolates differing in morphological appearance was selected and were streaked onto new plates until pure cultures were obtained. Pure cultures of bacterial isolates were maintained on NA slants and were stored at 4°C [21].

## 2.4 Efficiency of Surface Sterilization for Bacteria Isolation

The efficiency of surface sterilization procedure was necessarily checked by imprint method, also success of the surface sterilization method was confirmed by the absence of any microbial growth on media plates impregnated with 50 µl aliquots of the final rinse water. This was done to avoid isolation of epiphytic organisms, the surface sterilization was considered successful as there was no growth [22].

## 2.5 Identification of Bacteria Isolates from Leguminous Plants

Preliminary identification of the bacterial isolate where based on their morphological

characteristics and result from various biochemical tests carried out on them. Each of the endophytic isolate were cultured on media and observed after 24 h of incubation for their morphological characteristics. Biochemical characteristics were determined according to the methods of [23] and examined according to the Bergey's Manual of Determinative Bacteriology [24]. This was done to confirm the identity of the endophytic bacteria isolates.

### 2.5.1 Morphological characterization bacteria isolates from leguminous plants

The appearance of the colony of each isolate on the agar media was studied and characteristics such as shape, edge, pigment, opacity, elevation and surface were observed as described by [23].

### 2.5.2 Gram staining bacteria isolates from leguminous plants

This test was carried out on 24h old cultures of the isolate in order to determine their gram reaction and cellular morphology. The gram reaction differentiates bacteria into gram positive and gram-negative bacteria. A smear of the culture between 18 to 24 hours was prepared on a clean grease free microscope slide with a drop

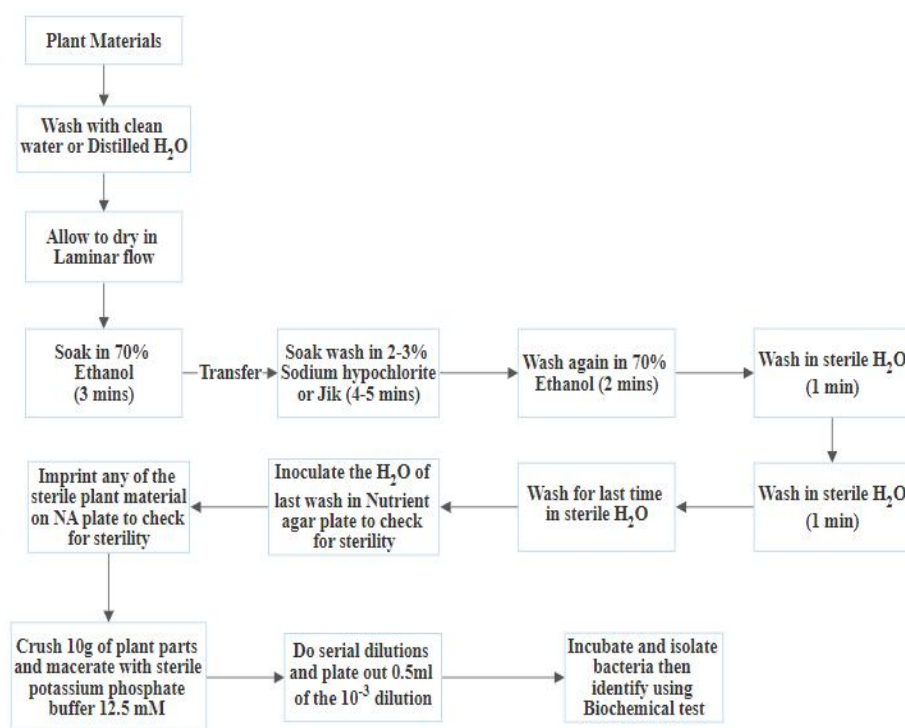


Plate 1. Schematic diagram showing procedure for Isolation of endophytic bacteria

of sterile distilled water and mixed. The smear was allowed to dry and then heat-fixed by passing the slide over a spirit flame once or twice. The heat-fixed smear was flooded with crystal violet and allowed to stain for 60 s after which the stain was poured off and rinsed with water, the slide was flooded with iodine solution and will be allowed to stand for another 60 s and then poured off and rinsed with water, the smear was decolorized with 95% ethanol and rinsed immediately after 10 s with water. The smear was further counter stained with safranin for another 60 s after which was gently rinsed off with water. It was air-dried and examined under the oil immersion objectives of 100x light microscope. The gram reaction, shape and arrangement of cell were then recorded [25].

### **2.5.3 Biochemical characterization of bacterial isolates from leguminous plants**

#### *2.5.3.1 Catalase test*

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide with the release of oxygen as bubble. With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide. 1- 2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative [25].

#### *2.5.3.2 Oxidase test*

This was carried out using oxidase reagent as described by Baker et al. (2001). Two drops of freshly-prepared oxidase reagent (1% aqueous tetramethyl-p-phenylene diamine hydrochloride solution) were placed on a piece of filter paper. A part of the colony of the bacterial isolate was collected using one end of sterile grease free glass slide and smeared across the filter paper impregnated with the oxidase reagent and observed for deep purple colour within 10s [26].

#### *2.5.3.3 Citrate test*

The agar used in citrate test is Simmon's Citrate Agar, it is used to test an organism ability to utilize citrate as a source of energy. The medium contain citrate as the sole carbon source and inorganic ammonium salts ( $\text{NH}_4\text{HPO}_4$ ) as the source of nitrogen. It was done by dissolving the agar and gently heat with mixing and boiling until

it dissolved. A 5 ml was dispensed into each tube and autoclaved at 121°C for 15 min. It was cooled and slanted before the test organism was streaked with a light inoculum. It was then be incubated aerobically at 37°C for 5 days and examined. Those that changed from original colour (green) to blue or yellow were considered positive. While those that retained the green colour were considered negative. Uninoculated control tubes were included then the results were then recorded [25].

#### *2.5.3.4 Indole test*

Some microbes are capable of hydrolyzing the amino acid tryptophan and one of the end products is indole. The latter reacts with 4-dimethyl amino benzylaldehyde to form a dark red dye. Each of the bacterial isolates were cultured in sterile nutrient broth for 48h at 37°C. 2mls of chloroform were added to the broth culture and mixed gently. About 2mls of Kovac's reagent were later added, shaken gently and allowed to stand for 20min. a red colour at the reagent layer indicates indole production [26].

#### *2.5.3.5 Starch hydrolysis*

A plate containing 1% starch agar was streaked with the test organisms across its surface. The plate was incubated at 37°C for 18-24 hours. The plate was flooded with Grant's iodine after incubation and left for about 30 seconds unhydrolyzed starch formed a blue coloration with the iodine while hydrolyzed starch appears as a clear zone along the streak resulting from alpha-amylase activity [27].

#### *2.5.3.6 Sugar fermentation test*

A situation whereby carbohydrates are utilized in the partial or total absence of oxygen is referred to as fermentation. But sugar is utilized in the presence of oxygen such reactions are known as oxidation. Sterilization of the basal medium was done using an autoclave at 121°C for 15min. Ten percent (10%) sterile solution of the test sugar (glucose, fructose, mannitol, dextrose and galactose) was added, inverted Durham tubes were put into each tube. Different isolates were inoculated into each test tube according to the labeling using a sterile inoculating loop. Un-inoculated tubes serve as controls. The results were examined daily for up to 7days in which methyl red indicator changed to yellow. A yellow coloration indicated growth and acid production. Also, the upper part of the Durham tubes was

examined to detect any accumulated gas which indicated gas production. [25] Results were then recorded.

## 2.6 Antibiotic Susceptibility Test for the Bacterial Isolates

The test was performed to determine the phenotypic resistant of the bacterial isolates to commonly used antibiotics. These tests were carried out following the Kirby-Bauer disc diffusion method of [28]. Inoculum from culture of bacteria isolates on nutrient agar slants were inoculated into test tubes containing sterilized nutrient broth and incubated at 37°C for 18h which serve as the stock for the test. Mueller-Hinton agar was prepared and sterilized, then dispensed into sterilized Petri dishes. The plates were allowed to gel and cool for about 15min so as to allow it to gel and excess surface moisture to be absorbed. The inoculum was introduced into plates by streaking before applying the antibiotics impregnated discs. Two types of discs were used; Cephalosporin antibiotic discs (Oxoid); Cefuroxime (30 µg), Cefprozil (30 µg), Cefoxitin (30 µg), Cefpodoxime (10 µg), Cefepime (30 µg) and Multi-test Predetermined commercial Gram negative and Gram positive discs which were applied to the surface of the well labeled inoculated agar plated aseptically using sterile forceps. The discs were then placed firmly by slightly pressing on the inoculated plates with the sterilized forceps to ensure complete contact with the agar. After 24h of incubation, each plates was examined, susceptibility to each antibiotics were indicated by a clear zone. The zone of inhibition were measured using a calibrated ruler was held on the back of the inverted petri plate and was recorded [29].

