

**The Phytochemical and Antimicrobial Potential of The Crude Extracts of
Bridelia ferruginea and the extracellular biosynthesized silver Nanoparticles**

Temitayo Omotola AKINSETE, Bukola Christianah ADEBAYO-TAYO and Abimbola Olumide
ADEKANMBI

Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria

Corresponding author: bukola.tayo@gmail.com; temitayoakinsete79@gmail.com;

bimboleen@yahoo.com

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ABSTRACT

Bridelia ferruginea has been used in African folk medicine for the treatment of several ailments especially throat infections. The phytochemical analysis, fractioning using different solvents, antimicrobials and silver nanoparticles (SNPs) produced by the ethanolic extract of the bark of *B. ferruginea* was investigated. Flavonoids (987mg/100g), alkaloids (762mg/100g), saponins (437mg/100g) and tannins (209mg/100g) were the major bioactive compounds present in this plant while phenols and terpenes were absent. All the test organisms were found to be susceptible to the *B. ferruginea* with *Klebsiella pneumonia* (23mm) having the highest susceptibility against the crude extract of the plant. Also, all the test pathogens were susceptible to the ethyl acetate fraction of *B. ferruginea* in which *Actinobacillus* sp. had the highest susceptibility (18mm). The bark extract was used for extracellular biosynthesis of SNPs which were characterized by using visual observation, UV-Visible Spectrophotometer, Scanning Electron Microscope and FTIR analysis. The SNPs has pentagonal shape and the size ranged from 0.0 – 9.4 nm. The antimicrobial potential of the characterized SNPs against some

pathogenic microorganisms was determined. The antibacterial activities of the crude and synthesized SNPs of *B. ferruginea* ranged from 15 – 23mm and 13 – 21mm respectively with *Klebsiella pneumonia* having the highest susceptibility. The crude extract exhibited higher antibacterial activities against the pathogens more than the SNPs from the extract. In conclusion, the crude ethanolic extract, the partitioned fractions, and the SNPs of the bark extract of *B. ferruginea* had a good potential use as antibacterial against pathogenic microorganisms.

Keywords: *Bridelia ferruginea*, phytochemical analysis, antimicrobials, silver nanoparticles,

INTRODUCTION

Several infections like the Gastro intestinal tract (GIT) infection, mouth infections, throat infections and skin infections have become rampant in so many people based on reported cases in hospitals. This may due to the prevalence of antibiotic resistance as the antibacterial agents used in treating this several bacterial infections are becoming less potent against the causative pathogenic microorganisms. This is as a result of the common misuse of these antibiotics by physicians and as a consequence of this widespread and injudicious use of antibacterial agents, there has been an accelerated emergence of antibiotic-resistant pathogens which has resulted into a serious threat to the global public health. From knowledge, it is widely known that traditional herbalists use plants to treat infectious diseases and many of these plants have been investigated scientifically for antimicrobial activity and some plant products have been shown to inhibit the growth of pathogenic microorganisms. These plants are generally referred to as medicinal plants[1].

Bridelia ferruginea, commonly called “*Epo Ira*” in Yoruba is a popularly known plant in many African countries which has played a major role in the treatment of several bacterial infections

and it has gained importance due to its unique constituents and versatile applicability in the treatment of various infections of the mouth, throat, gastro intestinal tract and even the skin [2]. Based on this knowledge, *Bridelia ferrugineacan* therefore be applied in various field of research especially nanotechnology in place of the common antibiotics used for the treatment of bacterial infections.

Nanotechnology is a modern field of research that basically deals with the synthesis, strategy and the manipulation of particles ranging from approximately 1 to 100 nm in size. These particles are generally referred to as Nanoparticles which are well known and recognized as they have 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 and also in the pharmaceutical industries for the production of soaps, detergents, toothpastes and other pharmaceutical products and are hence directly encountered by human systems. Various approaches available for the synthesis of silver nanoparticles include the chemical and physical methods which have been widely employed to prepare silver nanoparticles of various sizes and shapes [4 - 6]. These methods are quite expensive and potentially dangerous to the environment, involve more than one step, have a high energy requirement, low material conversion, difficulty in purification, emit hazardous chemicals, occupies a large space and employs a great deal of energy and time while raising the environmental temperature around the source material and succeeding thermal stability[4].

