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Characterization and Biological Activities of Silver Nanoparticles from Mangrove Rhizophora Apiculata Extracts

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The study explores the biosynthesis as well as characterization of AgNPs using mangrove Rhizophora apiculata extracts, focusing on their potential applications in therapeutic, food, and agricultural fields due to their biological properties. The biosynthesized AgNPs were characterized using UV-Vis, FTIR, and SEM-EDS techniques, revealing colour changes from yellow to dark brown, indicative of nanoparticle formation. Ultra Visible spectroscopy showed absorbance in the range of 430 to 4880 nm, while FTIR analysis displayed strong absorption bands at various wavelengths (576.03 cm-1 to 1623.84 cm-1). SEM imaging revealed oval-shaped particles with a size range of 8 to 10 nm and a smooth surface with ridges and grooves, corroborated EDS analysis showing a major emission of silver at 42.7%. The antioxidant activity of the Rhizophora apiculata extract-based AgNPs was evaluated using the DPPH scavenging assay, demonstrating concentration-dependent scavenging activity with values ranging from 17.5% to 51.8%.Nitric oxide in mangrove extract concentration of a minimum 25µg/ml value of percentage 19.5 ±2.4% compared to the standard value of 28.5 ± 2.4%. Furthermore, the haemolytic activity of the AgNPs was assessed, showing higher activity at higher concentrations (19 ± 0.5% at 100 µg/ml) and lower activity at lower concentrations (14 ± 1.4% at 80 µg/ml, 8 ± 1.2% at 60 µg/ml). These findings highlight the rich antioxidant and haemolytic properties of the Rhizophora apiculata-based AgNPs, suggesting their promising role in various biomedical and environmental applications.

Keywords: Silver nanoparticles, Biosynthesis, Rhizophora apiculata, Characterization, Antioxidant activity.

1. Introduction

Nanotechnology is the emerging field of contemporary investigation dealing with synthesis, and particle structure ranging from around 1-100nm. NPs find extensive applications in various sectors including biomedical science, chemical industries, environmental health, electronics appliances, single electron transistors, photoelectrochemical applications, and catalysis, among others. The biosynthesis of silver nanoparticles exploits potential therapeutic plant extracts due to the growing field of biomedical applications, therapeutic purpose for its properties, increased bioavailability, limited toxicity, and biodegradability [1-2]. Silver has been usedin ethnobotanical medicines of Siddha, Unani, and Ayurveda it attracted the modern advanced medical field. AgNPs were proven to be more effective in their antimicrobial activities against microorganisms, bacteria, fungi, and viruses. Silver nanoparticles interrelate easily with biological systems due to their small size [3]. AgNPs can be synthesized in various paths a natural green synthesis has several advantages compared to artificial chemical synthesis [4]. AgNPs are used in the packaging sector to extend the shelf life of food products because of their antimicrobial qualities [5]. The unique and complex ecosystems known as mangroves can be found in subtropical and tropical intertidal zones. They provide a wide range of ecosystem functions and are a rich source of bioactive chemicals. The mangrove plant Rhizophora apiculata is a member of the Rhizophoraceae family and is popularly reported for its therapeutic potential and has been the subject of extensive research into its various bioactive components [6]. The bioactive compounds found in R. apiculata, silver nanoparticles (AgNPs), have attracted attention for their distinctive physical and chemical properties of silver nanoparticles contributing to their diverse range of potential applications such as environmental remediation, biotechnology, and medicine [7]. AgNPs can be produced chemically, biologically, or physically among other methods, biological synthesis of AgNPs using plant extracts is a useful and environmentally safe technique. Mangrove has medicinal properties present in entire plant leaves, stems, fruit, and roots. It contains high phytochemicals like flavonoid, alkaloid, tannin, and saponins, clinically tested with plant extracts are has antibacterial, antiemetic, antidiarrheal, anticancer, and haemostatic activity [8]. Similarly, mangrove R. apiculata has against of antibacterial activity, cytotoxic activity, and hepatoprotective activity [9]. The AgNPs from R. apiculata plant extracts serve as reducing and stabilizing agents, suspending the nanoparticles' agglomeration and maintaining them in solution [10]. Mangrove R. apiculata extracts have revealed a variety of biological characteristics, such as anti-inflammatory, antioxidant, antibacterial, and anticancer activity. The bio-synthesized AgNPs' antibacterial action originate from their ability to interact with bacterial cell membranes, causing structural damage and blocking bacterial growth. The Gram-positive and Gram-negative AgNPs made from R. apiculata extracts are sensitive to Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus [11]. The antioxidant and haemolytic properties of AgNPs from R. apiculata extracts highlight their potential applications in biotechnology and medicine. The present research work is focused on the characterization and biological activities of AgNPs from mangrove Rhizophora apiculata

extracts.