## 2.7 Preparation and Synthesis of *Ocimum gratissimum* Mediated Nanoparticles

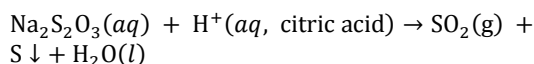
### 2.7.1 Preparation of leaf extract of *Ocimum gratissimum*

Fresh leaves of *Ocimum gratissimum* were collected from campus of Adekunle Ajasin University, Akungba, and used as source of nanoparticles preparation with deionized water used throughout the experiments. The fresh leaves of *O. gratissimum* were removed from their stalks and washed thoroughly with running tap water to remove any attached particles or debris, and finally washed with deionized

water. Subsequently, the leaves were allowed to dry for 3-4 weeks at room temperature to remove the adsorbed moistures. The dried leaves were ground into fine powders in a clean agate mortar and stored in a tight container for further use. Biomolecule contents of the leave were extracted by adding 20 g of the powdered leaves to 100 mL deionized water in a 250 mL beaker, and heated to 100°C for 60 min. The crude greenish extract was filtered through Whatmann No. 1 filter paper and stored in the refrigerator at -10 prior to the preparation of sulfur nanoparticles [30].

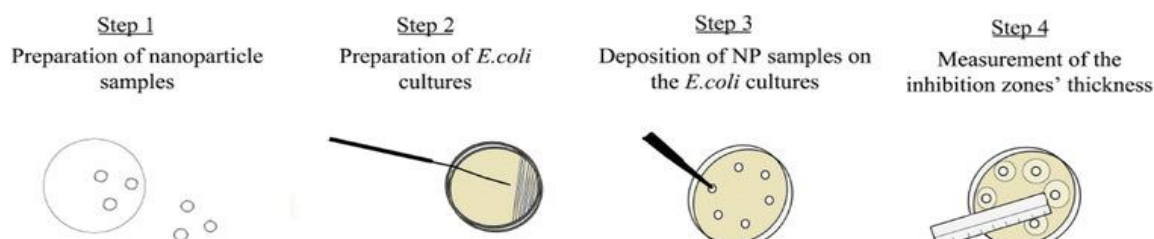
### 2.7.2 Biosynthesis of sulfur/*Ocimum gratissimum* mediated nanoparticles (SNP-1/ SNP-2), Scheme 1

Synthesis of sulfur nanoparticles in the presence or absence of *O. gratissimum* plant extract is as follows: sodium thiosulfate pentahydrate (0.403 M) and 50 mL of *O. gratissimum* leaf extract were to 150 mL deionized water and allowed to stir on a magnetic stirrer at room temperature for 30 min. Then aqueous solution of citric acid (2.42 M, 50 mL) was added drop wise under stirring to allow the precipitation of sodium thiosulfate as sulfur nanoparticles and SO<sub>2</sub> Scheme 1, in accordance with previous report [31]. The mixture was stirred for additional 1 hour and was allowed to stand undisturbed for 5 h for complete disproportionation of sodium thiosulfate. Biosynthesized sulfur nanoparticles was collected by centrifugation, washed with deionized water and EtOH, and dried in an oven at 50 °C for 24 h. For comparison, sulfur nanoparticles in the absence of leaf extract was also prepared using Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> and citric acid but without leaf extract. Sulfur nanoparticles prepared in the presence and absence of plant extract are named SNP – 1 and SNP – 2 , respectively [30].



### 2.7.3 Material characterizations using UV-VIS-NIR spectrophotometer Uv-3100

The solid reflectance spectra of prepared SNP – 1 and SNP – 2 were recorded on a Shimadzu UV-VIS-NIR Spectrophotometer UV-3100 with a MPCF-3100 sample compartment with samples mounted between two quartz discs which fit into a sample holder coated with barium sulfate. The spectra were recorded over the wavelength range of 800-250 nm, and the scans were



**Plate 2. Diagram showing susceptibility test using Sulfur/*Ocimum gratissimum* Mediated Nanoparticles (SNP-1/ SNP-2) (Modified Kirby-Bauer Method) [32]**

conducted at a medium speed using a 20 nm slit width. Infra-red spectra were recorded on a Thermo Fisher Scientific FTIR spectrophotometer, using pressed KBr pellets. Transmission electron microscope (TEM) image was recorded using a JEOL JEM-2010 electron microscope operating at 200 KV. The XRD spectra were obtained with Bruker D8 ADVANCE diffractometer (Germany) using Cu K $\alpha$  (1.5406 Å) radiation. Surface morphology and elemental composition of sulfur nanoparticles were analysed using scanning electron microscope (SEM) equipped with energy dispersive analysis of X-ray equipment (EDAX) (XL 30 FEG ESEM) [30].

### 2.8 Antibiotic Susceptibility Test for Bacterial Isolates Using Sulfur/*Ocimum gratissimum* Mediated Nanoparticles (SNP-1/ SNP-2)

The antibacterial effect of two sulfur nanoparticles; prepared in the presence and absence of *O. gratissimum* plant extract was tested against both endophytic bacteria and selected clinical isolates using the modified Kirby-Bauer method [32]. In the modified Kirby-Bauer method, 0.5 cm diameter wells were made on a Muller Hinton agar plates using a sterile cork borer after inoculating the microorganism using the swab technique. A known concentration of the sulfur nanoparticles was introduced into the wells and incubated at 37°C for 24 h. Antimicrobial activity was calculated by measuring the zone of clearance in the agar plate.

### 3. RESULTS

Table 1 shows the description of the three (3) leguminous plant specimens collected for isolation of Endophytic bacteria. All the plant specimen (100%) collected were mature at the time of sample collection.

Table 2 shows the colony morphology of the isolated leguminous endophytic bacteria based on parameter of Pigment, shape, surface, opacity, edge and elevation.

Table 3 shows the Gram staining of the isolated leguminous endophytic bacteria. All the isolates (100%) were gram positive and rod shaped.

Table 4 shows biochemical characteristics of isolated leguminous endophytic bacteria. All the isolates tested positive to Catalase, Citrate and Starch hydrolysis; END 4 from *Vigna unguiculata* was Oxidase positive, END 3 from *Calopogonium mucunoides* was Indole positive while the other endophytic bacteria tested negative to Oxidase and Indole respectively.

Table 5 shows Sugar fermentation and gas production of the leguminous endophytic bacteria isolates. All isolates except END 3 tested positive to fermentation of Mannitol, D-Glucose, Fructose and Dextrose while only END 1 (*Mucuna pruriens*) and 5 (*Calopogonium mucunoides*) tested positive to fermentation of Galactose with differences in gas production.

Table 6 shows the probable identity of the leguminous endophytic bacteria isolates.

**Table 1. Description of leguminous plant specimen collected**

Plant Specimen	Description
<i>Mucuna pruriens</i>	Mature, Long vines, Tap root, Slender stem, ovate leaves.
<i>Calopogonium mucunoides</i>	Mature, Hairy, Trailing, succulent stem, Trifoliolate leaves, Hairy leaflets.
<i>Vigna unguiculata</i>	Mature, Glabrous, Taproot, Roots with large nodules, Procumbent stem.

