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Effective in Vitro Antioxidant and Anti-Inflammatory Strategy Displayed by Gold Nanoparticles Using Anacardium Occidentale Leaf Extract

Anirban Majumder[1], Surya Kanta Dey[2], Sounik Manna[1], Angsuman Das Chaudhuri[1], Dibyendu Giri[1], Rumi Mahata[1], Pralay Maiti[1], Madhubanti Bepari[3], Gouri Karan[1], Ananya Pradhan[1], Sujata Maiti Choudhury[1*]

[1]Biochemistry, Molecular Endocrinology, and Reproductive Physiology Laboratory, Department of Human Physiology, Vidyasagar University, Midnapore, West Bengal, India.
[2]Department of Allied Health Sciences, Braineware University, Barasat, Kolkata, West Bengal.
[3]Department of Physiology, Midnapore College (Autonomous), Midnapore, West Bengal, India
Email: sujata_vu@mail.vidyasagar.ac.in

The study investigates the in vitro antioxidant and anti-inflammatory efficacy of gold nanoparticles synthesized through a green, plant-mediated approach using aqueous Anacardium occidentale leaf extract (AuNPs-WEAOL). Characterization using dynamic light scattering (DLS) determined the average sizes of AuNPs-WEAOL to be 56.74 ± 3.17 nm, with polydispersity index (PDI) of 0.183. In vitro antioxidant activity was evaluated through the assays for DPPH, nitric oxide, hydroxyl radical, peroxynitrite, hypochlorous, lipid peroxidation, and super oxide dismutase (SOD) scavenging using standard antioxidant ascorbic acid, and potent in vitro antioxidant efficacy of AuNPs-WEAOL was exhibited. In vitro anti-inflammatory potential was determined through human red blood cell (HRBC) membrane stabilization and protein denaturation assay. About 76.28±0.17 % membrane stabilization and 69.45±0.12 % inhibition of protein denaturation were demonstrated by AuNPs-WEAOL at 500 µg/ml dose level compared to standard drug diclofenac sodium. These findings highlight the possibility of AuNPs-WEAOL as a potential antioxidant and anti-inflammatory agent.

Keywords: Anacardium occidentale, Gold nanoparticles, Antioxidant and anti-

inflammatory efficacy, HRBC membrane stabilization.

1. Introduction

Nanotechnology has emerged as a powerful approach in biomedical applications, with metal nanoparticles, especially gold nanoparticles (AuNPs), garnering significant attention due to their unique properties, biocompatibility, and potential therapeutic applications (Shankar et al., 2005). Gold nanoparticles synthesized using plant extracts are particularly promising, as they align with eco-friendly, cost-effective, and sustainable methods. This green synthesis approach not only minimizes hazardous reagents but also imparts the nanoparticles with enhanced bioactivity due to the phytochemicals from the plants used (Uzma et al., 2022). By using gold nanoparticles as a delivery vehicle, the extract's bioavailability and targeted cellular uptake can be enhanced, potentially increasing its therapeutic effectiveness against oxidative stress. Oxidative stress, a condition marked by an imbalance between free radicals and antioxidants in the cell (Kumar & Yadav, 2011). Gold nanoparticles have advantages making them ideal for carrying therapeutic agents like plant extracts directly to targeted tissues. This strategy combines the benefits of traditional medicine with modern nanotechnology, providing a promising avenue for the development of more effective antioxidant and anti-inflammatory therapies (Uzma et al., 2022).

Anacardium occidentale (cashew) leaf extract is rich in bioactive compounds, including phenolics, flavonoids, and other antioxidants, known for their anti-inflammatory and antioxidant properties (De Brito et al., 2007). Leveraging this phytochemical-rich extract for the synthesis of gold nanoparticles could enhance their medicinal properties, especially for applications requiring antioxidant and anti-inflammatory effects (Hemshekhar et al., 2012). Reactive oxygen species (ROS) and inflammatory mediators are critical in the pathology of many chronic diseases, and agents that can counteract oxidative stress and inflammation have immense therapeutic potential (Ozcan et al., 2014; Salehi et al., 2019 ; Patil et al., 2023).

In this study, we report the green synthesis of AuNPs using Anacardium occidentale leaf extract and investigate their in vitro antioxidant and anti-inflammatory activities. The nanoparticles were characterized using advanced techniques to confirm their size, morphology, and elemental composition. The antioxidant and potential of the synthesized AuNPs was evaluated via established radical scavenging assays, while anti-inflammatory effects were assessed by different in-vitro models. Our results demonstrate that AuNPs synthesized with Anacardium occidentale exhibit robust antioxidant and anti-inflammatory properties, highlighting their potential as a therapeutic agent for oxidative stress-related and inflammatory diseases. This study provides new insights into the biomedical applications of plant-synthesized nanoparticles and establishes a foundation for further preclinical studies.

