

Systematic Comparative Study of Selected Antibiotics and Sulphur/ Medicinal Plant Mediated Nano-particles against Non-Leguminous Endophytic Bacteria and Clinical Isolates

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Authors' contributions

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

Article Information

DOI: 10.9734/JALSI/2020/v23i1030192

Editor(s):

(1) Dr. Muhammad Kasib Khan, University of Agriculture, Pakistan.

Reviewers:

(1) Duha S. Ahmed, University of Technology, Iraq.

(2) Azhar Jabar Bohan, University of Technology, Iraq.

(3) Deepti Gulati, Dolphin (PG) Institute of Biomedical & Natural Sciences, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/61516>

Original Research Article

Received 06 August 2020
Accepted 12 October 2020
Published 03 November 2020

ABSTRACT

The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, therefore, there is a need for a systematic approach to the menace of resistant bacteria. Green synthesized nanoparticle (NPs) of medicinal plant based as become an alternative way out

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to total eradication of resistant microorganisms, Therefore, the search for new, effective bactericidal agents is imminent significantly, for combating drug resistance microorganism. This research work aims to isolate, identify and characterize endophytic bacteria from five non-leguminous plants, namely *Carica papaya*, *Helianthus annuus*, *Talinum fruticosum*, *Phoenix dactylifera*, and *Solanum lycopersicum*. The surface of the plants were sterilized, Isolation, characterization and identification using biochemical characterization of the endophytic bacteria were examined according to Bergey's manual of Systemic Bacteriology. The sulfur/medicinal plant mediated Nanoparticle with and without *Ocimum gratissimum* were tested against the endophytic bacteria and selected clinical isolates, for their antimicrobial susceptibility test as described Kirby-Bauer Disc diffusion method. SNP1 was prepared from sodium thiosulfate pentahydrate, citric acid, with fresh leaves of *O. gratissimum* and characterized by using Shimadzu UV-VIS-NIR Spectrophotometer UV-3100 with a MPCF-3100 sample compartment while SNP2 was prepared using the same method but without *O. gratissimum*. The endophyte showed resistant to cephalosporin antibiotics family and SNP2, while all the endophytic bacteria were susceptible to ciprofloxacin (100%), pefloxacin (100%). *Streptococcus infectinalis* and *Cellumonas flavigena* showed high susceptibility to sulfur/ plant nanoparticle mediated with *Ocimum gratissimum* extract (SNP1). The study showed that sulfur/medicinal plant mediated nanoparticle can be a promising antimicrobial agent against a wide range of pathogenic and multiple drug resistance bacteria including both clinical isolates, its uses and practice should be encouraged especially against multiple drug resistance bacteria.

Keywords: Selected antibiotics; *Ocimum gratissimum*; biosynthetic sulfur nanoparticles; non-leguminous endophytic bacteria.

1. INTRODUCTION

An endophyte is an endo-symbiont, often a bacterium or fungus that lives within a plant for at least part of its life cycle without causing apparent disease. Microbial endophytes are present in all known plant species and are ubiquitous in nature [1]. The ability to enter and thrive in plant tissues makes endophytes unique, showing multidimensional interactions within the host plants. A comprehensive definition of endophytes does not specify their functional relationship and apart from commensalistic symbionts, they can exist from latent pathogens or saprotrophs to mutualistic associations [2].

It has been proposed that endophytes have originated from the rhizosphere microbes or seed-borne microbial communities, but genome studies and their correlation showed that these microbes are far more versatile and may contain genes for novel traits beneficial to the host plant Ali et al. [3]. In order to sustain the stable symbiosis, endophytes manufacture or induce the host plant to produce metabolites that promote plant growth and help them adapt better to the environment Das and Varma [4]. Endophytes plays an imperative role to maintain the health of plants, as they can protect or prepare the plant against abiotic and biotic stresses and help in enhancing growth and yields Lugtenberg et al. [5].

Over the years there has been increasing importance placed on the discovery of endophytes natural products, also referred to as bioprospecting. Many of these novel compounds produced by endophytes have been shown to have important medical applications such as antimicrobial, anti-parasitic, cytotoxic, neuro protective, antioxidant, insulin mimetic and immunosuppressant properties, an example of the discovery of chemicals derived from endophytic fungi is from the fungus *Taxomyces andreanae* isolated from the pacific yew *Taxus bervifolia*.

Nanoparticles (NPs) are increasingly used to target bacteria as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial infections. Examples include the utilization of NPs in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics and in antibacterial vaccines to control bacterial infections. The antibacterial mechanisms of NPs are poorly understood, but the currently accepted mechanisms include oxidative stress induction, metal ion release and non-oxidative mechanisms. The multiple simultaneous mechanisms of action against microbes would require multiple simultaneous gene mutations in

the same bacterial cell for antibacterial resistance to develop; therefore, it is difficult for bacterial cells to become resistant to NPs.

Nanoparticles had been tested with their toxicity to living tissues especially human tissues because the major point of any antimicrobial agent is its toxicity to human system. The earliest nano-therapeutics was approved based on their efficacy, but lower toxicity than their free-drug counterparts. The first nanomedicine to gain approval was Doxil, which is a liposomal formulation of doxorubicin Barenholz [6]. Low toxicity of nanoparticles to human body or tumor tissue is the enhanced permeability and retention (EPR) effect; whereas small molecules can freely pass through the vasculature of any tissue, the movement of nanoparticles is more restrictive. Another mechanism by which nanoparticles can reduce drug toxicity is associated with the administration of hydrophobic therapeutics; nanoparticles can serve as an alternative to toxic solubilizing agents [7]. 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 [8]. 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. However, NPs can also promote the emergence of bacterial resistance in certain cases [9].