**Table 2. Colony morphology of bacterial isolates from endosphere of leguminous plant**

Isolate code	Plant specimen	Pigment	Shape	Surface	Opacity	Edge	Elevation
END 1	<i>Mucuna pruriens</i>	White to yellow	Irregular	Smooth	Opaque	Undulate	Flat
END 2	<i>Mucuna pruriens</i>	White spreading	Irregular	Smooth	Opaque	Undulate	Flat
END 3	<i>Calopogonium mucunoides</i>	Creamy	Circular	Dull	Transparent	Lobate	Raised
END 4	<i>Vigna unguiculata</i>	Creamy	Irregular	Smooth	Opaque	Undulate	Flat
END 5	<i>Calopogonium mucunoides</i>	Thick white	Circular	Smooth	Transparent	Entire	Low convex
END 6	<i>Calopogonium mucunoides</i>	White spreading	Irregular	Smooth	Opaque	Undulate	Flat
END 7	<i>Vigna unguiculata</i>	White spreading	Irregular	Smooth	Opaque	Undulate	flat

Key: END 1 - *Mucuna pruriens*; END 2 - *Mucuna pruriens*; END 3 - *Calopogonium mucunoides*; END 4 - *Vigna unguiculata*; END 5 - *Calopogonium mucunoides*; END 6 - *Calopogonium mucunoides*; END 7 - *Vigna unguiculata*

**Table 3. Gram staining of bacteria isolated from endosphere of leguminous plants**

Isolate Code	Plant Specimen	Gram	Microscopy	Inference
END 1	<i>Mucuna pruriens</i>	+ ve	rod	Gram + rod
END 2	<i>Mucuna pruriens</i>	+ ve	rod	Gram + rod
END 3	<i>Calopogonium mucunoides</i>	+ ve	rod	Gram + rod
END 4	<i>Vigna unguiculata</i>	+ ve	rod	Gram + rod
END 5	<i>Calopogonium mucunoides</i>	+ ve	rod	Gram + rod
END 6	<i>Calopogonium mucunoides</i>	+ ve	rod	Gram + rod
END 7	<i>Vigna unguiculata</i>	+ ve	rod	Gram + rod

**Table 4. Biochemical test of bacterial isolates from endosphere of leguminous plant**

Isolate code	Plant specimen	Catalase	Oxidase	Indole	Simmons citrate	Starch hydrolysis
END 1	<i>Mucuna pruriens</i>	+ ve	- ve	- ve	+ ve	+ ve
END 2	<i>Mucuna pruriens</i>	+ ve	- ve	- ve	+ ve	+ ve
END 3	<i>Calopogonium mucunoides</i>	+ ve	- ve	+ ve	+ ve	+ ve
END 4	<i>Vigna unguiculata</i>	+ ve	+ ve	- ve	+ ve	+ ve
END 5	<i>Calopogonium mucunoides</i>	+ ve	- ve	- ve	+ ve	+ ve
END 6	<i>Calopogonium mucunoides</i>	+ ve	- ve	- ve	+ ve	+ ve
END 7	<i>Vigna unguiculata</i>	+ ve	- ve	- ve	+ ve	+ ve

Key: END 1 - *Mucuna pruriens*; END 2 - *Mucuna pruriens*; END 3 - *Calopogonium mucunoides*; END 4 - *Vigna unguiculata*; END 5 - *Calopogonium mucunoides*; END 6 - *Calopogonium mucunoides*; END 7 - *Vigna unguiculata*



**Table 5. Sugar fermentation result of leguminous endophytic bacteria isolates**

Isolate code	Plant Specimen	Mannitol	D-Glucose	Fructose	Galactose	Dextrose
END 1	<i>Mucuna pruriens</i>	+ve ++	+ve +	+ve +	+ve -	+ve ++
END 2	<i>Mucuna pruriens</i>	+ve +	+ve +	+ve -	-ve -	+ve -
END 3	<i>Calopogonium mucunoides</i>	-ve -	-ve -	-ve -	-ve -	-ve -
END 4	<i>Vigna unguiculata</i>	+ve ++	+ve -	+ve ++	-ve -	+ve ++
END 5	<i>Calopogonium mucunoides</i>	+ve +	+ve +	+ve +	+ve +	+ve -
END 6	<i>Calopogonium mucunoides</i>	+ve +	+ve -	+ve -	-ve -	+ve ++
END 7	<i>Vigna unguiculata</i>	+ve +	+ve +	+ve +	-ve -	+ve -

Key: Subscript – Gas production; END 1 - *Mucuna pruriens*; END 2 - *Mucuna pruriens*; END 3 - *Calopogonium mucunoides*; END 4 - *Vigna unguiculata*; END 5 - *Calopogonium mucunoides*; END 6 - *Calopogonium mucunoides*; END 7 - *Vigna unguiculata*

**Table 6. Probable identity of leguminous endophytic bacteria isolates**

Isolate Code	Plant Specimen	Probable Organism
END 1	<i>Mucuna pruriens</i>	<i>Bacillus pumilus</i>
END 2	<i>Mucuna pruriens</i>	<i>Bacillus subtilis</i>
END 3	<i>Calopogonium mucunoides</i>	<i>Microbacterium lacticum</i>
END 4	<i>Vigna unguiculata</i>	<i>Bacillus amylolyticus</i>
END 5	<i>Calopogonium mucunoides</i>	<i>Cellulomonas flavigena</i>
END 6	<i>Calopogonium mucunoides</i>	<i>Bacillus subtilis</i>
END 7	<i>Vigna unguiculata</i>	<i>Bacillus subtilis</i>

Key: END 1- *Bacillus pumilus*; END 2- *Bacillus pumilus*; END 3- *Microbacterium lacticum*; END 4- *Bacillus amylolyticus*; END 5- *Cellulomonas flavigena*; END 6- *Bacillus subtilis*; END 7- *Bacillus subtilis*

Fig. 1 shows the weight in Grams (g) of the three (3) leguminous plants specimen collected for endophytic bacteria isolation. All the plants specimen (100%) weighed 10,9 and 7g.

Fig. 2 shows the enumeration of isolates from Endophytic leguminous Bacteria ( $10^3$ )Cfu. (number of isolates) from the three (3) leguminous plant. Twenty-two (22) isolates were obtained from *Mucuna pruriens* and was highest number of isolates. Nine (9) and sixteen (16) isolates were obtained from *Calopogonium mucunoides* and *Vigna unguiculata* respectively.

Fig. 3 shows the diameter (in mm) of the zone of inhibition of the conventional antibiotics (oxid) against the tested isolates. All the isolates (100%) were resistant to all of the antibiotics of the cephalosporin family used.

Fig. 4 shows the diameter (in mm) of the zone of inhibition of the multiple susceptibility disc antibiotics against endophytic bacteria isolates. All the isolates (100%) were resistant to Ampiclox, Zinnacef and Amoxicillin but susceptible to Pefloxacin. All the isolates except *Bacillus pumilus* were susceptible to

Cirpofloxacin. *Microbacterium lacticum*, *Bacillus amylolyticus* and *Cellulomonas flavigena* were susceptible to Streptomycin, Septrin and Pefloxacin.

Fig. 5 shows the diameter (in mm) of the zone of inhibition of the multiple susceptibility disc antibiotics against selected clinical isolates. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to Pefloxacin at diameter zone of 20.00mm. *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to Streptomycin, Septrin, Erythromycin, Ampiclox and Rocepin with diameter zones ranging from 17.00-20.00mm. *Salmonella pullorum* was susceptible to Streptomycin and Septrin with diameter zone of 17.00mm while *Klebsiella pneumoniae* was resistant to 90% (9 of 10) of antibiotics.

Fig. 6. shows the diameter (in mm) of the zone of inhibition of the sulfur nanoparticles against endophytic bacteria isolates. *Bacillus amylolyticus* and *Cellulomonas flavigena* isolates showed susceptibility diameter zone of 14.00mm to sulfur nanoparticles (SNP1) mediated with *Ocimum gratissimum* plant extract. All the isolates

(100%) were resistant to sulfur nanoparticles (SNP2) synthesized in the absence of *Ocimum gratissimum* plant extract.

mediated with *Ocimum gratissimum* plant extract with diameter of 20.00mm; *Escherichia coli* and *Salmonella pullorum* showed the susceptibility diameter zone of 18.00mm; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed resistance. All tested clinical isolates were resistant to the other sulfur nanoparticles (SNP2) synthesized in the absence of *Ocimum gratissimum* plant extract.

