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In vitro and in vivo anti-inflammatory activity of green synthesized silver nanoparticles from the aqueous bark extract of *Mangifera indica* Linn. (Anacardiaceae)

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Abstract: The increasing demand to produce secure and cost-effective healthcare nanodevices is at the forefront of research in nanotechnology. The present work reports a simple and eco-friendly synthetic method for silver nanoparticles using the bark aqueous extract of *Mangifera indica* (MI-AgNPs) for the management of inflammation. MI-AgNPs were obtained following incubation of the mixture of the plant extract with silver nitrate solution (10:50 v/v). The sample was biophysically characterized using Ultraviolet Visible spectroscopy (UV-Vis), Fourier Transform Infrared spectroscopy (FTIR), Powder X-Ray Diffraction (PXRD) and Dynamic Light Scattering (DLS) technics while egg albumin denaturation and carrageenan-induced rat paw edema model were used to evaluate its anti-inflammatory activity. The changing colour of the reaction mixture and UV-Vis absorbance spectrum validated the formation of MI-AgNPs, showing a characteristic peak at 410 nm. FTIR spectra showed the main reducing groups from the bark extract and PXRD highlighted the crystalline nature of the MI-AgNPs. The hydrodynamic diameter was found to be 104 nm with a polydispersity index of 0.27. Anti-inflammatory activity of MI-AgNPs was observed in a dose-dependent manner. In heat-induced egg albumin denaturation method, MI-AgNPs exerted maximum protection of 80 % at 200 µg/mL (highest tested concentration) while aspirin used as reference drug exhibited 67 % protection. Similarly, the oral pre-treatment of rats with MI-AgNPs caused significant ($P<0.001$) inhibition of carrageenan-induced rat paw edema with a maximum inhibition of 89 % at the dose of 200 µg/kg. These findings infer that *Mangifera indica* bark aqueous extract-mediated silver nanoparticles could be considered as an interesting solution for the development of therapeutic strategies against inflammation related diseases.

Keywords: *Mangifera indica*, aqueous bark extract, silver nanoparticles, green synthesis, nanocharacterization**,** anti-inflammatory activity

INTRODUCTION

Inflammation is known to be the primary physiological defence mechanism that helps the body to protect itself against pathogens, toxic compounds or damage cells [1]. It is a defensive biological response characterized by redness, pain, heat, swelling and sometimes loss of function in the injured site [2]. Inflammatory reaction is a complex process frequently accompanied with an overproduction of free radicals which damage biological molecules and a body of events including increase in vascular permeability, protein denaturation and membrane alteration [3,4]. Furthermore, in the injured site the release of kinins, prostaglandins and histamine which act as chemical messengers attract some of the body's natural defence cells thereby, triggering many common diseases such as diabetes, obesity or cancer [5,6]. Therapeutic management of inflammation is a real health issue since the process involves various mechanisms [7]. Although non-steroidal anti-inflammatory drugs, steroidal agents or immnuno-suppressants administered by oral or topical route remains the main and effective drugs therapy, their usage is however limited by side effects and insufficient efficacy against chronic inflammation [8]. Again, concerns have been expressed about their safety on long-term administration, development of tolerance, addiction, and drug diversion, indicating a clinical need for a novel strategy that can resolve inflammation in a way that is homeostatic, modulatory, efficient, and well tolerated by the body [9,10].

Nanotechnology is a science encompassing the fabrication, manipulation and application of structures by controlling shape and size at nanoscale level. It is an area of extensive research in recent years which plays an important role in manufacturing new materials for application in the Nano health delivery

system. Nanostructures, especially nanoparticles (NPs) have drawn increasing interest, particularly in nanomedicine for their ability to deliver drugs in the optimum dosage range often resulting in increased therapeutic efficiency of the drugs, reduced side effects and improved patient compliance [11]. Among other metallic NPs, silver nanoparticles (AgNPs) have immense potential for diagnosis and treatment of diseases [12]. Studies indicate that AgNPs have proven to be among the most efficient in possessing strong antimicrobial, antioxidant, anti-cancer and anti-inflammatory properties [13,14]. However, NPs produced by various chemical and physical methods are relatively expensive, and the production methods are often hazardous to human and environment [15]. The green synthesis method for the production of NPs using biological materials such as ionic liquids and plant extracts have proven to be more cost effective than other processes [16]. These synthetic methods are simple, not expensive, not cumbersome, eco-friendly, efficient and the products are safe for therapeutic applications [10,17]. In addition, molecular components of plant extracts have strong affinity for the surface of nano structures, stabilize and prevent aggregation while improving the biological effects of NPs [18].

