Journal of Advances in Medical and Pharmaceutical Sciences

14(3): 1-13, 2017; Article no.JAMPS.34172
ISSN: 2394-1111

The Phytochemical and Antimicrobial Potentials of the Crude Extracts of *Bridelia ferruginea* and the Extracellular Biosynthesized Silver Nanoparticles

Temitayo Omotola Akinsete[1], Bukola Christianah Adebayo-Tayo[1*] and Abimbola Olumide Adekanmbi[1]

[1]Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author BCAT designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors TOA and AOA managed the analyses of the study, managed the literature searches and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2017/34172
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/20399

Original Research Article

Received 17th May 2017
Accepted 12th July 2017
Published 7th August 2017

ABSTRACT

Bridelia ferruginea has been used in African folk medicine for the treatment of several ailments especially throat infections. In this study, the phytochemical analysis, fractioning using different solvents, antimicrobials and silver nanoparticles (SNPs) produced by the ethanolic extract of the bark of *B. ferruginea* were investigated. The bark extract of the plant was used for the extracellular biosynthesis of SNPs; and characterized using visual observation, UV-Visible Spectrophotometer, Scanning Electron Microscope (SEM) and FTIR analysis. The antimicrobial potential of the characterized SNPs against some pathogenic microorganisms was also determined.
Flavonoids (987 mg/100 g), alkaloids (762 mg/100 g), saponins (437 mg/100 g) and tannins (209 mg/100 g) were the major bioactive compounds present in the plant while phenols and terpenes

**Corresponding author: E-mail: bukola.tayo@gmail.com;*
E-mail: bimboleen@yahoo.com

were absent. All the test organisms were sensitive to the crude extract of *B. ferruginea* with the highest zone of inhibition observed for *Klebsiella pneumoniae* (23 mm). In addition, all the test organisms were susceptible to the ethyl acetate fraction of *B. ferruginea* with *Actinobacillus* sp. having the highest zone of inhibition (18 mm). The SNPs has a pentagonal shape and the size ranged from 0.0 – 9.4 nm. The antibacterial activities of the crude and synthesized SNPs of *B. ferruginea* showed that the crude extract exhibited higher activity than the SNPs on the test bacteria with a range of 15-23 mm as against 13-21 mm observed for the SNPs.
In conclusion, the crude ethanolic extract, partitioned fractions, and SNPs of the bark of *B. ferruginea* had very good potential as antibacterial against some test bacteria.

Keywords: Bridelia ferruginea; phytochemical analysis; antimicrobials; silver nanoparticles.

1. INTRODUCTION

Several infections like the Gastro intestinal tract (GIT) infection, mouth infections, throat infections and skin infections have become rampart based on cases reported in patients attending hospitals. This may due to prevalence of antibiotic resistance, as the antibacterial agents used in treating these several bacterial infections are becoming less potent against the causative pathogenic microorganisms, partly as a result of antibiotics misuse. The widespread and indiscriminate use of antibiotics, has led to an accelerated emergence of antibiotic-resistant pathogens which has resulted in a serious threat to global public health. From literature, it is widely known that traditional herbalists use plants to treat infections and many of these plants have been scientifically proven to possess antimicrobial activity, thereby inhibiting the growth of pathogens. These plants are generally referred to as medicinal plants [1].

Bridelia ferruginea, commonly called *"Epo Ira"* by the Yoruba people of South-western Nigeria is a popularly plant known to many African countries. It has played a major role in the treatment of several bacterial infections and has gained importance due to its unique constituents and versatile applicability in the treatment of various infections (mouth, throat, gastro intestinal tract and skin infections) [2]. Based on this knowledge, *Bridelia ferruginea* can therefore be applied in various field of research especially nanotechnology as a probable alternative to common antibiotics in the treatment of bacterial infections.

