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***RESEARCH ARTICLE***

# AN ASSESSMENT OF THE ANTIBACTERIAL EFFICACY OF METHANOL EXTRACT OF *MITRACARPUS SCABER* AND ITS BIOSYNTHESISED SILVER NANOPARTICLES

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**ABSTRACT**

Research on biosynthesis and biological application of Nanoparticles (NPs) has increased significantly in the last decades due to their eco-friendly nature and cost-effectiveness compared to other routes of nanoparticle synthesis. Recently, significant growth has been observed in the utilisation of medicinal herbs for nanoparticle synthesis due to their numerous advantages and fewer complications. *Mitracarpus scaber* is a medicinal plant widely employed on both humans and animals in herbal medicine. This study used standard procedures to conduct qualitative and quantitative phytochemical analysis of *Mitracarpus scaber* leaf extract. Furthermore, the biosynthesised silver nanoparticles (AgNPs) were characterised using scanning electron microscopy, Energy dispersive x-ray, UV-visible spectroscopy and Fourier-transform infrared spectroscopy,. The inhibitory properties of the methanol extract and biosynthesised nanoparticles were evaluated against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus vulgaris, Listeria monocytogenes* ATCC 19111, *Pseudomonas aeruginosa* and *Escherichia coli* ATCC 25922 employing the macro-broth dilution techniques. Findings of phytochemical screening indicated the existence of alkaloids, tannins, saponins, terpenoids, phenols and flavonoids. However, phenol was the most abundant phytochemical present (42.00±0.04 mg/100g). The minimum inhibitory concentration exerted by methanolic extract of *Mitracarpus scaber* leaf ranged from 2.25-4.5 mg/ml and 0.01-0.05 mg/ml for biosynthesised silver nanoparticles. The minimum bactericidal concentration of the methanolic extract and biosynthesised silver nanoparticles ranged from 2.25-18 mg/ml and 0.03-0.1 mg/ml, respectively. The findings from the antibacterial evaluation show that the biosynthesised nanoparticle is potent compared to the methanolic extract and its potential can be harnessed and used as an antibacterial agent for the treatment of diseases.

**KEYWORDS**

Antibacterial, Biosynthesis, *Mitracarpus scaber*, Phytochemicals, Silver nanoparticles

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## Introduction

The utilization of medicinal plants in biomedical applications has increased significantly over the past decade owing to their numerous benefits as well as fewer adverse effects over conventional antibiotics [1-3]. Medicinal plants contain excellent phytochemical compounds, which make them suitable for microbe-resisted synthetic medicines. Furthermore, they are comparatively cheaper and safer [4-5]. Extracts from medicinal plants have exhibited tremendous antimicrobial actions, and their development as a potential source of chemotherapeutic agents is on the rise worldwide, thereby reducing the mortality cases associated with antimicrobial resistance [6].

Nanotechnology, an aspect of science, deals with nanomaterials' production, processing and utilisation. It is an expanding field in the 21st century, engaging scientists and researchers from many fields, including material science, chemistry, physics, etc. [7,8]. Research on the synthesis and biological applications of nanoparticles has increased tremendously in the last decades as nanomaterials exhibit exceptional physicochemical and biological properties, making them outstanding compared to bulk materials [9,10]. Silver nanoparticles possess high surface area to volume ratio, which allow for close contact with microbial cells and enhance their antimicrobial effects. Furthermore, the tiny size of silver nanoparticles makes them capable to cross into bacterial cells and destroy them. The antibacterial effectiveness of silver nanoparticles against drug resistant pathogens is also well established [11,12]. Generally, nanoparticles can be synthesised through physical, chemical, or green method. Although, the physical and chemical method of nanoparticle synthesis are known to be costly, energy-intensive and lethal to the ecosystem. Developing a biogenic route of nanoparticle synthesis tends to be more favourable to the ecosystem and cheaper than other routes [13,14].

Several parts of plants are used for the production of metallic and alloy-based nanoparticles. Capparis spinosa, Desmodium gangeticum and Phoenix dactylifera were well-reported in nanoparticles synthesis [15,16]. Silver nanoparticles (AgNPs) are well established to possess antimicrobial properties and are the most widely used metallic nanoparticles in biomedical applications. They exhibit antifungal, antiviral, antioxidant and anti-larvicidal activities and have also been used in cancer treatment and wound healing [17,18]. The leaves of medicinal plants, including Carica papaya, Acalypha indica, Moringa oleifera, Jatropha curcas and Nelumbo nucifera, have been used to successfully synthesise AgNPs and their antimicrobial activities well studied [19-22].

