In-Vitro Antioxidant and Anti-Inflammatory Activity of Annona Muricata Leaf Extract Mediated Silver Nanoparticles

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The reactive oxygen species produced by the action of free radicals on molecular oxygen causes serious inflammation conditions. Non-steroidal anti-inflammatory drugs which are commonly prescribed for the treatment of inflammatory conditions are associated with side effects. Therefore, silver nanoparticles synthesized using *Annona muricata* leaf extract as a reducing agent was investigated for its *in-vitro* antioxidant and anti-inflammatory potentials. Aqueous extract of *Annona muricata* leaf was used to prepare silver nanoparticles using $AgNO_3$. The structure and surface morphology of the silver nanoparticles was characterized using UV-VIS spectrophotometer, scanning electron microscope (SEM), energy dispersed X-ray (EDX) and X-ray diffraction spectroscopy. SEM images confirmed that the particles were spherical in shape. The EDX showed that silver was the most abundant element in the samples. The biosynthesized silver nanoparticles showed low DPPH and ABTS antioxidant activities compared with the standard but its activity in scavenging hydrogen peroxide was high at 20 – 100 µg/mL. Also, the silver nanoparticles showed high anti-inflammatory activity on lypoxygenase inhibition, proteinase inhibition and red blood cell membrane stabilization. From the data obtained from the various analysis, it can be concluded that the silver nanoparticles synthesised with *Annoma muriata* leaf extract is efficient as antioxidant and anti-inflammatory agent which can serve as a suitable alternative to expensive products.

Keywords: *Annona*s, characterization, inhibition, nanoparticles, synthesis

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INTRODUCTION

The reactive oxygen species produced by the action of free radicals on molecular oxygen causes an imbalance between the oxidizing molecules and the antioxidant system of the body which in turns results in serious inflammation conditions (Amri *et al*., 2018). Non-steroidal anti-inflammatory drugs (NSAIDs) which are commonly prescribed for the treatment of inflammatory conditions are associated with side effects, such as gastrointestinal bleeding, cardiovascular disorder, renal problem and suppressed function of the immune system (Adebajo *et al*., 2015; Altman *et al*., 2015). Due to the complications associated with synthetic anti-inflammatory drugs, there is need for safe anti-inflammatory and antioxidants agents which could have better activities than the harmful synthetic ones. Among several noble metals, silver is widely used in nanoparticle synthesis because of their unique properties such as stability, good conductivity and bioactivity (Aritonang *et al*., 2019). Silver nanoparticles are used for eliminating microorganisms in medical devices, implants and hospital masks. They are also used for preventing infections (Ansari *et al*., 2015). Conventionally, silver nanoparticles are synthesized by chemical method using chemicals as reducing agents. These chemicals were found to constitute biological risk due to their toxicity as such biosynthesis approach is now adopted (Ahmed *et al*., 2016). Biosynthesis approach is environmentally friendly and cost effective, it involves the use of plant extracts as reducing agent. It has been reported that secondary metabolites in plant extract such as protein, phenols and flavonoids play significant role in the reduction of metal ions and capping the biosynthesized nanoparticles (Pirtarighat *et al*., 2019).

Annona muricata is of the family annonaceae and genus *Annona*. The plant is also called sour sop because the fruit has a characteristic sour taste and flavor. All parts of the tree are used in traditional medicine in the tropics including the bark, leaves, root, fruits and seeds. The plant has been found to be useful as antispasmodic, sedative, hypoglycemic, hypotensive and smooth muscle relaxant. *Annona muricata* has edible fruits which are normally eaten by mothers after child birth to boast breast milk (Abdulwahab *et al*., 2018; Moghadamtousi *et al*., 2015). The phytochemical constituents of the leaf extract include saponins, phenols, tannins, glycosides, steroids,
terpenoids and alkaloids (Nwaehujor *et al*., 2019). Considering the wide traditional applications of this

plant, this research was carried out to investigate the anti-inflammatory and antioxidant activity of silver nanoparticles synthesized using aqueous extract of the leaf.

