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RESEARCH ARTICLE OPEN ACCESS

Biosynthesis of silver nanoparticles using *Mitracarpus scaber* extracts for the treatment of infectious disease: synthesis, characterization, antibacterial and anti-inflammatory efficacy

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Abstract
Objective: Due to the need for better treatments for infectious disorders, the antibacterial and anti-inflammatory potential of silver nanoparticles (AgNPs) of *Mitracarpus scaber (M.scaber)* extracts were assessed in this study.
Methods: AgNPs were synthesized from 0.1M $AgNO_3$ solution using *M.scaber* aqueous and ethanolic extracts as capping and reducing agents. FTIR, UV-VIS, FESEM and XRD studies were used to analyze the generated AgNPs. The Agar well diffusion method was used to evaluate their antibacterial activity against *E. coli*, while egg albumin and carrageenan-induced paw oedema assays were used to measure the anti-inflammatory activity of silver nanoparticles of aqueous and ethanol extracts of *M.scaber* (A.AgNPs and E.AgNPs).
Result: The formation of A.AgNPs and E.AgNPs at λ max = 425 nm and 410 nm, respectively, were demonstrated by UV–vis spectroscopy. The XRD pattern showed that the crystalline phase of silver and the generation of A.AgNPs and E.AgNPs were well aligned. Agglomerated A.AgNPs and E.AgNPs with particle sizes ranging from 5 to 20 nm were seen in the FESEM micrograph. The antibacterial activity result showed that, in a dose-dependent manner, A.AgNPs had a significantly ($P<0.05$) higher antibacterial activity against *E.coli* than E.AgNPs.The anti-inflammatory activity result showed that, following the fourth hour of the experimental setup, 100 mg/kg of A.AgNPs with peak effect (24.46 % inhibition) and 50 mg/kg of E.AgNPs with peak effect (18.11 % inhibition) produced the greatest inhibition of oedema development caused by carrageenan and egg albumin injection respectively.
Conclusion: The present work, therefore, concludes that A.AgNPs and E.AgNPs have potential value in the management of infectious diseases.

Keywords: Cystitis, Dysuria, Polypharmacy, Poly-pharmacology, Silver nanoparticles

Plain English Summary

An emerging hallmark of drug development is the use of a single medication to treat several disease conditions. One basic mechanism of action is being deployed for the treatment of numerous conditions with a bid to provide better therapies and a more efficient research and development process. Silver

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nanoparticles (AgNPs) are among the metallic nanoparticles that have been investigated the most because of their many commercial applications. It is worth noting that the size and shape of nanosilver influence their biomedical activities. Several studies have shown that plant extracts are utilized in the extracellular biosynthesis of silver nanoparticles, and that plant extract concentration as well as the type of plant extract (alcoholic or aqueous) used in this process can change the size and shape of the resulting nanoparticles. The present investigation assessed the potential antibacterial and anti-inflammatory properties of silver nanoparticles, which were biosynthesized using *M. scaber* aqueous and ethanol extracts, for the management of infectious diseases. The study's findings showed that silver nanoparticles made with *M. scaber* aqueous extract had stronger antibacterial and anti-inflammatory properties than those made with *M. scaber* ethanol extract due to their smaller size.