Efforts are being made globally towards the production of several alternatives to conventional antibiotics based on the increase in the rate of drug resistance bacteria. The new drugs that are produced must be able to kill or inhibit all these pathogenic bacteria and it must also express selective toxicity to the host and that's why pharmacologist are diverting their attention towards nano-sized materials which have the ability to kill and inhibits all these pathogenic bacteria. In addition, nanoparticles can also be used in combination with conventional antibiotics against Gram positive bacteria and Gram-negative bacteria [10]. Hence, the aim of this study was to detect and isolate endophytic bacteria from non-leguminous plants (*Carica papaya*, *Helianthus annuus*, *Talinum fruticosum*, *Phoenix dactylifera*, *Solanum lycopersicum*), to compare their susceptibility to conventional antibiotics and sulfur nanoparticles and finally, compare susceptibility pattern of endophytic bacteria with that of selected clinical isolates.

2. MATERIALS AND METHODS

2.1 Collection of Non-leguminous Plants Samples

Matured plants specimen of five Non-leguminous plants namely *Carica papaya*, *Talinum fruticosum*, *Helianthus annuus*, *Phoenix dactylifera*, *Solanum lycopersicum* were randomly collected from the School farm of Federal College of Agriculture, Akure, Ondo State, Nigeria (7.2704° N, 5.2241° E).

2.2 Isolation of Non-leguminous Endophytic Bacteria

The samples were collected separately and 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 microorganisms. Ten grams of the samples each were cut to 2-3 cm pieces and macerated using sterilized mortar and pestle with 12.5 mM potassium phosphate buffer (pH 7.1), a 10-fold serial dilution were carried out where 0.5ml of the 10⁻³ 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 [11]. After the incubation time, the colony forming units (CFU) for each plate was estimated. Isolates differing in morphological appearance were selected and were streaked and subcultured onto new plates for purity of the isolates. Pure cultures of bacterial isolates were maintained on NA slants and were stored at 4°C [11,12]. The efficiency of surface sterilization procedure was 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 [12].

2.3 Identification of Non-leguminous Endophytic Bacteria

The bacterial isolates were identified using their colony morphological characteristics. The appearance of each colony on the agar media and characteristics such as shape, edge, colour, elevation, and texture were observed as

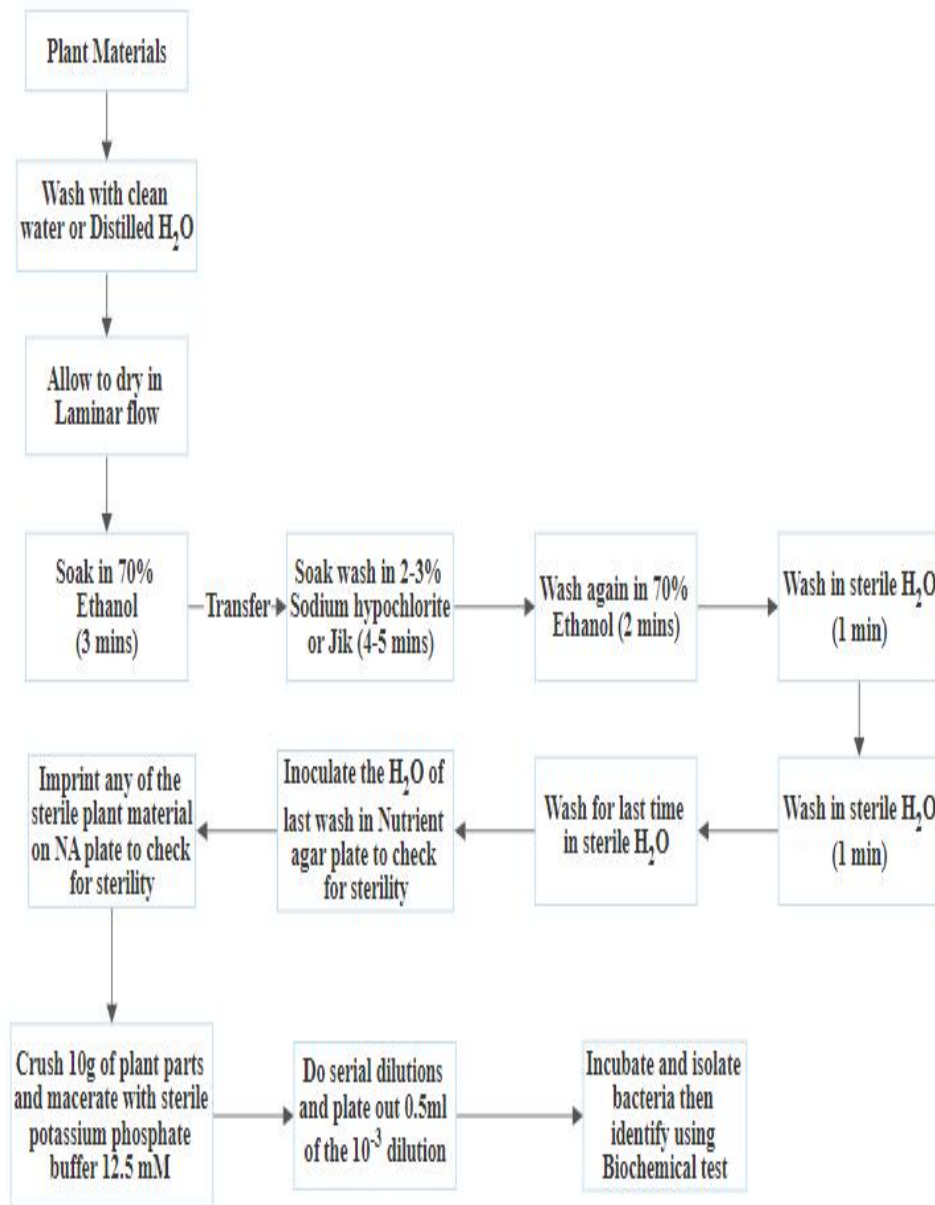


Plate 1. Schematic diagram showing procedure for isolation of non leguminous endophytic bacteria [12]

described by [13]. The isolates were thereafter subjected to biochemical tests and identified using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology.

