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Research Article

Potential of Green Synthesis Using a Nanotechnology Approach to Enhance the Biological Properties of Onion Extracts

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ABSTRACT

The use of onions as a natural source of medicinal compounds is on the rise globally. However, its therapeutic effectiveness is limited by several factors, including poor solubility, low bioavailability, etc. Hence, developing strategies to overcome these limitations and enhance their therapeutic potential is justified. This study therefore investigates the potential of green synthesis using a nanotechnology approach to enhance the biological properties of onion extracts. Three different onion varieties were used. The bulbs of each onion were sliced, air-dried, and separately extracted using an ethyl acetate and ethanol solvent mixture (1:1 v/v). Each extract obtained was divided into two: plain onion extract and synthesised silver nanoparticles (Ag-NPs) onion extract. This was obtained by mixing the extract with a solution of silver nitrate and heating for 5 hours at 60°C. DPPH (1,1 difenyl-2-picryl-hydrazyl) and hydrogen peroxide scavenging, total antioxidant capacity, red blood cell membrane stabilisation, protein denaturation, and heat-induced hemolysis were assayed. The results of the study showed enhanced DPPH scavenging abilities by the synthesised silver nanoparticles of onion extracts at ⩽ 81.01% in comparison to the value exerted by the plain onion extracts at ⩽ 75.61%. The total antioxidant capacities of the synthesised Ag-NPs ranged from 0.46±0.6 to 0.85±0.06 mg AAE/g dry extract, while the plain onion extracts ranged from 0.76±0.3 to 0.96±0.09 mg AAE/g dry extract. The synthesised Ag-NPs inhibited protein denaturation at 61.80±0.09–73.34±0.16%, compared to 42.25±0.20–55.08±0.12% by the plain onion extract. The study suggests that green synthesis using nanotechnology approaches can enhance the antioxidant and anti-inflammatory potential of onion extract, leading to improved therapeutic efficacy.

Keywords: *Antioxidant, Anti-inflammatory, Nanotechnology, Onion, Bulbs, Extract*

INTRODUCTION

The growing global demand for medicinal plant extracts for treating diverse health conditions is driven by their safety and rich presence of bioactive phytoconstituents (Okoduwa *et al.*, 2024; Mhya *et al.*, 2024). However, studies show that the therapeutic efficacy of plant extracts is limited because of their poor water solubility, lipophilic properties, and large particle sizes (Okoduwa *et al.*, 2016; Balram *et al.*, 2024). This has been a prominent concern for researchers in the past decades. Today, studies have shown that these limitations could be resolved by the green synthesis of plant extracts through nanotechniques (Alfei and Zuccari, 2024; Chettri *et al.*, 2024).

Nanotechnology offers promising solutions to overcome these challenges by encapsulating bioactive compounds within nanocarriers, thereby improving their stability, solubility, and targeted delivery to specific tissues or cells (Mahreen and Maham, 2023). Nanotechnology is one of the

current areas of study that uses less harmful technologies to synthesis nanoparticles that are firm, less toxic, compatible with tissues, and can be used for both biological and medical applications (Thompson, 2023). The most studied nanoparticle materials are metal nanoparticles, since little effort is needed in their production. Moreover, they have a broad spectrum of applications. Examples of metallic nanoparticles widely studied include silver (Ag) (Singh *et al.*, 2024), gold (Au) (Moreira dos Santos *et al.*, 2012), platinum (Pt) (Aritonang *et al.*, 2015; 2019), and palladium (Pd) (Jitae *et al.*, 2024).

The application of nanoformulations in drug delivery has enhanced their biological availability, water solubility, extended blood circulation, and specificity while minimising their adverse effects. Nanoformulations could be taken by inhaling, swallowing, dermal penetration, or being injected, and they are potentially linked with biological systems (Gupta and Xie, 2018). In the context of herbal medicine, nanotechnology offers innovative strategies for improving the bioavailability, stability, and targeted delivery of bioactive compounds. Several nanocarriers, including liposomes, nanoparticles, nanoemulsions, and nanofibers, have been explored for encapsulating bioactive compounds and enhancing their therapeutic efficacy (Nitthikan *et al.*, 2024). Nano formulation sketches aid in promoting the ability to reach the targeted site of action after administration, safeguarding the molecule from cytosolic degradation by changes in pH, enzyme activities, and biochemical catabolism. In addition, nanoformulations minimise administration doses through single-out delivery, site-specific secretion, and, in turn, enhanced therapeutic efficacy of agents (Jadhav *et al.*, 2024).