Introduction

Antibiotics and anti-inflammatory drugs are the principal drugs used for the treatment of infectious diseases, especially infections of the respiratory tract, ENT, and urinary tract, however, inflammation does not necessarily imply infection (1). Nonetheless, in any infection, when pathogenic bacteria are present in the body, an inflammatory response always occurs. Common symptoms are swelling, heat, redness and pain in the inflamed organ to varying degrees. There are instances where the inflammatory reaction is so severe that it can be lethal and compromise the functionality of organ systems. For example, even though three days of antibiotic treatment is effective in 90% of cases of acute uncomplicated cystitis (AUC), a minor condition frequently brought on by *E. coli,* nonetheless, patients experience difficulty during therapy due to a variety of symptoms (2, 3). The predominant indication of cystitis is dysuria, which is typified by frequent, urgent, copious amounts of hematuria and lower abdomen pain in some cases (4). Due to these symptoms, 54% of women say that their quality of life has decreased and that they have difficulty with daily tasks or duties at work (5). Controlling dysuria is therefore beneficial for the treatment of cystitis. Since anti-inflammatory medications are anticipated to lessen dysuria, consequently improving life quality, they are frequently prescribed in addition to antibiotics to treat infectious disorders. This results in polypharmacy, which is the word for when a patient is prescribed two or more drugs concurrently (6). Since every medication has its side effects it poses to the body; for instance, common antibiotics such as Amoxil or Cefuroxime used for the management of *E.coli* infections as well as conventional anti-inflammatory medicines have been linked to hypersensitivity, gastrointestinal, neurological, renal, cardiac and hepatic adverse effects (7, 8, 9), polypharmacy is frequently linked to a lower quality of life, which includes diminished mobility and mental capacity (10). Polypharmacy is also not cost-efficient (11, 12). This has led to the repurposing of many already existing drugs (13). Drug repurposing takes advantage of the fact that a single molecule can affect several biological targets. This is referred to as polypharmacology (14). Plants have been a major target for poly-pharmacology due to the wide variety of secondary metabolites contained in them, however, the use of herbal therapeutic molecules as drugs is difficult for a variety of reasons, including low plant yield, plant extracts' solubility in water and other solvents, the presence of cytotoxic components in the extract, the sample's limited bioavailability, and the improper use of readily available phytomedicines which can result in toxicity (15). The pharmacokinetics and efficacy of medicinal plants can thus be greatly enhanced if they are used to make nanoparticles (16). Amidst numerous available metal nanoparticles, silver nanoparticles have been acknowledged all over the world since they are very effective, less harmful, and best suited for medical uses (17). Silver nanoparticles are commonly made via chemical and physical techniques that require the use of harmful elements as reducing agents; nevertheless, the employment of these agents has resulted in several biological issues due to their overall toxicity (18). The biosynthesis of silver nanoparticles (AgNPs) utilizing medicinal plants is therefore a notable approach for several therapeutic improvements and efficient drug delivery (19).

Several researchers have studied the antimicrobial and anti-inflammatory properties of biosynthesized silver nanoparticles separately based on their interests. For example, the antimicrobial property of silver nanoparticles biosynthesized using *Cymbopogon citratus* leaf extract and *Carduus crispus* was studied alone by Rakib-Uz-Zaman et al., (20) and Urnukhsaikhan et al., (21) respectively while Xu et al., (22) and Khashan et al., (23) evaluated only the anti-inflammatory property of silver nanoparticles synthesized using aqueous extracts of *Ageratum conyzoides* and aqueous curcumin extract respectively. However, a growing amount of research has indicated that anti-inflammatory medications may have some antimicrobial and anti-biofilm effects on clinically significant microorganisms and vice versa (24, 25, 26). More so, silver nanoparticles have been noted to have the ability to directly combat

pathogens as well as associated inflammatory processes (27). This ability is due to their remarkable surface-to-volume ratio and enhanced bioavailability towards cells and tissues (28). Therefore, in a bid to find agents that can resolve infectious diseases in a way that is modulatory, efficient, and well-tolerated by the body, this investigation assessed the antibacterial and anti-inflammatory properties of silver nanoparticles that were biosynthesized using *Mitracarpus scaber* aqueous and ethanol extract.

Materials and Methods

Mitracarpus scaber (whole) plant was collected from the Federal Polytechnic Ilaro school field in Ogun State, Nigeria, and verified by Dr Nodza George, a botanist at the University of Lagos' Department of Botany and Microbiology. Every reagent utilized in this investigation was of analytical grade.

Preparation and extraction of the study plant material

For four weeks, the entire *Mitracarpus scaber* plant was air-dried at ambient temperature (25.00 ± 2.00 ^{o}C). Following drying, the *M. scaber* samples were carefully stored in sealed bottles for later use after being finely ground in a household blender. The milled plant (1 kg each) was then extracted by maceration in 7 Ltrs of distilled water and ethanol respectively for 72 days. Next, filtration was performed using cheesecloth and the filtrate was dried at 50 ^{o}C using a low-pressure rotary evaporator (29). Dried samples were stored for further studies.

Preliminary phytochemical analysis

The presence of active components was checked in the aqueous and ethanol extracts of the *Mitracarpus scaber* plant using conventional procedures (30).