Biosynthesis of nanoparticles is an alternative and eco-friendly method for 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 other biological agents since microbe mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions, the 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; it is environmental 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 biomolecules called phytochemicals which mediates the synthesis and stabilizes the nanoparticles formed in a desired size and shape[11]. Yugalet al.,[12] 2017 reported the antimicrobial, antioxidant and cytotoxic activity of silver nanoparticles from leaf extract of *Erythrina suberosa* (Roxb.) Ahmed et al., [13]; Mohanta et al., [14] reported the biosynthesis of AgNPs using different plant extract 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).

Materials and Methods

Collection of Plant

The fresh barks of *Bridelia ferruginea* were obtained from Oje market in Ibadan, Oyo state, Nigeria and were authenticated at the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan. This was air dried for 3 weeks and pulverized in a mortar and pestle.

Preparation of Extracts

The ground powdered sample of *Bridelia ferruginea* was weighed and 1500g (1.5kg) of the sample was poured into a macerating jar and then distilled ethanol was added and filled to the brim. The solvent was then stirred with the solute using a well sterilized glass rod and this was repeated every 7 hrs till the last day of maceration which lasted for 72 hrs. After, the extract was decanted and filtered using a Whatman's filter paper (No.1). This was then concentrated using a rotary evaporator under reduced pressure and low temperature. After concentration in vacuo at 50°C, 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.

Partitioning of Solvents

40g 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 100mLs ethanol and stirred to allow the extract to dissolve and 100mls of water was added to dilute the concentration. This solution was transferred into a separating funnel fixed to a retort stand and defatted sequentially using Hexane (5 × 200mL) to obtain the hexane fraction. Subsequently, the solution was

partitioned sequentially with Ethyl acetate ($5 \times 200\text{mL}$) to obtain the equivalent fraction and finally with Dichloromethane ($5 \times 200\text{mL}$) 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 later desiccated to obtain semi-liquid to dry fractions from each solvent. The fractions were stored air tight in a refrigerator for later use [15].

Testing for the Purity of Extracts

The purity of the extract was determined by streaking the extract on freshly prepared Nutrient and MacConkey agar plates. This was incubated at 37°C for 24 hrs. After incubation the plates were checked for the growth of contaminant microorganisms.

Collection of Test Organisms

Six bacterial isolates were collected for this study. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp. and *Actinobacillus* sp. were collected from The Department of Medical Microbiology, University College Hospital (UCH), Ibadan while typed organisms of *Streptococcus pyogenes* ATCC 19615 and *Klebsiella pneumonia* ATCC 10031 were obtained from The Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State. The isolates were sub-cultured on plates of nutrient agar and incubated for 24 hrs at $35-37^{\circ}\text{C}$. The desired colonies were then picked and stored in slants until when needed.

Phytochemical Analysis

Qualitative and quantitative phytochemical screening was carried out on the pulverized bark to determine the presence of active secondary metabolites present 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].

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 40 mL 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 (Ag^0) ions.

Characterization of Silver nanoparticles

Visual observation of the extracellular biosynthesized SNPs

The gradual colour change of the incubated plant extract and the AgNO_3 mixture was observed visually after incubation for 24 – 72 hrs.

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^0 by the plant extract. The absorption spectra of the samples were scan in UV-visible (vis) spectra, between a wave length of 200-800 nm in a spectrophotometer having a resolution of 1 nm was done. The UV-Vis spectra were recorded at intervals of 24 hrs, 48 hrs and 72 hrs.

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.

SEM analysis of the extracellular biosynthesized SNPs

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

Antibacterial estimation of the crude extract and of the extracellular biosynthesized SNPs from *Bridelia ferruginea*