Nanotechnology is a modern field of research that deals with the synthesis and manipulation of particles ranging from 1 to 100 nm in size. These particles are generally referred to as Nanoparticles and are well known and recognized owing to their significant applications in a large number of fields including medicine, manufacturing and materials, environment, energy and electronics, and pharmaceutical industries [3]. Among all the noble metal nanoparticles, silver nanoparticles has gained a boundless interest due to their unique properties such as chemical stability, anti-inflammatory activities, antifungal and antibacterial activities. It has been broadly applied in the health sector, mechanics, chemical industries, textile industries, drug-gene deliveries, pharmaceutical industries and also in the production of soaps, detergents, toothpastes and other products. Various approaches employed in the synthesis of silver nanoparticles of various sizes and shapes include the chemical and physical methods [4-6]. These methods are quite expensive and potentially dangerous to the environment, involve more than one steps, low material conversion, difficulty in purification, emit hazardous chemicals, occupy large space and employ a great deal of energy and time while raising the environmental temperature around the source material [4]. Biosynthesis of nanoparticles offers an alternative and eco-friendly method for the production of nanoparticles [7-9].

These drawbacks have led to the advancement of biological synthesis which involves the use of microorganisms, plant extracts or plant biomass for the production of nanoparticles. However, among the various biological methods of silver nanoparticles synthesis, the use of plant extracts has been observed to be more advantageous compared to the others since microbe-mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions, elaborate process of maintaining cell cultures and the gradual loss of the ability to synthesize nanoparticles due to mutations [10]. Plants are easier to scale up for a large scale production since they are readily available; environment-friendly, very affordable and easily accessible. The use of the bark extract of *Bridelia ferruginea*

for the synthesis of nanoparticles is as well advantageous due to the presence of bio-molecules called phytochemicals which mediate the synthesis and stabilize the nanoparticles formed into a desired size and shape [11]. Yugal et al. [12] reported the antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles from the leaf extract of *Erythrina suberosa* (Roxb.) while Ahmed et al. [13] and Mohanta et al. [14] reported the biosynthesis of AgNPs using different plant extracts as a reducing agent.

This research aimed at the phytochemical and antimicrobial evaluation of the crude, ethanolic extract and the partitioned fractions of the bark of *Bridelia ferruginea* and the silver nanoparticles (SNPs) produced.

2. MATERIALS AND METHODS

2.1 Collection of Plant

Fresh barks of *Bridelia ferruginea* were obtained from Oje market in Ibadan, Oyo state, Nigeria. They were authenticated at the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan.

2.2 Preparation of Extracts

The plant materials were air dried for 3 weeks at room temperature and pulverized in a mortar and pestle. 1.5 kg of the ground powdered sample of *Bridelia ferruginea* was weighed and poured into a macerating jar, filled to the brim with distilled ethanol. The solvent was then stirred with the solute using a sterilized glass rod. This procedure was repeated every 7 h till the last day of maceration which lasted for 72 h. Thereafter, the extract was decanted and filtered using a Whatman's filter paper (No.1) and concentrated using a rotary evaporator under reduced pressure and low temperature. After concentration in vacuo at 50℃, the percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

The extract was then stored air tight in a refrigerator prior to use.

2.3 Partitioning of Solvents

40 g of the crude extract of the plant was weighed separately and transferred into a sterile beaker and subjected to a bio-guided fractionation by solubilization in 100 mL ethanol. This was stirred to allow the extract to dissolve and 100 mL of water was added to dilute the concentration. The solution was thereafter transferred into a separating funnel fixed to a retort stand and defatted sequentially using Hexane (5 × 200 mL) to obtain the hexane fraction. Subsequently, the solution was partitioned sequentially with Ethyl acetate (5 × 200 mL) to obtain the equivalent fraction and finally with Dichloromethane (5 × 200 mL) to sunder out the DCM fraction. Each fraction with the relic from the final hydro-ethanolic fraction were siphoned into separate jars and concentrated using a rotary evaporator and desiccated to obtain semi-liquid to dry fractions. The fractions were stored in air tight containers under refrigeration for later use [15].

2.4 Testing for the Purity of Extracts

The purity of the extracts was determined by streaking the extract on freshly prepared Nutrient medium. This was incubated at 37℃ for 24 h to check for the growth of any contaminating microorganisms.

2.5 Test Organisms

Six test bacteria: *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp. and *Actinobacillus* sp. (obtained from Department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan) and typed strains of *Streptococcus pyogenes* ATCC 19615 and *Klebsiella pneumonia* ATCC 10031 (obtained from The Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State) were used in this study. The isolates were resuscitated on nutrient agar before use.