Biosynthesis of silver nanoparticles

Silver nitrate ($AgNO_3$, 0.1 M) was prepared. After which 90 ml of the $AgNO_3$ solution was added into two separate flacks containing aqueous and ethanol extracts of *Mitracapus scaber* (10 ml each). Both flasks were then allowed to stir continuously at 25 °C in a shaker for 24 hours. The resultant AgNPs were subsequently centrifuged for 20 minutes at 6000 rpm. The pellets were centrifuged and then repeatedly washed with distilled water to remove any remaining silver ion residues not converted and other unwanted impurities. The resulting AgNPs pellet was labelled as A.AgNPs for the aqueous extract and E.AgNPs for the ethanol extract. They were then stored for further characterization and analysis (31).

Characterization of biosynthesized silver nanoparticles

The generated silver nanoparticles were characterized using FESEM, FTIR, XRD and ultraviolet-visible spectroscopy (32). The reduction of pure Ag^{+} to AgNPs was tracked by monitoring the UV spectrum of the reaction media of silver nitrate solution and aqueous or ethanol extract of *M. scaber* with a UV-Vis spectrophotometer. FTIR was employed to identify potential functional groups involved in the production of silver nanoparticles. FESEM was used to determine the size and morphology of the biosynthesized silver nanoparticles while XRD was used to determine their crystalline structure and composition.

Estimation of total flavonoid and phenol contents of biosynthesized silver nanoparticles

The quantity of flavonoids was measured using the colourimetric technique with aluminium chloride while the Follins method was used to determine the phenol content as described by Elamawi et al., (33). To express the flavonoid and phenol contents in the samples, the rutin gallic acid concentration equivalents (mg/100 g of extracted component) were utilized.

In vitro antibacterial assay

The assay organism; *Escherichia coli* (NCTC 10418) was provided by the University of Lagos Microbiology Laboratory from their stock culture. It was then isolated on selective and diagnostic media, to suppress additional contaminants.

Sample preparation for antibacterial assay

Three working samples each of silver nanoparticles biosynthesized using aqueous and ethanol extracts of *M. scaber* (A.AgNPs and E.AgNPs), 25 mg/ml, 50 mg/ml and 100 mg/ml were achieved by weighing 1 g of either A.AgNPs or E.AgNPs dissolved in 10 ml of sterile distilled water which was then used as diluent for double diluting to obtain other working concentrations.

Screening of antibacterial activity

The antibacterial characteristics of the test substances (aqueous and ethanolic crude extracts of *Mitracarpus scaber*) and biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs) were evaluated using Agar well diffusion, following the methodology outlined by Sohail et al. (34). In a nutshell, standardized test organisms (1 ml) were seeded one at a time onto warm agar, and before plating, the mixture was well mixed using the roll-palm method. A cork borer was used to bore 10 mm-diameter wells in the nutritional agar plates after they had been set. Different concentrations of levofloxacin (standard

antibiotics), aqueous, ethanol extract of *Mitracarpus scaber*, and corresponding nanoparticles (A.AgNPs and E.AgNPs) were separately dispensed into the wells before incubation. Diffusion was allowed to occur for four hours. Following a 24-hour incubation period at 37 ^{o}C, the plates were checked for zones of inhibition.

Evaluation of anti-inflammation property

Egg albumin and carrageenan-induced paw oedema

The method of Azim et al., (35) was employed for the determination of anti-inflammatory properties. In brief, forty-eight (48) adult albino mice (20–30 g) were randomly assigned to eight groups for each analysis after being refused food for a full day. Egg albumin or carrageenan suspension (0.1 ml each) was injected into the sub-plantar tissue of the right hind paw of mice to create inflammatory responses one hour after administration of test samples. Subsequently, at 0, 1, 2, 3, and 4 hours, the diameter of inflammation was measured with a vernier caliper. % inhibition of carrageenan-induced paw oedema model in rats by the test samples was then calculated using the formula;

$$\frac{\text{mean paw volume (control group)} - \text{Mean paw volume (treated group)}}{\text{Mean paw volume (control group)}}$$