Mitracarpus scaber have been used extensively in herbal medicine on humans and animals due to its numerous medicinal values. It belongs to the family of family of Rubiaceae. This plant is believed to be native to tropical Africa's upland fields or savanna. It is an annual plant with a white-coloured flower, dicotyledonous broadleaved shaped and characterised by rough leaves [23-25]. Mitracarpus scaber leaves have been used to treat amenorrhea, skin diseases, leprosy, toothache, wounds, inflammation, bacterial infections, fungal diseases and as a cure for poison [26]. The aerial part of this plant is well reported to be used as creams and ointment to treat some fungal diseases [27,28]. Several authors have reported the antimicrobial activities of the extracts of M. scaber leaves [29,30]. Therefore, this present study investigated and compared the antibacterial efficacy of Mitracarpus scaber crude leaf extract and its biosynthesized silver nanoparticles on selected clinical isolates, thereby providing a prospective source of antimicrobial agents other than antibiotics to which microorganisms have developed resistance.

## Materials and Methods

### Sample Collection

Healthy and fresh leaves of Mitracarpus scaber were gathered from a farm in Odo Oba, Ogo-Oluwa Local Government Area, Oyo State, Nigeria (7.46670N, 4.13330E) in August 2023. The leaves were verified by Dr. Odewo at Forestry Research Institute Nigeria, Ibadan. After this, it was taken to the Microbiology Laboratory, Babcock University, for extraction and analysis. The gathered leaves were cleaned with rushing tap water to

flush out contaminants before being dried in a hot oven at 400C. Furthermore, the plant material were pulverised with a blender and refrigerated at 40°C until needed for extraction.

### Chemical Reagents

All chemicals and solvents utilised in this experimental study were of analytical quality.

### Extraction of Plant Material

The dried powdered Mitracarpus scaber was extracted using the cold maceration process to get a crude extract. This extraction process was performed by introducing 70 g of the pulverized material into 500 ml of 95% methanol in a tightly capped jar for three days. Afterwards, the jar's content was filtered via Whatman no. 1 filter paper. The extract was subsequently concentrated in a hot air oven to evaporate the methanol and obtain the dried extract [31].

### Phytochemical Screening

The extract was screened qualitatively and quantitatively for phytochemicals using conventional

Techniques [32,33].

### Collection of Test Bacterial Isolates

Bacterial isolates such as Gram-positive Staphylococcus aureus ATCC 25923, Staphylococcus aureus, Streptococcus pyogenes & Listeria monocytogenes ATCC 19111 and Gram-negative Pseudomonas aeruginosa, Proteus vulgaris and Escherichia coli ATCC 25922 were gotten from Department of Microbiology, Babcock University, Ilishan-Remo Nigeria. These clinical isolates were selected due to their prevalence and clinical relevance. The test isolates were initially cultured in a nutrient broth medium for 24 hours at 370C in an incubator for resuscitation.

### Biosynthesis of Silver Nanoparticles

One millimolar (1 mM) of Silver nitrate solution was prepared by dissolving 0.17 g of silver nitrate in 1 litre of distilled water. Afterwards, 10 ml of Mitracarpus scaber leaf extract was introduced into a conical flask containing 90 ml of 1 mM silver nitrate solution for the bioreduction process at room temperature. The colour changes from green to dark brown suggested the genesis of silver nanoparticles [34].

### Characterisation of Biosynthesised Silver Nanoparticles

An ultraviolet-visible (UV-visible) spectrophotometer (Cecil, USA) was utilised to verify the formation of silver nanoparticles. The formation was confirmed between wavelength 350-500 nm. Fourier transform infrared spectrometer (SHIMADZU FTIR-8400S) was used to establish the functional groups in the silver nanoparticles between spectrum 500-4000 cm-1. The shape and elemental compositions of the biosynthesised silver nanoparticles were established via a scanning electron microscope equipped with an energy dispersive X-ray spectroscopy.

### Evaluation of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The macro-broth dilution approach was used to estimate the minimal inhibitory concentration (MIC) of the biosynthesised AgNPs. [35]. Ten test tubes containing nutrient broth were sterilised correctly and were used for each bacterium isolate. Firstly, 2mL of a known crude extract/AgNPs concentration was introduced into the first tube for each isolate and mixed well. Then, 2 ml from the first tube was transfered into the second tube and mixed well. 2 ml was also withdrawn from the second tube and introduced into the third test tube, and this was repeated till the ninth tube to give half the original concentration each time. The crude extract/AgNPs was not added to the tenth tube which served as the control. After that, 0.1 ml of the standardised inoculum was introduced into each test tube. These tubes were placed in the incubator at 370C for 24 hours. The minimal inhibitory concentration were calculated and recorded as the minimum concentration at which the isolates had no visible growth. The tubes with no visible growth following the incubation for 24 hours were identified and inoculated into freshly prepared nutrient agar to determine the minimum bactericidal concentrations of the crude extract/nanoparticles.