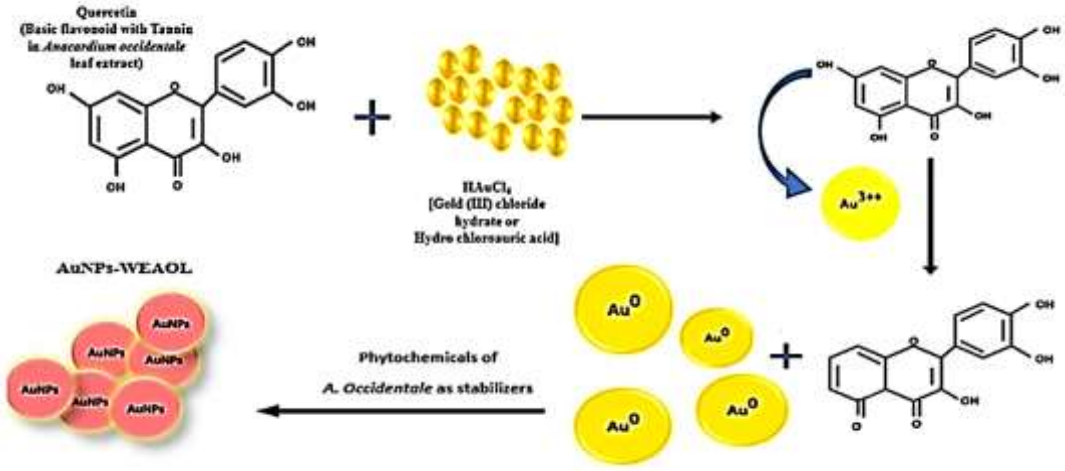

Figure 1 : Mechanisms of green synthesis of gold nanoparticles (AuNPs-WEAOL) using Anacardium occidentale leaf extracts.

2. Materials and methods

Chemicals

Thio-barbituric acid (TBA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxy-2-ribose, potassium chloride (KCl), acetone, ascorbic acid, hydro-chloroauric acid ($HAuCl_4$), bovine serum albumin (BSA), citric acid, dextrose, diclofenac sodium, diethylene tri-amine-penta acetic acid (DTPA), ethylene diamine tetra acetic acid (EDTA), Sulphanilamide, Evans blue, taurine, naphthyl ethylenediamine (NED)-dihydrochloride, ferric chloride ($FeCl_3$), ferrous sulphate ($FeSO_4$), hydrogen peroxide (H_2O_2), n-butane, nitro-blue tetrazolium (NBT), orthophosphoric acid, phenazine methosulfate (PMS), phosphate buffer, pyridine, reduced nicotinamide adenine dinucleotide (NADH), sodium chloride (NaCl), sodium citrate, sodium hypochlorite (NaOCl), sodium nitroprusside, trichloro acetic acid (TCA), sulphuric acid (H_2SO_4), tris-HCl buffer, and other chemicals were purchased from SRL chemicals, India, Merck India, Ltd., Mumbai, India for the experimentation.

Methods

Green synthesis of biogenic gold nanoparticles of Anacardium occidentale

Collection of the plant material and preparation of the plant extract (WEAOL).

Between the month of March and May, the leaves of Anacardium occidentale was collected from the Vidyasagar University campus in Midnapore, West Bengal, India, The leaf was approved by the Botanical Survey of India in Howrah. Then, Anacardium occidentale foliage material was desiccated and finely powdered. After this, 750 ml of double-distilled water was combined with 500 gms of dried leaf powder and left to steep for 72 hours at room temperature. Then, the mixture of aqueous extract was filtered, and the filtrate was

lyophilized. This Anacardium occidentale (WEAOL) freeze-dried aqueous leaf extract was stored at 4°C (Choudhury et al., 2015).

Green synthesis of biogenic gold nanoparticles of Anacardium occidentale (WEAOL)

A magnetic stirrer was used to mix 0.02 mg/ml of water-based leaf extract of Anacardium occidentale (WEAOL) with 0.5 millimolar hydrochloroauric acid ($HAuCl_4$) in double-distilled water for 45 minutes at 40°C. After allowing the time of settlement for 2 hours at room temperature, solution colour was examined to confirm wheather nanoparticles are formed from the preparation of Anacardium occidentale leaves. The biosynthesis of gold nanoparticles (AuNPs-WEAOL) from Anacardium occidentale is appreciated when the fluid turns violet. Newly synthesized (Figure 2), nanoparticle (AuNPs-WEAOL) was centrifuged at 12,000 rpm and stored at 4°C (Garg and Garg, 2017).