Table 1: Experimental design

Groups	Carrageenan-Induced Oedema	Number of Mice	Egg Albumin-Induced Oedema	Number of Mice
1	Carrageenaan + Distilled water	3	Egg Albumin + Distilled water	3
2	Carrageenaan + A.AgNPs (50 mg/kg)	3	Egg Albumin + A.AgNPs(50 mg/kg)	3
3	Carrageenaan + A.AgNPs (100 mg/kg)	3	Egg Albumin + A.AgNPs(100 mg/kg)	3
4	Carrageenaan + A.AgNPs (200 mg/kg)	3	Egg Albumin + A.AgNPs(200 mg/kg)	3
5	Carrageenaan + E.AgNPs (50 mg/kg)	3	Egg Albumin + E.AgNPs(50 mg/kg)	3
6	Carrageenaan + E.AgNPs (100 mg/kg)	3	Egg Albumin + E.AgNPs(100 mg/kg)	3
7	Carrageenaan + E.AgNPs (200 mg/kg)	3	Egg Albumin + E.AgNPs(200 mg/kg)	3
8	Carrageenaan+Diclofenac (50 mg/kg)	3	Egg Albumin + Diclofenac (10 mg/kg)	3

Statistical analysis

Mean ± standard error of the mean (SEM) was used to depict all the data. The Data were analysed using Graph Pad, Prism 6, IL, USA). The statistics carried out included an analysis of variance (ANOVA). A multiple-range test was employed to ascertain a significant difference, defined at $p<0.05$.

Result

Qualitative phytochemical screening

The result of the qualitative phytochemical screening of the aqueous and ethanol extracts of *Mitracarpus scaber* is presented in Table 2 below.

Table 2: Qualitative phytochemical screening of aqueous and ethanol extracts of *Mitracarpus scaber*

Phytocompound	Aqueous Extract	Ethanol Extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
C.glcosides	+	-
Steroids	+	+
Terpenoids	+	+
Anthraquinones	-	-
Reducing Sugars	+	+
Phlobatanins	-	-

Note: + =present, - =Not present

Synthesis and characterization of silver nanoparticles

UV-Visible spectroscopy

After twenty-four hours, it was confirmed by UV-visible spectroscopy that the Ag ions in the solution mixtures had reduced to A.AgNPs and E.AgNPs, respectively (Figure 1). The spectra consisted of absorption peaks in the visible

region of the electromagnetic spectrum. Absorption peaks of 425 nm and 410 nm were observed for A.AgNPs and E.AgNPs respectively, both of which were characteristic of silver nanoparticles.

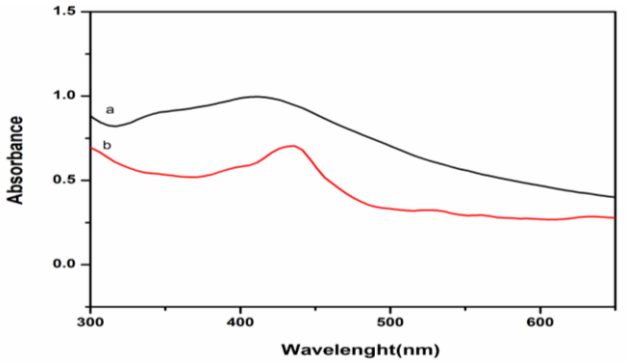

Figure 1: UV-VIS absorbance spectra of the colloidal silver nanoparticles prepared using aqueous and ethanol extract of *M.scaber* at 24hrs

FTIR spectroscopy

Possible biomolecules contained in aqueous and ethanol extracts of *Mitracarpus scaber* responsible for the synthesis and capping of A.AgNPs and E.AgNPs respectively were determined by FTIR spectroscopy analysis. The analysis's outcome is shown in Figure 2 below.

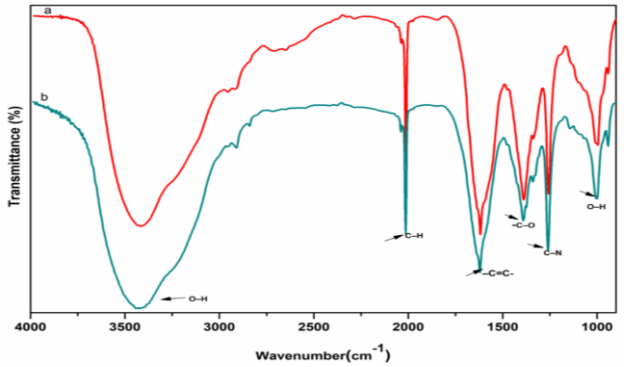

Figure 2: FTIR spectra of (a) biosynthesized A.AgNPs and (b) biosynthesized E.AgNPs

XRD analysis

The XRD patterns of the A.AgNPs and E.AgNPs synthesized are shown in Figure 3 below. The 2θ peaks observed at ~ 38.12°, ~ 44.30°, ~ 64.45° and 77.40° for both biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs) correspond to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflection planes of a fcc lattice of silver (ICSD No. 04-003-2941 and ICDD:04-006-2775 respectively.