A literature survey showed that the bioactivities of plant extracts can be enhanced via nanoformulation. For instance, enhanced antimicrobial activity of alcohol extracts from the leaves of *Vernonia amygdalina* and *Impatiens balsamina,* as well as the fruit of *Lantana camara L, was* reported by Sivalingam *et al.* (2024) and Kang *et al.* (2013). A comparative study of the DPPH scavenging ability of nano-synthesised and non-nano-synthesised particles of a plant extract reported significant antioxidant activity portrayed by the nano-synthesised molecule over the non-nano-synthesised element (Riaz *et al.*, 2020).

A recent study carried out by Shabbir *et al.* (2023) also showed an improvement in the substantial antioxidant activity of *Madhuca indica* extract subjected to nanosynthesis. The authors reported 81% DPPH scavenging and 96.20% protection of egg albumin protein denaturation. In addition, 73.26% inhibition of α-amylase was also reported, which was more than that of the control. Anwar *et al.* (2021) have successfully reported the potential anti-inflammatory effect of nanosynthesised *Tamarix articulate* leaf extract. A study performed by Helmy *et al.* (2020) showed enhanced free radical scavenging, anti-inflammatory, and anti-microbial activities of a green silver nanoparticle synthesis of aqueous extracts from three different plants: parsley, corn silk, and gum Arabic.

Onions (*Allium cepa*) have been traditionally used as both food and medicine across various cultures for centuries. It is primarily planted as a vegetable crop for consumption worldwide (FAO, 2012). The therapeutic properties of onions are attributed to their rich content of bioactive compounds, including flavonoids (quercetin, kaempferol), phenolic acids (caffeic acid, ferulic acid), and sulphur-containing compounds (allyl sulphides, thiosulfinates) (Surh, 1999; Ani *et al.*, 2022). These bioactive compounds exhibit a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Nicastro *et al.*, 2015). Consequently, onion extract has garnered significant interest in the field of complementary and alternative medicine as well as pharmaceutical research (Forney *et al.,* 2018; Fraga *et al.*, 2019; Grzelak-Baszczyk *et al.*, 2020; Chakraborty *et al.*, 2022). For instance, Cordeiroa *et al.* (2023) have recently demonstrated the antioxidant and anti-inflammatory activities of onions in rats fed high-fat diets.

Despite its potential therapeutic benefits, the practical application of onion extract in therapeutics faces several challenges. The bioavailability of onion phytocomponents is limited due to poor solubility and stability, leading to suboptimal therapeutic outcomes (Samota *et al.*, 2022). Moreover, the targeted delivery of bioactive compounds to specific tissues or cells remains a significant hurdle. To address these challenges, researchers are increasingly turning towards nanotechnology-based approaches for enhancing the therapeutic efficacy of onion extract (Ghatak and Iyyaswami, 2019). This study explores the potential of a nanotechnology approach to augmenting the therapeutic efficacy (antioxidant and anti-inflammatory activities) of onion extract.

MATERIALS AND METHODS

Sample collection

Ten (10) fresh onion (*Allium cepa*) bulbs of three different varieties (purple, white, and yellow) were each obtained from Maiduguri, Borno State, Nigeria. They were taxonomically identified and authenticated at the Herbarium Unit of the Biological Sciences Department of Abubakar Tafawa Balewa University, Bauchi, Bauchi State.

Sample processing and extraction

The onion's bulbs were freed of extraneous materials by washing thoroughly with tap water, sliced, and air-dried for seven days, then pulverised into powdered form using a pestle and mortar. The extraction was performed using the methods described by Al-Ansari (2023). One gramme of the dried onion bulbs was mixed with 50 ml of ethyl acetate/ethanol (1:1 v/v) and shaken continuously for twelve (12) hours in a laboratory shaker. The extract was filtered and concentrated using the rotary evaporator under vacuum at 35 °C, and the extracts obtained were stored at 4 °C.

Biosynthesis of silver nanoparticles of onion extracts

The biosynthesis of silver nanoparticles using onion extract was carried out as described by the method of Aritonang *et al.* (2019). A 10 mM $AgNO_3$ solution was prepared and used to prepare the Ag-NPs of the onion extract. Exactly 2 mg of the onion's bulb extract was mixed with 1 mL of $AgNO_3$ solution. The test tube containing the mixture was sealed with aluminium foil and heated at a temperature of 60°C in a test tube heating block for 5 hours. The synthesised Ag-NPs of onion extracts were kept in a refrigerator at 4°C until needed.