Mangifera indica Linn. commonly known as 'Mango' is a tropical fruit tree in the flowering plant family of Anacardiaceae and traditionally used to treat asthma, cough, dysentery, diarrhea, pain, leucorrhoea and malaria [19]. Studies on the phytochemical composition have led to the identification and isolation of many specific compounds such as mangiferin that was show to be endowed with various biological activities [20]. Also, numerous pharmacological investigations have demonstrated that the aqueous extract can advance haematological parameters in rats [21], possess anti-inflammatory, antioxidant, analgesic, immunomodulatory and hypoglycemic effect in man and mammalian experimental animals [22,23,1]. Based on the aforementioned, the present work was undertaken to synthesize and characterise AgNPs using *Mangifera indica* aqueous bark extract as a reducing and stabilizing agent and their anti-inflammatory activities evaluated in both *in vitro* and *in vivo* systems are also discussed.

Highlights

- Mangifera indica aqueous bark extract was used for silver nanoparticles synthesis (AgNPs-MI)
- AgNPs-MI showed significant anti-inflammatory activity in both systems
- Developed bottom-up process is of low-cost and environmentally friendly for obtaining nanopharmaceuticals

2. MATERIALS AND METHODS

2.1 Chemicals and reagents: All chemicals used in this study were of analytical grade. Silver nitrate ($AgNO_3$) and lambda carrageenan were purchased from Sigma Aldrich Co Ltd Germany. Aspirin and indomethacin were obtained commercially and used as received without further purification. Distilled water was used throughout the reactions.

2.2 Plant collection and extract preparation: The stem bark of *Mangifera indica* **(Figure 1)** was harvested in August 2018 at Ndogbong, Littoral region, Cameroon and authenticated at the National Herbarium of Cameroon, in comparison with a voucher specimen previously deposited (N°32875 HNC). The harvested material was cut into small pieces, air-dried for 14 days before crushing using an electric grinder. 10 g of the powdered plant was introduced into a conical flask containing 100 mL preheated distilled water (80 °C) and stirred for 5 min using a hot plate equipped with magnetic stirrer. After cooling at room temperature, the solution was filtered using Whatman paper n°1 and stored at 4 °C and used within one week due to gradual loss of plant extract viability over prolonged storage. 10

mL of the freshly prepared aqueous extract was introduced into a Petri dish and left overnight in an oven at 45 °C resulting in the complete evaporation of the solvent. The extract was weighed and the amount of plant extract present in the initial solution was calculated.

Figure 1: *Mangifera indica* tree (A) and grounded stem bark (B)

2.3 Silver nanoparticle synthesis: Aqueous bark extract of *Mangifera indica* was used as a source for the synthesis of silver nanoparticles following a protocol described previously [10]. Briefly, to 10 mL aqueous extract, 50 mL silver nitrate aqueous solution (1 mM) was added and the mixture was left at room temperature for the bioreduction process. The mixture was incubated in the dark to minimize the photoactivation of silver nitrate under static conditions until the appearance of a color change, then centrifuged (Hettich, D-7200 Tuttlingen, and Germany) at 6000 rpm for 20 min and washed twice with distilled water and once with ethanol 95°. Purified pellets were kept into a Petri dish, dried in the oven at 60 °C for 24 h and used for characterization and anti-inflammatory studies.

2.4 Characterization of silver nanoparticles:

2.4.1 UV-Visible spectroscopic measurement (UV-Vis): The reduction and formation of silver ions was monitored by recording the UV-Visible spectrum of the reaction mixture (plant extract and silver nitrate solution) at 5 min, 30 min, 1 h, and 2 h using a UV–visible spectrophotometer (UV-line 9100, single beam, halogen light source, 1 nm resolution with a wavelength ranging from 320 to 1100 nm). Distilled water was used as the blank.

2.4.2 Fourier transform infrared spectroscopy (FTIR): The Fourier transform infrared spectrum was recorded at room temperature with the potassium bromide pellet method. Samples were ground with KBr, pressed into pellets placed in the infrared path and the spectrum was obtained using a Nicolet IS5 model of Thermo Scientific operating at a resolution of 0.4 cm^{-1}.

2.4.3 Powder X-ray diffraction (PXRD): Powder X-ray diffraction measurements of purified silver nanoparticles were carried out at ambient temperature using a BRUKER D2 Phaser diffractometer (Cu Kα, λ = 1.54182 Å, 30 kV) by preparing a film of the silver-organic nanopowder on a flat, low-background silicon sample holder.