2. Materials and Methods

2.1 Collection and processing of the sample:

The mangrove Rhizophora apiculate samples were taken from the kalpakam coastline, Chengalpattu District, Tamil Nadu State, India. The collected mangrove was washed and shade-dried 2 to 3 days. when leaves loss its moisture content after that grind the samples and stored at room temperature for future analysis.

2.2 Preparation of seaweed extract:

20g Dried and powder sample was weighed & the samples were mixed with 100 ml of D.H_2O and kept in the shaker for 48 hours. The Whatman filter paper No.1 was used to filter the extract. Crude extract was prepared with 200 ml of 70% methanol and 20g of powdered sample was mixed. After two days of shaking the mixture, and samples was filtered through the filter paper. Then, the filtrate sample were heated at 55˚C in a water bath to evaporate the solvent, finally the crude was stored in the refrigerator for future experiments.

2.3 Synthesis of AgNPs:

The AgNPs was synthesized using mangrove Rhizophora apiculata aqueous extract. 10 mM Silver Nitrate ($AgNO_3$) solution was prepared and added to 10 ml of the aqueous extract of mangrove, and it was kept at for 72 hours with an orbital shaker. Bio-reduced reaction extract was subjected to centrifugation at 5000 rpm for 30 minutes resulting pellet was dried at Hot air oven below 60˚C to make dry powderare shown in Figure.1.

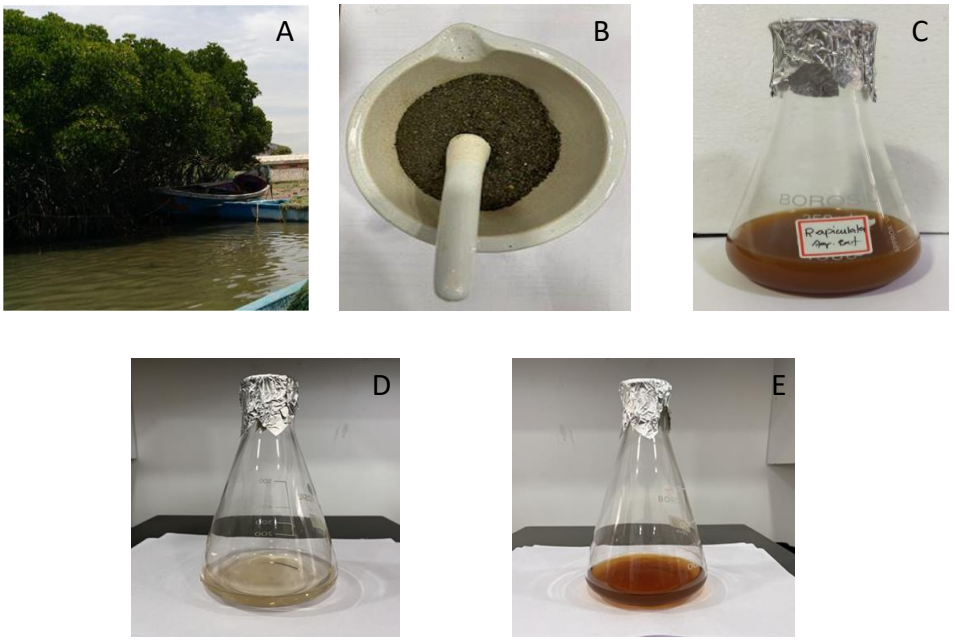

Figure 1: Mangrove Sample collection and pre-processing of the samples; A) Rhizophora

apiculata plant, B) Dried leaves, C) Extraction D) Initial synthesis E) Final synthesis

2.4 Characterization of Silver nanoparticle:

The biosynthesis of AgNPs was characterized by a UV wavelength range between (200 to 700 nm). FTIR nanoparticles were involved in the analysis of functional groups and the transmission mode from wavenumbers (450–400 cm^{-1})the aq. extract of Rhizophora apiculata Morphological characterization was analysed by (SEM) using the high resolution. EDS detector was to identify various elemental compositions of the AgNPs [12].