DPPH-scavenging effects of plain and synthesised AgNPs of onion extracts

The DPPH scavenging activity of both the plain and synthesised AgNPs of onion extracts was analysed using the method described by Kedare and Singh (2011). A varied concentration (0.06, 0.12, 0.25, 0.50, and 1.00 mg/mL) of ascorbic acid, the synthesised silver nanoparticles of onion extracts, and the plain onion extracts were separately mixed with 4 mL of freshly prepared methanol solution of DPPH (0.004%). The mixture was incubated at 25°C for 20 min; thereafter, absorbance was read against blank (methanol) at 517 nm. The DPPH scavenging activity of each onion's extract (synthesised silver nanoparticles of onion extracts and plain onion extracts) was determined from the absorbance difference of DPPH solution without a test sample or ascorbic acid and DPPH solution treated with either the test samples or ascorbic acid using the formula below according to Valko *et al.* (2007).

$$\text{DPPH scavenging \%} = \text{A (Control)} - \text{A (sample)} \ / \ \text{A (control)} \times 100$$

Where, A (control) = absorbance of DPPH solution without onion extracts/ascorbic acid, and A (sample) = absorbance of DPPH treated with either the onion extracts or ascorbic acid.

Hydrogen peroxide scavenging activity of plain and synthesized AgNPs of onions extracts

The ability of the plain and synthesised AgNPs of onion extracts to scavenge hydrogen peroxide was performed using the method described by Ruch *et al.* (1989). A 40 mM solution of hydrogen peroxide was prepared with phosphate buffer (pH 7.4), where 1.2 mL of H_2O_2 solution was mixed with 2 mL of the synthesised silver nanoparticles of onion extracts, the plain onion extracts, and ascorbic acid (a standard drug) (2 mg/mL in ethanol). Immediately, the absorbance of the mixture was recorded at 230 nm against a blank solution that contained the phosphate buffer without H_2O_2. The percentage scavenging activity of H_2O_2 was determined by the formula below:

$$\text{H2O2 scavenging (\%)} = \text{A (control)} - \text{A (sample)} / \ \text{A (control)} \times 100$$

Where A (control) = absorbance of the H_2O_2 solution before addition of onion extracts, and A (sample) = absorbance of the H_2O_2 solution after addition of the onion extracts or standard drug (ascorbic acid).

Determination of antioxidant capacity (TAC) of plain and synthesized Ag-NPs of onion extracts

According to Prieto *et al.* (1999), the transformation of molybdate (vi) into molybdate (v) and the subsequent formation of a green phosphate/molybdate (v) complex allowed for the determination of the plain onion extracts and Ag-NPs onion extracts' total antioxidant capacity. The molybdate reagent solution was made by combining 20 mL of distilled water with 0.1 mL of sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 M). The volume was increased to 50 mL with the addition of more distilled water. In six different test tubes, 10 µL of the plain onion extracts or the synthesised silver nanoparticles of onion extracts at 1 mg/mL each were added to 1 mL of the molybdate reagent solution. It was incubated at 95°C in a water bath for 90 min. Cool at room temperature for about 30 min, and absorbance was read at 695 nm against the reagent blank containing 300 µL of methanol mixed with 2700 µL of reagent solution. Ascorbic acid was used as a reference to draw a standard curve. The antioxidant activity was expressed as mg/g ascorbic acid equivalent (AAE) of dry extract.

RBC membrane stabilising effects of plain and synthesised Ag-NPs of onion extracts

The membrane stability effect of both plain and synthesised Ag-NPs of onion extracts was determined using the method described by Shinde *et al.* (1999) as modified by Sikder *et al.* (2012). Erythrocyte suspension (RBC) was prepared from EDTA-treated blood collected from experimental rats and then washed and separated using an isotonic solution (154 mM NaCl, 10 mM Na_3PO_4, pH 7.4). A 0.5 mL aliquot of the suspension was mixed with 4.5 mL of hypotonic solution (50 mM NaCl, 10 mM Na_3PO_4, pH 7.4). Then the mixture was mixed separately with 2 mg/mL of the synthesised silver nanoparticles of onion extracts, the plain onion extracts, or 200 µg/mL of the diclofenac-sodium standard drug, kept at 25 °C for 10 minutes, and spun at 3000 x g. The absorbance of the supernatant from each was read at 540 nm. RBC membrane stabilisation was determined using the equation below:

$$\text{Membrane stabilization \%} = 100 - [\text{Acontrol} - \text{Asample}] / \ \text{Acontrol} \times 100$$

Where, A (control) = absorbance of RBC suspension without onion extracts or standard drug, and A (sample) = absorbance of the RBC suspension treated with onion extracts or standard drug.