MATERIALS AND METHODS

Preparation and Extraction of Plant Material

The plant materials were collected from a local garden in Ilorin, Kwara State, Nigeria. The sample was identified and documented at the Herbarium of Plant Biology Department, University of Ilorin, with voucher number UIH001/1106. The leaves of *Annona muricata* were dried at room temperature and ground to powder using a mill.

Synthesis of Silver Nanoparticles

One liter of 1 mM $AgNO_3$ was prepared by dissolving 0.169 g of $AgNO_3$ in one liter of distilled water. 10 g of *Annona muricata* leaf powder was poured into a 500 mL round bottom flask and 200 mL of water was added to the flask. The flask was heated on a heating mantle at 60℃ for 15 minutes to extract the components of the leaf. The extract was allowed to cool after which it was filtered with Whatman No. 1 filter paper and stored in a sample bottle for further analysis. 50 mL of aqueous extract was added to 500 mL of 1 mM $AgNO_3$ (ratio 1: 10) in a conical flask. The flask was placed on a magnetic stirrer and heated at 60℃ with stirring at 700rpm for one hour (Kharat & Mendhulkar, 2016). A colour change was observed from wine red to dark brown which signified the formation of nanoparticles.
The reaction was repeated using same quantities of materials at ambient temperature (25℃) for comparison. The formation of nanoparticles was confirmed by measuring the UV-VIS spectrum. The UV-VIS spectral analysis was done by taking an aliquot of the nanoparticle solution (0.5 mL) and dissolving it in 3 mL of distilled water. The spectral data was taken using UV-VIS spectrophotometer at the range of 200 – 600 nm. The nanoparticle solution was centrifuged at 10, 000 rpm for 15 minutes to obtain the nanoparticles which was washed repeatedly with distilled water to obtained pure nanoparticles. The silver nanoparticles were dispersed in water and dried at 50℃. It was observed that the set up at 60℃ yielded more nanoparticles than the set up at 25℃. Silver nanoparticles synthesised at 60℃ was labelled AMW-N3 while the silver nanoparticles synthesized at 25℃ was labelled AMW-N4.

Characterization of the Silver Nanoparticles

Synthesis of Silver nanoparticles was confirmed with UV-VIS spectroscopy. The particle size and surface morphology of the nanoparticles was analysed using scanning electron microscope (SEM), energy dispersed X-ray (EDX) and X-ray diffraction spectroscopy (Jyoti *et al*., 2016).

Anti-oxidant Assays

AMW-N3 was analysed for its antioxidant activities. The parameters that were considered include; 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging, 2,2-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) radical scavenging and hydrogen peroxide scavenging activities. The experiments were carried out as described by Nisha *et al.*, (2012). Vitamin C was used as a standard for the antioxidant assays due to their potent antioxidant activity and it has also been used in several antioxidant studies (Padayatty *et* a*l*., 2003; Pisoschi & Negulescu, 2011).

Anti-inflammatory Assays

Anti-inflammatory assays were carried out on the silver nanoparticles prepared at 60℃ (AMW-N3) because it had finer particle sizes than the silver nanoparticles synthesized at 25℃. The anti-inflammatory assays include, Inhibition of lipoxygenase, Proteinase inhibitory activity, Albumin denaturation inhibition and Red blood cell (RBC) membrane stabilization. The assays were carried out using the procedures described by Leelaprakash and Das (2011).

RESULTS AND DISCUSSION

The silver ions were reduced to silver nanoparticles by the aqueous extract of *Annona muricata* leaf. It was observed that the colour of the solution changed from wine red to dark brown after one hour, indicating the formation of silver nanoparticles. This change took place at the same time for the set up at 60℃ and the set up at 25℃.

Results of Characterisation of the Silver Nanoparticles

UV-VIS spectroscopy analysis

UV-VIS spectrophotometer recorded maximum absorption peak at 426nm for AMW-N3 and 411nm for AMW-N4 which are characteristic of silver nanoparticles (Ahmed & Ikram, 2015).