2.6 Phytochemical Analysis

Qualitative and quantitative phytochemical screening was carried out on the pulverized bark to determine the presence of active secondary metabolites in the plant. The phytochemical assays for alkaloids, flavonoids, saponins, tannins, cardiac glycosides, anthocyanins, phenols and terpenes were carried out according to established procedures [16-22].

2.7 Production of Silver Nanoparticles (SNPs)

The bark extract of *Bridelia ferruginea* was used for the biosynthesis of silver nanoparticles. 100

mL of 1 mM of the aqueous solution of silver nitrate ($AgNO_3$) was prepared in a 250 mL Erlenmeyer flask and 40ml of the dissolved *Bridelia ferruginea* bark extract was added into a well labeled conical flask for the bio-reduction of the silver nitrate ($AgNO_3$) into silver atoms.

2.8 Characterization of Silver Nanoparticles

2.8.1 Visual observation of the biosynthesized SNPs

Visual observation of the extracellular biosynthesized SNPs was carried out by visually observing the gradual colour change of the incubated plant extract and the $AgNO_3$ mixture during the 72 h incubation period.

2.9 UV-Visible Spectroscopy of the Extracellular Biosynthesized SNPs

The SNPs were characterized using UV-Visible Spectrophotometer for the verification of the reduction of the $AgNO_3$ to Ag^o by the plant extract. The absorption spectra of the samples were scanned in UV-visible (vis) spectra, between a wave length of 200-800 nm in a spectrophotometer having a resolution of 1 nm. The UV-Vis spectra were recorded at intervals of 24 h, 48 h and 72 h.

2.10 FTIR Analysis of the Extracellular Biosynthesized SNPs

FTIR analysis of the dried SNPs was carried out using potassium bromide (KBr) pellet (FTIR grade) method in a ratio of 1:100. The spectrum was recorded using Jasco FT/ IR-6300 Fourier Transform Infrared Spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm^{-1}.

2.11 SEM Analysis of the Extracellular Biosynthesized SNPs

The size and morphological analysis of the SNPs was done using SEM. The samples were gold coated using a coater (JEOL, Akishima-shi, Japan, and Model No. JFC-1600). The images of SNPs were obtained in a SEM (ZEISS EVO-MA 10, Oberkochen, Germany). The details regarding applied voltage and magnification used were also implanted on the images.

2.12 Determination of the Antibacterial Activity of the Crude Extract and Extracellular Biosynthesized SNPs from *Bridelia ferruginea*

The antibacterial potential of the crude extract and extracellular biosynthesized SNPs from *Bridelia ferruginea* against some test microorganisms was done using the agar well diffusion method. The indicator strains used were: *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp. and *Actinobacillus* sp. *Streptococcus pyogenes* ATCC 19615 and *Klebsiella pneumonia* ATCC 10031. An 18-24 h old culture of each test isolate was inoculated into 5ml normal saline in a test tube and standardized to 0.5 MacFarland. A sterile swab stick was used to apply the suspension to the surface of already prepared Mueller Hinton Agar (MHA) plates. A sterile 8 mm cork borer was used in boring wells on the agar and a micropipette was used in dispensing 100 µL of the crude extract and 100 µL of the silver nanoparticles (SNPs) into the respective labeled wells. Dimethyl sulfoxide (DMSO), water, silver nitrate ($AgNO_3$) and ciprofloxacin discs were also used as control. The antimicrobial activities were then determined by measuring the diameter of the zones of inhibition.

2.13 Minimum Inhibitory Concentration (MIC) Determination

The MIC of the bark extract and SNPs on the test isolates was determined using two-fold dilution method [23]. Sterile 8 mm cork borer was used to bore five wells onto prepared MHA plates seeded with the test isolates. Different concentrations of the extract and SNPs (100%, 80%, 60%, 40% and 20%) were dispensed into each well and labeled. The preparation was left to diffuse before incubating at 37ºC for 24 h. The lowest concentration of the agent that prevented the growth of the bacteria was taken as the minimum inhibitory concentration (MIC). The zones of inhibition were observed and recorded.

3. RESULTS AND DISCUSSION

The phytochemical analysis of the bark of *Bridelia ferruginea* revealed that alkaloids, flavonoids, saponins, tannins, anthocyanins, cardiac glycosides and steroids were present while phenols and terpenoids were absent (Table 1).