## Results and Discussion

### Quantitative and Qualitative Phytochemical Screening

The result of the qualitative phytochemical analysis of the methanol extract of Mitracarpus scaber revealed the existence of a good number of phytochemicals, including alkaloids, tannins, phlobatannins, saponins, phenols, reducing sugars, steroids, cardiac glycosides, terpenoids and flavonoids. Phenols and flavonoids were abundantly present in the methanol extract, while alkaloids, tannins, phlobatannins, saponins, phenols, reducing sugars, steroids, and cardiac glycosides were moderately present, as shown in Table 1. The quantitative phytochemical analysis of Mitracarpus scaber leaf methanol extract was determined using a UV Spectrophotometer. Phenol had the highest content in the methanol extract (42.00±0.04 mg/100g), while phlobatannins had the lowest quantity (21.44±0.02 mg/100g), as shown in Figure 1. The analysis also revealed a high amount of flavonoids and a moderately high presence of reducing sugars and alkaloids. The antimicrobial potentials of alkaloids, flavonoids, phenols, saponins and tannins are well reported [36-41]. All these secondary metabolites, flavonoids, phenols, saponins, alkaloids, and tannins, could also be responsible for the antimicrobial potential of the methanol extracts of Mitracarpus scaber.

**Table 1. Qualitative phytochemical screening of Mitracarpus scaber leaf methanol extract.**

| Phytochemicals | Mitracarpus scaber methanol extract |
|---|---|
| Alkaloids | + |
| Tannins | + |
| Phlobatannins | + |
| Saponins | + |
| Phenols | ++ |
| Reducing sugars | + |
| Steroids | + |
| Cardiac glycosides | + |
| Terpenoids | ++ |
| Flavonoids | |

key: + = present; ++ = much present.

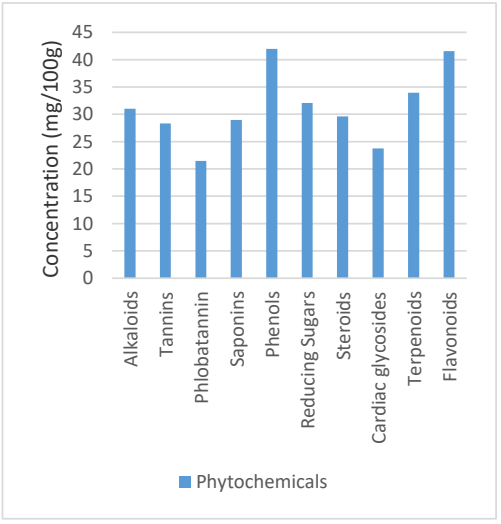


Figure 1. Quantitative phytochemical screening of Mitracarpus scaber leaf methanol extract.

**Biosynthesis and Characterisation of The Biosynthesised Silver Nanoparticles**

The biosynthesized silver nanoparticles mediated from methanol leaf extract of Mitracarpus scaber were confirmed upon adding leaf extract to the prepared silver salt solution, and a visible change of colour was observed. This was further confirmed through the use of UV-visible spectrophotometer, which indicated the absorbance peak for the nanoparticles at around 510nm, as shown in Figure 2, which is similar to the absorbance peak reported by previous researchers [42]. In this study, Fourier-transform infrared spectroscopy (FTIR) identified biomolecules present in the silver nanoparticles. As illustrated in Figure 3, the FTIR spectrum for the biosynthesised AgNPs showed peaks which are recognised as the O-H stretching vibration of alcohols, O=C=O stretching vibration of CO2, N-H bend of amines, which is similar to the spectra studies of biosynthesised AgNPs by Aina et al. [43]. These functional groups have been reported to play significant roles in the bioreduction and capping of the biosynthesized silver nanoparticles. The morphology of the biosynthesised AgNPs was identified via Scanning Electron Microscopy, the particles formed were polydisperse, such as spherical, triangular and decahedral, and the sizes were determined using advanced software named "ImageJ" as showed in Figure 4 to be within 2-10 nm which falls within the typical nanoparticles ranges of 1-100 nm [44,45]. EDX analysis

was used to verify the presence of elements in the synthesised nanoparticles. Elemental peaks, including silver, potassium, calcium, chlorine and magnesium, were observed as shown in Figure 5.