2.4 Antibiotic Susceptibility Testing for the Non Leguminous Endophytic Bacteria Isolates

Mueller-Hinton agar was prepared according to manufacturer's specification and instructions,

and sterilized at 121°C for 15 mins. The medium was then poured into appropriate Petri dishes aseptically and were allowed to gel. Antibiotics susceptibility was determined according to Clinical and Laboratory Standard Institute (CLSI) using the disc diffusion method [14].

With the aid of sterile swab stick, the swab stick was deepened into the inoculums and spread evenly on the plate, then the antibiotic disc were placed on the inoculated plates, incubated at

37°C for 24 hours. The growth and zone of inhibition after 24 hrs were recorded. Susceptibility of the isolates was determined by using the following single and multiple discs (Cephalosporin family); Cefuroxime (CXM, 30 µg), Ceftazidime (CAZ, 30 µg), Cefoxitin (FOX, 30 µg), Cefepim (FEP, 30 µg), Cefepidime (CPD, 10 µg), Streptomycin (S, 30 µg), Septrin (SXT, 30 µg), Erythromycin (E, 10 µg), Pefloxacin (PEF, 10 µg), Gentamycin (CN, 10 µg), Ampiclox (APX, 30 µg), Zinnacef (Z, 20 µg), Amoxicillin (AM, 30 µg), Rocephin (R, 25 µg), and Ciprofloxacin (CPX, 10 µg). The negative discs used were; Streptomycin (S, 30 µg), Septrin (SXT, 30 µg), Tarivid (OFX, 10 µg), Pefloxacin (PEF, 10 µg), Gentamycin (CN, 10 µg), Chloramphenicol (CH, 30 µg), Augmentin (AU, 30 µg), Amoxicillin (AM, 30 µg), Sparfloxacin (SP, 10 µg), and Ciprofloxacin (CPX, 10 µg).

2.5 Collection of Clinical Isolates Sample

Pure cultures of Five selected clinical isolates which include both Gram negative and Gram positive; *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.

2.6 Preparation and Synthesis of Sulfur / Medicinal Plant Mediated Nano Particles

2.6.1 Preparation of *Ocimum gratissimum* leaf extract

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 grounded into fine powders in a clean agate mortar and stored in a tight container for further use. Biomolecule contents of the leaf were

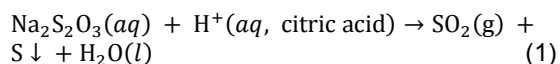
extracted by adding 20 g of the powdered leaves to 100 mL deionized water in a 250 mL beaker, and heated to 100°C for 60 min. The crude greenish extract was filtered through Whatmann No. 1 filter paper and stored in the refrigerator at -10°C prior to the preparation of sulfur nanoparticles [15].



Plate 2. *Ocimum gratissimum* leaf [12]

2.7 Biosynthesis of Sulfur/ Medicinal Plant Nanoparticles (SNP-1 and SNP-2)

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 added 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 dropwisely under stirring to allow the precipitation of sodium thiosulfate as sulfur nanoparticles and SO₂, in accordance with previous report [16]. The mixture was stirred for additional 1 hour and was allowed to stand undisturbed for 5 h for complete disproportionation of sodium thio sulfate. 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 Na₂S₂O₃ 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 [15].



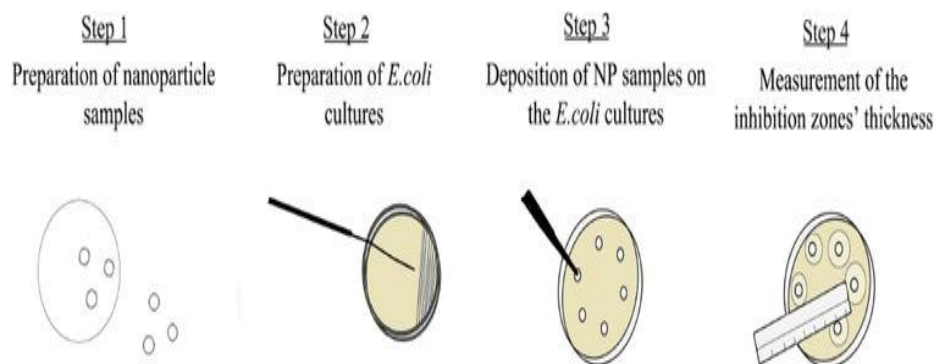


Plate 3. Diagram showing susceptibility test using nanoparticles (Modified Kirby-Bauer Method) [12]

2.8 Material Characterization of Sulfur/Medicinal Plant Nanoparticles (SNP-1 and SNP-2) Using UVVIS-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 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 α (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) [15].

2.9 Antimicrobial Susceptibility Test for Non Leguminous Endophytic Bacteria Isolates Using Sulfur Nanoparticles

The antibacterial effect of two sulfur nanoparticles; prepared in the presence and absence of *O. gratissimum* plant extract was tested against both Non Leguminous endophytic bacteria and selected clinical isolates using the modified Kirby-Bauer method [17]. 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

The description and weight of the five non-leguminous plant specimens collected for isolation of endophytic bacteria is depicted in Table 1. The entire plant specimens collected were mature at the time of sample collection.