2.4.4 Dynamic Light Scattering measurement (DLS): Particle sizes and size distributions were determined using a Zetasizer (Malvern Nano S Zetasizer) operating with a He-Ne laser at a wavelength of 633 nm. 10-15 measurements were made in each run and each DLS analysis was performed in triplicate.

2.5 Anti-inflammatory activity assessment: The synthesized AgNPs were screened for their anti-inflammatory potency using inhibition of albumin denaturation and carrageenan-induced rat paw oedema as *in vitro* and *in vivo* model respectively.

2.5.1 Animal experimental and ethical considerations: Wistar albinos rats of 9 ± 1 week old, weighing 150 - 180 g housed in standard polypropylene cage at room temperature (24 ± 2°C) and relative humidity under light and dark cycle (from 6-am to 6-pm) were used for this study. They were bred at the animal laboratory facility of the Department of Pharmaceutical Science, Faculty of Medicine and Pharmaceutical Science, University of Douala, Cameroon. They had free access to standard laboratory diet and tap water *ad libitum*. All experimental procedures were in strict compliance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985). Further, ethical authorization for this study was issued by the Institutional Ethical Committee of the University of Douala (Protocol approval number 1739IEC-UD/03/2019/T).

2.5.2 Heat-induced egg albumin denaturation assay: The method reported by [24] with slight modifications was used in the present study. The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of varying concentrations of AgNPs (50, 25, 12.5, 6.25 and 3.125 µg/mL). Similar volume of distilled water served as control. The mixture was incubated at 37°C in a biochemical oxygen demand incubator for 15 min then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm using distilled water as blank. Aspirin was used as reference drug and treated similarly for determination of absorbance. All experiments were performed in triplicate. The anti-inflammatory activity was estimated in percentage inhibition of protein denaturation and calculated using the Equation (1):

$$\% \text{ inhibition} = 100 \text{ X } \frac{Abs\ sample}{Abs\ control} - 1 \qquad (1)$$

2.5.3 Carrageenan-induced rat paw edema method: A standard procedure previously described by [25] with slight modifications where paw size was measured using a calliper ruler (No G5020045, Germany) was followed. Briefly, after one week of acclimatization, experimental animals were randomly divided into 5 groups of 6 rats each and treated as follow:

Group I: 10 mL/kg of distilled water, (Control)

Group II: 10 mg/kg of indomethacin, (Standard)

Group III: 400 µg/kg of AgNPs, (Test 1)

Group IV: 200 µg/kg of AgNPs, (Test 2)

Group V: 100 µg/kg of AgNPs, (Test 3)

Inflammation was provoked by sub-plantar injection of 0.1 mL of carrageenan (1 % carrageenan suspended in 0.9 % NaCl) in the right hind paw of each rat. The injection was made one hour following oral administration of various substances (silver nanoparticles, indomethacin or distilled water). Measurement of paw size was done immediately before carrageenan injection and 30 min, 1 h, 2 h, 3 h, 4 h and 5 h after the carrageenan injection. The anti-inflammatory activity was evaluated as percentage of inhibition of edema in each treated group as compared to control using the Equation (2):

$$\% \text{ Inhibition} = 100 \times \frac{(\text{Dt}-\text{Do})\ \text{control} - (\text{Dt}-\text{Do})\ \text{treated}}{(\text{Dt}-\text{Do})\ \text{control}} \qquad (2)$$

Where Dt is the average diameter for each group after treatment and Do is the average diameter obtained for each group before any treatment [26].

2.6 Statistical analysis: Results are expressed as the mean ± SEM. The difference between treated groups and control group was compared using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test to make pairwise comparisons. The statistical analyses were performed using GraphPad Prism Software version 5.01 (Inc., San Diego, CA). Probability values less than 0.05 were considered statistically significant. Concentration for 50% inhibition (IC_{50}) values were determined by nonlinear regression analysis.

3. RESULTS

3.1 Yield of the extract: The yield of the extract obtained from *Mangifera indica* bark using distilled water as solvent was found to be 39 % (w/v). The oven dried crude extract was deep brown in colour with good flavour and odour indicating its valid contribution for pharmaceutical products.

3.2 Visual observation of synthesized silver nanoparticles: The biological synthesis of AgNPs using *Mangifera indica* aqueous bark extract was first recorded through visual observation of the colour of the reaction mixture. When the bark extract of *Mangifera indica* was mixed with silver nitrate solution, darkish brown colour of aqueous extract changed to brownish golden colour immediately within 5 min, indicating the formation of AgNPs as shown on **Figure 2**. No further change was observed indicating the stabilization of the synthesized nanoparticles (MI- AgNPs).