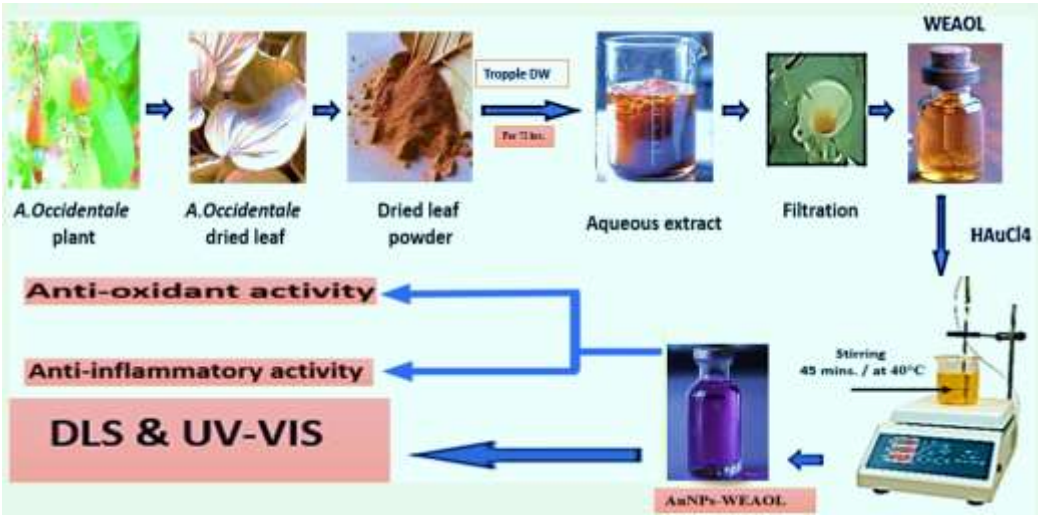

Figure 2: Schematic diagram showing the synthesis of AuNPs-WEAOL from aqueous leaf extract of Anacardium occidentale.

Characterization of gold nanoparticles (AuNPs-WEAOL)

The particle size of AuNPs-WEAOL including polydispersity was analysed by Dynamic Light Scattering (DLS) studies (Malvern Instruments, U.K.) (Maity et al., 2017).The UV-Visible absorption spectrum of AuNPs-WEAOL was measured using an UV–Visible spectrophotometer (Shimadzu, Columbia, MD, USA), with readings taken from 200 to 800 nm and a resolution of 0.5 nm, using distilled water as a reference.

In-vitro Antioxidant activity

DPPH scavenging activity

Specific volume (2.8 ml) of WEAOL and AuNPs-WEAOL were mixed with 0.2 ml of 1, 1-diphenyl-2-picrylhydrazine (DPPH) solution at concentrations ranging from 50 to 200 µg/ml. These were contrasted with the same concentrations of standard ascorbic acid. A spectrometer set to 517 nm wavelength was used to measure the mixtures' optical density

after they had been incubated for 30 minutes at 37°C in the dark. (Gaber et al., 2021). The percentage inhibition of DPPH radical was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\mathbf{C-T}}{\mathbf{C}} \times \mathbf{100}$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

Nitric oxide scavenging activity

One millilitre of 10 mM sodium nitroprusside and one millilitre of WEAOL, AuNPs-WEAOL as well as standard ascorbic acid in phosphate buffer (pH 7.4) at different concentrations (50, 100, 150, and 200 µg/ml) were all included in the experiment. For two and a half hours, these mixtures were incubated at 25°C. Afterward, 1 ml of the incubated mixture was treated with Griess reagent, which contains sulphanilamide, naphthyl ethylene diamide (NED) in phosphoric acid solution to produce a chromophore. The absorbance of this chromophore was measured at 546 nm, and the percentage inhibition was calculated using a specific formula (Jamali et al., 2021).

$$\text{Percentage inhibition} = \frac{\mathbf{C-T}}{\mathrm{C}} \times \mathbf{100}$$

Where, C = Absorbance of the control and T = Absorbance of the test sample.