Scanning electron microscope (SEM) and energy dispersive X-ray (EDX)

The SEM studies revealed the spherical nature of particles synthesized from silver metals. The particles were not of uniform in size. Apart from the nano size particles, larger sizes were also observed which may be due to agglomeration of the smaller ones. The average diameter of AMW-N3 was 64 nm while that of AMW-N4 was 75 nm. AMW-N3 had smaller sizes and better surface morphology. EDX spectrum showed strong signal for silver at 3 KeV and confirmed the formation of silver nanoparticles.

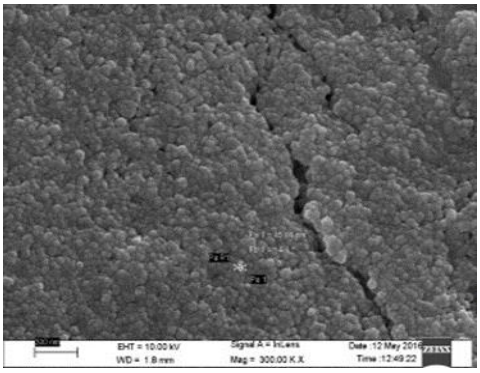

Figure 1: SEM image of AMW-N3

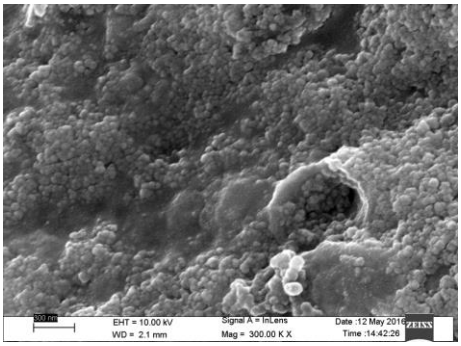

Figure 2: SEM image of AMW-N4

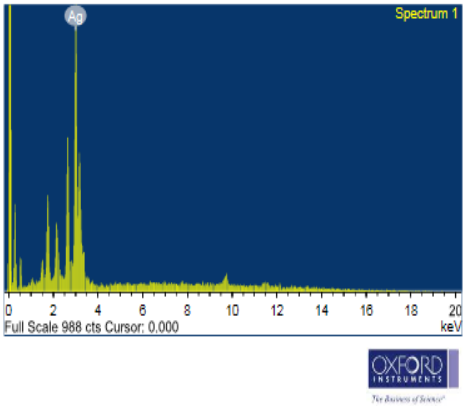

Figure 3: EDX Spectrum of AMW-N3

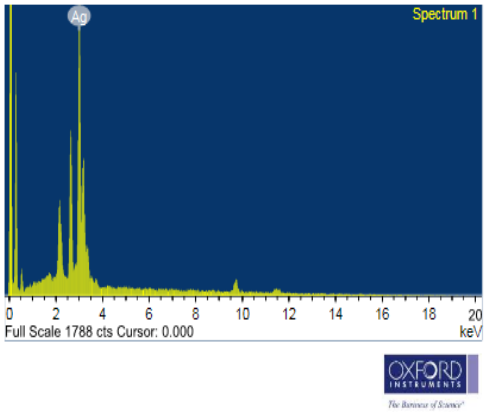

Figure 4: EDX Spectrum of AMW-N4

X-ray diffraction (XRD)

The exact nature of the silver nanoparticles formed was deduced from the XRD spectrum of the samples. The diffracted intensities were recorded from 0^0 to 100^0. The XRD spectrum showed three intense peaks with 2θ values 27.60^0, 32.04^0 and 46.06^0 and interplanar spacing (d-value) 3.229, 2.791 and 1.969 respectively for AMW-N3. For AMW-N4, three intense peaks were recorded at 2θ values 27.74^0, 32.14^0 and 46.18^0 with inter planar spacing 3.213, 2.782 and 1.964 respectively. There were other peaks at 38, 54, 57, 67, 76 and 85^0 for both AMW-N3 and AMW-N4. The values are characteristic of silver nanoparticles and are consistence with the report of Jyoti *et al*. (2016) on *Urtica dioica* leaf.