Fig. 7 shows the diameter (in mm) of the zone of inhibition of the sulfur nanoparticles tested against selected clinical isolates. *Staphylococcus aureus* was observed to have the highest susceptibility to sulfur nanoparticles (SNP1)

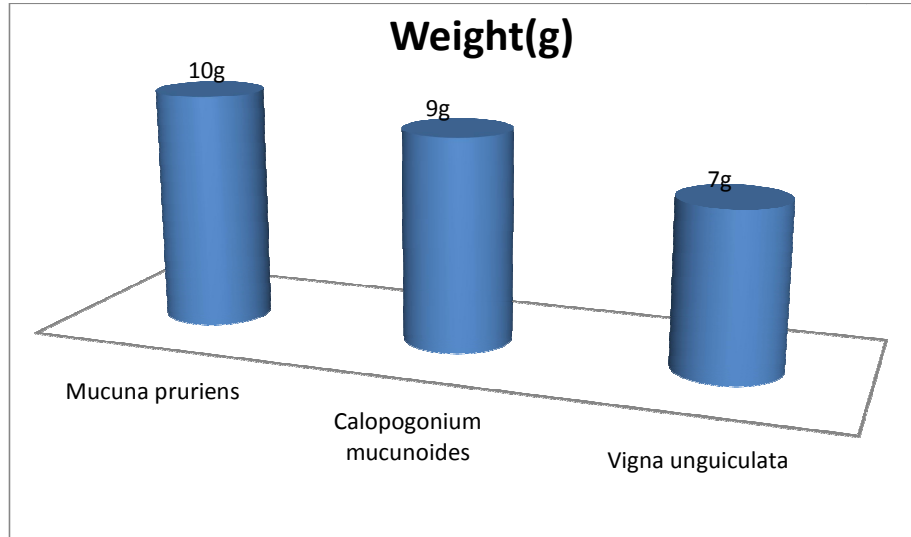


Fig. 1. Weight of Leguminous Plant Specimen (g)

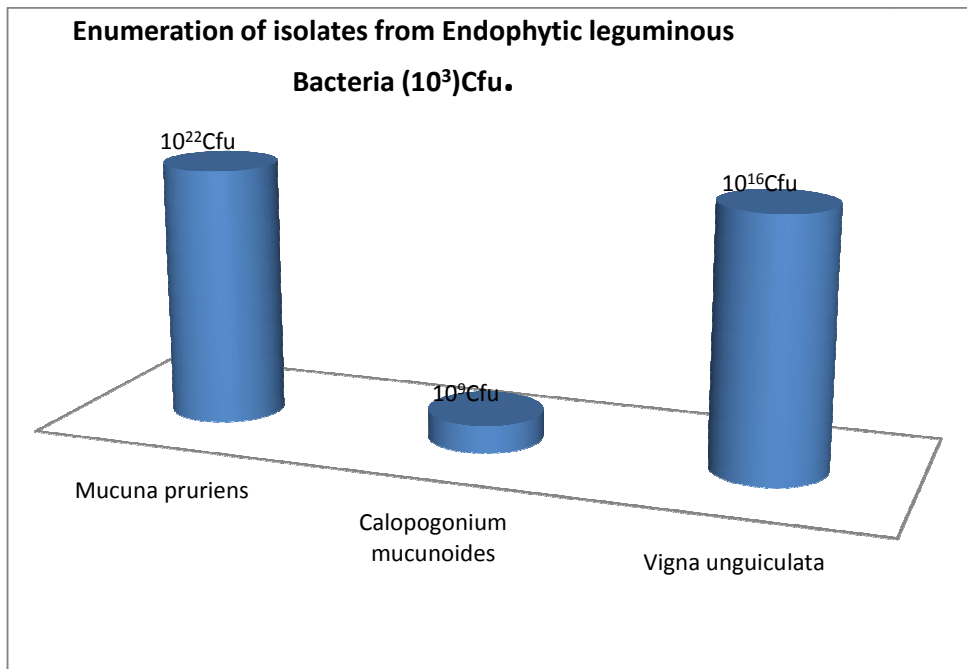
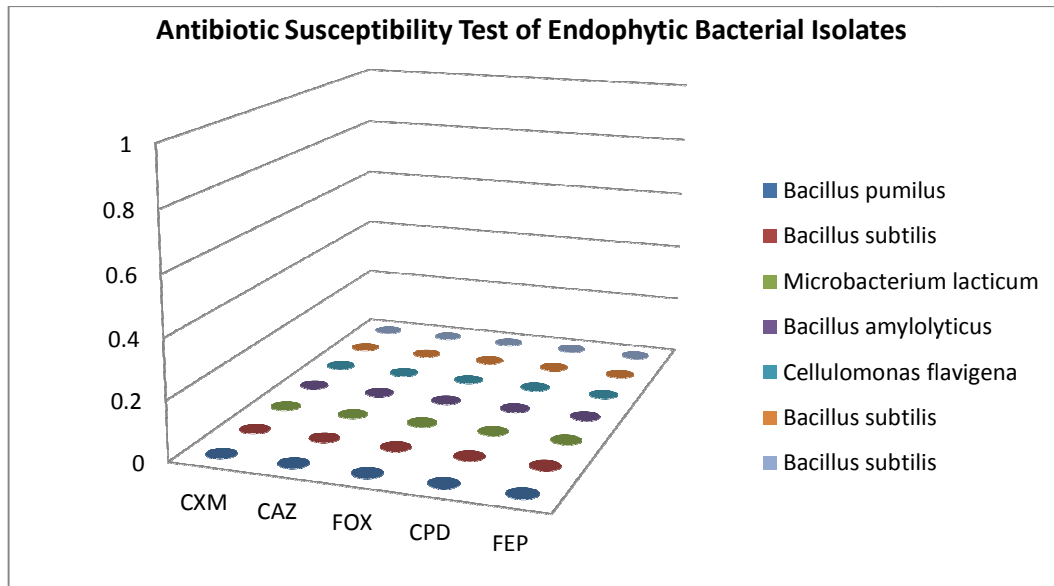
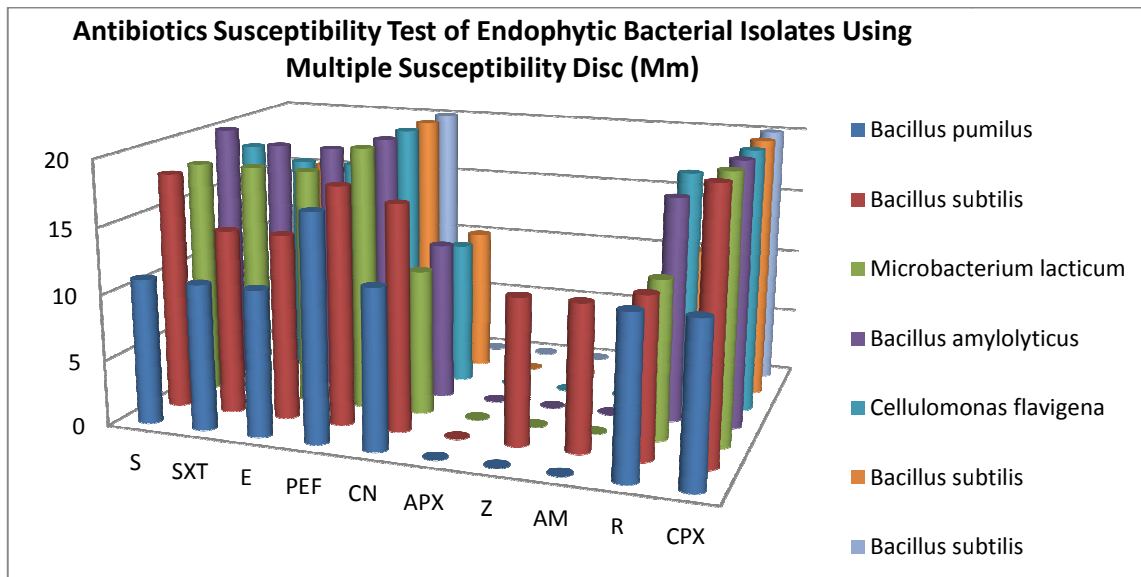


Fig. 2. Enumeration of isolates from Endophytic leguminous Bacteria (10<sup>3</sup>)Cfu



**Fig. 3. Antibiotic susceptibility test of endophytic bacterial isolate using cephalosporins (Oxoid) (mm) (Resistant isolates)**

Key: CXM – Cefuroxime (30 µg); CPD – Cefpodoxime (10 µg); CAZ – Ceftazidime (30 µg)  
FEP – Cefepime (30 µg); FOX – Cefoxitin (30 µg); 0.00 – No Inhibition