The antibacterial potential of the crude extract and the extracellular biosynthesized SNPs from *Bridelia ferruginea* against some pathogenic microorganisms was done using Agar Well Diffusion method. The indicator strains used are: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus* sp. and *Actinobacillus* sp. *Streptococcus pyogenes* ATCC 19615 and *Klebsiella pneumonia* ATCC 10031. Plates of nutrient agar were prepared and the isolates stored in slants were picked and sub-cultured on the nutrient agar plates and incubated for 24 hours at 37°C. A colony of each isolate was picked into McCartney bottles containing 10 mL of sterile water. Following the MacFarland standard, a sterile swab stick was placed in each

suspension and used in spreading each pathogenic isolate on plates of Mueller Hinton Agar (MHA) for sensitivity tests using the Agar Well Diffusion method. A sterile 8mm cork borer, was used in boring wells on the agar and a micropipette was used in dispensing 100µl of the crude extract and 100µl of the silver nanoparticles (SNPs) into the respective labeled wells. Dimethyl sulfoxide (DMSO), water, silver nitrate (AgNO₃) and Ciprofloxacin discs were also used as control. The antimicrobial activities were then determined by measuring the diameter of the zones of inhibition in mm that was produced.

Minimum Inhibitory Concentration (MIC) Determination

The MIC of different concentrations of the bark extract and SNPs was determined using two-fold dilutions method [23], that is, half strength (10mg/mL) and quarter strength (5mg/ml) of the extract; in addition to the normal strength (20mg/mL) while AgNO₃, DMSO and ciprofloxacin discs were used as controls. The sterilized 8mm cork borer was used to bore five wells in the already solidified media inoculated with the microorganisms. Different concentrations of the extract and SNPs (100%, 80%, 60%, 40% and 20%) were dispensed into each well and properly labeled. The preparation was left to diffuse before incubating at 37°C for 24 hours for the bacterial strains. The lowest concentration of antimicrobial agent that completely prevented the growth of the bacteria was taken as the minimum inhibitory concentration (MIC) of the extract. The zones of inhibition were then observed and recorded.

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/100g)
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 has revealed that the bark of *Bridelia ferruginea* contain medicinally important biological active substances known as phytochemicals which contributes to the unique characteristics of the plant and its antimicrobial potentials against GIT infections, mouth infections, throat infections 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, pain relief

223 and valuable tranquilizers respectively and so can be used in the development of powerful
224 painkiller medications [24].

225 Flavonoids have antimicrobial agent against a wide array of microorganisms which indicates
226 *Brideliaferruginea* as a plant that has anti-inflammatory, anti-allergic, anti-thrombotic or
227 vasoprotective and antibacterial effects. This activity may be due to the ability of flavonoids to
228 form complexes with extracellular and soluble proteins and to complex with bacterial cell walls
229 [25 - 26]. Saponins and tannins are also known to be effective anti-oxidants, antimicrobial and
230 anti-carcinogenic agents [27].

231 However, cardiac glycosides are generally known to be used medicinally for the treatment of
232 heart problems, chest problems like chest pain and cough and they also serve antioxidant and
233 antimicrobial purposes [28].

234
235 The antimicrobial activities of crude ethanolic extract of *Brideliaferruginea* against some test
236 pathogens is shown in Table 2. It was also observed that all the test pathogens were susceptible
237 to the crude ethanolic bark extract of *Brideliaferruginea*.

238
239 The antimicrobial activities of partitioned fractions obtained from the extract of
240 *Brideliaferrugineabark* against some test pathogens is shown in Table 3. Of all the pathogenic
241 isolates, only *K. pneumonia* and *Actinobacillus* sp. were susceptible to the hexane fraction of
242 *Brideliaferrugineawhile4* were susceptible to the dichloromethane fraction. All the pathogenic
243 isolates showed maximum susceptibility against the ethyl-acetate fraction with the highest
244 susceptibility observed against *Actinobacillus* sp. For the controls used, 66.67% of the test
245 pathogens only showed susceptibility with the highest zone of inhibition observed against

246 *Actinobacillus* sp. while all the test organisms were resistant to DMSO that was used as a solvent
247 for the crude fractions.