The probable identity of the non-leguminous endophytic bacteria isolates is depicted in table 2. The bacteria recovered were *Micrococcus kristinae*, *Serratia mercerscens*, *Streptococcus infectinalis*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus amylolytica* and *Cellulomonas flavigena*. All the endophytic bacterial isolates were highly resistant (100%) to all conventional antibiotics used in the cephalosporin family. None of the bacteria was sensitive to the antibiotics.

Table 3; Colony morphology of the isolated non-leguminous endophytic bacteria based on parameter of Pigment, shape, odour, surface, opacity, edge and elevation.

Table 4; Gram stain of the isolated non-leguminous endophytic bacteria. The isolated bacteria were both Gram positive and Gram negative.

Fig. 1; Number of bacteria isolated from the five non-leguminous plants. Twenty-two (22) isolates recovered from *Talinum fruticosum* has the highest percentage of isolates. *Carica papaya* has seventeen isolates (17), with *Helianthus annuus* (13), eleven (11) isolates from *Solanum*

lycopersicum, and nine (9) from *Phoenix dactylifera* which had the least number of isolates recovered from each plant specimen.

The diameter of zone of inhibition of the endophytic bacteria and selected clinical isolates was depicted in Fig. 2. All the endophytes were

susceptible to ciprofloxacin (100%), pefloxacin (100%). Each of the bacteria showed diverse reaction to the antibiotic tested. *Bacillus subtilis* was susceptible to all the antibiotics used. *Micrococcus kristinae* showed resistance to Seprin and Streptomycin while *Serratia marcescens* showed resistance to ampiclox,

Table 1. Description and weight of non-leguminous plants specimen collected

Plant specimen	Description of the plant	Weight(g)
<i>Carica papaya</i>	Matured, young, large and spirally arranged leaves without fruit, succulent stem with hairy roots.	15
<i>Talinum fruticosum</i>	Matured, young and fleshy leaves, succulent stem with hairy roots.	12
<i>Helianthus annuus</i>	Matured, high and broad leaves, hairy stem and hairy roots.	8
<i>Phoenix dactylifera</i>	Matured, young, high and singly isolated leaves, clump stems and singly roots.	9
<i>Solanum lycopersicum</i>	Matured, young, hairy, strongly odorous, and pinnately compound leaves, succulent stem without fruit, and hairy roots.	8

Table 2. Probable identity of non-leguminous endophytic bacteria isolates

Isolates code	Plant specimen	Probable organism
A	<i>Carica papaya</i>	<i>Micrococcus kristinae</i>
B	<i>Talinum fruticosum</i>	<i>Serratia marcescens</i> <i>Streptococcus infectinalis</i> <i>Bacillus subtilis</i>
C	<i>Helianthus annuus</i>	<i>Bacillus cereus</i> <i>Bacillus amylolytica</i>
D	<i>Phoenix dactylifera</i>	<i>Cellulomonas flavigena</i>
E	<i>Solanum lycopersicum</i>	<i>Cellulomonas flavigena</i>

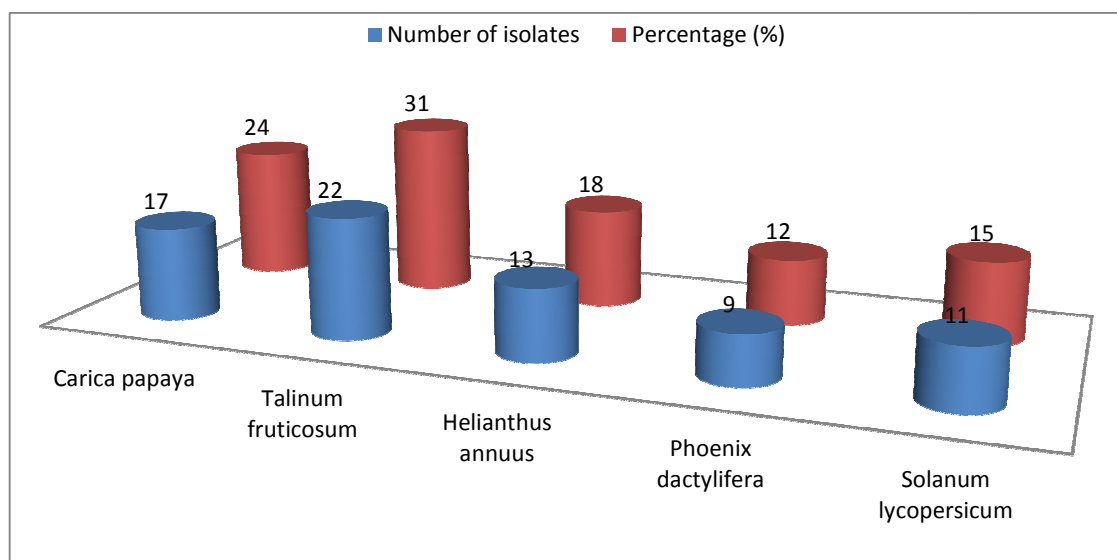


Fig. 1. Number of endophytic bacteria isolates from non leguminous plant

Table 3. Colony morphology of the bacterial isolates from endosphere of non leguminous plant