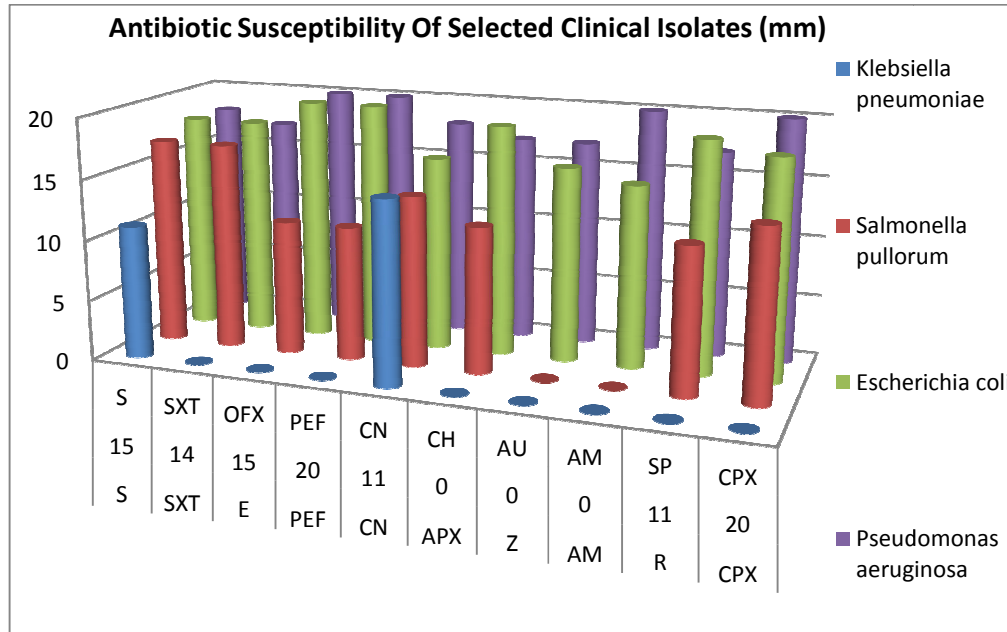
**Fig. 4. Antibiotics susceptibility test of endophytic bacterial isolates using multiple susceptibility disc (mm) (Susceptible Isolates)**

#### 4. DISCUSSION

The problem of antibiotic resistance continues to be a menace both in the clinical setting and in the environment, which is mainly attributed to excessive agricultural usage, the inappropriate and excessive use of antimicrobials

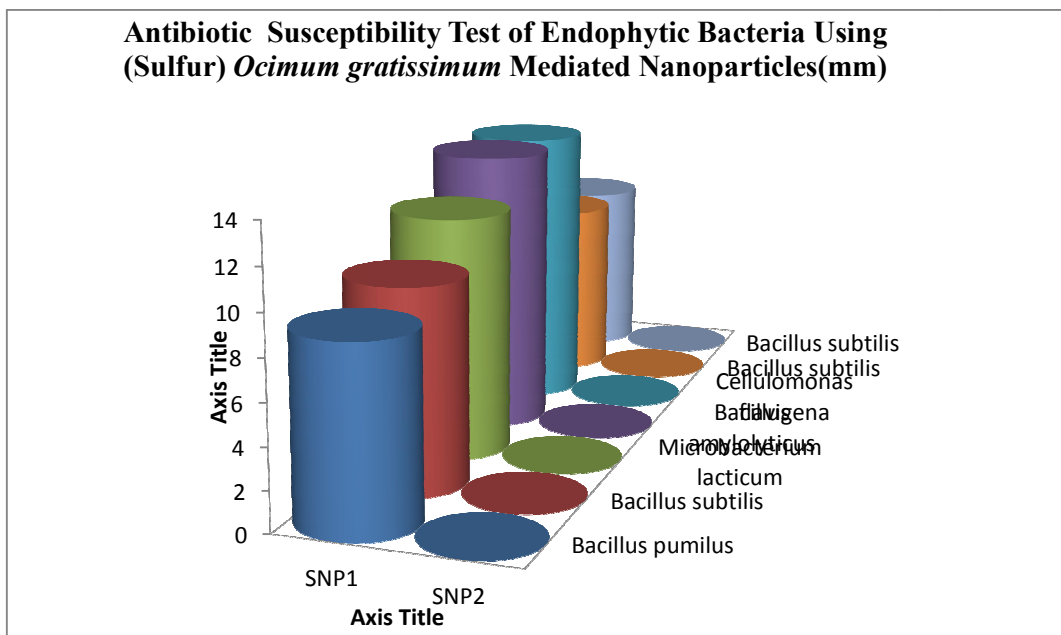
in humans and animals, and many other factors. The search for novel antimicrobials requires crucial action, antibacterial applications of nanotechnology are gaining importance to prevent the catastrophic consequences of antibiotic resistance through the use of nanoparticles [33]. The purpose of this research

work is to compare the antimicrobial activities of conventional antibiotics and *Ocimum gratissimum* mediated nanoparticle against microorganisms isolated from endosphere of leguminous plants and selected clinical organisms.

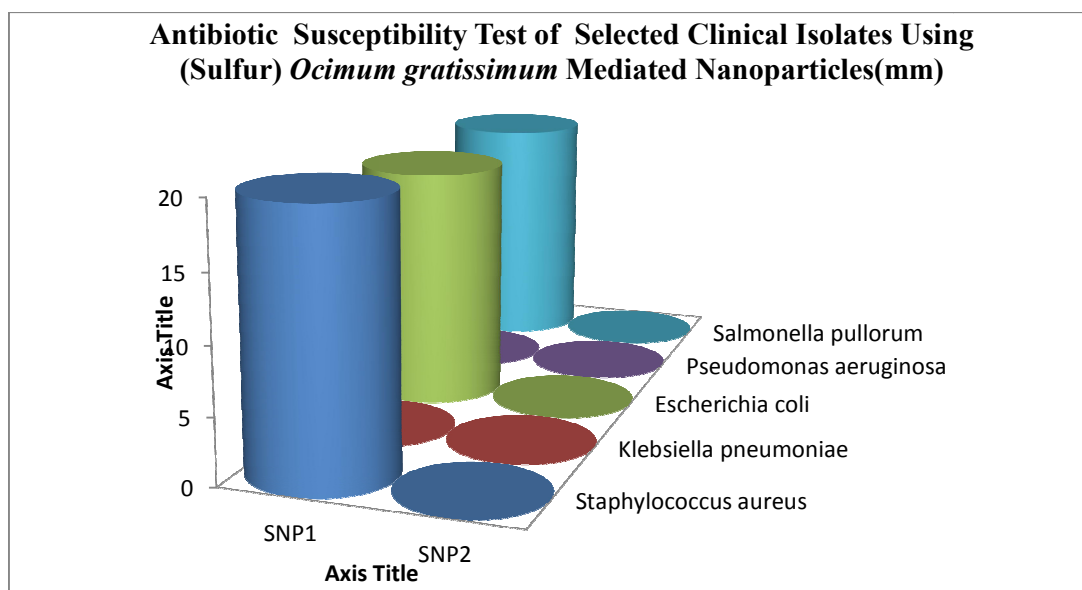


**Fig. 5. Antibiotic Susceptibility of Selected Clinical Isolates (mm) (Susceptible Isolates)**

Key:  $\geq 17$  Sensitive, 16-12 Intermediate  $\leq 11$  Resistant; S – Streptomycin 30 $\mu$ g  
 APX – Ampiclox 30 $\mu$ g; SXT – Septrin 30 $\mu$ g; Z – Zinnacef 20 $\mu$ g; E – Erythromycin 10 $\mu$ g; AM – Amoxicillin 30 $\mu$ g  
 PEF – Pefloxacin 30 $\mu$ g; R – Rocepin 25 $\mu$ g; CN – Gentamycin 10 $\mu$ g; CPX – Cirpofloxacin 10 $\mu$ g  
 OFX – Tarivid; 10 $\mu$ g CH – Chloranphenicol; 30 $\mu$ g AU – Augmentin 30 $\mu$ g; SP – Sparfloxacin 10 $\mu$ g; 0.00 – No Inhibition



**Fig. 6. Antibiotic susceptibility test of endophytic bacteria (Sulfur) *Ocimum gratissimum* mediated nanoparticles (mm) (Susceptible Isolates)**



**Fig. 7. Antibiotic Susceptibility Test of Selected Clinical Isolates Using (Sulfur) *Ocimum gratissimum* Mediated Nanoparticles (mm) (Susceptible Isolates)**

Key: SNP 1 - Sulfur Nanoparticle 1 SNP 2 - Sulfur Nanoparticle 2 0.00 - No inhibition

In a logical approach, the use of leguminous endophytic bacteria and selected clinical isolates to explore (sulfur) *Ocimum gratissimum* mediated nanoparticle (SNPs) as an alternative antimicrobial agent as well as comparing its efficacy with antibiogram of the same organisms.