248
249
250
251 **Table 2: Antimicrobial activities of crude ethanolic extract of *Brideliaferruginea* against**
252 **some test pathogens**

S/N	Pathogens	Zones of Inhibition (mm)	
		<i>Brideliaferruginea</i>	Ciprofloxacin
1	<i>Streptococcus pyogenes</i> ATCC 19615	19	-
2	<i>Klebsiella pneumonia</i> ATCC 10031	23	21
3	<i>Bacillus</i> sp.	18	-
4	<i>Actinobacillus</i> sp.	22	26
5	<i>Pseudomonas aeruginosa</i>	15	12
6	<i>Staphylococcus aureus</i>	20	19

254
255
256 **Table 3: Antimicrobial activities of partitioned fractions obtained from the extract of**
257 ***Brideliaferruginea* bark against some test pathogens.**

Partitioned fractions	Zones of Inhibition for (mm)					
	<i>S. pyogenes</i>	<i>K. pneumoniae</i>	<i>Bacillus</i> sp.	<i>Actinobacillus</i> sp.	<i>P. aeruginosa</i>	<i>S. aureus</i>
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
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259

260 All the selected pathogens were highly sensitive to the bark extract of *Brideliaferruginea* with
 261 zones of inhibition ranging from 15 - 23mm in diameter. These findings is in agreement with the
 262 previous works of Irobi, *et al.* [29] who reported antimicrobial potential of the ethanolic stem
 263 bark extracts of *Brideliaferruginea* against some pathogens. Also, some of these test pathogens
 264 which were susceptible to ciprofloxacin which is a broad spectrum antibiotic, were highly
 265 susceptible to the extract. Some pathogen that shows resistance to Ciprofloxacin was found to be
 266 highly susceptible to *Brideliaferruginea* extract(19mm and 18mm respectively). This has proved
 267 that *B. ferruginea* can also exhibit broad spectrum activities.

268 *Brideliaferruginea* bark extract also showed varying antibacterial activities when the ethanolic
 269 extract was partitioned into fractions. From this study, it was observed that of all the partitioned
 270 fractions obtained, the ethyl-acetate fraction had the highest zone of inhibition against all the
 271 pathogenic organisms used in this study with zones ranging from 11–18mm in diameter followed
 272 by the dichloromethane fraction having activity on four pathogens (6-11mm) and the least zones
 273 were observed by the hexane fraction on two pathogens (7-11mm).

274 From these work, it can be deduced that apart from the effective pure ethanolic extract of the
 275 bark of *Brideliaferruginea* that had antibacterial activities against these test pathogens, the ethyl-
 276 acetate fraction of the plant can still be used for the treatment of some infections.

277

278 The production of SNPs from *Brideliaferrugineais* shown in Plate 1 (a-c). As the crude ethanolic
 279 extract of *Brideliaferruginea* was added to the prepared aqueous silver nitrate solution, there was
 280 a gradual colour change of the solution from a faint light brown colour to golden brown and
 281 finally to permanent colloidal brown after 24 hours of incubation whichwas an indication of the

formation of silver nanoparticles. It was also observed that the colour intensity increased with the duration of incubation which indicates 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 *Brideliaferruginea* bark extract is shown in Figure 1. The formation of silver nanoparticles using *Brideliaferruginea* 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 hours due to the reduction of the aqueous silver nitrate to silver ions which was further reduced to silver nanoparticles. Different absorption peaks of 400nm, 550nm and 600nm was observed after incubation for 24, 48 and 72hrs respectively during biosynthesis. Also, it was denoted that these nanoparticles had an increasing intensity with time [30] with the nanoparticles analyzed at 72 hours 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 SNPssynthesized from the bark of *Brideliaferruginea* is shown in Figure 2. The FTIR spectrum of *Brideliaferruginea* were observed between 459.07cm^{-1} and

3441.12cm⁻¹ which represents various functional groups found in the SNPs formed. The peak observed at 3441.12cm⁻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 2812.31cm⁻¹ and 2360.95cm⁻¹ represents the C-H stretching bonds and prominent bands were also observed around 1888.37cm⁻¹, 1622.19cm⁻¹, 1539.25cm⁻¹ and 1456.30cm⁻¹ denoting N-H and C=C bonds. The peaks at 777.34cm⁻¹, 694.40cm⁻¹, 520.08cm⁻¹ and 459.07cm⁻¹ shows the C-O stretching of the carbonyl bonds and the prominent and sharp peaks observed at 1116.82cm⁻¹ and 619.17cm⁻¹ denotes the bio-reduction of silver nitrate (AgNO₃) to silver ions (Ag⁰).

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 X1000, X250, X100 and X25 is shown in Figure 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 the size ranged from 0.0 – 9.4nm.