2.5 DPPH Antioxidant Activity

In the investigation, the DPPH method was elaborate on the antioxidant capability of mangrove Rhizophora apiculata crude extract by methodology and a systematic procedure was followed [13]. The experiment commenced by combining 4.5 mL of the mangrove extract with 0.5 mL of 1 mM DPPH, followed by vigorous vortexing for 30 seconds. Concurrently, a control was prepared with 4.5 mL of ethanol and 0.5 mL of the DPPH solution. Both the sample and blank mixtures were then subjected to a 30-minute incubation period at 37˚C in a dark place. Subsequently, a spectrophotometer was used to measure each mixture's absorbance at a wavelength of 517 nm .The experimental range of sample concentrations 10, 20, 30, 40, and 50 ppm. The positive control was varying concentrations of ascorbic acid, specifically 1, 2, 3, 4, and 5 ppm. Furthermore, the IC_{50} with the insertion of 50 as the Y value and the antioxidant potential, the % of inhibition was calculated using the formula

$$\text{Inhibition of DPPH radical percentage} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

2.6 Nitric Oxide Radical Scavenging

In assessing the antioxidant activity of Rhizophora apiculata leaf extract (100 µg/mL) with slight modification [14]. The extract underwent treatment with 3 mL of 10 of mM $[Na_2Fe(CN)_5(NO)]$ in PBS, followed by incubation at 25˚C for 150 minutes. Subsequently, 0.5 mL of the resulting solution was combined with one mL of 0.33% C6H7NO3S, incubated at 37˚C for five minutes, and then the addition of one mL of 0.1% Napthylethy lenediamine dihydrochloride, with a further incubation period at 25˚C for 30 minutes. The UV spectrophotometer at 546 nm determined the absorbance of the pink chromophore formed during diazotization. Blank solutions devoid of sodium nitroprusside were prepared, and control experiments using distilled water used as a control. All experiments were performed in triplicates, and a standard bar, utilizing L-ascorbic acid con. ranging from 10 to 100 µg/ml, was plotted. The % of Nitric Oxide capability was subsequently calculated based on the established standard bar.

$$\text{Percent (NO)Scavenging} = \frac{\text{(A)control} - \text{(A)sample}}{\text{(A) control}} \times 100$$

2.7 Haemolytic activity assay

To determine the hemolytic activity assay, RBC cells were utilized. Aliquots of seven milliliters of blood were centrifuged at 180 grams for five minutes in order to prepare the cells. The samples were then exposed to three washes with a saline solution (0.89%, w/v, NaCl,

pyrogen-free). The pellet was diluted to a concentration of 0.5% in a saline solution to form the prepared cell suspension. Then, 0.5 mL of the cell suspension was mixed with 0.5 mL of a diluent that included extracts from the mangrove Rhizophora apiculata, separately, at varying concentrations (5, 10, 25, 50, 100, 250, 500, and 1000 µg/mL) in a saline solution. The mixtures were centrifuged at 70g for 10 minutes after being incubated for 30 minutes at 37°C. At 412 nm measured the amount of free haemoglobin in the supernatants using spectrophotometry. Saline and D.H2O were used as the minimum and maximum hemolytic controls, respectively, to create a baseline. All experimental groups had the conventional saline control's hemolytic percentage deducted. The whole investigation was carried out in triplicate, and the mean ± standard deviation (S.D.) was computed in accordance with the protocol described by [15].

$$\text{Haemolysis percentage} = \frac{\text{sample} - \text{blank}}{\text{positive control}} \times 100$$

3. Results and discussion:

3.1 Biosynthesis of AgNPs

In current studies synthesis AgNPs of mangrove extract was mixed with silver nitrate at normal room temperature. The mixed solution color changes observed Yellow to dark brown color confirmed the NPs formation [16]. The sample's change to yellowish color is an indication of the synthesis process of silver nanoparticle particles, which are colloidal nanoparticles.