From literature, clinical organisms are known to possess the predisposed factors of antimicrobial resistant, due to multiple use of antibiotic during and after treatment of infection, there is need to isolate pure microorganism void of antibiotics treatments. The basic reason during this research work, the microorganisms were isolated from endophyte of leguminous plant.

In this study, a total of seven (7) bacteria were isolated from the endosphere of three leguminous plants; *Mucuna pruriens*, *Calopogonium mucunoides* and *Vigna unguiculata*. The endophytic bacteria isolates were biochemically and microbiologically characterized through grain staining and biochemical test which include citrate, indo, oxidase etc. the isolated organism were *Microbacterium lacticum*, *Cellulomonas flavigena* and *Bacillus* spp including; *B. pumilus*, *B. subtilis* and *B. amylolyticus*. *Bacillus subtilis* was common to all three leguminous plant. The organisms were all (100%) Gram positive rods affirming that bacteria endophyte are present in

legumes and can be isolated by proper surface sterilization method [34].

The first set of antibiotics used in this study for susceptibility test were Cephalosporins (oxid); Cefuroxime (30 µg) Cefpodoxime (10 µg), Ceftazidime (30 µg), Cefepime (30 µg) and Cefoxitin (30 µg). All bacterial endophytes (100%) were resistant to these cephalosporins. These may be due to the fact that most antibiotics are made from strains of fungi and bacteria that occur naturally in all environments. Most antibiotic-producing strains transfer genes encoding resistance to the antibiotics that they yield, and these genes typically originated in the same gene cluster as the antibiotic biosynthesis pathway genes [35].

In addition, antibiotics produced in the environment may apply selective pressure on neighboring organisms. With the result obtained later in this study, most isolated endophytic bacteria could be considered multidrug resistant (MDR), supporting the emerging body of evidence that the environment including endosphere (plant environment) constitutes a vast reservoir of organisms that are rapidly becoming antibiotic resistant [36,37].

Due to the general resistant pattern of all bacterial endophyte to the Cephalosporins (oxid), another set of antibiotics (commercial

multiple susceptibility disc) were again used against the endophytic bacteria. For the second set of antibiotics (multiple susceptibility disc). All the endophytic isolates (100%) were resistant to Ampiclox, Zinnacef and Amoxacillin but susceptible to Pefloxacin. All the endophytic isolates except *Bacillus pumilus* were susceptible to Cirpofloxacin. *Microbacterium lacticum*, *Bacillus amylolyticus* and *Cellulomonas flavigena* were susceptible to Streptomycin, Septrin and Pefloxacin. While for the clinical isolates; *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to Pefloxacin at diameter zone of 20.00mm. *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to Streptomycin, Septrin, Erythromycin, Ampiclox and Rocepin with diameter zones ranging from 17.00-20.00mm. *Salmonella pullorum* was susceptible to Streptomycin and Septrin with diameter zone of 17.00mm while *Klebsiella pneumoniae* was resistant to 90% (9 of 10) of the antibiotics.

Evaluation of antimicrobial activity of nanoparticles was done using agar well diffusion assay [38]. For the endophytic isolates; *Bacillus amylolyticus* and *Cellulomonas flavigena* isolates showed susceptibility diameter zone of 14.00mm to sulfur nanoparticles (SNP1) mediated with *Ocimum gratissimum* plant extract. All the endophytic isolates (100%) were resistant to sulfur nanoparticles (SNP2) synthesized in the absence of *Ocimum gratissimum* plant extract. While, for the clinical isolates; *Staphylococcus aureus* was observed to have the highest susceptibility to sulfur nanoparticles (SNP1) mediated with *Ocimum gratissimum* plant extract with diameter of 20.00mm; *Escherichia coli* and *Salmonella pullorum* showed the susceptibility diameter zone of 18.00mm; *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed resistance. All tested clinical isolates were resistant to the other sulfur nanoparticles (SNP2) synthesized in the absence of *Ocimum gratissimum* plant extract [38].

The result obtained also showed that *Ocimum gratissimum* extract increased the efficacy of sulfur nanoparticle as antimicrobial agent. The antimicrobial properties of elemental sulfur have long been recognized, and the use of Sulfur nanoparticles (SNPs) as antimicrobial agents was first proposed by [39]. Since then, [40] demonstrated that SNPs of about 150 nm in size exhibited antimicrobial activity against various microorganisms.

Two sulfur nanoparticles were used in this study; SNP1 was mediated with *Ocimum gratissimum* plant extract while SNP2 was synthesized in the absence of *O. gratissimum* extract. *O. gratissimum* is commonly called 'Efinrin nla' and is widely cultivated in Nigeria for its medicinal use [41].

*Ocimum gratissimum* phytochemical screening has indicated the presence of various phytoconstituents which has been shown to be active against several bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* [42].

SNP2 showed no antibacterial effect on all the bacteria (endophytic and clinical isolates) used in this study while SNP1 showed potential antibacterial effect on three of the five selected clinical isolates; *Staphylococcus aureus* were observed to have the highest susceptibility with diameter of 20.00mm; *Escherichia coli* and *Salmonella pullorum* showed the susceptibility diameter zone of 18.00mm. The endophytic bacterial isolates were also susceptible to the nanoparticles with lower diameter zones ranging from 9mm-14mm.

Furthermore, comparing the efficacy of Sulfur/*Ocimum gratissimum* mediated nanoparticles (SNP1) with multiple disc antibiotics used, the endophytic bacterial isolates; *Microbacterium lacticum* and *Bacillus amylolyticus* were found to be more susceptible to SNP1 than 40% (4 of 10) of antibiotics used while *Cellulomonas flavigena* was found to be more susceptible to SNP1 than 50% of antibiotics used. The selected clinical bacterial isolates; *Staphylococcus aureus* was found to be more susceptible to SNP1 than 80% (8 of 10) of antibiotics used, while *Escherichia coli* and *Salmonella pullorum* were found to be more susceptible than 30% and 100% of antibiotics used respectively.

The Sulfur/*Ocimum gratissimum* mediated nanoparticles (SNP1) showed potential antibacterial activity, the amount of nanoparticle used maybe increased to achieve even better outcome. [40] demonstrated that SNPs has no toxicity to human cells, thereby making it more considerable as an alternative antimicrobial agent. It should be mentioned here that the nanoparticle efficacy over conventional antibiotics should be used to combat multiple resistant organism, due to their unique mode of action. it emerged as a novel alternative to overcome bacterial multidrug resistance

