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Antidiabetic, Anti-Inflammatory and Antioxidant Potential of Green Synthesized Silver Nanoparticles Using Fresh Aqueous Leaf Extract of *Chromolaena odorata*

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ABSTRACT

The current research study is to develop an easy and eco-friendly method for the synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract of *Chromolaena odorata* and evaluate the extract to know its effects as an antioxidant, and anti-inflammatory agent. The synthesized AgNPs were characterized using UV-Vis spectroscopy and Scanning Emission Microscopy (SEM). The antioxidant (DPPH, ABTS, Reducing Power, Hydrogen Peroxide), anti-diabetic (alpha-glucosidase inhibition) and anti-inflammatory (Albumin denaturation and Protein inhibition) activities were carried out under standard conditions. The synthesized AgNPs were noticed through a visual color change from yellow to brown. The surface plasmon resonance confirmed the formation of AgNPs with maximum absorbance at 419nm and the SEM result shows that the AgNPs size ranged from 23nm to 31nm. The reducing power and ABTS radical scavenging activity of the AgNPs is low and partially significant compared to the control. The DPPH and Hydrogen peroxide radical scavenging activity is similar to that of the control. The ability of the synthesized AgNPs to inhibit protein denaturation, alpha glucosidase was similar to that of the standard. It can be concluded that *C. odorata* leaf extract can be used in the production of potential antioxidant, anti-diabetic and anti-inflammatory AgNPs which could be useful in various fields such as in healthcare, industrial purposes, food, etc.

Keywords: *C. odorata;* AgNPs; SEM; FTIR; antioxidant; anti-inflammatory.

INTRODUCTION

Nanoparticles are referred to as particles with a size of 1-100 nm in at least one dimension (Chen et al., 2013). The surface area to volume ratio increases as the size of the nanoparticles decreases and leads to minor changes in their physiochemical and biological properties. In past years, nanoparticles had many biomedical applications such as antimicrobial, antioxidant, anti-inflammatory, antiviral, cytotoxic, anticancer, antidiabetic, anti-HIV, and so on (Abdel-Aziz et al., 2014). Presently, several metallic nanomaterials are being synthesized using copper, zinc, titanium, magnesium, gold, alginate, and silver. Nanoparticles are being used for several purposes, from medical treatments, using different branches of industrial production such as solar and oxide fuel batteries for energy storage, to wide incorporation into diverse materials of everyday use such as cosmetics or clothes (Dubchak et al., 2010).

Silver nanoparticles (AgNPs) are extensively used for the detection and treatment of diseases, drug delivery, and many other biomedical purposes due to their

eco-friendly properties. Many methods have been used in the preparation of AgNPs, among them are chemical and physical methods but usage of a huge amount of toxic chemicals, and also requires high temperature (Quaresma et al., 2009). Synthesis of nanoparticles by biological methods using micro-organisms, enzymes, plants or plant extracts are suggested as an eco-friendly method to the alternative to chemical and physical methods (Schultz et al., 2000). Biological agents (plants, fungi, bacteria and virus) that can be able to absorb and accumulate metals can be used as reducing agents and control the structural nano topography of the metal ions. The green synthesis of AgNPs using natural organisms is described as a simple, reliable, nontoxic and eco-friendly reaction process (Narayanan and Sakthivel, 2010).

The use of AgNPs as medicinal remedies in the prevention and treatment of disease has increased due to its discovered advantages and its efficient relationship with phytoconstituents present in plant materials. Medicinal plants are known to be composed of several medicinal properties used in the treatment of several ailments. An example of such a plant is *Chromolaena odorata* (Siam weed). *Chromolaena odorata* is one of the oldest cultivated plants to man and it is commonly known as Siam weed (Chakraborty et al., 2011). All parts of the plant are known to be actively used in traditional medicine. The floral portion of the plant is known to be biochemically composed of constituents that are actively used in the treatment of ulcers and dysentery. Several reports on *C. odorata* indicate that the plant has several biochemical activities which expresses the anti-oxidative, anti-inflammatory, anticancer, antidiabetic, antimicrobial and anti-hemorrhagic properties of the plant (Sirinthipaporn and Jiraungkoorskul, 2017). This study focuses on the anti-oxidative and anti-inflammatory properties of aqueous leaf extract of fresh *Chromolaena odorata*.