3.2 Characterization of AgNPs

3.2.1. UV–visible spectroscopy

The UV spectrophotometer is used to detect the synthesized AgNPs and the absorption band is called surface plasmon resonance (SRP). The reduction of silver nanoparticles in the aqueous solution of the silver compound reaction with Rhizophora apiculata leaf extract was identified by the UV–visible spectrum. The peak of silver nanoparticle Rhizophora apiculata can be seen in the figure.2 at 430 to 4880 nm. Likewise, the current studies results are shown similar to the silver nanoparticle are categorized by a absorption peak at 435 nm, which is detected by the UV–Vis spectrum of silver nanoparticle showing the reduction of silver salts with C. serrulata leaf extract [17]. UV spectroscopic absorption peaks was reported from the extracts of various algae species, The absorption peaks of silver nanoparticle observed by U. rigida 424 nm, and G. foliifera 415 nm are respectively [18]. Compared our results to Previous studies are shown UV-visible absorption spectra of colloidal AgNPs Acacia cyanophylla as a absorption peaks in the visible region at 460 nm [19]. The synthesized NPs combined electron vibration in the conduction band, with light wavelength, are easily confirmed with UV–Vis spectrophotometers, an SPR peak shown at the 400 to500 nm range [20].

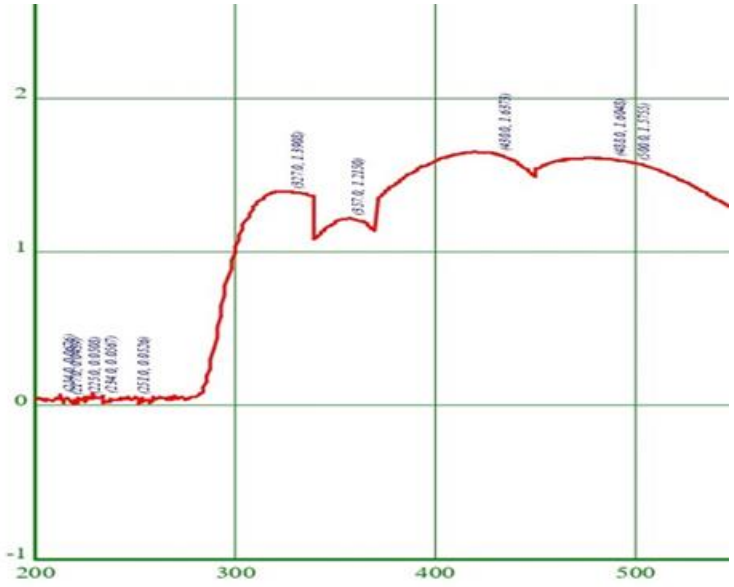

Figure 2: UV -vis aqueous extract of Ag nanoparticles from Rhizophora apiculata

3.2.2. Fourier Transform Infrared Spectroscopy (FTIR):

A FTIR analysis with mangrove Rhizophora apiculata leaves extract to find the biomolecules answerable for the reduction of Ag ions into AgNPs. The result FTIR spectrum of silver nanoparticle absorption bands at the various wavelengths of 576.03 cm^{-1} to 1623.84 cm^{-1} Figure.3. A high absorption peak at 576.03cm^{-1} has been associated with the C0–I stretching halo compound leading to the absorption peak. From the peak showed 93.76 cm^{-1} Strong C=C Bending could be indicative of a double bond in a substituted alkene, the bending frequency is expected to be strong.788.86 cm^{-1} Medium C=C Bending stretching vibration in a tri-substituted alkene. The Strong Broad peak 1034.57 cm^{-1} is associated with Co-O-Co Stretching vibration of the carbonyl group in an anhydride. The peak 1403.80 cm^{-1} with s=o Stretching vibration of a sulfonyl chloride functional group. In 1623.84 cm^{-1} Strong peak associated with C=C Stretching Alpha & Beta unsaturated ketone compounds in the Rhizophora apiculata leaf extract plays a major role in both the silver nanoparticle stability. Similar results were shown by FTIR analysis functional groups of the organic compounds in the biosynthesis of AgNPs in R. mucronata silver nanoparticle FTIR results observed peaks at 3371.96 to 1585.05 cm^{-1} [21-22]. Previous studies showed 1623.84 cm^{-1} Strong peak associated with C=C Stretching Alpha & Beta unsaturated ketone compounds in the Rhizophora apiculata leaf extract [23].