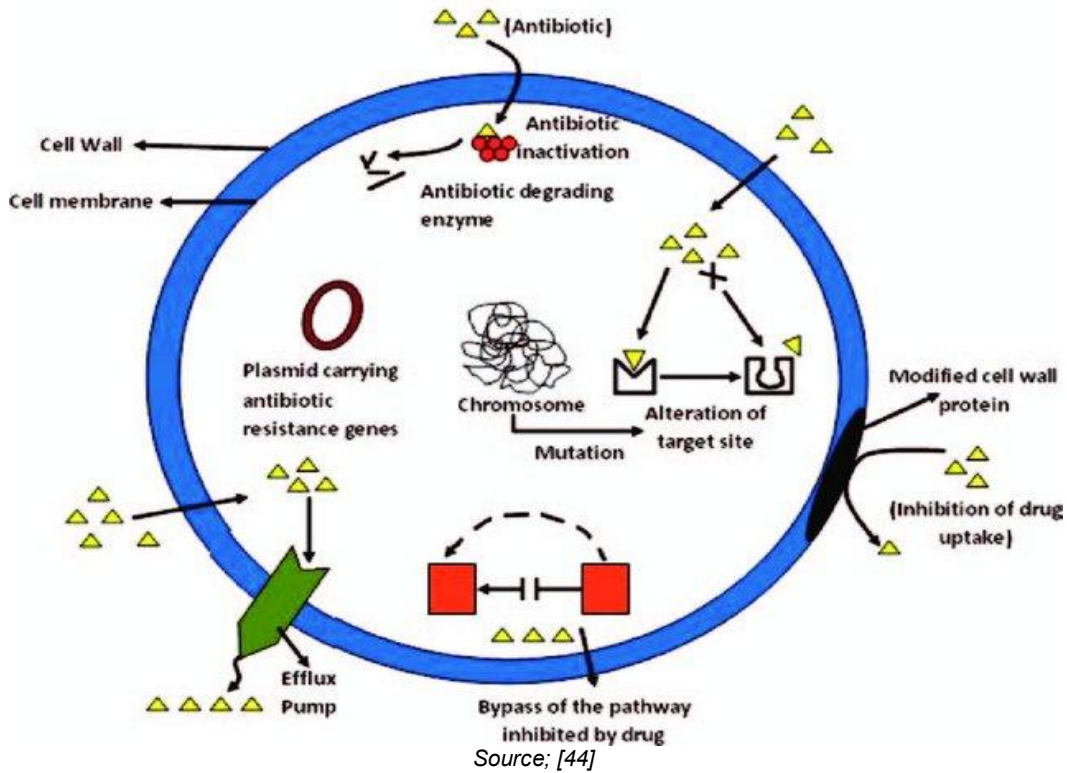


Fig. 8a. Schematic representation of possible mode of action of antibiotics against multiple resistant organism

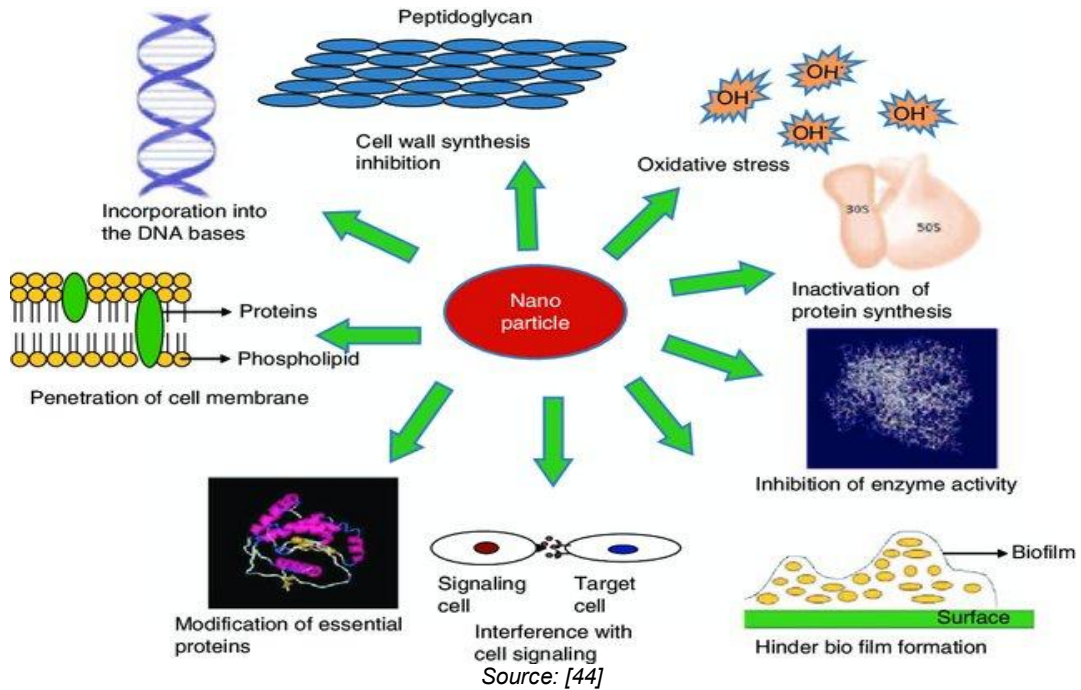


Fig. 8b. Schematic representation possible mode of action of nanoparticles against multiple resistant organism

encountered globally due to misuse of antibiotics. Use of nanoparticles as antimicrobial agents could overcome mechanisms of bacterial resistance as the microbicidal nature of nanoparticles result from direct contact with the bacterial cell wall, without the need to penetrate into the cell [43]. The development of antibacterial resistance to NPs are therefore less likely when compared to antibiotics. In the next figure, the mode of action of nanoparticle and antibiotics on antibacterial resistance is schematically representation demonstrated below in comparism, to show the efficacy of nanoparticle over conventional antibiotics.

It should be clearly stated that the physical structure of the nanoparticle itself may have inherent antibacterial properties due to its membrane damaging abrasiveness, as seen in Graphene oxide nanoparticles. Enhanced release of antibacterial metal ions from the surface of nanoparticles is another mechanism which has been suggested. The low surface to volume ratio of the nanoparticles can increase the antimicrobial activity allowing greater interaction of the nanomaterial with the surrounding environment. Chemistry, particle size, particle shape, and zeta potential are among the most relevant variables affecting antibacterial activity [43].

## 5. CONCLUSION

The global problem of antibiotic resistant organisms requires urgent action. There is a strong demand to develop novel antimicrobial materials, and the emergence of nanotechnology is creating a variety of options in this respect. Today, nanomaterials are a promising platform to control bacterial infections in a broad range of applications. In this study, *Ocimum gratissimum* mediated Sulfur nanoparticles (SNP1) were found to exhibit antibacterial activity against various bacterial species. The result of the antimicrobial susceptibility assay showed promising evidence for the antimicrobial effect of sulfur nanoparticles (SNP1) against multidrug resistant bacterial endophyte and even more profound effect on *Staphylococcus aureus*, *Escherichia coli* and *Salmonella pullorum*. Though, the efficacy of some antibiotics used in this study can also not be disregarded. The combination of sulfur nanoparticles and antibiotic may show better antibacterial activity as compared to nanoparticles alone or antibiotics alone. However, this topic deserves more attention and investigation regarding better

standardization for comparative analysis, selective toxicity, clinical trials and dosage of sulfur nanoparticles.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENTS

All the technical staffs of the laboratory unit of Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria and Centre for Bio Computing and Drug Development (CBDD), Adekunle Ajasin University, Nigeria for their support and all the technical assistance rendered during the course of this research work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* 2015;79:293–320.
2. Krell V, Jakobs-Schoenwandt D, Patel AV. Application of formulated endophytic entomopathogenic fungi for novel plant protection strategies. In *Endophytes for a Growing World*, ed. T. R. Hodkinson, F. M. Doohan, M. J. Saunders and B. R. Murphy. Cambridge: Cambridge University Press, Chapter 3; 2019.
3. Wilson D. Endophyte: The evolution of a term, and clarification of its use and definition. *Oikos.* 1995;73:274–276.
4. Dobereiner J. History and new perspectives of diazotrophs in association with non leguminous plants. *Symbiosis.* 1992;13:1–13.
5. Schulz B, Boyle C. What are endophytes? *Microbial Root Endophytes.* 2006;9:1–14.
6. Sessitsch A, Hardoim P, Döring J, Weilharter A, Krause A, Woyke T, Mitter B,