Hydroxyl radical scavenging activity

A mixture comprising EDTA, $FeCl_3$, H_2O_2, deoxyribose, ascorbic acid, phosphate buffer, and different concentrations of WEAOL and AuNPs-WEAOL was incubated for one hour at room temperature. After adding TCA and incubating in a hot water bath, a pink chromogen was formed. At 412 nm, the absorbance was measured, and a particular formula was used to determine the percentage of deoxyribose degradation inhibition (Giri et al., 2024).

Peroxynitrite scavenging assay

A reaction mixture was prepared with phosphate buffer, DTPA, NaCl, KCl and different doses of WEAOL and AuNPs-WEAOL along with newly synthesized peroxynitrite. Ascorbic acid at different concentrations was used as a standard. The mixture was incubated at 25°C for 30 minutes and then analysed spectrophotometrically at 611 nm (Karmakar et al., 2011).

Hypochlorous acid scavenging activity

Hypochlorous acid (HOCl) was freshly prepared using a solution of NaOCl and H_2SO_4, and its concentration was measured at 235 nm. Then, HOCl and various concentrations of WEAOL and AuNPs- WEAOL, along with ascorbic acid, were mixed and incubated for 1 hour at 37°C. Afterward, taurine was added, and the mixture was incubated for another 30 minutes at 37°C, followed by the addition of 5-thio, 2-nitro benzoic acid (TNB). The absorbance was measured at 412 nm, and the percentage of scavenging was calculated using previously described formula (Starzak et al., 2021).

Lipid peroxidation inhibition assay

The homogenised rat liver was separated into a supernatant by centrifuging it in a Tris-HCl buffer. This supernatant was mixed with various concentrations of WEAOL and AuNPs-WEAOL, along with KCl, $FeSO_4$, and ascorbic acid, and incubated at 37°C for 1 hour. The reaction was stopped with TCA and TBA, and the test-tubes were placed in hot water bath for 30 minutes. After cooling, n-butanol:pyridine solution were added, and the mixture was centrifuged. The organic layer containing the colored malondialdehyde (MDA)-TBA complex was measured at 532 nm and percentage inhibition calculated using previously mentioned method (Janarny et al., 2021).

Superoxide anion radical scavenging activity.

In order to measure the superoxide anion scavenging activity, superoxide radicals were created in a PMS-NADH system that contained PMS, Tris-HCl buffer, NADH, and NBT. This system was supplemented with different concentrations of WEAOL and AuNPs-WEAOL, to evaluate free radical scavenging. The mixtures were incubated at room temperature for 5 minutes, and then the absorbance was measured at 560 nm. A control was also prepared by substituting the sample with Tris-HCl buffer. (Rao, 1989). The scavenging effect percentage was calculated using below memtioned formula :

Scavenging effect (%) = $(1 - A_{\text{sample 560}} / A_{\text{control 560}})$ X 100 μg/ml

Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. IC_{50} value (μg/ml) is the concentration at which the scavenging activity is 50%. The IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half.

In-vitro anti-inflammatory activity

HRBC membrane stabilization method

Blood from healthy volunteers, free from certain medications and systemic diseases, was collected under medical supervision with ethical approval (VU / IHEC-3 / 5-22 dated 22. 04. 2022). It was mixed with Alsever's solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm for 10 min, and the packed cells were washed and suspended in iso-saline. Various concentrations of extracts and nano-preparation (50-500 μg/ml) were mixed with phosphate buffer, hyposaline, and HRBC suspension, then incubated and centrifuged. The haemoglobin content was measured spectrophotometrically at 560 nm. Diclofenac was used as a reference drug, and a control without extracts was included. Experiments were done in triplicates, and the percentage of HRBC membrane stabilization or protection was calculated (Nagaharika et al., 2013), using the following formula:

$$\text{Percentage inhibition} = \frac{C-T}{C} \times 100$$

Where, C = Absorbance of the control and T = Absorbance of the test sample

Inhibition of protein denaturation

BSA solution (1%) was added to various concentrations of WEAOL and AuNPs-WEAOL. The pH was adjusted to 6.3 with HCl, and the mixture was incubated at 37°C for 20 minutes, then heated at 51°C for 20 minutes and cooled. The turbidity of the samples was measured at 660 nm. Diclofenac sodium was used as a standard, and the experiment was repeated three times. (Deshpande et al., 2009). Percent inhibition of protein denaturation was calculated as follows:

$$\text{Percent Inhibition} = 100 - \frac{\text{OD of test } \times 100}{\text{OD of control}}$$

Statistical analysis

Results of antioxidant and anti-inflammatory activity were expressed as Mean ±SEM. Results were analysed using one-way ANOVA. Differences were considered as statistically significant $p<0.05$ are compared to control.