The synthesis of AgNPs follows several conventional techniques such as laser ablation, gamma irradiation, photochemical methods and microwave processing which are known to be labor-intensive and relatively expensive. Other relative chemical methods are known to involve processes that are toxic or require the use of hazardous solvents and reductants which make them unsuitable for the synthetic process of AgNPs (Dubchak et al., 2010). Furthermore, the development of simple and environmentally friendly processes for the preparation of nanoparticles using non-toxic reagents and the cost-effective procedure becomes a priority. In this context, green synthesis of nanoparticles using microbes, marine organisms, and plant extracts has become popular as these methods are biologically compatible, environmental and economically friendly (Quaresma et al., 2009). Plant-mediated synthesis of AgNPs is a widely accepted approach for the green synthesis of the particle among many other greener synthetic approaches considering the cost of the process and the environmentally friendly nature of the reaction process which promotes the rapid production of the particle (Krutyakov et al., 2008). This study utilizes the synthesis of AgNPs using fresh *C. odorata* leaf extracts were analyzed for their antioxidative, antidiabetic, and anti-inflammatory potentials.

METHODOLOGY

Preparation of *Chromolaena odorata* extract

C. odorata leaves were procured from Badagry, Lagos State, Nigeria. The leaves were rinsed, left to dry for some minutes and ground fresh to obtain a fresh leaf aqueous extract. The aqueous extract (8% w/v) of *C. odorata* was prepared using a 500ml beaker containing 8g powder and 100 ml deionized water and heated at 70^0C using an oven for 2hours. The extract was obtained by centrifuging the mixtures at 3000 revolutions per minute (rpm) for 5 minutes followed by filtration using Whatman no. 1 filter paper. These filtrates

were stored in the refrigerator for further use (Lin and Simon, 2016).

Synthesis of silver nanoparticles (AgNPs)

To synthesize AgNPs, 20ml of the extract was added to the 180 ml silver nitrate solution (1mM) in a 250 ml beaker and stirred two times for 5 minutes using a stirrer at room temperature. The change in color of the solution after 1 week indicated the reduction of silver nitrate into AgNPs. Then 2 ml aliquots were taken every 5 h till 24 h and absorbance (200-600 nm) was analyzed using UV visible spectrophotometer. After the completion of the reaction, the solution was centrifuged at 5000 rpm for 15 minutes and the pellet was collected. Then pellets were washed three times using the 5 ml deionized water and centrifuged at 5000 rpm for 15 min. Further, the pellets were dried in the oven at 80^0C for 10 minutes (Krutyakov et al., 2008).

Characterization of AgNPs

The AgNPs were powdered and characterized by Scanning Electron Microscopy (SEM) and Fourier Transform Infra-Red spectroscopy (FTIR). SEM was used to analyze the shape and size of AgNPs and FTIR was used to check the capping agents on the surface of AgNPs.

Antioxidant activity of AgNPs

3.2.4.1 DPPH radical scavenging assay

A solution of the radical (DPPH) is prepared by dissolving 2.4mg DPPH in 100 ml of methanol. 50 µl of test samples (silver nanoparticles, plant extract, and silver nitrate) was added to 2 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 minutes in the dark. The absorbance of the reaction mixture was measured at 515 nm spectrophotometrically (Azeez et al., 2017). Ascorbic acid was used as standard and water was used as control. The percentage of DPPH radical scavenging activity was calculated using:

Formula 1:

Percentage Inhibition = [(Control – Test) / (Control) * 100]

Hydrogen Peroxide scavenging activity

0.1 ml of test samples was mixed with 0.3 ml phosphate buffer (50 mM, pH 7.4) and 0.6 ml hydrogen peroxide solution (2 mM in phosphate buffer, 50 mM, pH 7.4). The mixture was vortexed and after 10 minutes the absorbance was recorded at 230 nm using UV Visible spectrophotometer (Yu et al., 2009). Vitamin C was used as a standard while phosphate buffer (50 mM, pH 7.4) was used as blank. The percentage of hydrogen peroxide scavenging activity was calculated using Formula 1.

ABTS cation radical scavenging activity

The ABTS cation radical scavenging activity of silver nanoparticles and the extract were measured. 50 ml of 7 mM ABTS solution was mixed with 50ml of 2.45 mM potassium persulphate solution. The mixture was stored in the darkroom at room temperature for 12 to 16 hours. The solution was then diluted with methanol until the absorbance at 743 nm becomes 0.7. 2 ml of the prepared solution was added to 50 µl of test samples then incubated at room temperature for 6 minutes and absorbance was measured at 734 nm using a spectrophotometer (Dong et al., 2009). Water was used as control and ascorbic acid was used as a standard. The percentage of ABTS cation radical scavenging activity was calculated using Formula 1