Table 1. Qualitative and quantitative phytochemical composition of *B. ferruginea*

S/N	Phytochemical parameters	Qualitative	Quantitative (mg/100 g)
1	Alkaloids	+++	762
2	Flavonoids	+++	987
3	Saponins	+++	437
4	Tanins	++	209
5	Phenols	-	-
6	Anthocyanins	++	98
7	Cardiac glycosides	++	19
8	Terpenoids	-	-
9	Steroids	+	-

Keys: - = Absent; + = Trace; ++ = Present; +++ = Prominent

The results obtained from this study revealed that the bark of *Bridelia ferruginea* contains medicinally important bioactive substances known as phytochemicals which contribute to the unique characteristics of the plant and its reported antimicrobial potentials against GIT, mouth, throat and skin infections.

These phytochemicals also act as good reducing and capping agents for the stability of silver nanoparticles. The phytochemical screening of this plant has shown the presence of alkaloids, flavonoids, saponins, tannins, anthocyanins, cardiac glycosides and steroids. Some of these phytochemicals contain quinine, morphine and resperine which are used for malaria treatment, pain relief and valuable tranquilizers respectively and so can be used in the development of powerful painkiller medications [24].

Flavonoids have been reported to have antimicrobial activity against a wide array of microorganisms. This activity may be due to the ability of flavonoids to form complexes with extracellular and soluble proteins and to complex with bacterial cell walls [25-26]. Saponins and tannins are also known to be effective anti-oxidants, antimicrobial and anti-carcinogenic agents [27]. The presence of these compounds in *Bridelia ferruginea* is a further confirmation of its medicinal potentials. In addition, cardiac glycosides are generally known to be used medicinally for the treatment of heart problems, chest problems like chest pain and cough and they also serve antioxidant and antimicrobial purposes [28].

The antimicrobial activities of crude ethanolic extract of *Bridelia ferruginea* against some test bacteria is shown in Table 2. It was also observed that all the test bacteria were susceptible to the crude ethanolic bark extract of *Bridelia ferruginea*. The antimicrobial activities of partitioned fractions obtained from the extract of *Bridelia ferruginea* bark against some test bacteria is shown in Table 3. Of all the pathogenic isolates, only *K. pneumonia* and *Actinobacillus* sp. were susceptible to the hexane fraction of *Bridelia ferruginea* while four of the test bacteria were susceptible to the dichloromethane fraction. All the pathogenic isolates showed maximum susceptibility against the ethyl-acetate fraction with the highest susceptibility observed against *Actinobacillus* sp. (18 mm). For the controls used, 66.67% of the test bacteria showed susceptibility with the highest zone of inhibition observed against *Actinobacillus* sp. while all the test organisms were resistant to DMSO that was used as a solvent for the crude fractions.

All the selected bacteria were highly sensitive to the bark extract of *Bridelia ferruginea* with zones of inhibition ranging from 15 – 23 mm. These findings is in agreement with the work of Irobi, et al. [29] who reported antimicrobial potential of the ethanolic stem bark extracts of *Bridelia ferruginea* against some pathogens. Also, some of the test bacteria which were susceptible to ciprofloxacin which is a broad spectrum antibiotic, were highly susceptible to the extract. Some of the test organisms that showed resistance to ciprofloxacin were observed to be highly susceptible to the extract of *Bridelia ferruginea*. This is a further confirmation of the broad spectrum activity of *B. ferruginea* on bacterial isolates.

Bridelia ferruginea bark extract also showed varying antibacterial activities when the ethanolic extract was partitioned into fractions. From this study, it was observed that of all the partitioned fractions obtained, the ethyl-acetate fraction had the highest zone of inhibition against all the pathogenic organisms used in this study with zones ranging from 11 - 18 mm followed by the

dichloromethane fraction having activity on four bacteria (6 - 11 mm) and the least zones were observed with the hexane fraction on two of the organisms (7 - 11 mm).

From this study, it can be deduced that apart from the effective pure ethanolic extract of the bark of *Bridelia ferruginea* that had antibacterial activities against these test organisms, the ethyl-acetate fraction of the plant can still be used for the treatment of some infections.

The production of SNPs from *Bridelia ferruginea* is shown in Plate 1(a - c). As the crude ethanolic extract of *Bridelia ferruginea* was added to the prepared aqueous silver nitrate solution, there was a gradual colour change of the solution from a faint light brown colour to golden brown and finally to permanent colloidal brown after 24 h incubation which was an indication of the formation of silver nanoparticles. It was also observed that the colour intensity increased with the duration of incubation which indicated the formation of more nanoparticles.