3. Results and discussion

Characterization:

Dynamic light scattering (DLS) measurement

The size distribution of synthesized nanoparticles was determined by dynamic light scattering. Based on Figure 3A, the average diameter of AuNPs-WEAOL was observed to be 56.74 ± 3.17 nm with a polydispersity index (PDI) of 0.18. AuNPs-WEAOL exhibited negative surface charge of –24 mV which provides the electrostatic stabilization and effectively inhibiting rapid agglomeration through the repulsive forces acting between the negatively charged particles (Dey et al., 2022)

UV-Vis spectral analysis

Gold nanoparticles (AuNPs) functionalized with Anacardium occidentale (cashew) leaf extract can alter their UV-Vis absorbance spectrum due to the interaction between the nanoparticles and the bioactive compounds in the extract. The absorption peak for gold nanoparticles typically appears in the visible region, around 520–580 nm, due to the surface plasmon resonance (SPR). This is the characteristic peak for AuNPs. When Anacardium occidentale leaf extract, which contains polyphenols, flavonoids, and other bioactive compounds, is used to functionalize gold nanoparticles, the UV-Vis spectrum can show additional features (Figure 3B). (Balavigneswaran et al., 2014).

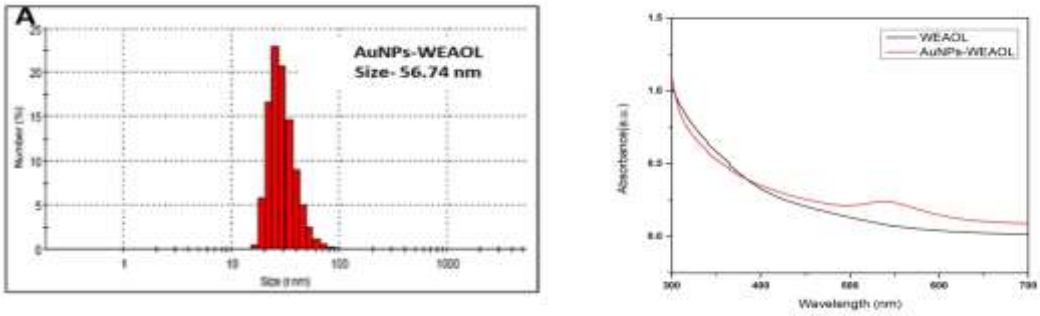

Figure 3: Figure 3A shows the Particles size distribution analysis of biogenic AuNPs-WEAOL was measured by dynamic light scanning (DLS) and Figure 3B presents UV-vis spectra and comparative study of WEAOL and AuNPs-WEAOL for their size and stability.

Antioxidant scavenging assays

DPPH scavenging

The DPPH color change indicates a strong antioxidant response (Moncada and Higgs, 1993) from AuNPs-WEAOL, potentially surpassing that of WEAOL. Both AuNPs-WEAOL and ascorbic acid showed similar effectiveness in scavenging DPPH radicals (Figure 5). The IC_{50} values, indicating the concentration required to scavenge 50% of DPPH radicals, were found to be 119.54 µg/mL for ascorbic acid, 135.25 µg/mL for WEAOL, and 135.65 µg/mL for AuNPs-WEAOL, respectively. These results suggest that both WEAOL and AuNPs-WEAOL exhibit effective DPPH scavenging abilities, with comparable potency to ascorbic acid. According to Kim and Lee (2004) as well as Baumann et al. (1979), the impacts of antioxidants on DPPH are assigned to their hydrogen-contributing functions. The antioxidant activity is likely attributed to the functional groups present in the nanoparticles of leaf extract (Vanitha and Urooj 2013; Blois, 1958).

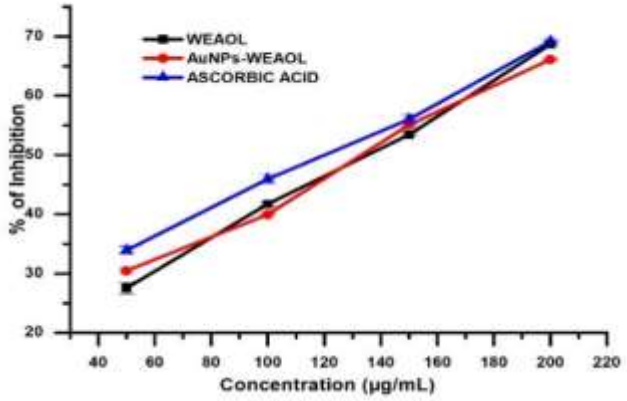

Figure 5: DPPH scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Nitric oxide (NO) scavenging