Reducing power activity of AgNPs

In brief, 50 µl of test samples were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^0C for 20 minutes and then cooled rapidly. Subsequently, 2.5 ml of 10% TCA was added with the abovementioned solution and centrifuged at 3000 rpm for 8 minutes. Finally, 1 ml of 0.1% ferric chloride was added with the upper layer and the absorbance was measured

spectrophotometrically at 700 nm (Lin and Simon, 2016). The obtained results were compared with BHT which was used as a standard and water was used as control. The percentage inhibition of reducing power was calculated by formula1

Anti-inflammatory activity of AgNPs
Protein inhibitory action of AgNPs

The reaction mixture (2 ml) was containing 1 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH 7.4) and 50 µl of test samples. The reaction mixture was incubated at 37^0C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank and water were used as control (Sheikpranbabu et al., 2009). The percentage of inhibition of proteinase inhibitory activity was calculated using formula 1.

Inhibition of albumin denaturation

1% stock of bovine serum albumin (BSA) was prepared with double distilled water. A stock solution of clove nanoparticles was prepared at the concentration of 10 mg/ml. The reaction mixture contained 0.5 ml of BSA and 50 µl of test samples which were incubated at room temperature for 15 minutes. Denaturation was induced by heating the reaction mixture at 60°C for 10 minutes and the absorbance was measured at 660 nm using a UV-visible spectrophotometer (Jang et al., 2012). Only BSA was considered negative control. Percentage inhibition of denaturation was calculated using the formula given below:

Percentage Inhibition = [(Negative control – Test) / (Negative control) * 100

∝-Glucosidase Inhibition Activity

The a-glucosidase inhibition was determined according to a modified method (Kim *et al*., 2005). The assay mixture consisted of 150 µl of 0.1 M sodium phosphate buffer (containing 6 mM NaCl, pH 6.9), 0.1 units of a-glucosidase, and dried AgNPs and extract (50 µl each). The assay mixture was pre-incubated at 37ºC for 10 minutes. After incubation, 25 µL of 2 mM para-nitrophenyl-a-D-glucopyranoside (PNPG) in 25 µl of 0.1 M sodium phosphate buffer was added to the mixture and incubated at 37ºC for 20 minutes. The reaction was terminated by adding 50 µl of 0.1 M sodium carbonate (Na_2CO_3). The absorbance was measured at 405 nm. The tube with a-glucosidase but without AgNPs served as the control with 100% enzyme activity and acarbose served as the positive control. The percent inhibition was calculated using;

Percentage inhibition (%) = $\frac{Ac-As}{Ac} \times 100$

Where: Ac represents the absorbance of control (all the reagents except the test sample) and
As is the absorbance of each sample (extract and nanoparticles).

RESULTS

The result below is the UV-Visible absorption spectra of AgNPs and the formation of AgNPs is observed at the highest absorbance peak between 400-419nm.

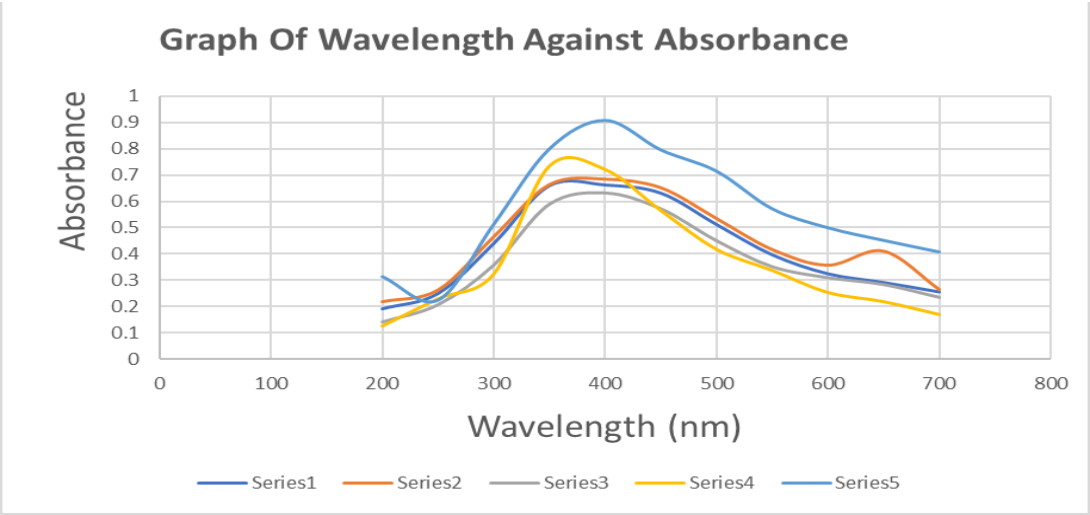

Fig. 1: UV-Vis absorption spectra of obtained silver nanoparticles at different time intervals using aqueous leaf extract *C. odorata* as a reduction agent.