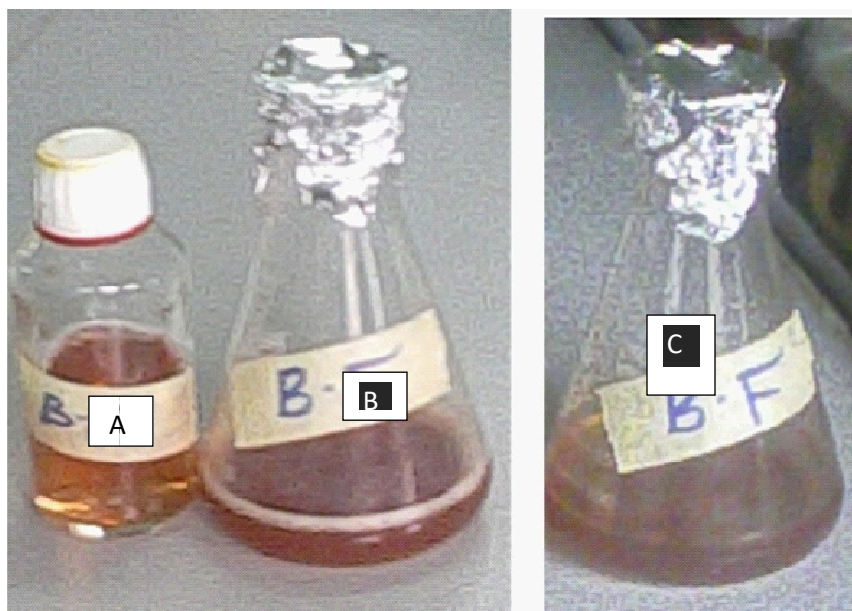


Plate 1 : Production of SNPs from *Bridelia ferruginea* bark extract

A- Before addition of AgNO_3 , B- After addition of AgNO_3 , C- Final colour change

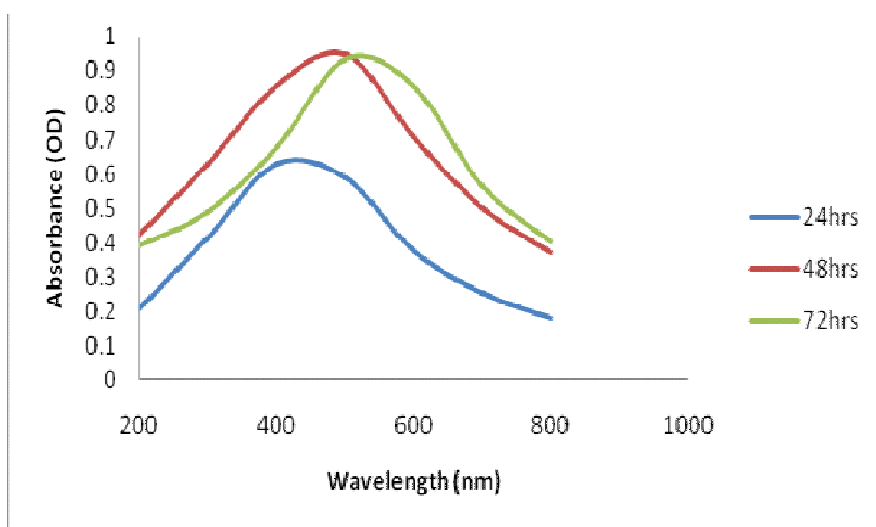


Fig. 1: UV-Vis Spectra of SNPs synthesis using *Bridelia ferruginea* bark extract

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 Plasmons [31] caused by the reduction of silver ion to silver nanoparticles.

Boraseet al. [32] reported the active components of plants that are responsible for the reduction of silver ions to SNPs and according to these authors, the list of active components 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].