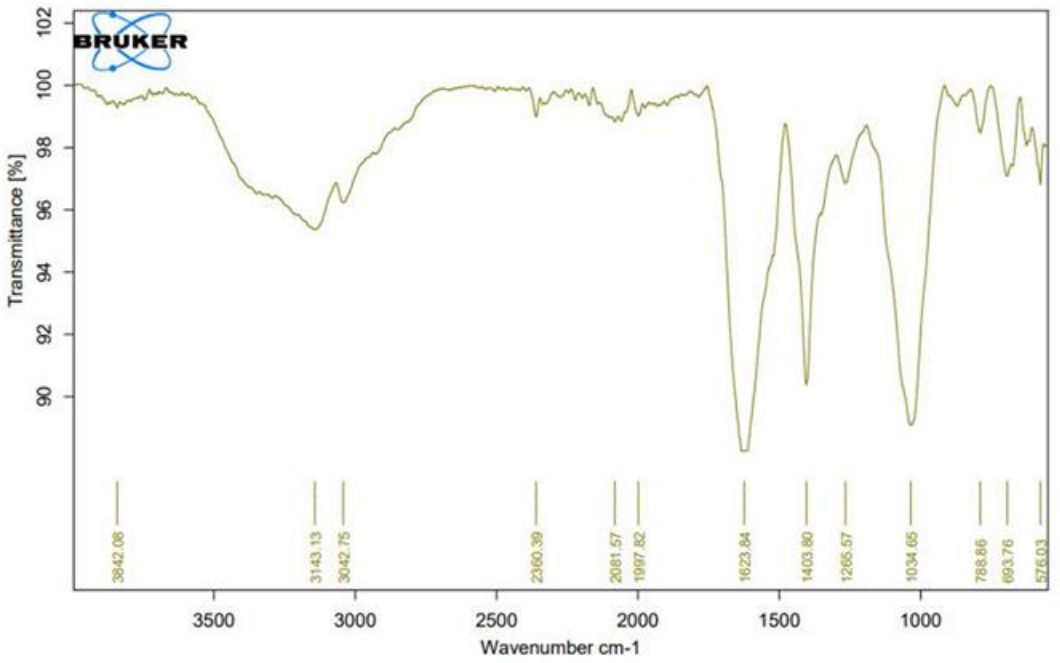

Figure 3: FTIR spectrum biosynthesized Silver Nanoparticle Rhizophora apiculata

3.2.3. SEM with analysis of silver nanoparticles:

SEM analysis is to identify the morphology of the biogenically synthesized silver nanoparticle, which are not visible to the naked eye. In our research mangrove Rhizophora apiculata leaf extract (SEM) image is oval-shaped 8 to 10 nm a smooth, relatively fine network of ridges and grooves surface is shown in Figure. 4. Likewise mangrove Rhizophora mucronata SEM analysis also showed silver nanoparticles with a size range from 10 to 19 nm [22]. In previous studies an average particle size of 15 and 43 nm for AgNPs leaves extract of mangrove A. marina [24]. SEM investigations morphology part of the silver nanoparticle Rhizophora apiculata images revealed with an uneven form and their diameter between 35 and 100 nm [23].

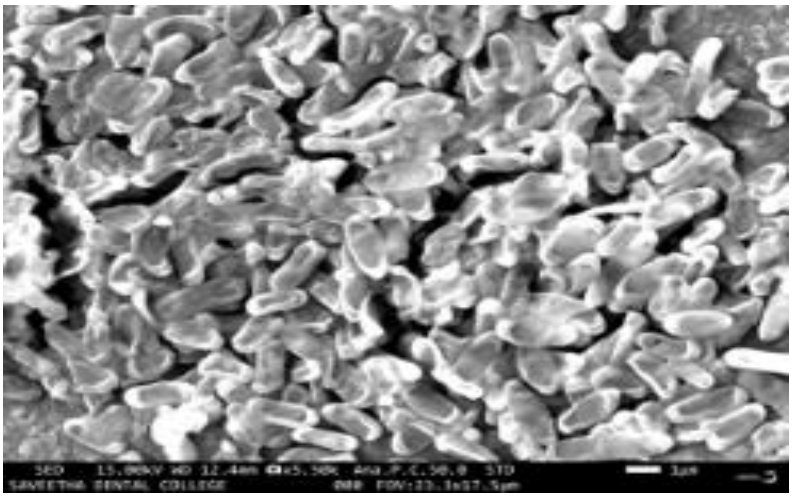

Figure 4: SEM images of biosynthesized silver nanoparticles using Rhizophora apiculata extracts confirming the spherical shaped nanocrystal