The color change from yellow to brown solution in fig. 2 confirms the existence of AgNPs in the solution due to the reduction of silver ions to AgNPs

Fig. 2: The visual observation of color changes at different time intervals (0h to 24h) as a result of the reduction of silver ions to silver nanoparticles.

The result below shows the reducing power radical scavenging activity of AgNPs and the nanoparticles scavenging ability is lower when compared to the control.

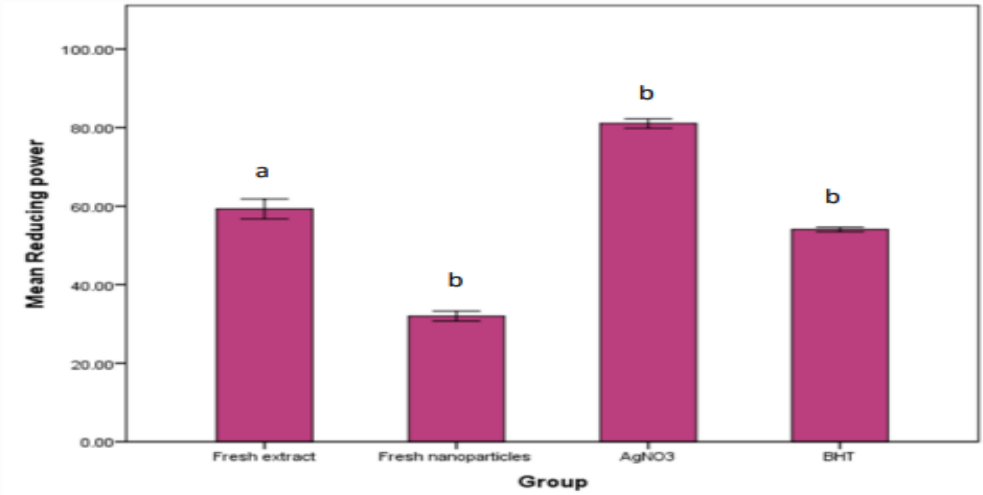

Fig. 3: Reducing power radical scavenging activity of AgNPs synthesized using leaf aqueous extract of *C. odorata* when compared with that of standard antioxidants

The result below shows the hydrogen peroxide radical scavenging activity of AgNPs and the nanoparticles scavenging ability is lower when compared to the control.

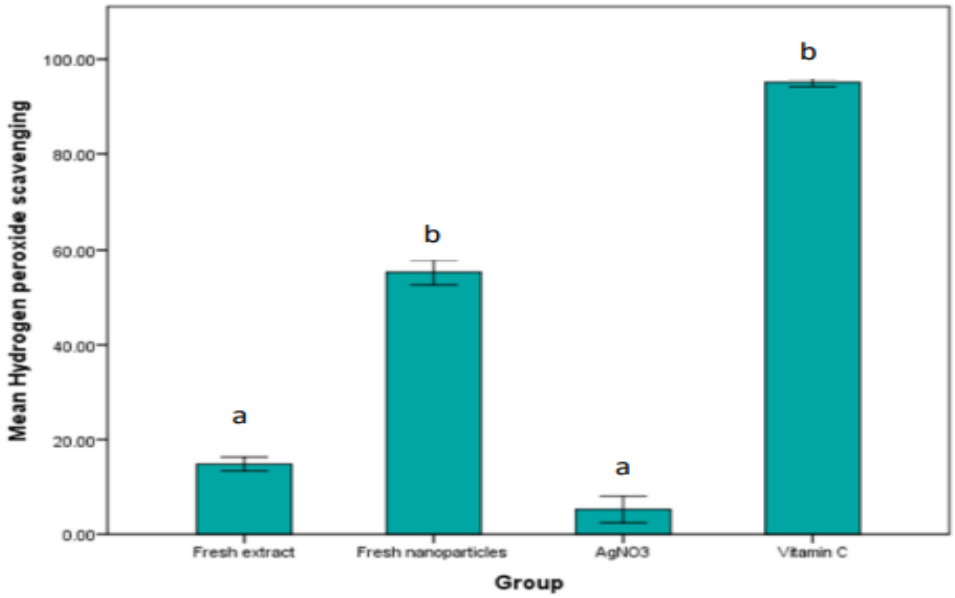

Fig. 4: Hydrogen Peroxide radical scavenging activity of AgNPs synthesized using leaf aqueous extract of *C. odorata* when compared with that of standard antioxidants

The result below shows the DPPH radical scavenging acti b of AgNPs and the nanoparticles scavenging ability is similar to that of the control.