Plant samples	Pigment	Shape	Odour	Opacity	Surface	Edge	Elevation
<i>Carica papaya</i>	Yellow	Tetrad	Foul smell	Opaque	Slimery	Rounded edge	Slightly convex
<i>Talinum fruticosum</i>	Orange pigment	Rod	Fruity odour	Translucent	Sticky	Confluent	Umbonate elevation
<i>Talinum fruticosum</i>	White	Dome	Caramel odour	Translucent	Slimery	Entire edge	High convex
<i>Talinum fruticosum</i>	Fuzzy white	Circular	Foot odor	Opaque	Rough matted	Jagged edge	Umbonate elevation
<i>Helianthus annuus</i>	Grey-yellow	Granular	Foot odour	Opaque	Rough matted	Distal edge	Flat convex elevation
<i>Helianthus annuus</i>	Fuzzy white	Circular	Foot odor	Opaque	Sticky surface	Irregular edge	Flat convex elevation
<i>Phoenix dactylifera</i>	Yellow	Circular	Foul odour	Opaque	smooth	Irregular edge	Slight elevation
<i>Solanum lycopersicum</i>	Yellow	Circular	Foul odour	Opaque	Smooth	Irregular edge	Slight elevation

Table 4. Gram staining of bacteria isolated from endosphere of non leguminous plants

Isolate code	Plant specimen	Gramreaction	Microscopy	Inference
<i>Micrococcus kristinae</i>	<i>Carica papaya</i>	+ve	Cocci	Gram + cocci
<i>Serratia mercerscens</i>	<i>Talinum fruticosum</i>	-ve	Cocci	Gram – cocci
<i>Streptococcus infectinalis</i>	<i>Talinum fruticosum</i>	+ve	Cocci	Gram + cocci
<i>Bacillus subtilis</i>	<i>Talinum fruticosum</i>	+ve	Rod	Gram + rod
<i>Bacillus cereus</i>	<i>Helianthus annuus</i>	+ve	Rod	Gram + rod
<i>Bacillus amylolytica</i>	<i>Helianthus annuus</i>	+ve	Rod	Gram + rod
<i>Cellulomonas flavigena</i>	<i>Phoenix dactylifera</i>	+ve	Rod	Gram + rod
<i>Cellulomonas flavigena</i>	<i>Solanum lycopersicum</i>	+ve	Rod	Gram + rod

amoxicillin and zinnacef. The Gram-negative bacterium among them (*Streptococcus infectinalis*) was susceptible to all the antibiotics except for streptomycin, augmentin and septrin.

Staphylococcus aureus, *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to pefloxacin with diameter zone of 20.0 mm. *Escherichia coli* and *Pseudomonas aeruginosa* were susceptible to streptomycin, septrin, erythromycin, ampiclox and rocepin with diameter zones ranging from 17.0-20.0 mm. *Salmonella pullorum* showed susceptibility to streptomycin and septrin with diameter zone of 17.0 mm while *Klebsiella pneumoniae* was resistance to 90% of the antibiotics. The zone of inhibition of the sulfur nanoparticles against endophytic bacteria isolates and selected clinical isolates is showed in Fig. 3.

Streptococcus infectinalis and *Cellulomonas flavigena* isolates showed susceptibility diameter zone of 12.0 mm and 15.0 mm respectively to sulfur nanoparticles (SNP1) mediated with *Ocimum gratissimum* plant extract. All the isolates were 100% resistant to sulfur nanoparticles (SNP2) synthesized with the absence of *Ocimum gratissimum* plant extract. *Staphylococcus aureus* was observed to have the highest susceptibility to sulfur nanoparticles

(SNP1) mediated with *Ocimum gratissimum* plant extract with diameter of 20.0 mm; *Escherichia coli* and *Salmonella pullorum* showed the susceptibility diameter zone of 18.0 mm; while *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. 4; Diameter (in mm) of the zone of inhibition of the sulfur nanoparticles against non leguminous endophytic bacteria isolates. *Streptococcus infectinalis* and *Cellulomonas flavigena* isolates showed susceptibility diameter zone of 12.00 mm and 15.00 mm respectively to sulfur nanoparticles (SNP1) mediated with *Ocimum gratissimum* plant extract. All the isolates (100%) were resistant to sulfur nanoparticles (SNP2) synthesized with the absence of *Ocimum gratissimum* plant extract.

Fig. 5; 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) mediated with *Ocimum gratissimum* plant extract with diameter of 20.00 mm; *Escherichia coli* and *Salmonella pullorum* showed the susceptibility diameter zone of 18.00 mm; *Klebsiella pneumoniae* and