3.2.4. EDS analysis of silver nanoparticles

The EDS energy-dispersive spectroscopy is to analysis and confirms the presence of elements in selected mangrove leaves Rhizophora apiculata nanoparticles as shown in Figure.5. The major emission energies for sodium2.1%, aluminum3.3, oxygen 13.1%, carbon 24.6%, chloride 14.1% and sliver 42.7% are observed which reveals the abundance of organic materials like sliver in the mangrove aqueous extract. The EDS elements in the bio-nanoparticles derivative from the leaf extract of R. mucronata Silver (73.5%) was the high level showed [22]. In our investigation relevant to EDS the nanoparticles from leaf extract results confirmed the presence of element silver maximum 51.6% formed [24].

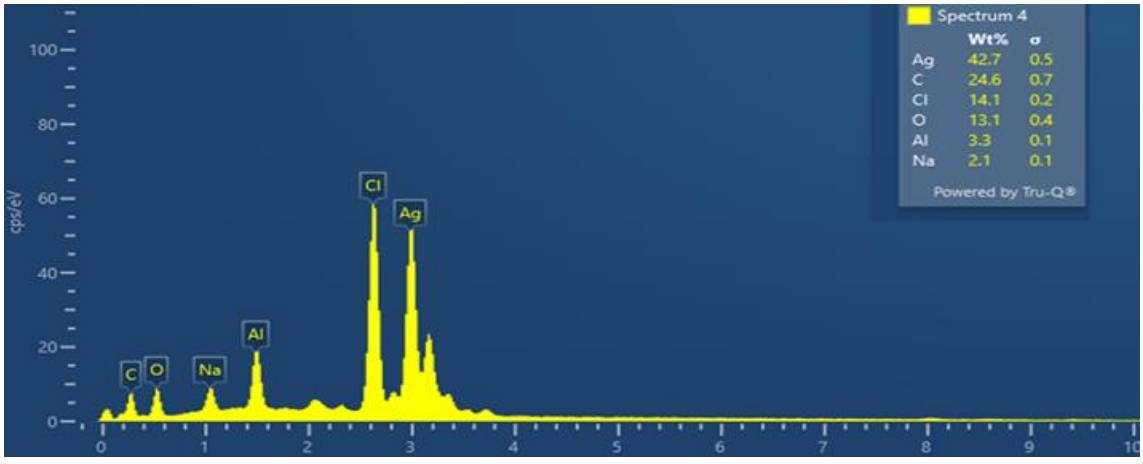

Figure 5: EDS analysis of Ag NPs synthesized using Rhizophora apiculata extracts showed the signal of Silver

3.3 DPPH Radical Scavenging Activity

The scavenging capability of the crude extract from mangrove was evaluated using the DPPH assay Figure.6. The results indicated a concentration of DPPH-dependent increase in the scavenging ability of the mangrove, standard Ascorbic acid was used. In our result, DPPH scavenging activity mangrove Rhizophora apiculata extract showed a concentration of a minimum 25µg/ml value of 17.5 ±1.8% compared to the standard value of percentage 25.5 ± 1.5%, and a maximum concentration of 100µg/ml value of percentage 51.8 ± 2.7 compared to the standard value 71.6 ± 2.2%. similar studies are The IC50 for C. elongata was 19.4 µg/mlcompared to the standard curve of ascorbic acid with an IC50 of 18.4 µg/ml[25].The values for an extract of E. antenna 70 µg/mL and E. linzaand 80 µg/mL it indicating they are natural antioxidants [26]. Compared to the aqueous extracts, the Rhizophora stylosa bark methanol extract had a greater antioxidant activity and a high phenol concentration of 85.5% [27]. Furthermore, Taonia atomaria exhibited increased inhibitory activity, reaching 43.23%, at a higher con. 150 µg/mL against the DPPH radical [28]. Currently, the discovery of natural antioxidants in silver nanoparticle mangrove Rhizophora mucronata furthermore high level of bioactive phytochemicals, that are harmless than chemical analogies, their protective role in improving diseases like atherosclerosis, diabetes, cancer, arthritis, and aging diseases, Alzheimer's [29].