The formation and stability of the colloidal solution of the chemically reduced SNPs formed using this plant extract was monitored using UV-Vis spectrophotometry analysis at different time intervals and wavelengths. The colour changes from the formation of silver nanoparticles were due to the formation of Surface Plasmon Resonance (SPR) in the silver nanoparticles.

The UV-Vis Spectra of SNPs synthesis using *Bridelia ferruginea* bark extract is shown in Fig. 1. The formation of silver nanoparticles using *Bridelia ferruginea* bark extract led to changes in colour from a faint yellow colour to a yellowish brown colour within two minutes and then to a permanent colloidal brown colour after 24 h due to the reduction of the aqueous silver nitrate to silver ions which was further reduced to silver nanoparticles. Different absorption peaks of 400 nm, 550 nm and 600 nm were observed after incubation for 24 h, 48 h and 72 h respectively during biosynthesis. Also, it was denoted that these nanoparticles had an increasing intensity with time [30] with the nanoparticles analyzed at 72 h having the highest intensity. The absorption spectra formation from the SNPs was due to the formation of Surface Plasmon Resonance (SPR) in the SNPs. Variation in absorption peak may be due to an increase in colour intensity as incubation time increases which was as a result of surface plasma resonance character which equally confirm the formation of AgNPs [14,9].

The FTIR analysis of the SNPs synthesized from the bark of *Bridelia ferruginea* is shown in Fig. 2. The FTIR spectrum of SNPs was observed between 459.07 cm^{-1} and 3441.12 cm^{-1} which represents various functional groups found in the SNPs formed. The peak observed at 3441.12 cm^{-1} indicates the N-H stretching of the secondary amides with a strong absorption of the O-H stretching of the hydroxyl group. Peaks observed at 2812.31 cm^{-1} and 2360.95 cm^{-1} represents the C-H stretching bonds and prominent bands were also observed around 1888.37 cm^{-1}, 1622.19 cm^{-1}, 1539.25 cm^{-1} and 1456.30 cm^{-1} denoting N-H and C=C bonds. The peaks at 777.34 cm^{-1}, 694.40 cm^{-1}, 520.08 cm^{-1} and 459.07 cm^{-1} shows the C-O stretching of the carbonyl bonds and the prominent and sharp peaks observed at 1116.82 cm^{-1} and 619.17 cm^{-1} denotes the bio-reduction of silver nitrate ($AgNO_3$) to silver atoms.

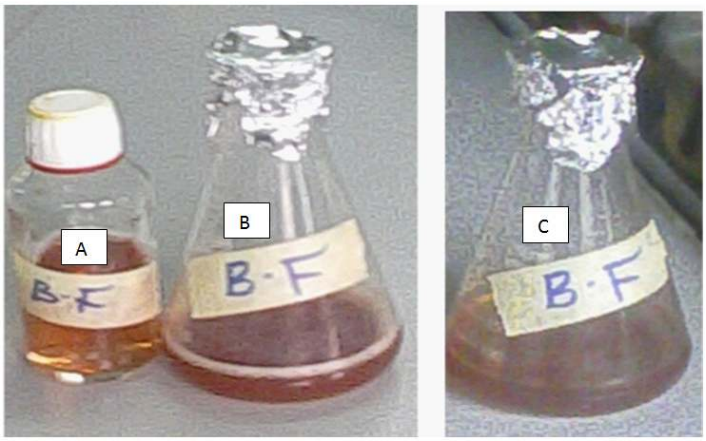

Plate 1. Production of SNPs from *Bridelia ferruginea* bark extract
A- Before addition of $AgNO_3$, B- After addition of $AgNO_3$, C- Final colour change

Table 2. Antimicrobial activities of crude ethanolic extract of *Bridelia ferruginea* against some test bacteria

S/N	Test bacteria	Zones of inhibition (mm)	
		Bridelia ferruginea	**Ciprofloxacin**
1	*Streptococcus pyogenes* ATCC 19615	19	-
2	*Klebsiella pneumonia* ATCC 10031	23	21
3	*Bacillus* sp.	18	-
4	*Actinobacillus* sp.	22	26
5	*Pseudomonas aeruginosa*	15	12
6	*Staphylococcus aureus*	20	19