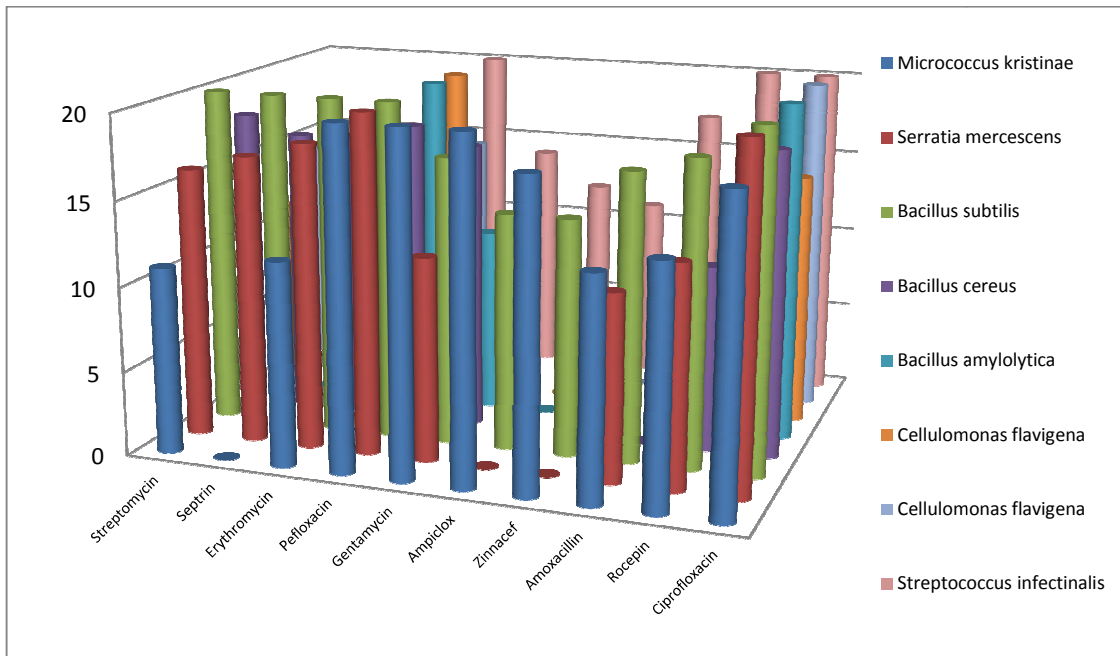


Fig. 2. Antibiotics susceptibility profile against non-leguminous endophytic bacterial isolates

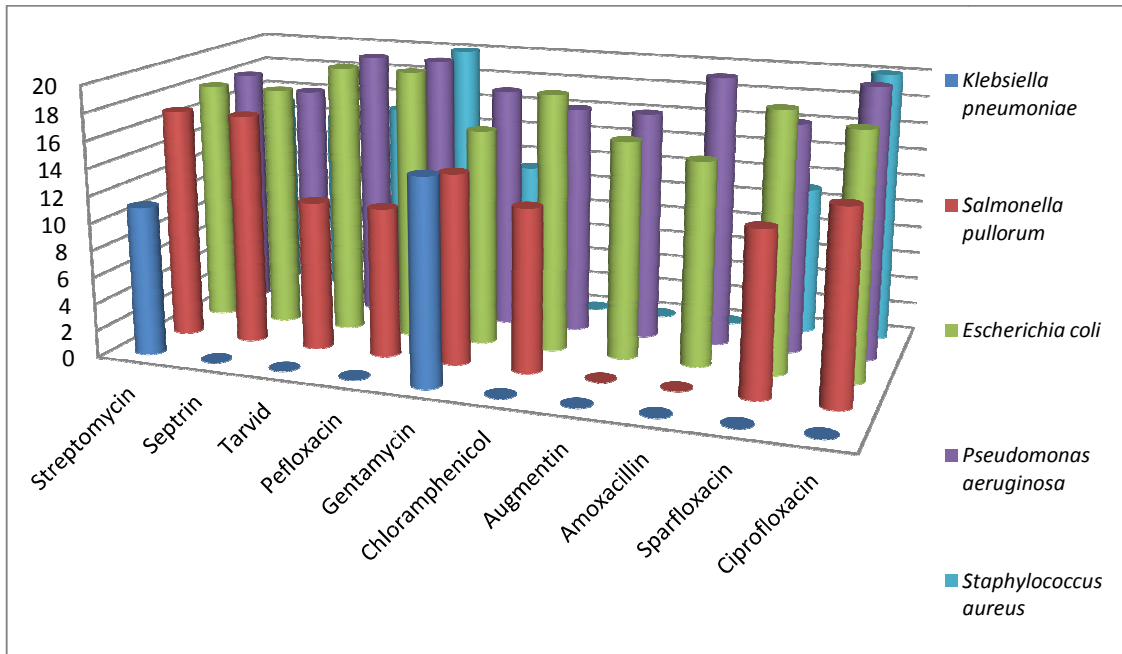


Fig. 3. Antibiotics susceptibility profile against selected clinical bacterial isolates