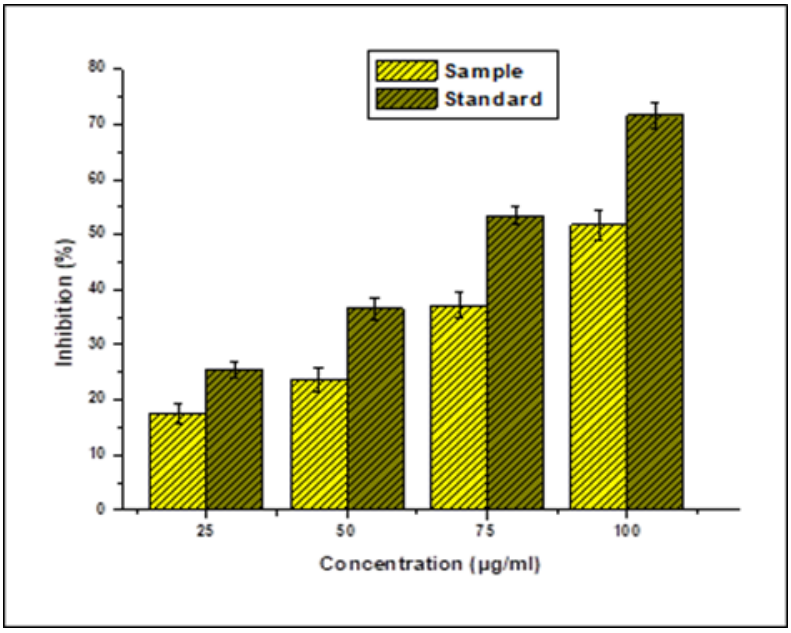

Figure 6: Graphical Representation DPHH activity from Rhizophora apiculata

3.4 Nitric Oxide Radical Scavenging Activity

All mangrove extracts increased NO_2 produced by the degradation of Na_2 Fe $(CN)_5NO$ in vitro, the suppression of Nitric oxide release may be elucidated by a direct scavenging activity explained by a direct NO scavenging effect. Figure.7 Mangrove extract showed a concentration of a minimum 25µg/ml value of percentage 19.5 ±2.4 %compared to the

standard value of 28.5 ± 2.4%, and a maximum concentration of 100µg/ml value of percentage 55.4 ± 2.6 compared to the standard value of 70.5 ± 1.8%.Antioxidant properties of E. antenna and E. linza, their nitric oxide scavenging abilities were found to be 77% and 31% [26]. These values were notably lower than the radical scavenging activity of the standard antioxidant, ascorbic acid, which exhibited an 87% efficacy in neutralizing nitric oxide. The present results suggest mangrove Rhizophora apiculata have a novel therapeutic approach for scavenging Nitric Oxide. Previous study showed the highest scavenging activity (85%) at a concentration of 1000 µg/mL, while the lowest activity (28%) was recorded at 100 µg/ml [30].