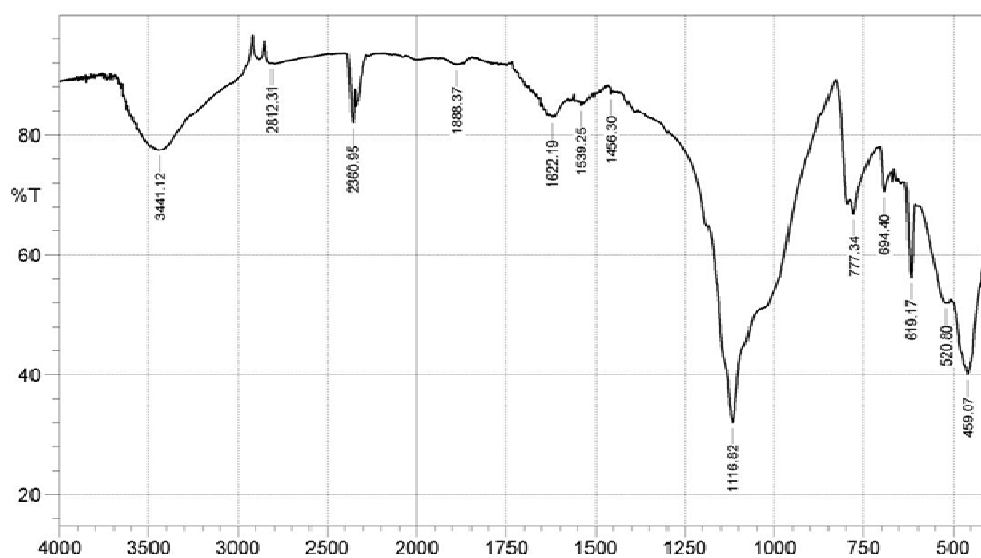


Fig. 2: FTIR analysis of SNPs synthesized from *Bridelia ferruginea* bark extract.

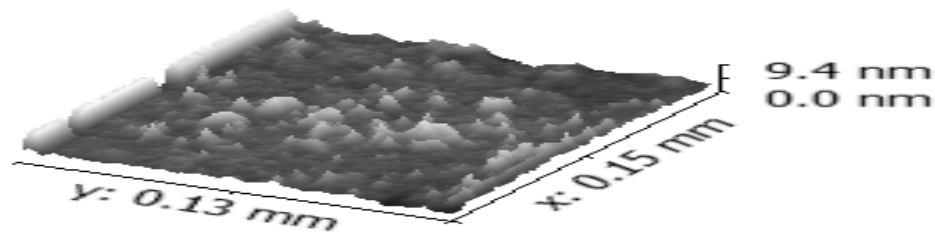
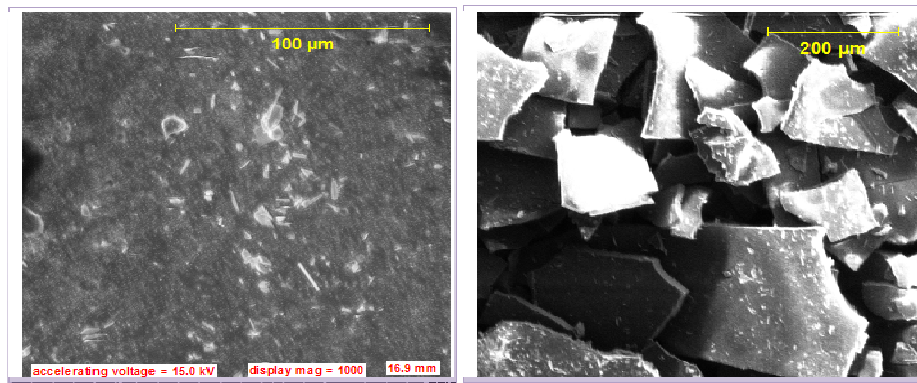


Fig. 3: SEM micrographs of SNPs synthesized from *Bridelia ferruginea* bark extract

It was observed that the crude extract of the bark of *Bridelia ferruginea* and the biosynthesized SNPs had activities against all the 6 pathogenic organisms with the highest activity observed against *Klebsiella pneumonia* (23mm and 21mm resp.) The aqueous solution of silver nitrate used as control had activity on 2 of the organisms with the highest zone of inhibition observed

against *Streptococcus pyogenes* (6mm), Ciprofloxacin also had activity on four of the six organisms with the highest zone of inhibition recorded against *Actinobacillus* sp. (26mm) 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).