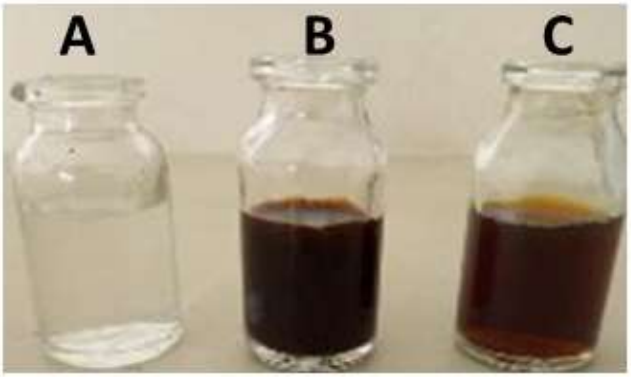

Figure 2: Silver nitrate (A), *Mangifera indica* aqueous bark extract (B), and silver nanoparticles solutions (C)

3.3 UV- Visible spectral analysis: UV-visible spectroscopy was used to monitor the bio-reduction of silver ions in the aqueous solution. Periodic sampling of the reaction mixture and subsequent recordings of the absorption spectra as a function of time at different wavelengths ranging from 300-1000 nm in distilled water revealed a peak at 410 nm (**Figure 3).** It is noticeable that the characteristic peak of AgNPs known as surface Plasmon resonance confirming the presence of NPs in the sample occurred 5 min following incubation. No further change was observed, confirming the synthesis and stability of the as-prepared MI-AgNPs.

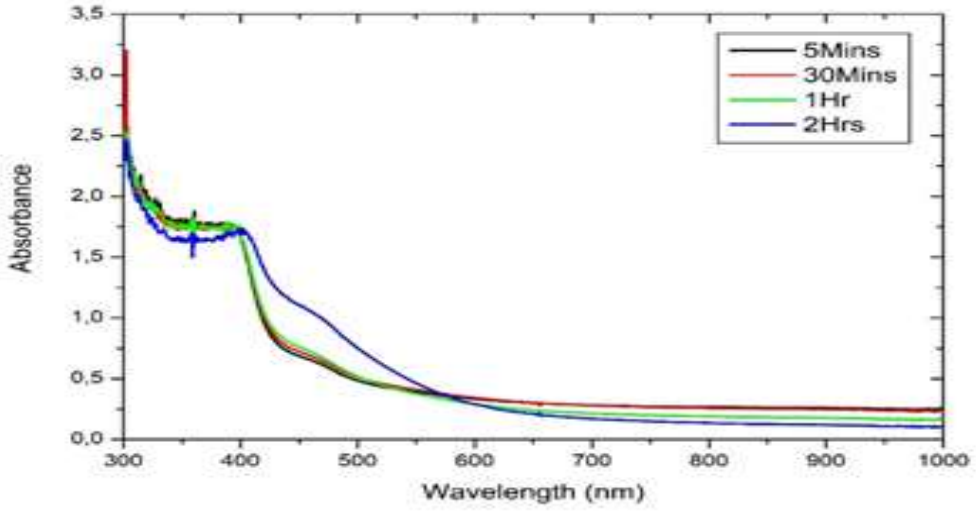

Figure 3: UV Visible spectrum of MI-AgNPs at different time.

3.4 Fourier transform infrared spectroscopy results: The infrared spectrum of MI-AgNPs recorded over the spectral range from 500 cm^{-1} to 4000 cm^{-1} is depicted in **Figure 4** and functional groups which acted as capping agents are listed in **Table 1**. The characteristic IR peak centred at 767 cm^{-1} can be attributed to the C-H bending of monosubstituted benzene derivative compounds. The vibrational band at 1020 cm^{-1} is associated with a strong sharp C-O stretching of alcohol, ester, ether or carboxylic acid compounds. The peak centred at 1283 cm^{-1} indicated the stretching mode of C-O bonds of aromatic ester. The absorption band at 1452 cm^{-1} is due to bending vibration of C-H of alkane. The absorption occurring at 1610 cm^{-1} can be attributed to C=C stretching of α, β-unsaturated ketone. The band at 2916 cm^{-1} is attributed to the stretching vibration mode of C-H bond of alkane. The strong broad absorption band at 3338 cm^{-1} is due to the presence of O-H stretching of alcohol.