- Hauberg Lotte L, Friedrich F, Rahalkar M, et al. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Mol. Plant Microbe Interact.* 2012;25:28–36.
7. Baker CJ, Orlandi EW. Active oxygen in plant pathogenesis. *Annu. Rev. Phytopathol.* 1993;33: 299–321
  8. Chen Z, Silva H, Klessig DF. Active oxygen species in the induction of plant systemic Acquired resistance by salicylic acid. *Science.* 1993;262:1883–1886.
  9. Mehdy MC. Active oxygen species in plant defense against pathogens. *Plant Physiol.* 1994;105: 467–472.
  10. Krause A, Ramakumar A, Bartels D, Battistoni F, Bekel T, Boch J, Böhm M, Friedrich F, Hurek T, Krause L, et al. Complete genome of the mutualistic, N<sub>2</sub>-fixing grass endophyte *Azoarcus* sp. strain BH72. *Nat. Biotechnol.* 2006;24:1385–1391.
  11. Taghavi S, van der Lelie D. Genome sequence of the plant growth-promoting endophytic bacterium *Enterobacter* sp. 638. *Mol. Microb. Ecol. Rhizosph.* 2017;2:899–908. *Microorganisms* 5, 77 23 of 26
  12. Mitter B, Petric A, Shin MW, Chain PSG, Hauberg-Lotte L, Reinhold-Hurek B, Nowak J, Sessitsch A. Comparative genome analysis of *Burkholderia phytofirmans* PsJN reveals a wide spectrum of endophytic lifestyles based on interaction strategies with host plants. *Front. Plant Sci.* 2013;4:1–15.
  13. Ali S, Charles TC, Glick BR. Amelioration of high salinity stress damage by plant growth promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol. Biochem.* 2014;80:160–167
  14. Alquéres S, Meneses C, Rouws L, Rothballer M, Baldani I, Schmid M, Hartmann A. The bacterial superoxide dismutase and glutathione reductase are crucial for endophytic colonization of rice roots by *Gluconacetobacter diazotrophicus* PAL5. *Mol. Plant Microbe Interact.* 2013;26:937–945.
  15. Quinn, J. (2014). Cowpea. A Versatile Legume for Hot, Dry Conditions. Jefferson Institute. Available at: <http://www.hort.purdue.edu/newcrop/article/s/ji-cowpea.html>.
  16. US Forest Service, (2011). *Mucuna pruriens* (L.) DC. Pacific Island Ecosystems at Risk (PIER)
  17. Flora of China Editorial Committee, (2014). *Flora of China*. St. Louis, Missouri and Cambridge, Massachusetts, USA: Missouri Botanical Garden and Harvard University Herbaria. [http://www.efloras.org/flora\\_page.aspx?flora\\_id=2](http://www.efloras.org/flora_page.aspx?flora_id=2)
  18. Cook BG, Pengelly BC, Brown SD, Donnelly JL, Eagles DA, Franco MA, Hanson J, Mullen BF, Partridge IJ, Peters M, Schultze-Kraft R. *Tropical forages*. CSIRO, DPI&F(Qld), CIAT and ILRI, Brisbane, Australia; 2005.
  19. Ali S, Duan J, Charles TC, Glick BR. A bioinformatics approach to the determination of genes involved in endophytic behavior in *Burkholderia* spp. *J. Theor. Biol.* 343:193–198.
  20. Martinez JL, Baquero F. Mutation frequencies and antibiotic resistance. *Antimicrob. Agents Chemother.* 2000;44:1771–1777.
  21. Olayemi O, Odedara O. Screening of endophytic plant growth-promoting bacteria isolated from two Nigerian rice varieties. *Nigerian Journal of Biotechnology.* 2017;33(1).
  22. Schulz B, Wanke U, Draeger S, Aust HJ. Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. *Mycological Research.* 1993;97(12):1447–1450.
  23. Olutiola PO, Famurewa O, Sonntag HS. *An introduction to general microbiology (A practical Approach)*. 2000;157-237.
  24. Bergey DH, Holt JG, Noel RK. *Bergey's Manual of Systematic Bacteriology*, 19<sup>th</sup> ed Baltimore. Williams & Wilkins. 1994;1935.
  25. Cappuccino JG, Sherman N. *Microbiology: A laboratory manual*. Pearson Education Incorporated; 2002.
  26. Oludare Temitope Osuntokun MA. Yusuf-Babatunde, Oladele, Olasumbo Fasila. Components and Bioactivity of Ipomoea batatas (L.) (Sweet Potato) Ethanolic Leaf Extract, *Asian Journal of Advanced Research and Report.* 2020;10(1):10-26. [ISSN: 2582-3248] Available: [https://doi.org/10.9734/ajarr/2020/v10i1\\_30232](https://doi.org/10.9734/ajarr/2020/v10i1_30232)
  27. Oludare Temitope O, Thonda Oluwakemi A, Adeleye Bukola M. Exploring the efficacy of medicinal plants (*Moringa oleifera* and *Tamarindus indica* seeds) in the treatment of well water in two major cities in southwestern part of Nigeria, West Africa. *Drug Des Int Prop Int J.* 2019;3(2). DDIPIJ.MS.ID.000159.

- DOI: 10.32474/DDI.PIJ.2019.03.000159
28. Sigmon J. The Starch Hydrolysis Test. American Society for Microbiology. 2011;156-160.
  29. CLSI. Clinical and Laboratory Standards Institute; Wayne, PA: Methods for dilution antimicrobial susceptibility testing for bacteria that grew aerobically. M7-A10; 2009.
  30. Oludare Temitope Osuntokun. Evaluation of Inhibitory Zone Diameter (IZD) of crude *Spondias mombin* (Linn.) extracts (root, leaf and stem bark) against thirty infectious clinical and environmental isolates. J BacteriolInfec Dis. 2018;2(1):8-16.
  31. Bankole OM. Phytochemical and antimicrobial studies of colloidal silver nanoparticles mediated by *Laportea aestuans* extract. International Journal of Biomedical Engineering and Clinical Science. 2018;4(2):58-65.
  32. Thakur S, Barua S, Karak N. Self-healable castor oil based tough smart hyper branched polyurethane nanocomposite with antimicrobial attributes, RSC Adv. 2015;5:2167-2176.
  33. Karaman, et al. Karaman DS, Manner S, Fallarero A, Rosenholm JM. Current approaches for exploration of nanoparticles as antibacterial agents, antibacterial agents, Ranjith N. Kumavath, Intech Open; 2017.
  34. Rangeswaran R, Raj J, Sreerama Kumar P. Resistance and susceptibility pattern of chickpea (*Cicer arietillum* L) endophytic bacteria to antibiotics. Journal of Biological Control. 2008;22(2):393–403.
  35. Hopwood, Hopwood DA. How do antibiotic-producing bacteria ensure their self-resistance beforeantibioticbiosynthesis incapacitates them? Mol Microbiol. 2007;63(4):937-40.
  36. Arunachalam C, Gayathri P. Studies on bioprospecting of endophytic bacteria from the medicinal plant of *Andrographis paniculata* for their antimicrobial activity and antibiotic susceptibility pattern. Int. J. Curr. Pharm. Res. 2010;2:63–68.
  37. Pal A, Paul AK. Bacterial endophytes of the medicinal herb *Hygrophila spinosa* T. Anders and Their Antimicrobial Activity. Journal of Pharmaceutical Research International. 2013;3(4):795-806.
  38. Ramalingam V, Rajaram R, PremKumar C, Santhanam P, Dhinesh P, Vinothkumar S, et al. Biosynthesis of silver nanoparticles from deep sea bacterium *Pseudomonas aeruginosa* JQ989348 for antimicrobial, antibiofilm, and cytotoxic activity: biosynthesis of silver nanoparticles. J Basic Microbiol. 2014;54(9):928–36.
  39. Lawson GB. The inhibitory action of sulfur on the growth of *tubercle bacilli*. American Review of Tuberculosis. 1934;29:650-651.
  40. Schneider T, Baldauf A, Ba LA, Jamier V, Khairan K, Sarakbi MB, Reum N, Schneider M, Roeseler A, Becker K, Burkholz T, Winyard PG, Kelkel M, Diederich M, Jacob C. Selective antimicrobial activity associated with sulfur nanoparticles. Journal of Biomedical Nanotechnology. 2011;7:395-405.
  41. Aguiyi JC, Obi CI, Gyang SS, Igweh AC. Hypoglycaemic activity of *Ocimum gratissimum* in rats. Fitoterapi. 2000;71:444–6.
  42. Rishikumar et al. Rajeshkumar S, Malarkodi C, Gnanajobitha G, Paulkumar K, Vanaja M, Kannan C, Annadurai G. Seaweed-mediated synthesis of gold nanoparticles using *Turbinariaconoides* and its characterization. J. Nanostruct. Chem. 2013;3(1):1–7.
  43. Hsueh PR. New Delhi metallo-β-lactamase-1 (NDM-1): an emerging threat among Enterobacteriaceae. J Formos Med Assoc. 2010;109(10):685–687.
  44. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Int J Nanomed. 2017;12:1227-1249. DOI: <https://doi.org/10.2147/IJN.S121956>

© 2020 Osuntokun et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<http://www.sdiarticle4.com/review-history/59147>