Table 3. Antimicrobial activities of partitioned fractions obtained from the extract of *Bridelia ferruginea* bark against some test bacteria

Partitioned fractions	Zones of inhibition (mm)					
	S. pyogenes	***K. pneumoniae***	***Bacillus* sp.**	***Actinobacillus* sp.**	***P. aeruginosa***	***S. aureus***
Hexane	-	11	-	7	-	-
Ethyl-acetate	16	13	13	18	11	12
Di Chloro Methane (DCM)	6	11	10	-	-	9
Ethanol relic	10	9	8	-	6	14
DMSO	-	-	-	-	-	-
Ciprofloxacin	-	21	-	26	12	19

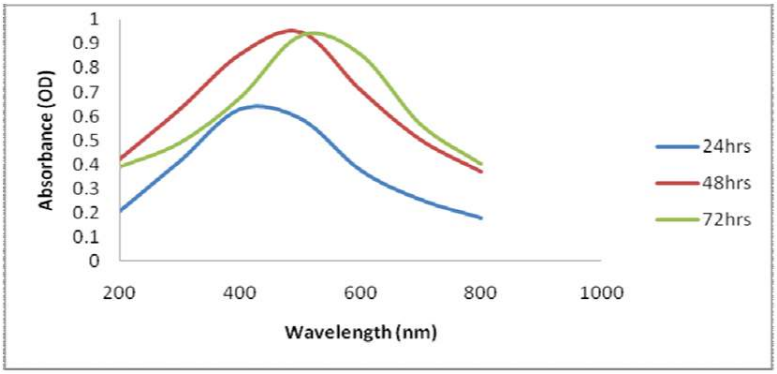

Fig. 1. UV-Vis Spectra of SNPs synthesis using *Bridelia ferruginea* bark extract

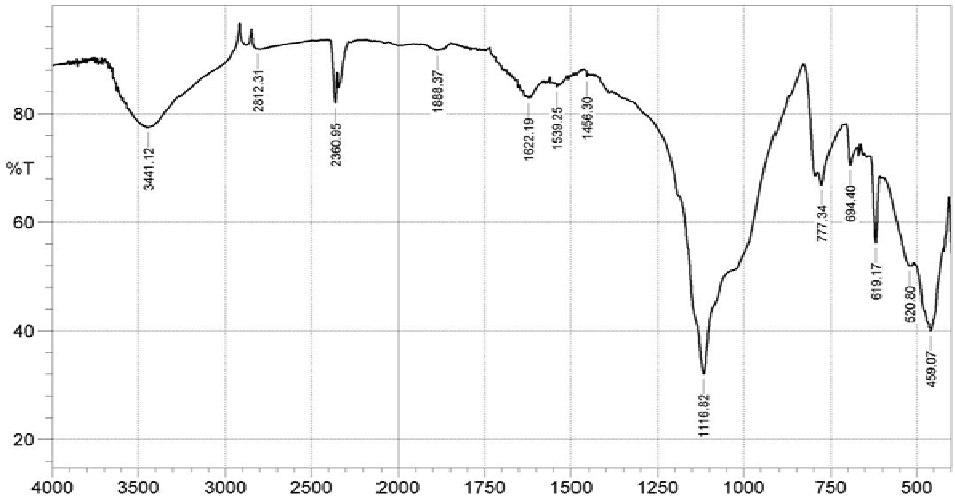

Fig. 2. FTIR analysis of SNPs synthesized from *Bridelia ferruginea* bark extract

There were various functional groups found in this range which includes the carboxyl group, the hydroxyl group, the carbonyl group, secondary amides and the aromatic conjugates while the SEM showed varying shapes of the extract from square to triangular to pentagonal shapes.

The SEM micrographs of the SNPs biosynthesized from *Bridelia ferruginea* at magnifications of ×1000, ×250, ×100 and ×25 is shown in Fig. 3. This provided further insight into the morphology and size details of the SNPs. From the SEM images, it was evident that the silver nanoparticles have a pentagonal shape and size ranging from 0.0 – 9.4 nm.