Effects of plain and synthesized Ag-NPs of onion extracts against protein denaturation

The effect of synthesised Ag-NPs and plain onion extracts against protein denaturation was assayed following the method described by Shenoy *et al.* (2010). Exact 1.0 mL of egg albumin solution was separately mixed with 1.0 mL of the synthesised silver nanoparticles of onion extracts (2 mg/mL), plain onion extracts (2 mg/mL), or diclofenac sodium (200 µg/mL) standard drug. The mixtures were kept

at 27 oC for 15 minutes, then incubated at 60°C in a water bath for 10 minutes for denaturation to be induced. The contents of the test tubes were cooled to room temperature, and absorbance was measured at 660 nm. The percentage inhibition of protein denaturation was determined using the equation below:

Percentage inhibition = A (control) − A sample/
A (control) x 100

Where, A (control) = absorbance of albumin solution without onion extracts or standard drug, and A (sample) = absorbance of the albumin solution treated with onion extracts or standard drug.

Effects of plain and synthesized Ag-NPs of onions extract against heat induced haemolysis

The preventive capability of the synthesised Ag-NPs and the plain onion extracts against haemolysis induced by heat was determined using the protocol described by Azeem *et al.* (2010). In brief, 1.0 mL of each of the synthesised silver nanoparticles of onion extracts, the plain onion extracts (2 mg/mL) or diclofenac sodium (200 µg/mL), was separately mixed with 1.0 mL of RBC (10%) suspension and incubated at 60 oC in a water bath for 30 minutes. After the incubation, the content in the test tubes was cooled by immersing them in tap water. The sample was centrifuged at 3000 x g for 5 minutes, and the absorbance of each supernatant was read at 560 nm. The percentage prevention of haemolysis was determined by using the equation:

$$\text{Percentage inhibition} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where, A (control) = absorbance of RBC suspension without onion extracts or standard drug, and A (sample) = absorbance of the RBC suspension treated with onion extracts of standard drug.

Statistical evaluation

The data obtained from the study were expressed as the mean ± standard deviation (SD). The statistical evaluation was carried out using one-way analysis of variance (ANOVA) and the Duncan multiple range test. This was performed using the Statistical Package for Social Sciences version 23 software. The significance difference was considered at $p \leqslant 0.05$.

RESULTS

Percentage yield and physical appearances of plain and synthesized Ag-nanoparticles of onion extracts

The percentage yield of plain onion extracts from three different varieties was as follows: white onion extract (WOE), purple onion extract (POE), and yellow onion extract (YOE) gave values determined at 30%, 31%, and 33%, respectively. The physical appearance of the extracts was pale in colour, powdered in nature, and partially soluble in aqueous solutions.

The percentage synthesis of Ag-NPs in the three different varieties of onion extracts was as follows: AgNPs of white onion extract (AgNP-WOE), AgNPs of purple onion extract (AgNP-POE), and AgNPs of yellow onion extract (AgNP-YOE) gave values estimated at 11.10%, 33.30%, and 40.00%, respectively. During the Ag-NPs onion extract synthesis, a change in colour from a silver-coloured solution to a brownish colour was observed.

DPPH-scavenging effects of plain and synthesised Ag-NPs of onion extracts

The DPPH-reducing ability of plain and the synthesised Ag-NPs of onion extracts investigated showed the ability to scavenge free radicals. The scavenging capacity of the synthesised Ag-NPs of onion extract was compared with that of the plain onion extracts and a known standard (ascorbic acid), as presented in Table 1. The synthesised Ag nanoparticles in onion extracts demonstrated greater efficiency in scavenging DPPH compared to the plain onion extracts. The rate of DPPH scavenging increases proportionally with the concentration of onion extract. The concentrations for 50 percent scavenging of DPPH by both the synthesised and the plain onion extracts in comparison to the standard drug (vitamin C) were also presented in Table 1. The IC_{50} results show the synthesised silver nanoparticles of onion extracts are lower than their counterparts, the plain onion extracts, except for the yellow variety, which showed a reversed effect.