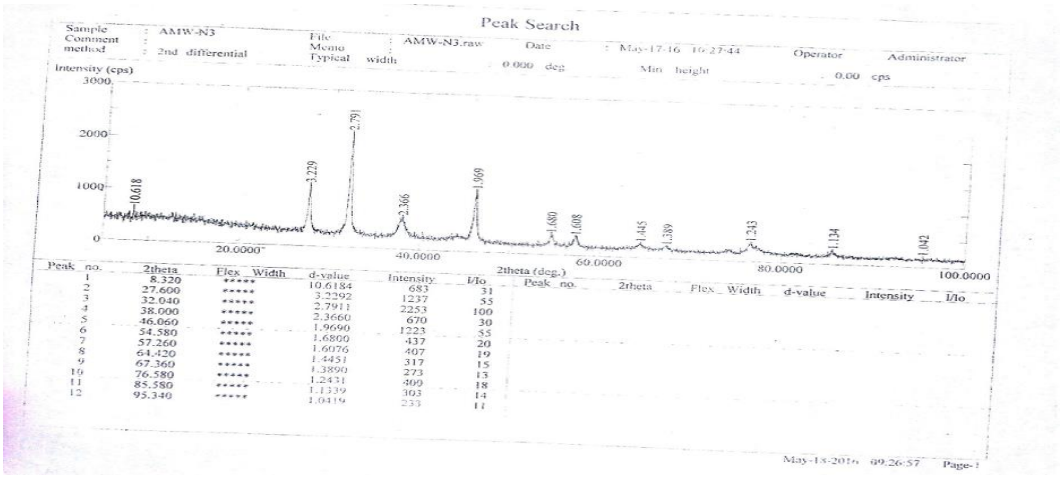

Figure 5: XRD Spectrum of AMW-N3

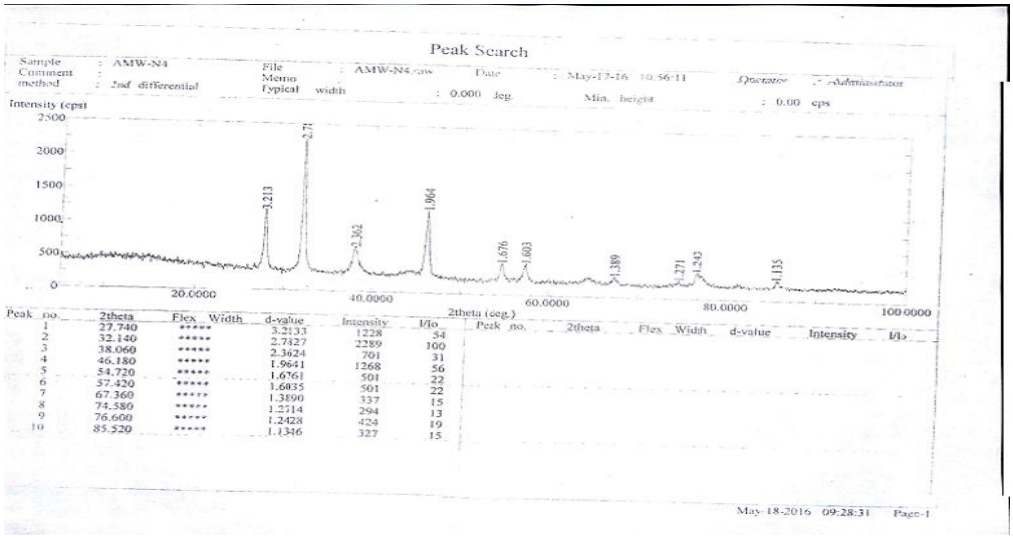

Figure 6: XRD Spectrum of AMW-N3

Results of Antioxidant Activity of *Annona Muricata* Leaf Extract Mediated Silver Nanoparticles