Excessive Nitric oxide (NO) production is linked with tissue damage and several diseases, while simultaneously playing a crucial role as a biochemical mediator synthesized by nerve tissues, macrophages, and endothelial cells. It has a part in the governance of numerous physiological processes (Moncada and Higgs, 1993). The study indicates that AuNPs-WEAOL exhibits a dose-dependent nitric oxide scavenging activity (Figure 6) and at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL, have concentration dependent inhibition on nitric oxide scavenging and the IC_{50} value were found to be 104.45, 136.21 and 138.97 µg/ml respectively. The nanoparticles' ability to scavenge nitric oxide was demonstrated by their power to prevent nitrite ion formation through a direct contest with oxygen and nitrogen oxides in the reaction mixture (Prabaharan et al., 2009).

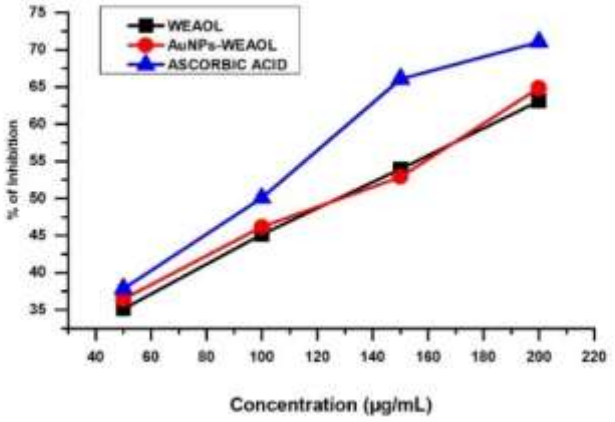

Figure 6: Nitric oxide (NO) scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Hydroxyl radical (OH) scavenging

The results obtained from the •OH assay demonstrate that AuNPs-WEAOL exhibit the highest inhibition due to their extensive surface area, whereas WEAOL exhibit less inhibition as even compare to the standard ascorbic acid in different concentration level. The result shows that hydroxyl radical (OH) scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL, have concentration dependent inhibition on Hydroxyl radical scavenging and the IC_{50} value were found to be 111.42, 128.98, and 126.12 µg/ml respectively. The maximum inhibition is attributed to the presence of several phytochemicals in the Anacardium occidentale leaf extract. These phytochemical compounds likely become adsorbed onto the active surface of the AuNPs-WEAOL. (Dauthal and Mukhopadhyay, 2012).

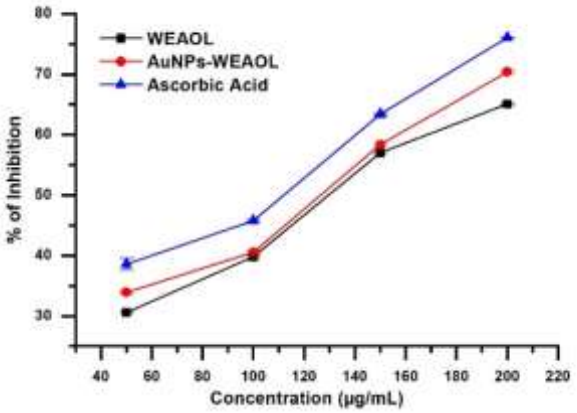

Figure 7: Hydroxyl radical (OH) scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Peroxynitrite scavenging

The peroxynitrite scavenging activity of Ascorbic acid, WEAOL, and AuNPs-WEAOL was evaluated at varying concentrations, showing a concentration-dependent inhibition (Figure 8). The IC_{50} values for peroxynitrite scavenging activity were found to be 142.50 µg/ml for Ascorbic acid, 170.38 µg/ml for WEAOL, and 160.95 µg/ml for AuNPs-WEAOL, indicating that all three compounds effectively scavenge peroxynitrite radicals, though with varying potencies (Ma et al.; 1997).