The hydrogen peroxide scavenging effect of plain and the synthesised Ag-NPs of onion extracts

The H_2O_2-reducing ability of the plain and the synthesised Ag-NPs of onion extracts investigated showed a varied antioxidant potential. The H_2O_2 scavenging effect of both synthesised Ag-NPs of onion extract and the plain onion extracts was compared with a known standard (ascorbic acid) as presented in Figure 1. The results reveal that both synthesised Ag-NPs and the plain onion extracts possess the ability to scavenge free radicals, but with a different trend. The H_2O_2 scavenging pattern of the synthesised Ag-NPs of the onion extract is as follows: AgNP-WOE, AgNP-POE, and AgNP-YOE scavenged H_2O_2 at 45.08±3.84%, 47.95±2.22%, and 52.56±5.88%, respectively, as against 76.92±3.85% exerted by the standard (L-ascorbic acid) antioxidant substance. The H_2O_2 scavenging effects of the plain onion extracts are as follows: WOE at 40.13±8.00%, POE at 34.62±7.69%, and YOE at 43.59±5.88%. The synthesised silver nanoparticles in the onion extracts showed a better scavenging effect in comparison with the plain onion extracts.

Total antioxidant capacity of plain and synthesized Ag-NPs of onion extracts

The total antioxidant capacity of onion extracts (synthesised Ag-NPs and plain) determined showed a difference in the antioxidant capacities exerted by the synthesised silver nanoparticles and the plain onion

Table 1. Free Radical Scavenging Effects of Onion Extracts (Plain and Synthesized Silver Nanoparticles) on DPPH

Conc. (mg/mL)	Percent (%) DPPH Scavenging Activity of Samples						
	Vit. C	AgNP-YOE	YOE	AgNP-WOE	WOE	AgNP-POE	POE
0.06	55.75 ± 0.01	52.44 ± 4.68	44.60 ± 1.49	39.30 ± 2.26	33.08 ± 0.68	48.22 ± 2.88	37.74 ± 11.60
0.12	79.09 ± 0.00	54.53 ± 5.97	47.74 ± 6.77	42.69 ± 0.90	48.38 ± 2.12	50.94 ± 3.65	49.83 ± 1.08
0.25	75.96 ± 0.01	69.16 ± 0.90	51.57 ± 5.83	58.57 ± 1.87	56.62 ± 0.68	54.25 ± 6.16	52.09 ± 4.87
0.50	87.11 ± 0.02	72.47 ± 1.37	66.90 ± 3.64	61.99 ± 2.37	65.51 ± 1.13	65.30 ± 1.26	69.34 ± 3.90
1.00	88.50 ± 0.01	81.01 ± 1.57	75.61 ± 2.47	73.14 ± 0.93	70.38 ± 1.32	74.39 ± 2.05	70.56 ± 2.36
IC_{50} (mg/mL)	0.41	0.48	0.25	0.23	0.27	0.10	0.15

Values are expressed as Mean ± Standard Deviation of three Determinations

AgNP-YOE = Synthesized-silver nanoparticles of yellow onions extract; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles of white onions extract; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles of purple onions extract; POE = Plain onions extract purple variety

extracts. An enhanced antioxidant capacity was recorded by the synthesised silver nanoparticles as compared to the plain onion extracts. The synthesised silver nanoparticles of onion extracts had total antioxidant capacities in the range of 0.46±0.06 to 0.85±0.06 mg AAE/g dry extract, with the yellow onion variety showing the best antioxidant capacity at 0.44±0.06 mg AAE/g extract. While the plain onion extracts had their total antioxidant capacities between 0.76±0.13 and 0.96±0.09 mg AAE/g extract (Figure 2).