DPPH radical scavenging activity

The results of the DPPH radical scavenging activity showed that the activity of AMW-N3 at 500 µg/mL had good antioxidant activity (Figure 7). The standard had higher activity than the silver nanoparticle and this is consistence with the report of Salari *et al*. (2019). The results also showed that at 500 µg/mL, AMN-N3 is capable of scavenging free radicals. It was revealed that silver nanoparticles had higher DPPH antioxidant activity than Vitamin C (Keshari *et al*., 2020). This disparity might be due to the compounds in the plant extracts that were involved in the formation of the silver nanoparticles. Also, Nagaich *et al*. (2016) documented high DPPH antioxidant activity of silver nanoparticles synthesized with apple extract. The DPPH antioxidant activity of silver nanoparticles synthesized from *Chenopodium murale* leaf was found to be higher than the activity of the plant extract itself (Abdel-Aziz *et al*., 2014). Kharat and Mendhulkar (2016) reported that DPPH antioxidant activity of silver nanoparticles was dose dependent.

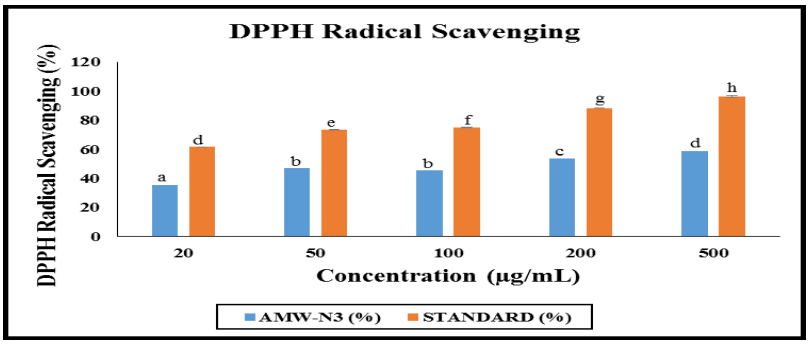

Figure 7: The *In-vitro* DPPH radical scavenging activity of *Annona muricata* leaf extract mediated silver nanoparticles compared with a standard

NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

ABTS radical scavenging activity

The ABTS radical scavenging activity of AMW-N3 was significantly (p = 5) lower than that of the standard (Vitamin C) at all concentrations (Figure 8). Low ABTS antioxidant activity was also documented by Das *et al*., (2019) in their study on antioxidant activity of silver nanoparticles synthesized using the extract of *Ananas comosus.* Fafal *et al*. (2017) compared the ABTS antioxidant activity of *Asphodelus aestivus* aerial part extract with silver nanoparticle synthesized using the plant extract. Their findings showed that the silver nanoparticles had higher ABTS radical scavenging than

the plant extract. Also, a dose dependent ABTS activity of *Cassia roxburghii* leaf extract mediated silver nanoparticles was reported (Moteriya *et al*., 2017).

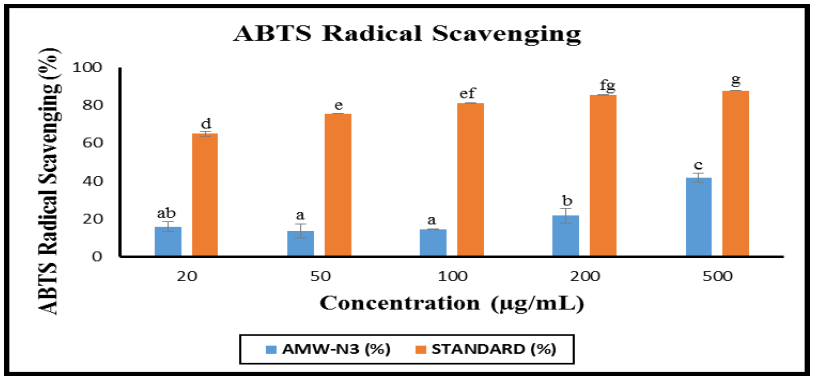

Figure 8: The *In-vitro* ABTS radical scavenging activity of *Annona muricata* leaf extract mediated silver nanoparticles compared with a standard.
NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

Hydrogen peroxide scavenging activity
The hydrogen scavenging activity of AMW-N3 at 20 µg/mL was higher than that of the standard (Vitamin C). The results also indicated that AMW-N3 performed better at lower concentration with optimum activity at 100 µg/mL (Figure 9).