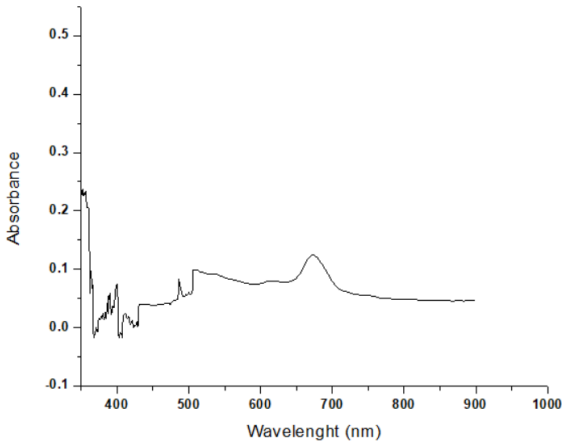


Figure 2. The UV-visible spectrum of the biosynthesised silver nanoparticles.

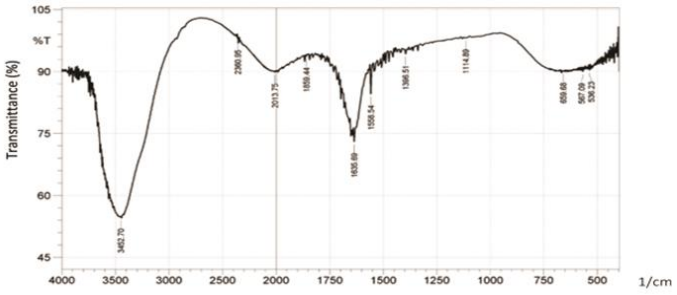


Figure 3. Fourier transform infrared spectroscopy spectrum of the biosynthesised silver nanoparticles.

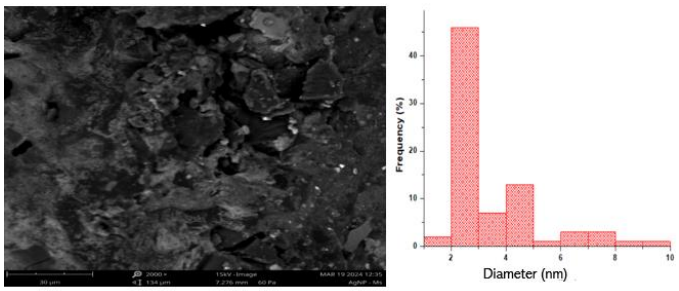


Figure 4. Scanning electron micrograph and the particle size of the biosynthesised silver nanoparticles.

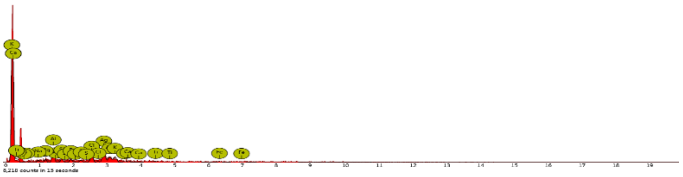

Figure 5. Energy dispersive X-ray analysis result of the biosynthesised silver nanoparticles.

## Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

As shown in Table 2, the minimum inhibitory concentration exhibited by the methanolic extract of Mitracarpus scaber leaves and the biosynthesised AgNPs ranged from 2.25-4.5 mg/mL and 0.01-0.05 mg/mL, respectively. The biosynthesised AgNPs exhibited the lowest MIC at a concentration of 0.01mg/mL against Streptococcus pyogenes and the highest MIC at a concentration of 0.05 mg/mL against Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa. The methanol extract exerted the lowest MIC at 2.25 mg/mL and the highest MIC at a concentration of 4.5 mg/mL against Proteus vulgaris. The differences observed could be linked to the structural makeup of the bacteria isolates. For instance, the presence of thick peptidoglycan layer which absorbs external materials, including antimicrobial agents, more quickly in Gram-positive bacteria.