It is known from studies that the use of medicinal plant extracts for the synthesis of silver nanoparticles enhances the reduction and stabilization of silver nitrates in an aqueous medium for the formation of silver nanoparticles SNPs) which resulted in colour changes as a result of the vibrations produced by surface plasmon resonance [31] caused by the reduction of silver ion to silver nanoparticles.

Borase et al. [32] reported the active components of plants that are responsible for the reduction of silver ions to SNPs include proteins, flavonoids and carboxylic groups of arabinose and galactose, reducing sugars, tannins, aliphatic amines, aliphatic alkenes of alkaloids, polysaccharides, aromatic amines, sec-alcohols, water-soluble heterocyclic components and saponins. Typically, plant extracts possess intrinsic biological activities, which may further manifest in the biological activities of SNPs as a result of combining the two materials. Therefore, plant extracts can potentially be developed into novel nano-materials with diverse biological activities [33].

Table 4. Antibacterial activity of *Bridelia ferruginea* crude extract, silver nanoparticles biosynthesized from *Bridelia ferruginea* bark, $AgNO_3$, DMSO and Ciprofloxacin controls against test bacteria

Test bacteria	Zone of inhibition (mm)				
	Bridelia ferruginea		Control		
	Crude extract	SNPs	$AgNO_3$	DMSO	Ciprofloxacin
Streptococcus pyogenes	19	17	6	-	-
Klebsiella pneumoniae	23	21	3	-	21
Bacillus sp.	20	16	-	-	-
Actinobacillus sp.	22	17	-	-	26
Pseudomonas aeruginosa	15	13	-	-	12
Staphylococcus aureus	20	17	-	-	24

It was observed that the crude extract of the bark of *Bridelia ferruginea* and the biosynthesized SNPs had activities against all the six test organisms with the highest activity observed against *Klebsiella pneumonia* (23 mm and 21 mm respectively). The aqueous solution of silver nitrate used as control had activity on two of the organisms with the highest zone of inhibition observed against *Streptococcus pyogenes* (6 mm), ciprofloxacin also had activity on four of the six organisms with the highest zone of inhibition recorded against *Actinobacillus* sp. (26 mm) while DMSO had no effect on any of the pathogenic organisms. *Bacillus* sp. and *Streptococcus pyogenes* were also susceptible to both the crude extract and the synthesized SNPs but were resistant to the aqueous solution of silver nitrate, DMSO and ciprofloxacin. *Pseudomonas aeruginosa* had the least susceptibility to both the crude extract of *Bridelia ferruginea* and the SNPs produced when compared to the other bacteria isolates. It was also susceptible to ciprofloxacin but resistant to silver nitrate and DMSO (Table 4 above).

One of the most remarkable and peculiar properties of silver nanoparticles is their antibacterial activities compared to $AgNO_3$. The silver nanoparticles biosynthesized from the bark extract of *Bridelia ferruginea* however revealed a very potent and effective activity of the nanoparticles against all the test organisms in this study with more pronounced zones of inhibition ranging from 13 mm on *Pseudomonas aeruginosa* to 21 mm on *Klebsiella pneumonia*. The SNPs produced from this bark extract showed higher toxicity to the test organisms when compared to $AgNO_3$ used as control in this study. However, from previous studies, the SNPs derived from the extract of *Tribulister restris* had a lesser activity against *Streptococcus pyogenes* compared to that of SNPs derived from *Bridelia ferruginea* [34].

The application of SNPs against some pathogenic microorganisms was also investigated by Parveen et al. [35]. In their study, there was antimicrobial activity against the organisms, with maximum zones of inhibition towards *Bacillus* sp. (7 mm) and *Klebsiella pneumonia* (7 mm), these values are however lower than what was obtained in this study for SNPs biosynthesized from *Bridelia ferruginea* bark extract, which had zones of 16 mm and 21 mm for *Bacillus* sp. and *Klebsiella pneumoniae* respectively.

Based on the report of Gavhane *et al*. [36], the zones of inhibition against Gram positive and Gram negative test pathogens ranged from 11 - 14 mm for Neem SNPs and 10 - 14 mm for Triphala SNPs, which were lesser than the range of zones of *Bridelia ferruginea* SNPs which was from 13 - 21 mm in in this study. This report is in contrast to that of Balashanmugam et al. [37] that SNPs biosythensized using *Cassia fistula* extract had greater antimicrobial potential against some pathogenic microorganisms than the extract from the same plant. The use of silver nanoparticles as antimicrobial agents and as potential drug carrier in treatment of cancer has gained worldwide attention [9,38].