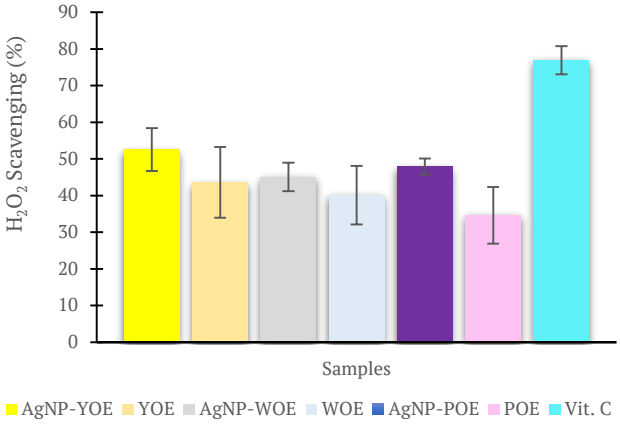

Figure 1. Free Radical (Hydrogen Peroxide) Scavenging Efficacies of Onion Extracts (Plain and Synthesized Silver Nanoparticles)

Values are expressed as Mean ± Standard Deviation of three Determinations.
AgNP-YOE = Synthesized-silver nanoparticles onions extract yellow variety; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles onions extract white variety; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles onions extract purple variety; POE = plain onions extract purple variety

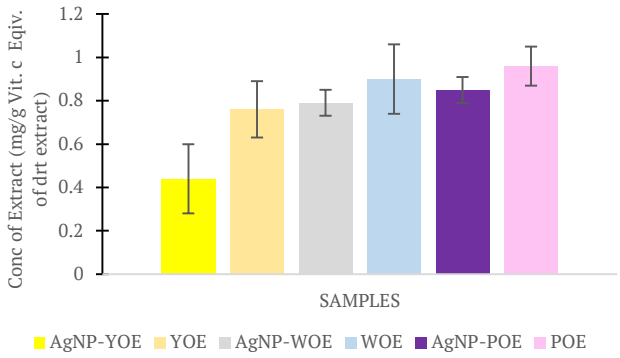

Figure 2. Total Antioxidant Capacity of Synthesized Silver Nanoparticles and Plain Onion Extracts.

Values are expressed as Mean ± Standard Deviation of three Determinations.

AgNP-YOE = Synthesized-silver nanoparticles onions extract yellow variety; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles onions extract white variety; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles onions extract purple variety; POE = plain onions extract purple variety.

RBC membrane stabilization ability of plain and synthesized Ag-NPs of onion extracts

The study found Ag NPs of onion extracts able to stabilize the RBC membrane better than their counterpart (the plain onion extracts). RBC membrane stabilization by the synthesized silver nanoparticles of different onion varieties is in the range of 17.02±0.35 to 33.56±0.69%, with synthesized silver nanoparticles of the purple onion variety being the highest at 37.02±0.35%. The study observed an improvement in the RBC membrane stabilization effects by the Ag-NPs of onion extracts compared to the plain onion extracts. The value of the RBC stabilizing effects of the plain onion extracts ranges from 07.61±0.35 to 21.34±1.04%. The membrane stabilization capacities of the Ag-NPs of the onion extracts, the plain onion's extracts, and the standard drug are presented in Table 2.

Table 2. Anti-inflammatory Abilities of Onion Extracts (Plain and Synthesized Silver Nanoparticles) on RBC Membrane Stabilization

Substances	% Inhibition of RBC Lysis/Stabilization
AgNP-YOE	33.56 ± 0.69^{e}
YOE	21.79 ± 1.04^{c}
AgNP-WOE	27.34 ± 0.69^{d}
WOE	11.07 ± 0.06^{b}
AgNP-POE	17.02 ± 0.35^{c}
POE	07.61 ± 0.35^{a}
Diclofenac Drug	50.05 ± 0.72^{f}

Values are expressed as Mean ± Standard Deviation of three Determinations. Values with different superscript letter(s) are statistically different at $p \leqslant 0.05$

AgNP-YOE = Synthesized-silver nanoparticles onions extract yellow variety; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles onions extract white variety; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles onions extract purple variety; POE = plain onions extract purple variety.

Effects of plain and synthesized silver NPs onion extracts on heat induced protein denaturation

As part of the evaluation in the quest for anti-inflammatory efficacy of synthesised silver nanoparticles of onion extracts, plain onion extracts and a standard drug (Diclofenac sodium) were investigated against heat-induced protein denaturation. The study found that both synthesised silver nanoparticles and the plain onion extracts inhibited heat-induced albumin denaturation, but to a different degree. The synthesised silver nanoparticles were able to inhibit heat-induced albumin denaturation at 61.80±0.09–73.34±0.16%, with the yellow onion variety exerting the better inhibitory effect. The abilities of plain onion extract to minimise heat-induced albumin denaturation are in the range of 42.25±0.20–55.08±0.12% as against the standard drug (Diclofenac sodium), with an inhibitory effect of 98.70±0.24% (Table 3).