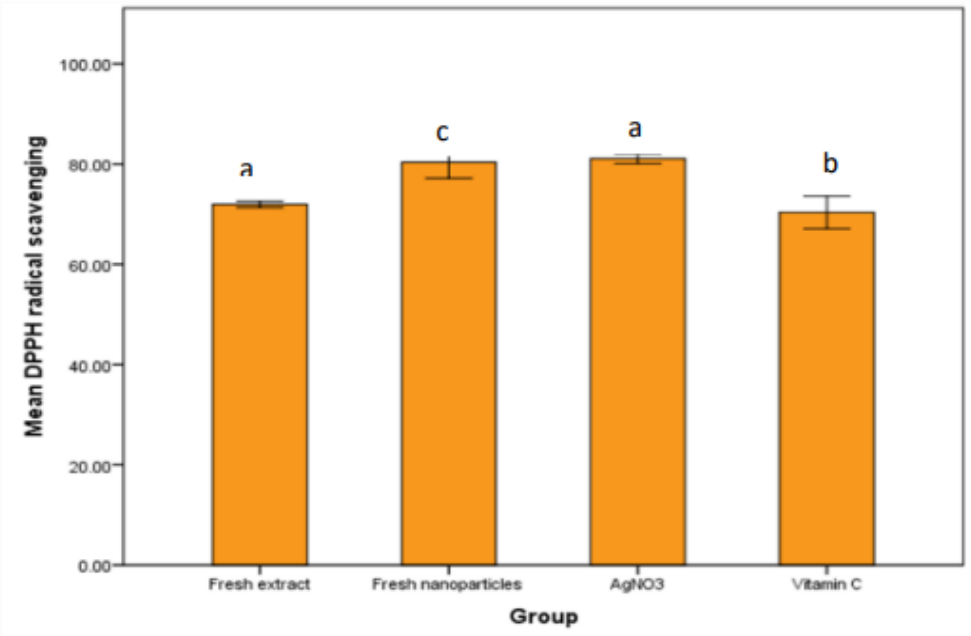

Fig.5: DPPH radical scavenging activity of AgNPs synthesized using leaf aqueous extract of *C. odorata* when compared with that of standard antioxidants.

The result below shows the ABTS radical scavenging activity of AgNPs and the nanoparticle's scavenging ability is moderate when compared to the control.

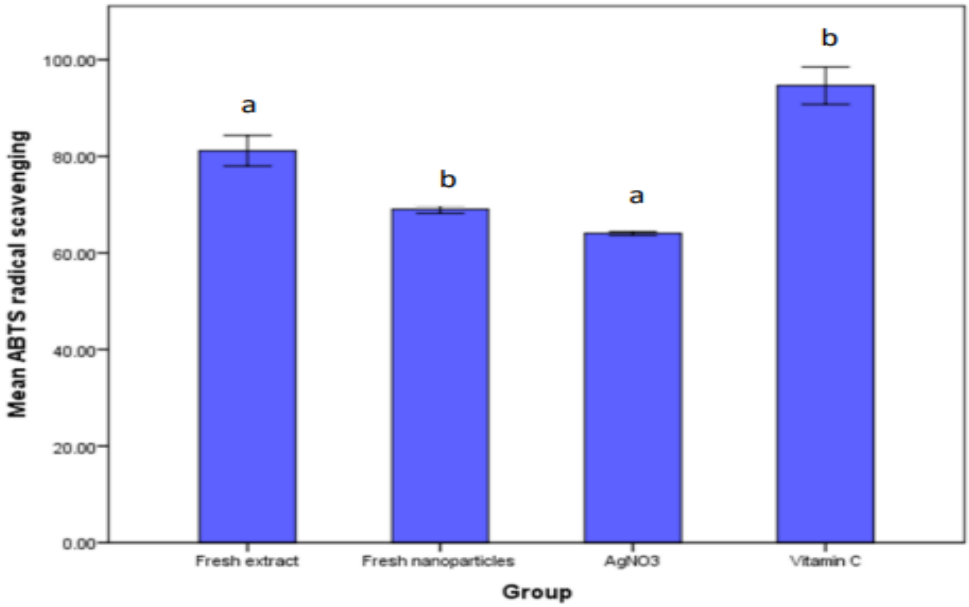

Fig. 6: ABTS radical scavenging activity of AgNPs synthesized using leaf aqueous extract of *C. odorata* when compared with that of standard antioxidants

The result below shows the inhibitory action of protein (albumin) denaturation of AgNPs and the nanoparticle's ability to inhibit protein denaturation is lower when compared to the control.