Table 4: Antibacterial activity of silver nanoparticles biosynthesized from *Bridelia ferruginea* bark against test pathogens

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

One of the most remarkable and peculiar properties of silver nanoparticles is their antibacterial activities compares to AgNO₃.

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 pathogens in this study with more pronounced zones of inhibition that ranged from 13mm on *Pseudomonas*

377 *aeruginosa* to 21mm on *Klebsiella pneumonia*. These SNPs produced from this bark extract
378 showed higher toxicity to the test pathogens when compared to AgNO₃ used as control in this
379 study. However, from previous works done, the SNPs derived from the extract of
380 *Tribulusterrestris* also has a lesser activity against *Streptococcus pyogenes* compared to that of
381 SNPs derived from *Brideliaferruginea*[34].

382 The application of SNPs against some pathogenic microorganisms was also investigated by
383 Parveen *et al.* [35] which showed efficient antimicrobial activity with maximum zones of
384 inhibition towards *Bacillus* sp. (7mm) and *Klebsiella pneumonia* (7mm) but not as potent as the
385 significant activity of SNPs biosynthesized from *Brideliaferruginea* bark extract which had
386 zones of 16mm and 21mm in respect to the pathogenic microorganisms.

387 Based on the works of Gavhane *et al.* [36], the zones of inhibition against the Gram positive and
388 Gram negative test pathogens ranged from 11 - 14mm for Neem SNPs and 10 - 14mm for
389 Triphala SNPs, which were lesser than the range of zones of *Brideliaferruginea* SNPs which was
390 from 13 - 21mm in diameter. This report contrast the report of Balashanmugam *et al.* [37] that
391 SNPs biosynthesized using *Cassia fistula* extract had greater antimicrobial potential against
392 some pathogenic microorganism than the extract from the same plant. The use of silver
393 nanoparticles as antimicrobial agent and as a potential drug carrier in treatment of cancer has
394 gained attention [38, 9].

395
396 The minimum inhibitory concentration of the crude extract of the bark of *Brideliaferruginea* and
397 the synthesized SNPs were also determined. Table 5 shows the minimum inhibitory
398 concentration of *Brideliaferruginea* extract and their SNPs. The minimum inhibitory
399 concentration (MIC) was determined using the agar well diffusion method. Against

Streptococcus pyogenes, *Klebsiella pneumonia*, *Actinobacillus* sp., *Staphylococcus aureus* and *Bacillus* sp. the MIC of both the crude extract and the biosynthesized SNPs were at 20% (20µl/mL). However, the MIC of the synthesized SNPs that prevented the growth of *Pseudomonas aeruginosa* was at 40% (40µl/mL) while it was 20% for the crude extract of the bark of *Bridelia ferruginea*. This is an indication that the bacteriostatic potential of crude extract and the SNPs was at different concentration against *Pseudomonas aeruginosa*.

Table 5: MIC of *Bridelia ferruginea* extract and their SNPs on the test pathogens

Pathogens	Zones of Inhibition (mm)									
	(100%)		(80%)		(60%)		(40%)		(20%)	
	Crude Extract	SNPs	Crude Extract	SNPs	Crude Extract	SNPs	Crude Extract	SNPs	Crude Extract	SNPs
<i>Strep.pyogenes</i>	19	16	15	10	9	6	5	3	2	1
<i>Kleb.pneumoniae</i>	22	19	17	13	11	8	4	3	2	2
<i>Bacillus</i> sp.	18	16	11	12	7	8	4	5	1	2
<i>Actinobacillus</i> sp.	21	16	15	10	8	7	5	5	2	2
<i>Pse.aeruginosa</i>	13	11	9	5	7	3	4	1	1	-
<i>Staph.aureus</i>	18	15	14	11	9	7	6	4	2	1

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 thickness of the layer of colonies on the streak but also a decrease in the number of colonies from low concentrations to high concentrations thus reflecting a dose-dependent sensitivity. This implies that 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.

Conclusion

This research work demonstrated that *Bridelia ferruginea* bark extract contain some bioactive compounds with varying antimicrobial potentials. The fractions and the SNPs also exhibit potent antimicrobial activity. The crude extract had a better antibacterial potential than the biosynthesized SNPs. Conclusively, the bark extract and the SNPs from *Bridelia ferruginea* 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

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