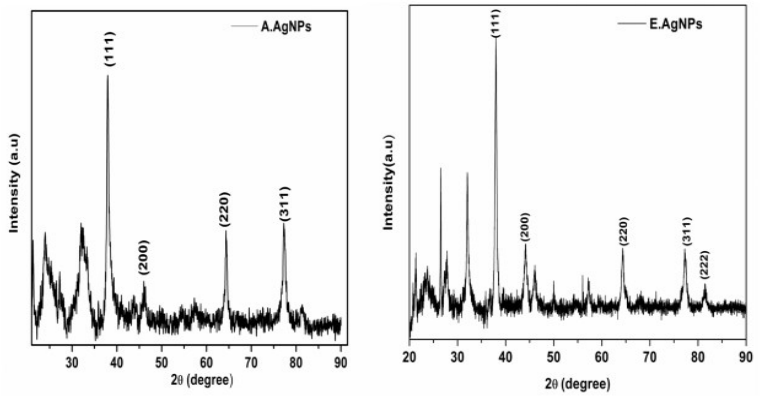

Figure 3: XRD pattern of A.AgNPs and E.AgNPs

FESEM

The size and structure of the final biosynthesized nanoparticles (A.AgNPs and E.AgNPs) are revealed in Figure 4 below.

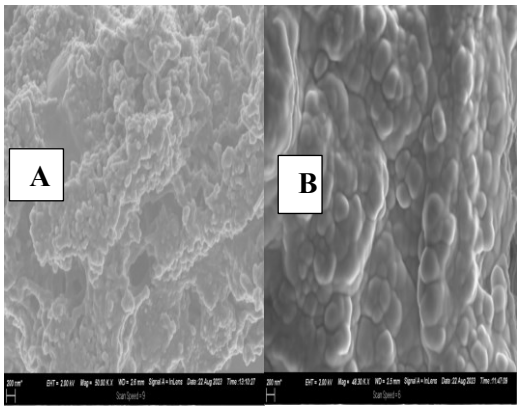

Figure 4: FESEM images of A (A.AgNPs) and B (E.AgNPs)

Determination of total phenol and flavonoid content

Table 3 below displays the results of the total flavonoid and phenol contents of the biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs). The outcome showed that the total flavonoid content of the silver nanoparticles made using the aqueous extract of *Mitracarpus scaber* was higher than that of the silver nanoparticles made with the ethanol extract of *Mitracarpus scaber* ($p < 0.05$), however, the phenol concentrations of the silver nanoparticles made using the ethanol extract of *Mitracarpus scaber* were significantly ($p < 0.05$) higher than those made with the aqueous extract of the same plant.

Table 3: Total phenol and flavonoid contents of biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs)

	Total Phenol (mg/100g)	Total Flavonoid (mg/100g)
A.AgNPs	139.11 ± 0.04	178.56± 0.18*
E.AgNPs	142.47± 0.02*	172.57 ± 0.02

Determination of antibacterial activity

Table 4 below lists the antibacterial activity of various concentrations of silver nanoparticles that were biosynthesized using *Mitracarpus scaber* aqueous and ethanol extracts against *E. coli*. The outcome showed that silver nanoparticles made using *Mitracarpus scaber* aqueous extract exhibited more antibacterial activity than those made with *Mitracarpus scaber* ethanol extract ($p < 0.05$). The antibacterial activity of both nanoparticles increased in a concentration-dependent manner.