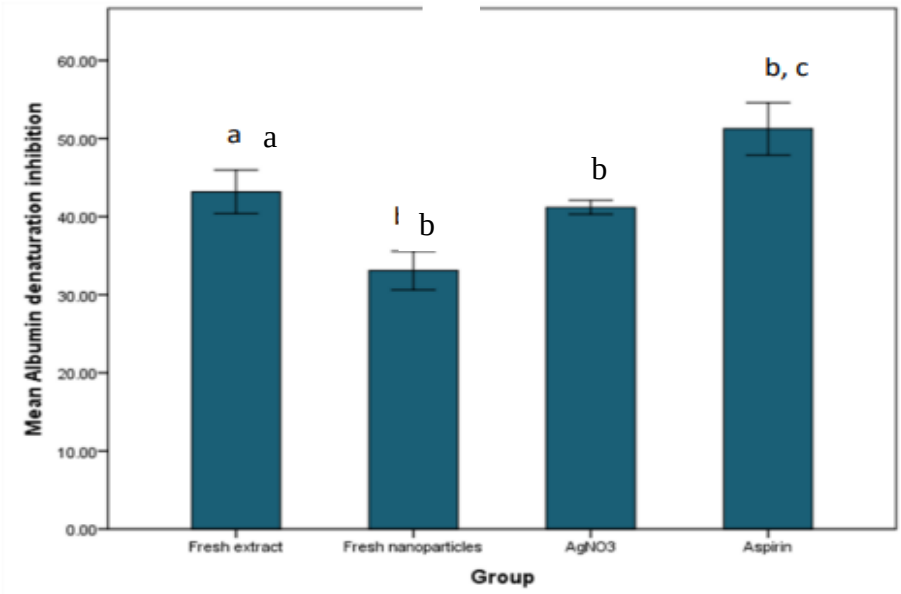

Fig. 7: Albumin denaturation of AgNPs synthesized from leaf aqueous extract of *C. odorata* when compared with that of standard (Aspirin).

The result below shows the protein (trypsin) inhibitory action of AgNPs and the nanoparticle's ability to inhibit protein denaturation is similar to the control.

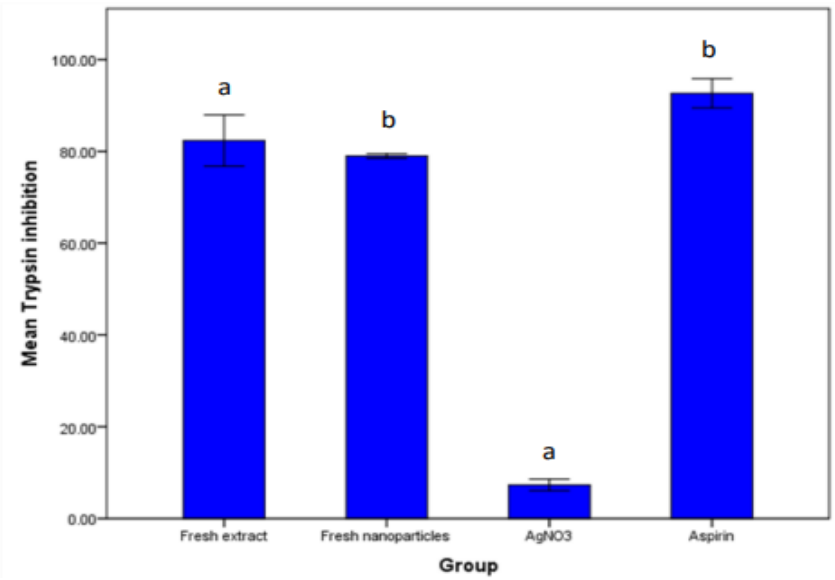

Fig. 8: Protein inhibitory action of AgNPs synthesized from *leaf aqueous extract of C. odorata when* compared with that of standard (Aspirin).