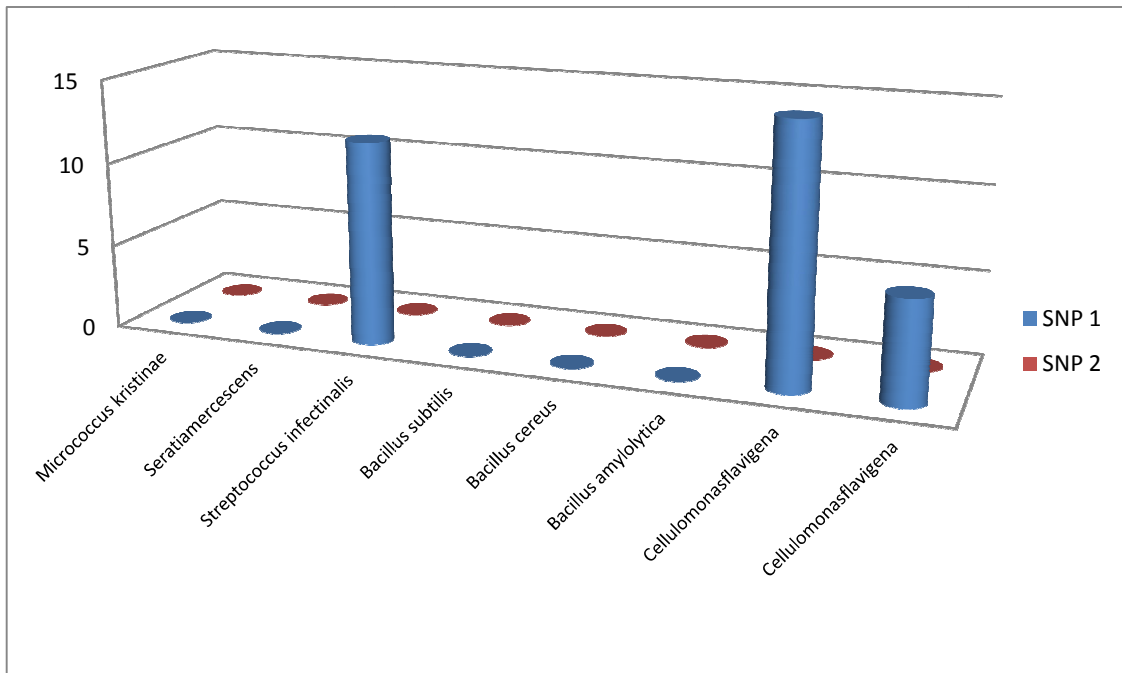


Fig. 4. Antimicrobial susceptibility test of non leguminous endophytic bacteria using sulfur /plant mediated nanoparticles

SNP 1 - Sulfur Nanoparticle 1 SNP 2 - Sulfur Nanoparticle 2

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.

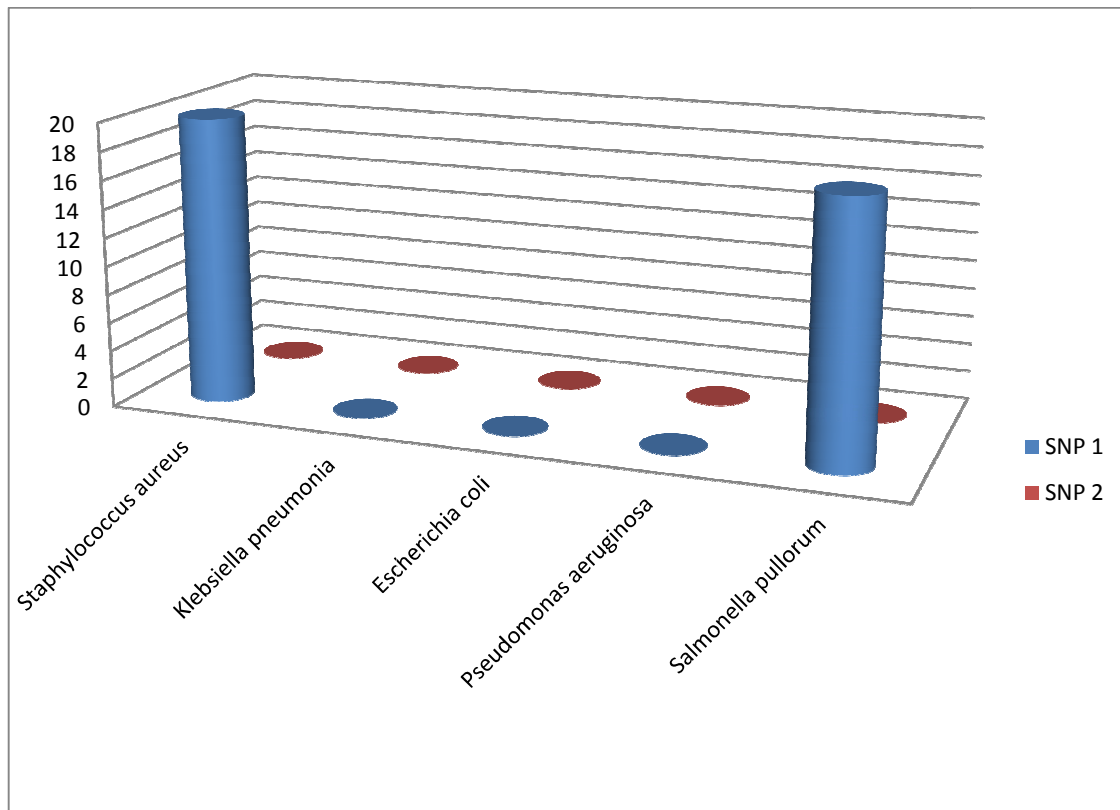


Fig. 5. Antimicrobial susceptibility test of clinical bacteria using sulfur/plant mediated nanoparticles

SNP 1 - Sulfur Nanoparticle 1 SNP 2 - Sulfur Nanoparticle 2

4. DISCUSSION

In this study, a number of non Leguminous endophytic bacteria were recovered from plant specimen such as *Micrococcus kristinae*, *Serratia mercenscens*, *Cellulomonas flavigena*, *Streptococcus infectinalis* etc. This study corroborates the studies of Ren et al. [18] who observed that the non-leguminous plant root endosphere could be dominated by a few bacterial groups which provides further evidence of the active and robust selection of bacteria from soil to plants which are Gamma proteobacteria of the genera *Enterobacter*, *Pseudomonas* and *Stenotrophomonas* that constituted the core bacterial operational taxonomic units (OTUs) in root endosphere of rice(Gamma proteobacteria, 30–98%) [18-20].

The endophytes were all resistant to cephalosporin family of antibiotics used. This may be due to the overuse or abuse of the antibiotics thus making all the bacteria to be resistance to the antibiotic. The resistance of

endophytes to cephalosporin may also be due to the horizontal transfer of resistant gene between bacteria in the environment, which can also make those bacteria to become resistant to such antibiotics [21]. Endophytic bacteria can also be classified among multi-drug resistance bacteria based on the outcome of the work done in this research work.