Table 4: Antibacterial activities (Zone of inhibition) of different concentrations of silver nanoparticles biosynthesized using aqueous and ethanol extracts of *Mitracarpus scaber* (A.AgNPs and E.AgNPs) against *E. coli*

Concentration	Sample	*E. coli*
25 (mg/ml)	A.AgNPs	1.75±0.01*
	E.AgNPs	1.50±0.01
50 (mg/ml)	A.AgNPs	1.95±0.01*
	E. AgNPs	1.73±0.01
100 (mg/ml)	A. AgNPs	2.33±0.01*
	E.AgNPs	1.93±0.01
6.25 (µg/ml)	Levofloxacin	1.05±0.00
12.5 (µg/ml)		1.10±0.00
25 (µg/ml)		1.40±0.00
50 (µg/ml)0		1.60±0.00

Values are mean ± SEM (n = 3)

Anti-inflammatory activity of biosynthesized silver nanoparticles

Carrageenan-induced inflammation (paw oedema) in mice

The result of the anti-inflammatory activity of biosynthesized silver nanoparticles against carrageenan-induced inflammation (paw oedema) in mice is shown in Table 4 below. The greatest inhibition of oedema development with peak effect (24.46 % inhibition) was produced by 100 mg/kg of biosynthesized silver nanoparticles using an aqueous extract of *Mitracarpus scaber* at the fourth hour ($p < 0.05$). This effect was comparable to that elicited by Diclofenac and 200 mg/kg of biosynthesized silver nanoparticles using an aqueous extract of *Mitracarpus scaber.*

Table 4: Effect of different doses of biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs) and Diclofenac on carrageenan-induced paw oedema in Mice

	Carr Grp+ Dist Water	Carr+A.AgNPs (50 mg/kg)	Carr+A.AgNPs (100mg/kg)	Carr+A.AgNPs (200 mg/kg)	Carr+E.AgNPs (50 mg/kg)	Carr+E.AgNPs (100 mg/kg)	Carr+E.AgNPs (200 mg/kg)	Carr+Diclo (50mg/kg)
0 hr	0.487±0.01	0.530±0.01	0.533±0.01	0.517±0.01	0.513±0.02	0.497±0.01	0.537±0.06	0.503±0.02
1 hr	0.973±0.08	0.903±0.01	0.803±0.02*	0.893±0.04	0.823±0.02*	0.7967±0.03*	0.827±0.09*	0.800±0.02*
2 hrs	1.030±0.07	1.007±0.05	0.870±0.03*	0.970±0.03	0.850±0.02*	0.903±0.01	0.860±0.03*	0.880±0.01*
3 hrs	1.163±0.11	0.940±0.04*	0.867±0.04*	0.897±0.02*	0.883±0.02*	0.9533±0.00*	0.917±0.03*	0.860±0.06*
4hrs	1.010±0.05	0.817±0.02 (19.11%)*\	0.763±0.02 (24.46%)*	0.807±0.03 (20.10 %)*	0.817±0.03 (19.11%)*	0.837±0.01 (17.13%)*	0.830±0.03 (17.82%)*	0.803±0.07 (20.50%)*

Values are mean ± SEM (n = 3).*P < 0.05, significant decrease in oedema development compared with control.% Inhibition is indicated by values in parenthesis

Egg albumin-induced inflammation (paw oedema) in mice

The result of the anti-inflammatory activity of biosynthesized silver nanoparticles against egg albumin-induced inflammation (paw oedema) in mice is shown in Table 5 below. The greatest inhibition of oedema development with peak effect (18.11 % inhibition) was produced by 50 mg/kg of biosynthesized silver nanoparticles using ethanol extract of *Mitracarpus scaber* at the fourth hour ($P < 0.05$). This effect was comparable to that elicited by the other treatment groups as revealed below.

Table 5: Effect of different doses of biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs) and Diclofenac on Egg albumin-induced paw oedema in Mice

	Egg Albumin +Dist.Water	Egg Albumin+A.AgNPs (50 mg/kg)	Egg Albumin+A.AgNPs (100 mg/kg)	Egg Albumin+A.AgNPs(200 mg/kg)	Egg Albumin+E.AgNPs (50 mg/kg)	Egg Albumin+E.AgNPs (100 mg/kg)	Egg Albumim+E.AgNPs (200 mg/kg)	Egg Albumin+ Diclo (50 mg/kg)
0hr	1.143±0.04	1.253±0.02	1.177±0.00	1.210±0.03	1.287± 0.01	1.290± 0.01*	1.220±0.02	1.183±0.01
1 hr	1.907± 0.01	1.917±0.01	1.863±0.03	1.873±0.01	1.760±0.02*	1.883±0.03	1.743±0.10*	1.703±0.07*
2 hrs	2.193± 0.07	1.650±0.02*	1.997±0.04*	2.053±0.08	1.857±0.02*	1.903±0.06*	1.823±0.05*	1.830 ±0.04*
3 hrs	1.910± 0.06	1.543±0.03*	1.713±0.03*	1.930± 0.01	1.610±0.03*	1.713±0.04*	1.697±0.05*	1.760±0.02
4 hrs	1.933±0.06	1.700±0.02 (12.05 %)*	1.610±0.02 (16.71%)*	1.623±0.02(16.03%)*	1.583±0.03 (18.11%)*	1.663±0.03 (13.97%)*	1.633±0.07 (15.52%)*	1.657±0.04 (14.28%)*