Table 3. Anti-inflammatory Effects of Onion Extracts (Plain and Synthesized Silver nanoparticles) on Heat-Induced Albumin Denaturation

Substances	% Inhibition of Protein Denaturation
AgNP-YOE	73.34 ± 0.16^{d}
YOE	42.25 ± 0.20^{a}
AgNP-WOE	67.50 ± 0.08^{c}
WOE	45.79 ± 0.16^{a}
AgNP-POE	61.80 ± 0.09^{c}
POE	55.08 ± 0.12^{b}
Diclofenac drug	98.70 ± 0.24^{e}

Values are expressed as Mean ± Standard Deviation of three Determinations. Values with different superscript letter(s) are statistically different at $p \leqslant 0.05$

AgNP-YOE = Synthesized-silver nanoparticles onions extract yellow variety; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles onions extract white variety; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles onions extract purple variety; POE = plain onions extract purple variety.

Effects of plain and synthesized Ag-NPs of onion extracts on heat induced haemolysis

The study recorded the abilities of both synthesised silver nanoparticles and plain onion extracts to suppress heat-induced hemolysis. The results show synthesised silver nanoparticles from onion extracts are able to minimise lysis of the erythrocyte membrane induced by heat in varying degrees, ranging from 42.63±2.32 to 66.67±1.55%. While the plain onion extracts also suppressed heat-induced hemolysis, but at a lower degree of 18.09±9.68 to 31.95±6.20%. The synthesised nanoparticles of the yellow onion's variety showed the highest percent inhibition of heat-induced hemolysis at 66.67±1.55% against Diclofenac sodium (the standard drug), which offered protection against hemolytic damage from heat by 77.52±0.78%, as displayed in Table 4.

Table 4. Protective Effect of Onion Extracts (Plain and Synthesized Silver nanoparticles) on Heat-Induced Haemolysis

Substances	% Inhibition of Heat Induced Haemolysis
AgNP-YOE	66.67 ± 1.55^{e}
YOE	18.09 ± 9.69^{a}
AgNP-WOE	58.14 ± 4.10^{d}
WOE	31.95 ± 6.20^{b}
AgNP-POE	42.63 ± 2.32^{c}
POE	29.46 ± 1.55^{b}
Diclofenac Drug	77.52 ± 0.78^{f}

Values are expressed as Mean ± Standard Deviation of three Determinations. Values with different superscript letter(s) are statistically different at $p \leqslant 0.05$

AgNP-YOE = Synthesized-silver nanoparticles onions extract yellow variety; YOE = Plain onions extract yellow variety; AgNP-WOE = Synthesized-silver nanoparticles onions extract white variety; WOE = Plain onions extract white variety; AgNP-POE = Synthesized-silver nanoparticles onions extract purple variety; POE = plain onions extract purple variety.

DISCUSSION

Medicinal plants remain one of the most common remedies in the management of diseases affecting both humans and animals. This is because they are safe, readily available, and affordable (Meenakshi *et al.*, 2011). However, studies have shown that the medicinal efficacy of these plants is limited because of their large particle sizes, lipophilic characteristics, and poor water solubility (Okoduwa *et al.*, 2016; Balram et al., 2024). This has been a prominent concern for researchers; however, recent studies have shown that these limitations could be resolved by the green synthesis of plant extracts through nanotechniques (Alfei and Zuccari, 2024; Chettri *et al.*, 2024). On this basis, the current study attempted to enhance the biological properties of onion extracts using silver nanoparticle synthesis. Results from the study showed that synthesised silver nanoparticles in the onion extracts exert better antioxidant and anti-inflammatory activities as compared to the effects exhibited by the plain onion extracts.

The colour shift from colourless to brownish observed when silver solution was heated in the presence of the onion extract but not in the onion extract untreated with silver

solution could suggest the silver nano-formation of the onion extracts. The underlying reason here is that the onion extract turns silver ions into silver nanoparticles. This is aligned with the report of Patel and Patel (2022), which states that this colour shift is an indication that nanoparticles were successful. As earlier reported by Marslin *et al.* (2018), the colour change might be an indication that silver nanoparticles have developed with the plant extract.