The result obtained in this work showed that sulfur nanoparticle mediated with *Ocimum gratissimum* plant extract showed antimicrobial activity on some multidrug resistant endophytic bacteria isolated from non-leguminous plants and some selected clinical isolates which have been posing a serious health to the populace. This result support the findings of Kumar et al. [22] who reported that synthesized silver nano particles have the potential antimicrobial efficiency against multidrug microorganisms. This showed more profound effect on the selected clinical isolates (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella pullorum*).

The antimicrobial activity of Nanoparticles is well known that nanoparticles opened a new field of nanomedicine in cancer therapy, where the structural and physical properties of SNPs gives a unique power for targeting and penetrating the abnormal cancer cell [23]. They can penetrate the abnormal cells causing DNA damage and determine defects in the genes. SNPs can also aid in drug delivery, imaging of abnormal cells, and monitoring of therapeutic drugs against cancer and bacterial infection [23].

Klebsiella pneumoniae, *Staphylococcus aureus* and *Salmonella pullorum* are highly susceptible to sulfur activated nanoparticle mediated with *Ocimum gratissimum* plant extract (SNP1). *Streptococcus infectinalis* and *Cellumonas flavigena* showed high susceptibility to sulfur activated nanoparticle mediated with *Ocimum gratissimum* plant extract (SNP1). Overall assessment of sulfur activated nanoparticles on non-endophytic bacteria and selected clinical isolates showed that sulfur nanoparticle have antimicrobial activities on the bacterial isolates than some of the conventional antibiotics used.

This corroborated the study of Tahmina et al. [24] and Osuntokun et al. [12] which reported that bacteria isolates are resistance to conventional antibiotics but synthesized SNP were found to be effective against the pathogenic organisms. This study also corroborates the study of Eman [25] who demonstrated that SNPs are an effective biocidal agent against a wide range of Gram-negative and Gram-positive bacteria, even

multidrug-resistant bacteria and fungal pathogens [25].

Antimicrobial properties of sulfur activated nanoparticle mediated with *Ocimum gratissimum* plant extract (SNP1) showed higher efficacy than sulfur activated nanoparticle in the absence of *Ocimum gratissimum* plant extract (SNP2). *Ocimum gratissimum* is a culinary herb in West Africa with local name 'Efirin in native Yoruba language. The presence of *Ocimum gratissimum* in SNP1 increases its antimicrobial activity than SNP2 reason due to the antimicrobial properties of *Ocimum gratissimum* [26].

Medicinal plants contain a variety of natural compounds, which have strong antibacterial and antiviral activity. Green synthesized SNPs can further improve the therapeutic applicability of plants and can be a source of new antibacterial agents [27]. These SNPs are safe and have multivalent functions, which make it less likely to encounter resistant organisms. However, many important approaches studied the antimicrobial effect of green synthesized SNPs using marine organisms, eg, Pugazhendhi et al. [23] studied the effect of SNPs synthesized from the red algae *Gelidium amansii* against pathogenic Gram-positive bacteria: *S. aureus*, *Bacillus pumilus*, and Gram-negative bacteria: *Escherichia coli*, *P. aeruginosa*, *Vibrio para haemolyticus*, *Aeromonas hydrophila*, which reduced the bacterial growth via exerting a bactericidal activity against the Gram-positive and Gram-negative bacterial pathogens.

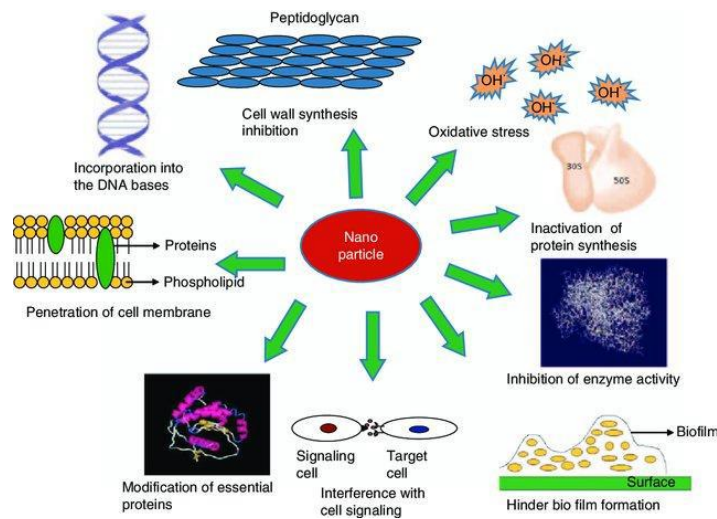


Fig. 6. Schematic representation possible mode of action of nanoparticles against multiple resistant organism (Osuntokun et al. [12])

The modus operandi of the nanoparticle should be mention for the clarity of purpose on the activity of nanoparticle over recalcitrant multiple resistant clinical and environmental organisms shown in Fig. 6. It can be seen that the nanoparticle has a deletions activity on cell wall of organisms, reduced oxidative stress, inactivation of protein synthesis and penetration of cell membrane. It can also modified the essential protein thereby increase the cell signal processes and hinder the formation of biofilm. The inclusion of medicinal plant, "*Ocimum gratissimum*" enhance the potency of nanoparticle because of the present of various arrays of secondary metabolites. Secondary metabolite like, flavonoids, tannins, Alkaloids, saponin, just to mention a few.

5. CONCLUSION

Sulfur nanoparticle mediated with *Ocimum gratissimum* plant extract can be a promising antimicrobial agent against a range of pathogenic and multidrug resistance bacteria including both clinical isolates than conventional methods. Scientists and pharmacologist should therefore focus on the use of nano-materials as an alternative to antibiotics in order to combat this problem. Combination of nano-particles with antibiotics and even with some medicinal plant extract can help to salvage the problem of antibiotic resistance.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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