Values are mean ± SEM (n = 3).*p < 0.05, a significant decrease in oedema development compared with control.% Inhibition is indicated by values in parenthesis

Discussion

Qualitative phytochemical evaluations of the aqueous and ethanol extracts of *M. scaber* showed the presence of alkaloids, tannins, saponins, steroids, flavonoids, reducing sugars and terpenoids in both extracts while the aqueous extract had C.glycosides additionally present (Table 3). These phytochemical constituents have been reported for their medicinal properties (36, 37, 38). However, since the report has shown that biosynthesized silver nanoparticles possess enhanced pharmaceutical properties than crude extracts (39), silver nanoparticles were biosynthesized using aqueous and ethanol extracts of *M. scaber*.

In this study, the generation of metallic AgNPs was verified using the UV-Vis characteristic absorption band of silver nanoparticles, which has a wavelength between 300 and 800 nm. The maximum absorbance peak of AgNPs of aqueous and ethanol extracts of *M. scaber* (Figure 1) was observed in the surface plasmon resonance region between 425 and 410 nm, respectively. Similar peaks were reported by Maheswari et al., (40) for biosynthesized silver nanoparticles using aqueous extracts of the leaf and bark of *Neolamarkia cadamba*. The existence of biomolecules including phenols, saponins, flavonoids, and terpenoids was confirmed by the functional groups shown by the FTIR spectrum (Figure 2), which suggests that these biomolecules served as capping and stabilizing agents for the synthesized nanoparticles. A similar finding has been reported in the literature (41). The crystalline character of the Ag nanoparticles produced by the extracts is amply demonstrated by the XRD measurements (Figure 3). The 2θ peaks

observed at ~ 38.12°, ~ 44.30°, ~ 64.45° and 77.40° for both biosynthesized silver nanoparticles (A.AgNPs and E.AgNPs) correspond to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) reflection planes of a fcc lattice of silver (ICSD No. 04-003-2941 and ICDD:04-006-2775 respectively. Similar XRD patterns of silver nanoparticles have been reported (42). Evident in the FESEM micrograph are agglomerated silver nanoparticles with particle sizes ranging from 5- 20 nm, with the silver nanoparticles biosynthesized using aqueous extract of *M. scaber* presenting the smallest particles (Figure 4). Silver nanoparticles with similar size ranges of 5-20 nm have been reported in the literature (43).

The result of the antibacterial activity revealed that A.AgNPs exhibited significant antibacterial activity against *E. coli* in a dose-dependent manner than E.AgNPs.This could be a result of the higher levels of flavonoids contained in A.AgNPs. Even though Stanković et al., (44) reported that there is little to no correlation between Teucrium species' antibacterial activity and their flavonoid content, Dzoyem et al., (45) reported that out of the eleven (11) compounds isolated from *Pseudarthria hookeri*, flavonoids from this plant showed the highest antibacterial activity against *E. coli.* The greater antibacterial activity of silver nanoparticles biosynthesized using aqueous extract of *M. scaber* against *E. coli* could also be a result of its smaller sizes in comparison to silver nanoparticles biosynthesized using ethanol extract of *M. scaber*. Several researchers have reported that the antibacterial activity of silver nanoparticles is greatly influenced by size and that the smaller the size of a nanoparticle, the greater the antibacterial activity it exhibits (46, 47). This is because compared to bigger size nanoparticles, the smaller size AgNPs aggregate more readily and affect the target organelle more (48). In all the antibacterial activity of A.AgNPs and E.AgNPs were greater than that exhibited by the different standard concentrations of Levofloxacin used against *E. coli.* This result is different from the findings of Sher et al., (49) who reported that Levofloxin had a slightly higher antibacterial effect against *E. coli* than silver nanoparticles biosynthesized using *Hippeastrum hybridum* extract. This could be a result of differences in plant extracts and concentrations used for synthesis and the antibacterial assay.