The in vitro free radical scavenging study carried out on DPPH revealed that green synthesis of onion extracts, achieved through a nanotechnology approach, enhanced the ability to scavenge free radicals. DPPH free radical scavenging has been an accepted mechanism for screening the antioxidant activity of plant extracts (Guchu *et al.*, 2020). This has been demonstrated by the various capacities of the onion extracts to scavenge free radicals by lowering the colour of DPPH. The enhanced capacity of DPPH scavenging suggests an enhancement in the scavenging effects of the synthesised silver nanoparticles of onion extract as compared to the non-synthesised extracts. Similar findings of enhanced antioxidant activity through green synthesis using silver metal have been reported in other studies, such as the silver-nano-synthesised aqueous leaf extract of *Solanum trilobatum* (Manimegalai *et al.*, 2022) and the nano-formulation of plant olive (*Olea Europeae*) fruit extract (Ghaffar *et al.*, 2023).

In an attempt to identify the most efficient extract, the study determines each onion extract's IC_{50} values. According to Abdel-Tawab's report (2021), a compound's antioxidant activity is inversely correlated with its IC_{50} levels. The antioxidant capacity increases with decreasing IC50 and vice versa. It can be inferred that a molecule is considered very strong if its IC_{50} is less than 50 µg/mL, strong if its IC_{50} is between 50 and 100 µg/mL, medium if its IC_{50} is between 101 and 150 µg/mL, and weak if it is larger than 150 µg/mL (Wahyuningsih *et al.*, 2020; Hussen and Endalew, 2023). Given the IC_{50} value of the Ag-NPs in purple onion extract, 0.1 mg/mL (that is, 100 µg/mL) falling within the ranges of 50–100 µg/mL could be regarded as potent antioxidants in this sense. Yang *et al.* (2004) have reported that the ability of onions to scavenge free radicals varies depending on the variety. The antioxidant effect of onion extracts may possibly be attributed to their capacity to scavenge H_2O_2. In light of this, the onion extracts' ability to scavenge hydrogen peroxide further supported their capacity to eradicate free radicals; however, as the three onion varieties that were employed demonstrate, varietal variation in H_2O_2 scavenging also plays a part. Furthermore, the production of silver nanoparticles in onion extract has improved the scavenging function of H_2O_2, which is a step towards enhancing the biological characteristics of plants.

Tissue proteins are frequently denatured as a result of inflammatory responses, according to Chatterjee *et al.* (2012). Synthetic anti-inflammatory medications can reduce inflammation, but they may also obstruct the body's normal repair and healing mechanisms. As a result, more effective healing agents made from natural sources are required. The quest for drugs with anti-inflammatory qualities frequently makes use of techniques such as protein denaturation inhibition and stabilisation of the erythrocyte membrane (Dhivya *et al.,* 2023). The present study's findings showed that onion extract-based silver nanoparticles may enhance the stability of the erythrocyte membrane and inhibit heat-induced protein denaturation and hemolysis. Similar to this, it was discovered that human red blood cell membrane stability was enhanced by silver nanoparticles derived from *Solanum trilobatum* aqueous leaf extract (Manimegalai *et al.*, 2022).

Nano-synthesised *Tamarix articulata* leaf extract has been shown to have improved anti-inflammatory properties (Anwar *et al.*, 2021). Inhibiting albumin denaturation and safeguarding membrane integrity from heat-induced haemolysis are two benefits of *Tamarix articulata* leaf-synthesised silver nanoparticles. The results of that study indicate that enhancing the antioxidant and anti-inflammatory properties of *Tamarix articulata* leaf extract using silver nanosynthesis appears to be a viable method. Additionally, *Terminalia neotaliala* bark extract was used to create silver nanoparticles that might stop albumin denaturation (Vernekar and Taranath, 2023). These data suggest that the bioactivities of onion extract, including its anti-inflammatory and antioxidant properties, may be amplified by the process of silver nanosynthesis.

CONCLUSION

The results of the study suggest that green synthesis through a nanotechnology approach successfully enhanced the antioxidant and anti-inflammatory capabilities of the different onion extracts. This suggests that the modified extracts may have increased therapeutic efficacy compared to regular onion extracts.

AUTHORS' CONTRIBUTIONS

Conceptualization and methodology: DHM; validation: MA, SIRO, and AGJ.; formal analysis: DHM.; investigation: MHS and HNM.; resources: MHS and HNM.; data curation: DHM; writing—original draft: MHS and HNM.; writing—review and editing: SIRO and MA.; supervision: DHM.; project administration: MA.; funding acquisition: MHS, HNM, and DHM. All authors have read and approved the final revised version for publication.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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