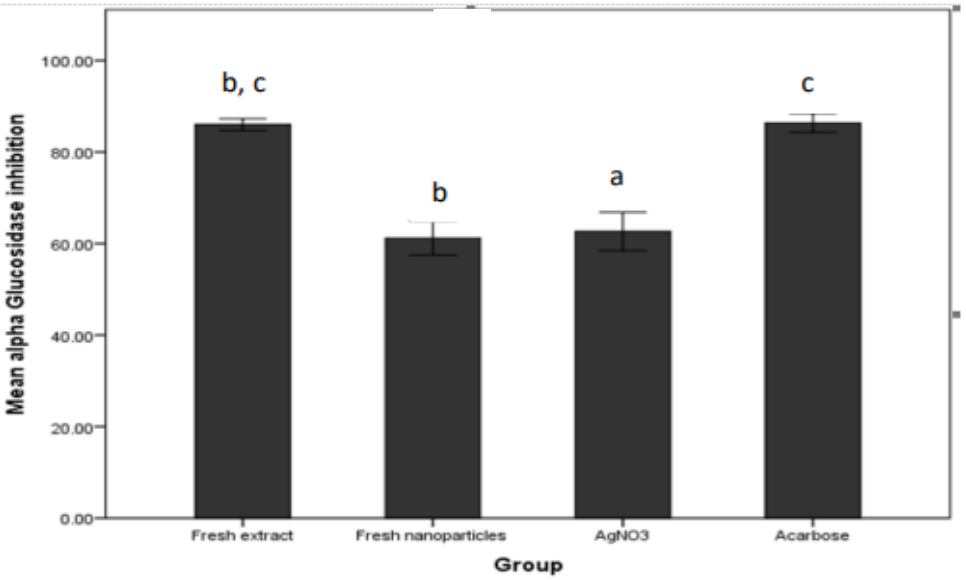

Fig 9: alpha-glucosidase inhibitory activity of AgNPs synthesized from *leaf aqueous extract of C. odorata when* compared with that of standard (Acarbose).

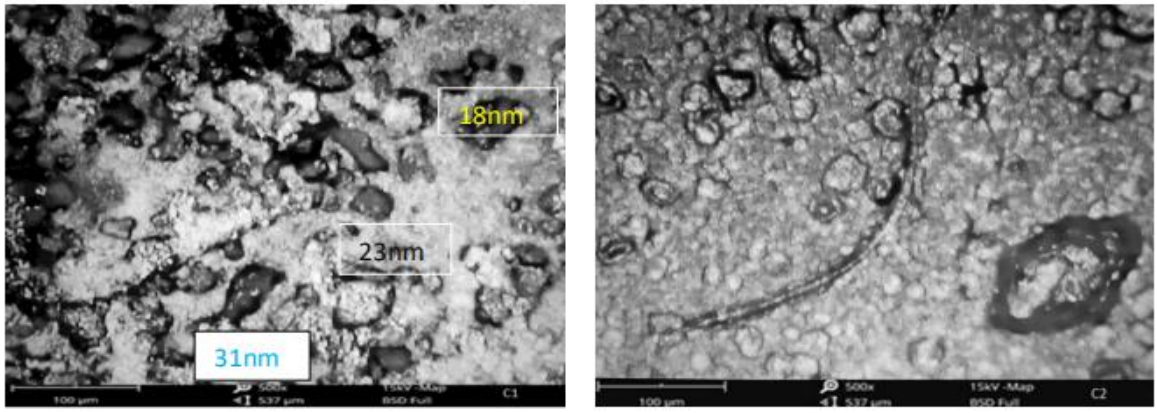

Fig 10: SEM image indicates the formation of variable size of silver nanoparticles

DISCUSSION

The leaf extract of *C. odorata* yielded flavonoids, saponins, tannins, alkaloids, glycosides, phenols, terpenoids and coumarins. Triterpenes, flavonoids, saponins, alkaloids, flavonoids, tannin, glycosides, saponins were recorded from leaves of *C. odorata* Medzhitov (2008). The extracted colour changed to brown due to the addition of 1 mM $AgNO_3$ within 30 mins and no further colour change was observed after 24 h. This may be due to the presence of active phytochemicals in the extract of *C. odorata* which are responsible for reducing silver metal to AgNPs due to excitation of surface Plasmon resonance of synthesized AgNPs. Bioreduction of Ag+ ions were observed in the solution of $AgNO_3$ into silver nanoparticles from *C. odorata* phytochemicals was using UV–visible spectroscopy. The highest absorbance peak was observed at 419 nm for *C. odorata*, which indicates the formation of AgNPs. The SEM result established that the variable size silver nanoparticle was synthesized (18-31nm) this is similar to the report of Bhakya et al. (2016) on the size of nanoparticle synthesized.

The biosynthesized AgNPs of leaves extract of *C. odorata* was dose-dependent in reducing powers. The AgNPs have shown distinct reducing power activity compared with that of standard BHT. The low reducing power activity might be due to inadequate expression of the phytochemicals in the extract Nathan (2002). However, this may also be due to the structure and characterization of the AgNPs. The obtained result was correlated with the results of Dipankar and Murugan (2012), (Bhakya et al., 2016).