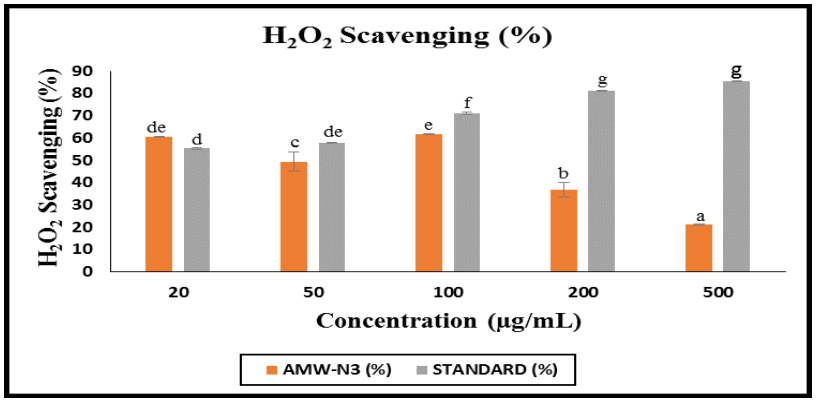

Figure 9: The *In-vitro* hydrogen peroxide scavenging activity of *Annona muricata* leaf extract mediated silver nanoparticles compared with a standard
NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

Results of Anti-Inflammatory Activity of *Annona Muricata* Leaf Extract Mediated Silver Nano-Particles
Lipoxygynase inhibition activity
The results showed that AMW-N3 had good lipoxygenase inhibition which increased with concentration (Figure 10). At 20 and 50 µg/mL the activity of AMW-N3 was higher than that of the standard (indomethacin) which is a nonselective inhibitor of cyclooxygenase 1 and 2 enzymes that participate in prostaglandin synthesis from arachidonic acid. (Suresha *et al*., 2012).

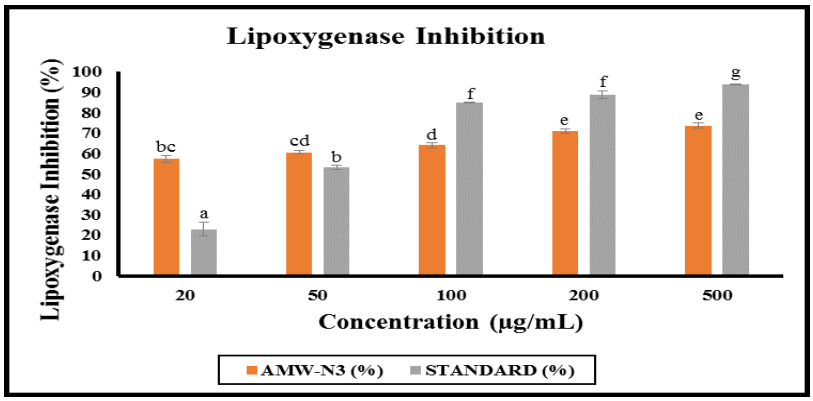

Figure 10: The *In-vitro* lipoxygenase inhibition activity of *Annona muricata* leaf extract mediated silver nanoparticles compared with a standard

NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

Proteinase inhibition activity

AMW-N3 showed good proteinase inhibition activity at all concentrations tested but the activity was not dose dependent (Figure 11). Protienases cause cleavage of proteins and they are involved in the control of key physiological processes such as cell cycle progression cell death, cell proliferation, DNA replication, haemostasis, immune response, tissue remodeling and wound healing. Inhibitors that can moderately control the activities of proteinase are important for drug development (Olivia and Sampaio, 2009).