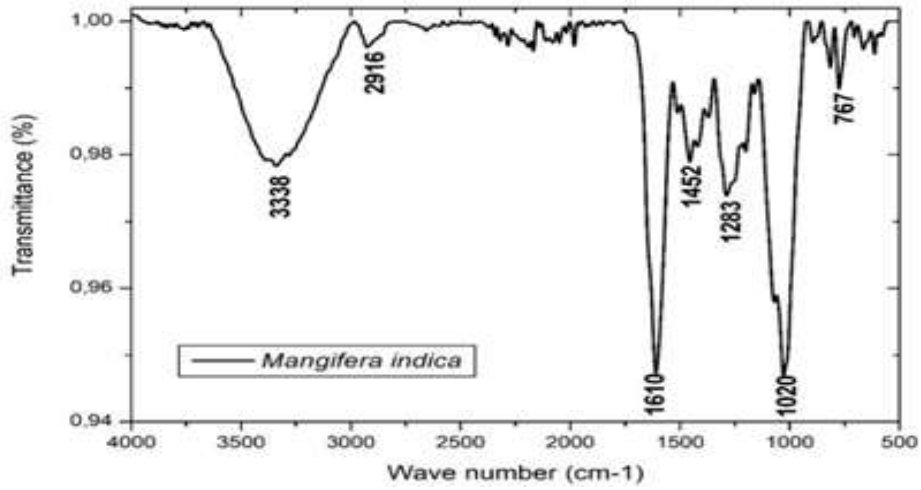

Figure 4: Fourier Transformed Infrared spectrum of MI-AgNPs

Table 1: Functional groups at a given wavenumber for the spectra of MI-AgNPs

Absorption (cm^{-1})	Appearance	Functional group	Compound class
3338	Strong broad	O-H stretching	Alcohol
2916	Medium	C-H stretching	Alkane
1610	Strong sharp	C=C stretching	α, β-unsaturated ketone
1452	Medium	C-H bending .	Alkane
1283	Strong	C-O stretching	Aromatic ester
1020	strong sharp	C-O stretching	Alcohol, Ester, Ether, carboxylic acid
767	strong	C-H bending .	monosubstituted benzene derivative

3.5 Powder X-ray Diffraction: The typical PXRD pattern of synthesized MI-AgNPs is shown on **Figure 5** and their principal characteristics are indicated in **Table 2**. The pattern is compatible with the cubic phase of silver with reflections at 2θ values of 38.07°, 44.16°, 64.33°, and 77.40° that can be indexed to the (111), (200), (220), and (311) planes, respectively of the face-centred cubic structure (JCPDS file: 65-2871). No other characteristic peaks were indicated by the XRD analysis suggesting the high purity of the as-prepared MI-AgNPs. The average crystalline size of synthesized particles was calculated using the Debye-Scherrer formula as follows:

$$Dv = \frac{K\lambda}{\beta\, cos\theta}$$

Where Dv is the average crystallite size; K is a dimensionless shape factor, with a value close to unity (0.99); λ is the wavelength of Cu Kα; β is the full width at half-maximum of the diffraction peaks, and θ is the Bragg angle.

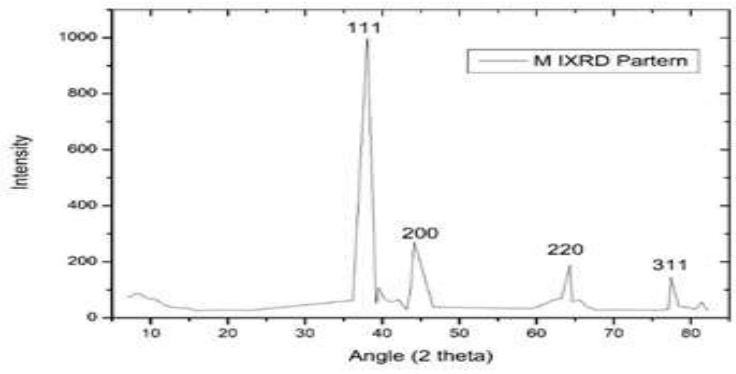

Figure 5: PXRD pattern of biosynthesized MI-AgNPs

Table 2: PXRD characteristics of biosynthesized MI-AgNPs

N	2θ	COSθ	HKL	FWHM (degree)	β (radian)	Interplanar spacing (d)	Lattice constant (a)	Cell volume ($A°^3$)	Crystallite size-D (nm)
1	38.07	0.9829	111	0.4380	0.0076	2.3620	4.0911	68.47	18.57
2	44.16	0.9961	200	0.4380	0.0076	2.0491	4.0982	68.83	18.32
3	64.33	0.7323	220	0.4380	0.0076	1.4469	4.0924	68.54	24.91
4	77.40	0.5400	222	0.4380	0.0076	1.2321	4.0864	68.24	33.79