Meanwhile, paw oedema in rats brought on by egg albumin or carrageenan is a well-known animal model used to assess how effective herbal remedies and prescription drugs are in reducing inflammation (50, 51). In the process of wanting to determine also the anti-inflammatory potential of the biosynthesized silver nanoparticles, the biphasic phase of oedema formation was achieved by applying carrageenan and egg albumin to different groups of rat paws. For the carrageenan assay, rats in the control group developed oedema (1.163±0.11 cm increase in paw circumference), which peaked three hours after injecting the phlogistic drug (carrageenan) into their right hind paw's subplantar tissue while for the egg albumin assay, rats in the control group developed oedema, which peaked two hours following subplantar injection of egg albumin into the right hind paw (2.193± 0.07 cm increase in paw circumference). These findings are consistent with earlier research that indicates the peak carrageenan and egg albumin effects occur 3 hours and 2 hours respectively after injection (52, 53). According to the literature, for one to one and a half hours, the first phase of oedema formation is characterized by non-phagocytic oedema which is followed by a second phase that lasts for two to four hours and has increased oedema production. Different mediators, such as histamine, serotonin, and bradykinin, operate on vascular permeability to initiate the initial phase of oedema, while overproduction of prostaglandins causes the late phase, or second phase (50).

The result of the anti-inflammatory activity of the synthesized silver nanoparticles (Table 4 and Table 5) revealed that the greatest inhibition of oedema development caused by carrageenan and egg albumin injection after the 4th hour of experimental set-up ($P < 0.05$) was produced by 100 mg/kg of biosynthesized silver nanoparticles using aqueous extract of *Mitracarpus scaber* with peak effect (24.46 % inhibition) and 50 mg/kg of biosynthesized silver nanoparticles using ethanol extract of *Mitracarpus scaber* with peak effect (18.11 % inhibition) respectively. These effects were comparable to those elicited by Diclofenac for both assays. This implies that biosynthesized silver nanoparticles using aqueous and ethanol extracts of *M. scaber* have antagonistic reactions to all inflammation mediators implicated in the vascular and cellular phases. However, the anti-inflammatory activity observed in this study is lesser than the anti-inflammatory activity reported by Das, Mondal, Patowary and Malipeddi, (54) for AgNPs biosynthesized using aqueous leaf extract of *Ipomoea eriocarpa*.

Conclusion

The outcome of the study showed that aqueous and ethanol extract of *M. scaber* can be utilised for the biosynthesis of silver nanoparticles. This is being reported for the first time. The result also demonstrated that silver nanoparticles biosynthesized using aqueous and ethanol extracts of *M. scaber* possessed significant

antibacterial and anti-inflammatory properties and could be utilized as an effective therapy in the management of infectious diseases especially those caused by *Escherichia coli* after proper pharmacological investigations.

List of Abbreviations

AgNPs: Silver nanoparticles
A. AgNPs: Silver nanoparticles biosynthesized using an aqueous extract of *Mitracarpus scaber*
$AgNO_3$: Silver nitrate
ANOVA: Analysis of Variance
E.AgNPs: Silver nanoparticles biosynthesized using ethanol extract of *Mitracarpus scaber*
E. coli: *Escherichia coli*
ENT: Ear, Nose and Throat Infection
FESEM: Field emission scanning electron microscopy
FTIR: Fourier-transform infrared spectroscopy
R and D: Research and Development
M. Scaber: *Mitracarpus scaber*
SEM: Standard Error of Mean
UV-VIS: Ultraviolet-visible spectroscopy
XRD: X-ray diffraction analysis

Declarations

Ethics approval and consent to participate
This research protocol was approved by the Ethics Committee of Covenant University, Nigeria (CU/HREC/OES/096/21). The current regulations of the Animal Protection Act (Law 27,265) were adopted.

Consent for Publication:
All the authors gave their consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license. Otherwise, all copyright ownership including all rights incidental thereto is conveyed to the journal when published.

Availability of data and materials
The study data is available upon request to the corresponding author.

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Authors' contributions
ATI came up with the concept under the guidance of AAH and IEJ, took care of the material preparation, data collecting, and analysis, and wrote the first draft of the manuscript. AAH and IEJ then proofread it. Every author participated in the full process of revision.

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