Table 5 shows the minimum inhibitory concentration of *Bridelia ferruginea* extract and their SNPs. The MIC of both the crude extract and the biosynthesized SNPs against *Streptococcus pyogenes*, *Klebsiella pneumonia, Actinobacillus* sp., *Staphylococcus aureus* and *Bacillus* sp. were at 20% (20 µl/mL). However, the MIC of the synthesized SNPs that prevented the growth of *Pseudomonas aeruginosa* was 40% (40 µl/mL) while it was 20% for the crude extract of the bark of *Bridelia ferruginea*.

Table 5. MIC of *Bridelia ferruginea* extract and their SNPs on the test bacteria

Test bacteria	Zones of inhibition (mm)									
	(100%)		(80%)		(60%)		(40%)		(20%)	
	Crude extract	SNPs	Crude extract	SNPs	Crude extract	SNPs	Crude extract	SNPs	Crude extract	SNPs
Strep. pyogenes	19	16	15	10	9	6	5	3	2	1
K. pneumoniae	22	19	17	13	11	8	4	3	2	2
Bacillus sp.	18	16	11	12	7	8	4	5	1	2
Actinobacillus sp.	21	16	15	10	8	7	5	5	2	2
P.aeruginosa	13	11	9	5	7	3	4	1	1	-
S. aureus	18	15	14	11	9	7	6	4	2	1

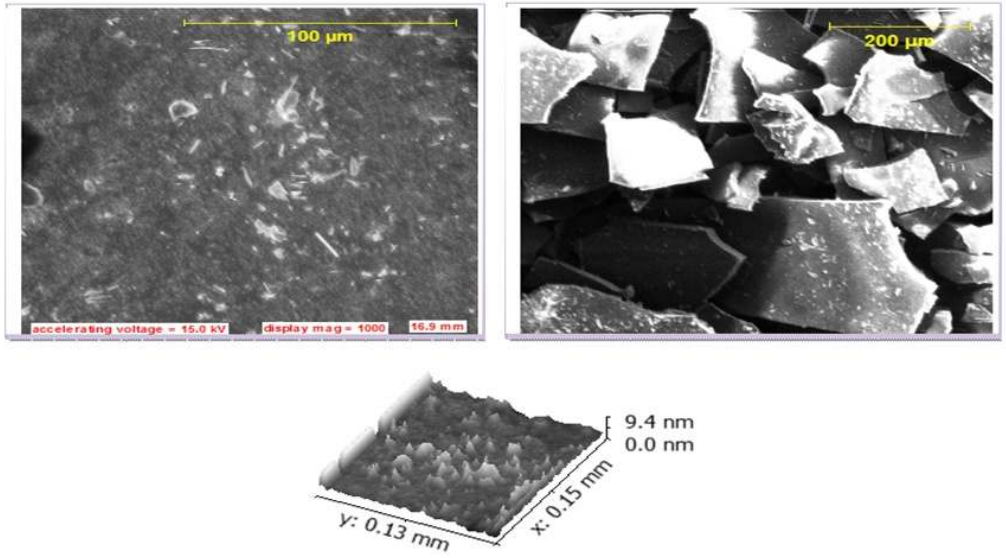

Fig. 3. SEM micrographs of SNPs synthesized from *Bridelia ferruginea* bark extract

The MIC illustrates a decreasing inhibitory effect of the crude extracts and its SNPs as the concentration decreases as there was a progressive decrease in the zone of inhibition suggesting a dose-dependent activity. This implies that the antimicrobial activity of a substance is concentration dependent, which is in concordance with the report of Dubey et al. [39] and Oboh [40], that antimicrobial activity is a function of the active ingredient reaching an organism.

4. CONCLUSION

This study demonstrated that *Bridelia ferruginea* bark extract contain some bioactive compounds with varying antimicrobial potentials. The fractions and the SNPs also exhibited potent antimicrobial activity. The crude extract had a better antibacterial potential than the biosynthesized SNPs. Conclusively, the bark extract and the SNPs from *Bridelia feruuginea* can therefore be seen as a good substrate for the production of useful drugs in place of antibiotics that are becoming less potent against pathogenic microorganisms

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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