3.6 Dynamic light scattering results: **Chart 1** shows particles size and size distributions by number (A), by intensity (B) and by volume (C) of synthesized NPs using DLS technique. The hydrodynamic diameter observed for MI-AgNPs was 104 nm with a polydispersity index of 0.266

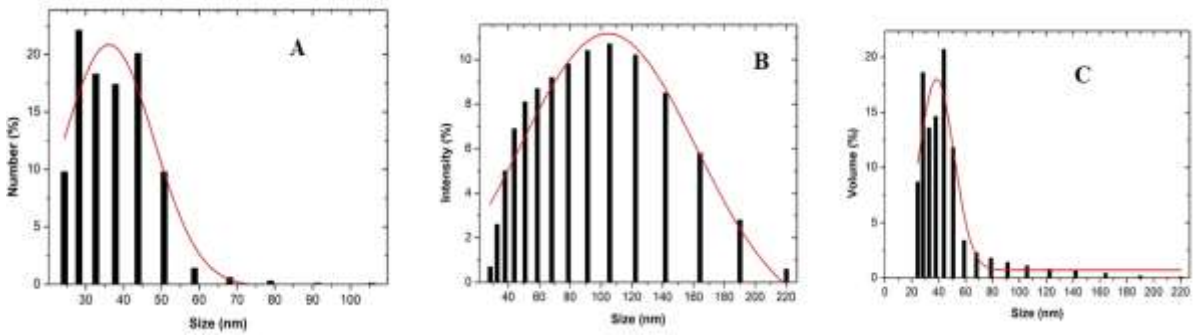

Chart 1: Histogram and Gaussian fit of MI-AgNPs size distributions by number (A), intensity (B) and volume (C) from DLS measurements

3.7 Inhibition effect of MI-AgNPs nanoparticle on egg albumin denaturation: The *in vitro* bioassay results of anti-inflammatory effects of MI-AgNPs assessed against heat-induced egg albumin denaturation are summarized in **Table 3.** All tested concentrations significantly inhibited the denaturation of egg albumin in a concentration-dependent manner. The maximum inhibition percentage was 79.93 % at the highest tested concentration (200 µg/mL) while aspirin, used as standard drug exhibited an inhibition of 67.46 % at the same.

Table 3: Influence of MI-AgNPs and aspirin against egg albumin denaturation

Sample	Concentration (µg/mL)	Optical density	% inhibition	IC_{50} (µg/mL)
Control	-	2.88±0.05	-	
MI-AgNPs	25	1.18±0.05***	59.04	
MI-AgNPs	50	1.06±0.01***	63.03	57.01
MI-AgNPs	100	0.96±0.05***	66.40	
MI-AgNPs	150	0.81±0.02***	71.87	
MI-AgNPs	200	057±0.00***	79.93	
Aspirin	25	2.23±0.03***	22.51	
Aspirin	50	1.88±0.02***	34.73	

Aspirin	100	1.70±0.04***	40.94	198.3
Aspirin	150	1.25±0.03***	56.69	
Aspirin	200	0.93±0.02***	67.46	

Values are expressed as means ± SEM in each group (n=3); ***P<0.001 compared with the control. MI-AgNPs, silver nanoparticles; IC_{50}, concentration for 50% inhibition.

3.8 Effect of MI-AgNPs on carrageenan-induced rat paw edema: As shown in **Table 4**, the injection of carrageenan in control animals produced local edema within 30 min that increased progressively to reach a maximum intensity after 4 hours following the carrageenan injection. Oral pre-treatment of animals with synthesized MI-AgNPs provoked a significant inhibition (P<0.01 and P<0.001) of paw edema when compared to control. Maximum inhibitions observed after 5 hours were found to be 89.50 %, 75.37 % and 69.47 % at doses of 200, 100 and 400 µg/kg respectively. Indomethacin (10 mg/kg) used as standard, inhibited the paw edema by 78.95 % at the fifth hour.