Accumulation of uninhibited H_2O_2 leads to the development of oxygen free radicals (Peroxide and hydroxyl) which causes heavy damage to cell membranes in living systems (Nathan, 2002). Using spectrophotometric quantification, the hydrogen peroxide scavenging activity of AgNPs inhibition was found to be 56.95% and 92.19% for the AgNPs and ascorbic acid, respectively. Interestingly, H_2O_2 free radical was consistently lower than those obtained for DPPH scavenging activity. In the presence of hydrogen peroxide, the dispersed AgNPs can induce reactive oxygen species such as hydroxyl radicals. Hydrogen peroxide inside a cell at a low dose can accelerate the dissolution of AgNPs and produce much stronger oxidative stress Nathan (2002). AgNPs can produce greater accounts of hydrogen peroxide and induce greater inflammasome formation because they can cause stronger leakage of cathepsins from impaired lysosomes and efflux of K+ ions may contribute to the production of superoxide and hydrogen peroxide in the membranes of mitochondria (Elsayed and Norredin, 2019). Our results are in good accordance with an earlier report on the H_2O_2 scavenging effect of leaf extract of Abutilon Indicum Pahwa et al. (2018).

DPPH is a stable free radical well-characterized synthetic solid radical for evaluation of the antioxidant potential of compounds. The DPPH will be reduced by accepting the hydrogen or electron, the DPPH reducing the ability of silver nanoparticles was quantified spectrophotometrically by changing the DPPH color from purple to yellow. Inhibition was found to be similar in silver nanoparticles when compared with control. AgNPs showed highest activities than *C. odorata* extract against hydrogen peroxide and DPPH. Also, *C. odorata* extract performed lower activity than AgNPs antioxidant activity according to ABTS and reducing power scavenging assay Ng et al. (2017).

Protein denaturation is a perfectly documented reason for the inflammation in conditions as rheumatoid arthritis (Viscido et al., 2014). The prevention of protein denaturation is the main mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, the ability of the studied extract and the greenly synthesized AgNPs to prevent the

denaturation of proteins could be responsible for their anti-inflammatory properties. The *C. odorata* synthesized AgNPs have the ability to inhibit protein denaturation (albumin) (35.62%) and its percentage inhibition is low when compared to standard aspirin (53.25%) (Figure 6). However, the ability of the AgNPs to inhibit protein (trypsin) action (76.54%) is similar to that of the control (85.29%) which might be a result of the secondary metabolites present in the extract used to synthesize the AgNPs. Our results are confirmatory with the reports of Pretsch et al. (2014) and Tomer et al. (2019).

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a long period and a therapeutic approach to decrease hyperglycemia is to inhibit the carbohydrate digestive enzyme. The anti-diabetic activity of the silver nanoparticles was evaluated using α-glucosidase inhibitory activity. The carbohydrate digestive enzyme α-glucosidase is responsible for the breakdown of carbohydrates into monosaccharides for absorption. Thus natural compounds using traditional medicinal plants that could inhibit the digestive enzyme would be useful for the treatment of non-insulin diabetes. From the result, *C. odorata* mediated nanoparticles showed significant inhibition for α-glucosidase which is similar to that of control-acarbose. The mechanism behind this is that the phytochemicals in *C. odorata* adhered to the surfaces of the silver nanoparticles, providing superior anti-diabetic potential. Comparable results were obtained in the study carried out by Saratale et al. (2018) where *Punica granatum* AgNPs potentially inhibited the carbohydrate digestive enzyme α-glucosidase. However, the foregoing result suggests the potential usefulness of synthesized silver nanoparticles using *C. odorata* leaf extract to treat diabetes and could be considered an effective approach for diabetes care.

CONCLUSION

Synthesis of nanoparticles using a biological agent is eco-friendly, of low cost and capable of phytochemicals acting as both reducing and stabilizing agents. We have characterized the green synthesized silver nanoparticles based on surface plasmon resonance study. The phytochemicals were responsible for reducing and capping of AgNPs, which was confirmed by FTIR spectra. SEM results revealed spherical and uniform-shaped silver nanoparticles. The AgNPs significantly showed antioxidant, anti-diabetic and anti-inflammatory activity. The outcome of the research confirms that the leaf phytochemicals of *C. odorata* are responsible for the formation of silver nanoparticles and also prove to be a good antioxidant, anti-diabetic and anti-inflammatory agent.

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