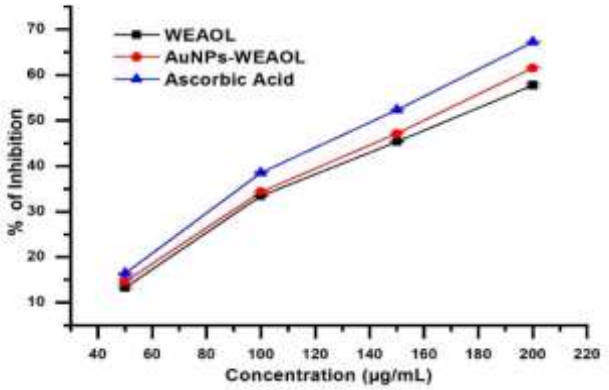

Figure 8: Peroxynitrite scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Hypochlorous acid (HOCL) scavenging

The activities of WEAOL, AuNPs-WEAOL and the standard ascorbic acid in scavenging hypochlorous acid demonstrate a dose-dependent manner. The results demonstrate that the AuNPs-WEAOL exhibits a greater efficiency in scavenging hypochlorous acid (IC_{50} -146.26

μg/ml) compared to ascorbic acid (IC_{50} – 117.43 μg/ml). But, specifically, at a concentration of 200 μg/ml, the plant extract, AuNPs-WEAOL scavenged 61.87 % inhibition of hypochlorous acid very adjacent value of ascorbic acid (68.62%), while WEAOL scavenged about 59.93%.

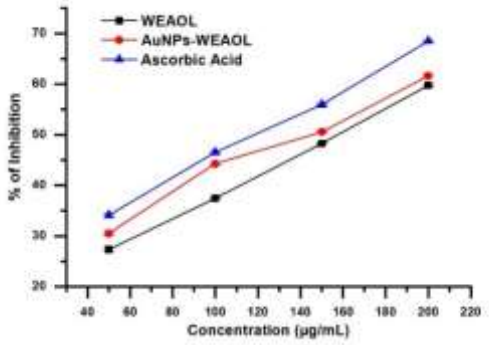

Figure 9: Hypochlorous acid (HOCL) scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Lipid peroxidation inhibition:

The primary measure of antioxidant activity is the inhibition of lipid peroxidation. Reactive oxygen species lead to lipid peroxidation by damaging membrane polyunsaturated fatty acids through hydrogen atom abstraction and resulting in cellular malformations (Klaunig et al., 1998; Catalá, 2010). The lipid peroxidation scavenging activity of ascorbic acid, WEAOL, and AuNPs-WEAOL was evaluated across different concentrations, demonstrating a concentration-dependent inhibition of lipid peroxidation. The IC_{50} values, indicating the concentration required to inhibit 50% of lipid peroxidation, were determined to be 141.69 μg/mL for ascorbic acid, 171.84 μg/mL for WEAOL, and 154.99 μg/mL for AuNPs-WEAOL. This study detected the lipid peroxidation scavenging activity of AuNPs-WEAOL (Figure 10), indicating their potency to mitigate free radical-induced cellular damage by disrupting lipid peroxidation chain reactions.

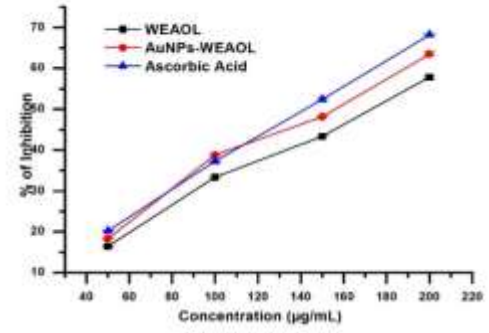

Figure 10: Lipid peroxidation inhibiting activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Superoxide anion (SOD) scavenging

The superoxide anion (SOD) scavenging activity of ascorbic acid, water extract of Anacardium occidentale leaves (WEAOL), and gold nanoparticles synthesized using A. occidentale leaf extract (AuNPs-WEAOL) was evaluated at varying concentrations. Each demonstrated a concentration-dependent inhibition of superoxide anion radicals. The IC_{50} values, indicating the concentration required to achieve 50% inhibition, were determined to be 142.13 µg/mL for ascorbic acid, 173.61 µg/mL for WEAOL, and 156.32 µg/mL for AuNPs-WEAOL. This suggests that AuNPs-WEAOL exhibits enhanced superoxide anion scavenging activity compared to WEAOL alone, although ascorbic acid remains the most potent among the tested samples.

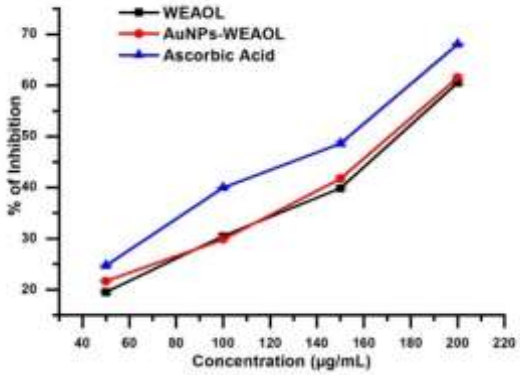

Figure 11: Superoxide anion (SOD) scavenging activity at different concentrations of Ascorbic acid, WEAOL and AuNPs-WEAOL NPs. Results are described as Mean ±SEM.