In contrast, Gram-negative bacteria possess an outer layer (membrane) that shields them from external agents. These outer layers are protective features that make them less sensitive than Gram-positive bacteria [46]. Koohsari et al. [47] further attested that Gram-negative bacteria's lipopolysaccharide layer and periplasmic space are pivotal to their comparative resistance. Another study by Masoumian & Zandi [48] indicated the higher in vitro antibacterial activity of some medicinal plants against Gram-positive bacteria such as Staphylococcus aureus than Gram-negative bacteria such as Escherichia coli, Pseudomonas aeruginosa and Salmonella spp. The results obtained from the MIC analysis showed that the biosynthesised AgNPs can inhibit bacteria growth at a much lower concentration than the crude extract. Several factors have been identified that contribute to the superior antimicrobial activity of AgNPs. One primary

reason for this difference lies in the inherent properties of AgNPs (high surface area-to-volume ratio), which allow for increased contact with microbial cells and enhance their antimicrobial effects [49]. Silver nanoparticles (AgNPs) are well-studied group of materials that have been proven to exhibit potent antimicrobial activity against a wide spectrum of microorganisms. The unique physicochemical properties of AgNPs, such as their high surface area-to-volume ratios, make them an attractive choice for developing antimicrobial agents. In addition, the discharge of silver ions from AgNPs upon interaction with microbial cells contributes to their bactericidal action, disrupting cellular processes, inducing cytotoxic effects and leading to microbial death [50]. The presence of silver ions in the silver nanoparticles influences its affinity for microbial membranes thereby influencing its antimicrobial effects. The antimicrobial efficacy of silver nanoparticles has been widely reported by several authors [51-54]. The methanolic extract's minimum bactericidal concentration (MBC) ranged from concentrations 2.25-18 mg/mL, while AgNPs had their MBC between concentrations 0.03-0.1 mg/mL, as shown in Table 3. Concentrations 2.25-18 mg/mL are the minimum concentrations of the methanolic extract of Mitracarpus scaber leaf needed to cause the test organisms' death, as revealed in the study. Streptococcus pyogenes and Escherichia coli had the lowest MBC at a concentration of 0.03 mg/mL, while the highest MBC was against Proteus vulgaris at a concentration of 18 mg/mL, as observed in this study. The AgNPs caused the test organisms' death between 0.03-0.1 mg/mL concentrations; however, no MBC of the AgNPS was observed against Pseudomonas aeruginosa, indicating that the minimal concentration of the AgNPs needed to lyse the organism is higher than 0.1 mg/mL. The MIC and MBC of silver nitrate solution used as the control was higher than that of the crude extract and this could be attributed to the inherent antibacterial properties of silver ions present.

| Test Isolates | Minimum Bactericidal Concentration (mg/ml) | |
|---|---|---|
| | Methanol extract | AgNPs AgNO3 |
| Staphylococcus aureus ATCC 25923 | 4.50 | 0.05 > 0.20 |
| Staphylococcus aureus | 4.50<br>2.25 | 0.05 > 0.20<br>0.03 0.20 |
| Streptococcus pyogenes | 18<br>4.50 | 0.05 > 0.20<br>> 0.20 |
| Proteus vulgaris | | |
| Listeria monocytogenes ATCC 19111 | 9<br>4.50 | - > 0.20<br>0.03 0.20 |
| Pseudomonas aeruginosa | | |
| Escherichia coli ATCC 25922 | | |

**Table 2. The minimum inhibitory concentration of methanol extract of Mitracarpus scaber leaves and biosynthesised AgNPs against test bacterial isolates.**

| Test Isolates | Minimum Inhibitory Concentration (mg/mL) | |
|---|---|---|
| | Methanol extract | AgNPs AgNO3 |
| Staphylococcus aureus ATCC 25923 | 2.25 | 0.05 0.20 |
| Staphylococcus aureus | 2.25 | 0.03 0.10 |
| Streptococcus pyogenes | 2.25<br>4.5 | 0.01 0.10<br>0.03 0.10 |
| Proteus vulgaris | 2.25 | |
| Listeria monocytogenes ATCC 19111 | 2.25 | 0.05 0.20<br>0.03 0.10 |
| Pseudomonas aeruginosa | 2.25 | |
| Escherichia coli ATCC 25922 | | |

**Table 3. The minimum bactericidal concentration of methanol extract of Mitracarpus scaber leaves and biosynthesised AgNPs against test bacterial isolates.**

## Conclusion

This study has further proved the importance of plant extracts in the green synthesis of nanoparticles as a cost-effective, ecosystem-friendly and less hazardous means of nanoparticle synthesis. As revealed in this study, the biosynthesised silver nanoparticles proved to be the more effective antibacterial agent against the test organisms. Therefore, this study has revealed the potentials of the biosynthesized silver nanoparticles that is further harnessed and used as alternative medicines in the treatment of antibiotic-resistant infections.

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## Conflict of Interest

The authors declare no conflict of interests

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