Table 4: Influence of MI-AgNPs on rat hind paw edema induced by carrageenan

Treatment	Dose (µg/kg)	Edema (ΔD in cm)						
		0 min	30 min	1h	2 h	3 h	4 h	5 h
Control		0.21±0.01	0.32±0.01	0.35±0.01	0.39±0.01	0.39±0.01	0.41±0.01	0.37±0.01
MI-AgNPs	400	0.23±0.01	0.31±0.01 (23.07)	0.30±0.01 (50.60)	0.33±0.03 (45.37)	0.31±0.02** (58.18)	0.31±0.03** (62.18)	0,28±0,01*** (69.47)
MI-AgNPs	200	0.24±0.02	0.30±0.01 (47.7)	0.30±0.02 (62.70)	0.30±0.02** (71.30)	0.29±0.01** (73.60)	0.28±0.01*** (82.40	0.26±0.01*** (89.50)
MI-AgNPs	100	0.22±0.01	0.27±0.01 (6.92)	0.28±0.02** (59.64)	0.29±0.02** (51.39)	0.27±0.01*** (70.45)	0.27±0.01*** (60.92)	0.25±0.01*** (75.37)
indomethacin	10,000	0.22±0.02	0.30±0.01 (26.31)	0.28±0.01* (53.01)	0.30±0.02** (56.48)	0.29 ±0.01*** (60,91)	0.28±0.01*** (69.75)	0.25±0.01*** (78.95)

Values are expressed as means ± SEM in each group (n=6); *P<0.05 **P<0.01, ***P<0.001 compared with control. Values between parentheses represent the percentage of inhibition. MI-AgNPs, silver nanoparticles

DISCUSSION

Mangifera indica (Mango tree) is a well-known evergreen broad canopy tree with various pharmacological and ethnomedical properties. A literature review indicated that, in addition to the common use of mango fruit as a food item, various parts of mango trees have been used in folk medicine for the treatment of numerous illness, representing a promissory source of bioactive secondary metabolites [19]. Consequently, several research groups have demonstrated empirically many biological activities of Mango tree. However, to our knowledge, this is the first report of the anti-inflammatory activity of *Mangifera indica* aqueous bark mediated AgNPs.

Thus, eco-friendly and cost-effective biosynthetic approach for AgNPs production using *Mangifera indica* aqueous bark extract as an effective anti-inflammatory agent was demonstrated. The generally used method for the synthesis of AgNPs through green route consisting in blending plant extract with silver nitrate aqueous solution has been retained for this work [27]. The formation of AgNPs is initially observed by the change in colour of the reaction mixture due to the excitation of free electrons in the NPs [28]. The darkish brown colour of *Mangifera indica* aqueous bark extract changed to brownish golden colour immediately within 5 min thereby indicating the formation of MI-AgNPs and no further colour change was observed after 2 hours, indicating the end of NPs synthesis reaction.

Formation and stability of AgNPs in aqueous colloidal solution were confirmed using UV-Vis spectral analysis. The technique is widely used to characterize the optical properties of NPs given indication such as formation, size growth or shape [29]. The absorption spectra band allowed to state the characteristic peak of MI-AgNPs at 410 nm. The peak appearance is due to the formation of plasmon at the colloid surface, due to coherent oscillation of conduction electrons on the surface and occurred 5 min following incubation confirming the presence of NPs in the sample [30] . The peak intensity did not increase with the reaction time indicating complete reduction as well as nucleation and stabilization of synthesized MI-AgNPs. Thus, *Mangifera indica* aqueous bark extract acts as both reducing and stabilizing agent necessary for the synthesis and stabilization of NPs. Such findings have been observed for *Azadirachta indica* leaf extract [31].

An FTIR analysis was carried out to identify functional groups of molecules responsible for the reduction of silver ions and coating of the bioreduced AgNPs. The typical FTIR spectrum of synthesized MI-AgNPs shows several absorption bands, indicating various stretching as well as bending modes including O-H, C-H, C=C and C-O. From the FTIR analysis, it is evident that the MI-AgNPs are capped with phytochemicals from plant extract with various functional groups of organic molecules such as mangiferin, mangocoumarin, mangiferolate, manglupenone, manghopanal, mangoleanone, hydroxymangiferonic acid, indicoside giving characteristic peaks in the spectrum [19]. Similar observations have been reported with *Stachytarpheta cayennensis* aqueous extract [32].

Synthesized AgNPs from *Mangifera indica* aqueous bark extract were examined using powder X-ray diffraction and the pattern shown in Figures 5 indicated that the NPs are face-centered cubic. The obtained peaks at 2θ value of 38.07°, 44.16°, 64.33° and 77.40° suggest crystalline and amorphous organic phases. No additionnal characteristic peaks were found, indicating the high purity of the as-prepared MI-AgNPs. The size of silver nanoparticles determined by the Scherrer equation ranged 18-34 nm. The obtained results agree with the findings previously reported by [14].