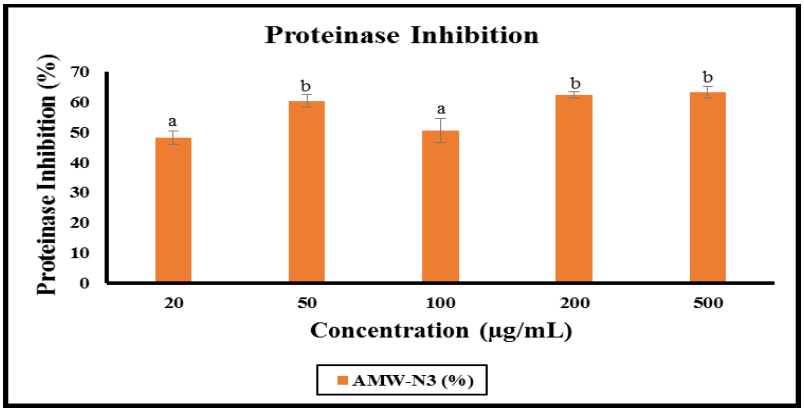

Figure 11: The *In-vitro* proteinase inhibition activity of *Annona muricata* leaf extract mediated silver nanoparticles

NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

Membrane stabilization activity

The red blood cell membrane stabilization activity of AMW-N3 was almost the same with that of the standard (diclofenac) at concentration of 50 and 100 µg/mL. Also, the sample showed high membrane stabilization potential at all the concentrations. The red blood cell membrane stabilization is as a result of anti-inflammatory agents inhibiting the rupture of the erythrocytes by stabilizing the cell membrane. Flavonoids and tannins have been reported to possess anti-inflammatory properties (Kumbhare *et al*., 2014).

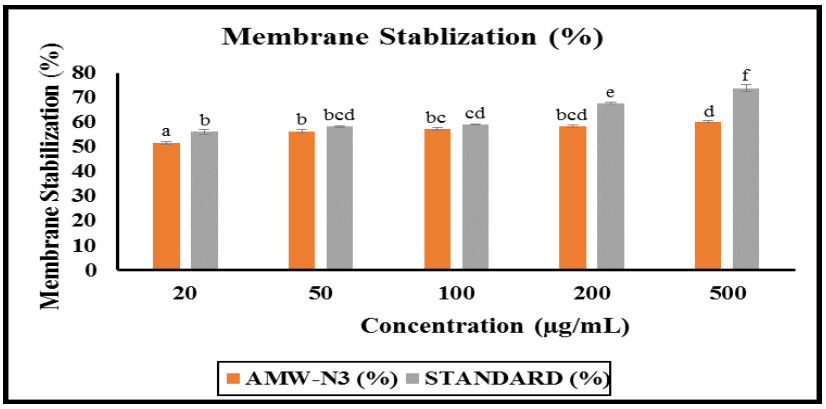

Figure 12: The *In-vitro* red blood cell stabilization activity of *Annona muricata* leaf extract mediated silver nanoparticles compared with a standard

NOTE: Each bar represents mean of triplicate readings; error bar represents the standard error of the mean. Bars with different letters are significantly different (p=0.05).

CONCLUSION

Silver nanoparticles were successfully synthesized using aqueous extract of the leaf of *Annona muricata*, this approach is simple, cost effective and eco-friendly. The silver nanoparticles were characterized with SEM, EDX and XRD. The leaf extract metabolites act as reducing agent in the synthesis process. SEM images confirmed the spherical nature of the silver nanoparticles. The biosynthesized silver nanoparticles exhibited remarkable in vitro antioxidant and anti-inflammatory activities. From the study, it is deduced that biosynthesized nanoparticles have outstanding attributes to transform drug synthesis as this silver nanoparticles can provide a suitable alternative in the treatment of various disorders.

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