Anti-inflammatory assays

The current study emphasize possible comparative anti-inflammatory properties of aqueous leaf extract of Anacardium occidentale (WEAOL) and gold nanoparticles of WEAOL (AuNPs-WEAOL) on in vitro model.

HRBC membrane stabilizing activity

This study evaluated the anti-inflammatory properties of a plant extract (WEAOL) and its gold nanoparticle conjugate (AuNPs-WEAOL) by assessing their ability to prevent hypotonic solution-induced lysis of human erythrocyte membranes (HRBCs). The extracts inhibit damaging enzymes released during inflammation, similar to the mechanism of NSAIDs like diclofenac sodium (Sandhya et al., 2010; Debnath et al., 2013). Both WEAOL and AuNPs-WEAOL demonstrated dose-dependent erythrocyte membrane protection, with AuNPs-WEAOL showing close efficacy to diclofenac sodium at higher concentrations (500 µg/ml), achieving 76.28% inhibition compared to 87.42% with diclofenac. These findings highlight the potential of AuNPs-WEAOL as a membrane-stabilizing agent in anti-inflammatory applications (Table 1).

Concentration (µg/ml)	HRBC membrane stabilizing activity		
	Diclofenac Sodium (%)	WEAOL (%)	AuNPs-WEAOL (%)
50	72.48±0.09	41.58±0.26**	43.08±0.19***
100	77.68±0.14	48.44±0.25**	52.3±0.16***
150	81.41±0.16	54.75±0.34**	61.11±0.21***
200	85.25±0.14	63.39±0.41**	67.89±0.15***
500	87.42±0.23	70.41±0.21***	76.28±0.17***

Table 1: Effect of WEAOL and AuNPs-WEAOL on human red blood cell (HRBC) membrane stabilization. Results are expressed as Mean ± SEM. (*p<0.05, **p<0.01, ***p<0.001, compared with control.

Protein denaturation assay

Protein denaturation is the primary attributes to understand anti-inflammatory mechanisms, as most biological proteins lose their function upon denaturation, which can trigger inflammation (Esho et al., 2021). Current study emphacise possible comparative anti-inflammatory properties of aqueous leaf extract of Anacardium occidentale (WEAOL) and gold nanoparticles of WEAOL (AuNPs-WEAOL) on in vitro model. It had been reported that Phenols, flavonoids and terpenoids and other bioactive molecules are responsible for acute anti-inflammatory effect (Manach et al., 2005; Akinpelu et al., 2015).

Table 2 indicating that, AuNPs-WEAOL exhibited concentration-dependent inhibition (50-500 µg/ml). In this range WEAOL and AuNPs-WEAOL inhibited protein denaturation from 14.61±0.24% to 61.89±0.29%, and 16.26±0.31% to 69.45±0.12%. At 500 µg/ml, AuNPs-WEAOL showed significant and near to similar inhibition (69.45±0.12 %), close to diclofenac sodium's inhibition at 81.22±0.32 % (Table 2).

Concentration (µg/ml)	Percentage of Protein Denaturation		
	Diclofenac Sodium (%)	WEAOL (%)	AuNPs-WEAOL (%)
50	20.87±0.18	14.61±0.24**	16.26±0.31***
100	36.27±0.12	28.11±0.25**	29.46±0.1***
150	52.92±0.47	36±0.06**	43.72±0.15***
200	69.1±0.45	47.24±0.11**	57.94±0.34***
500	81.22±0.32	61.89±0.29**	69.45±0.12***

Table 2: Effect of WEAOL and AuNPs-WEAOL on protein denaturation. Results are expressed as the Mean ± SEM. (***p<0.001, **p<0.01).

4. Conclusion

The current study highlights that gold nanoparticles (AuNPs-WEAOL), synthesized using the water extract of Anacardium occidentale leaves rich in flavonoids and phenolics, exhibit notable antioxidant, free radical scavenging, reducing, and chelating activities. This encapsulation enhances targeted delivery with minimal side effects. In vitro assays demonstrate the extract's potential as a natural antioxidant, potentially useful in mitigating oxidative stress-related diseases. The ROS-neutralizing effects of these nanoparticles can stabilize red blood cell membranes, offering potential as alternative anti-inflammatory agents. Further research is recommended to isolate the active components responsible for the

membrane-stabilizing and anti-inflammatory effects.

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