The particle size and particle size distribution of the synthesized MI-AgNPs was determined using DLS technique. The size distribution by number, intensity and volume of the synthesized silver nanoparticles is depicted in Chart 1. The particles hydrodynamic diameter was found to be 104 nm with a polydispersity index of 0.226. The hydrodynamic diameter is found similar to the maximum of the Gaussian plot of the size by intensity distribution. The maximum of the Gaussian plot of size by number distribution and the size by volume distribution are 36 and 38 nm respectively. These values are in the same range of those obtained using *Microsorum punctatum* plant leaf extract as bioreacting media with a size by number maxima of 28 nm and a size by volume maxima of 39 nm [33]. The relatively high polydispersity index is indicative of a low monodispersity index as previously reported [34]. This observation is corelated by the three different analytical runs due to aggregation. The interaction in aqueous solution can't separate the hydrodynamic nanospheres. This effect may result from the high concentration of the analytical sample. Sizes and shapes of metal nanoparticles are influenced by many factors including pH, precursor type and precursor concentration, reductant concentration, ionic forces, time of incubation, temperature as well as method of preparation. Similar observation has been made with red apple fruit and *Ricinodendron heudelotii* extracts [35,36].

Previous studies have evidenced that denaturation of proteins especially blood proteins as albumin is a major cause of rheumatoid arthritis and inflammation [37,38]. Protein denaturation is a process in which proteins lose their secondary and tertiary structure by application of external stress such as physical or chemical agents like heat shock. It is well known that proteins lose their biological function(s) upon denaturation [3]. As part of the investigation on the mechanism of the anti-inflammation activity, the ability of the synthesized MI-AgNPs to inhibit protein denaturation was investigated. Results shows

that MI-AgNPs were effective in inhibiting heat-induced albumin denaturation. Maximum inhibition of 79.93 % was observed at 200 µg/ml indicating the capability of the biogenerated nanosilver to prevent protein denaturation involved in the inflammatory process.

Carrageenan-induced rat hind paw edema model was used to assess the anti-inflammatory potential of MI-AgNPs. It is the standard experimental model of acute inflammation used to screen *in vivo* the anti-inflammatory potential of various compounds. The method exhibits a high degree of reproducibility and allow to ascertain action mechanisms of tested substances [39]. The inflammatory condition induced by the subplantar injection of carrageenan in rats involves step-wise release of vasoactive substances such as serotonin, histamine and kinins in the early phase (0-2 h) and prostaglandins and cyclooxygenase products in the late acute phase (>3 h) [40,41]. These chemical substances increase vascular permeability upon their production, thereby promoting accumulation of fluid in tissues that accounts for the edema [42]. Oral pre-treatment of animals with MI-AgNPs resulted in an effective inhibition of edema rate in all phases when compared with control. The maximum inhibition (89.50%) was obtained at the dose of 200 µg/kg, 5 hours after injection of the phlogistic agent. Based on this finding, it is possible to propose that synthesized nanoparticles may act by inhibiting the release of histamine, serotonin and kinins and/or by interfering with the synthesis of prostaglandins mediators as previously reported by [10]. Moreover, it is also reported that anti-inflammatory activities were exerted by many bioactive compounds from plant extracts [43,44]. Therefore, the anti-inflammatory properties exhibited by the NPs in the present study may be due to the individual or synergistic action of various phytochemical compounds coated to the nanoparticles.

CONCLUSION

Plant extract mediated synthesis amongst other biological methods is simple, environmental friendly and cost-effective. It is a bottom-up process of recent interest for obtaining metal nanoparticles. In this study, silver nanoparticles have been obtained via a completely green approach using *Mangifera indica* bark aqueous extract. The characterization shows that MI-AgNPs were crystalline in nature, coated with organics, polydispersed and of various size. The anti-inflammatory activity was assessed, and the results indicated their effectiveness against protein denaturation and edema formation. Thus, MI-AgNPs could have potential application as anti-inflammatory therapeutic agents. These findings contribute to the development of low-cost and resource settings, environmentally friendly, ease of use and available nanopharmaceuticals.

Competing interest: None

Authors' contributions: PBEK, BNE, JCNN, SDD and FEM conceived and designed the study. PBEK, BNE, AAN, HEAM, CCN, JYSF, EET, AMH, and YKDMKM carried out the experiment. PBEK, AAN, LPFK, APG analysed and interpreted the results with help of FEM, ABD and MM. PBEK drafted the manuscript and VD, BM, UKD, SDD, FEM, EAMM, ABD, CJ and MM revised the manuscript. ABD, FEM, PTN and MM supervised the work at all stages. All authors read and approved the final manuscript.

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