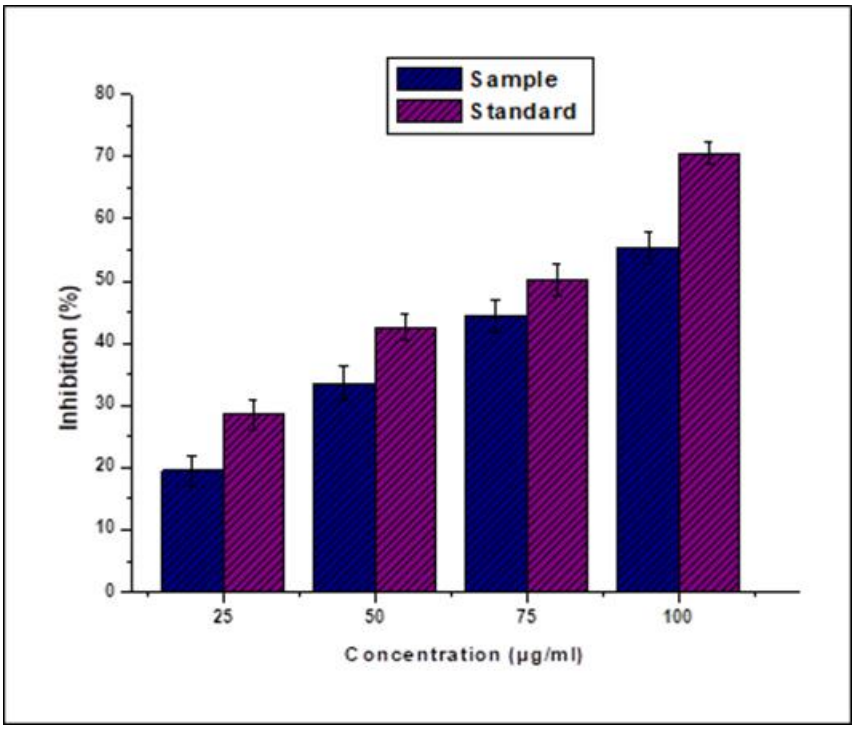

Figure 7: Graphical Representation Nitric oxide activity from Rhizophora apiculata

3.5 Haemolytic activity

In Our result showed Hemolytic Activity, in Rhizophora apiculata extract had the highest Figure.8 haemolytic activity (19 ± 0.5%) at 100 µg/ml concentration and the lowest haemolytic activity (14 ± 1.4%) at 80 µg/ml, and at 60 µg/ml concentration (8 ± 1.2%) and least percentage in 20,40 µg/ml of mangrove extract. Generally, the higher the concentration of extracts (20 to 100 µg/ml) showed the higher hemolytic activity. similarly, In Padina gymnospora Hemolytic activity of synthesized silver nanoparticles at different concentrations, 50 µg /ml concentration showed low haemolysis [31]. At a concentration of 1000 g/ml, Padina pavonica's ethanolic extract displayed higher haemolytic activity (63.4%) compared to Laurencia catarinensis (46.7%), indicating potentially distinct bioactive compounds in seaweed extract [32]. Similarly previous studies, B. gymnorrhiza demonstrated effective prevention of haemolysis [33].

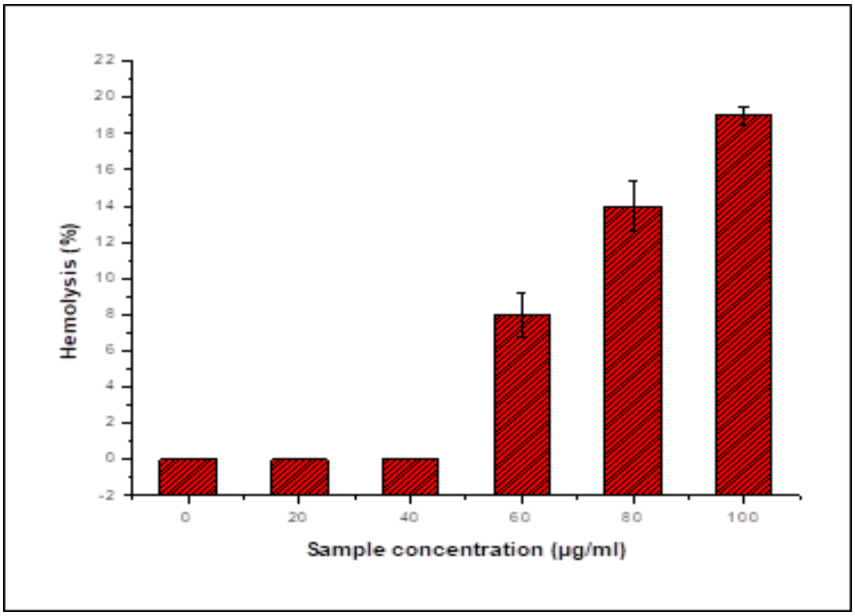

Figure 8: Graphical Representation Haemolytic activity from Rhizophora apiculata

4. Conclusion:

The reducing agent used in the current mangrove Rhizophora apiculata leaf aq. extract research to create AgNPs from AgNO3. The produced AgNPs were examined by UV-Vis, FTIR, and SEM-EDS, among other analytical techniques. All analytical methods confirmed that AgNPs were synthesized. The Antioxidant and Haemolytic Activity Assays against RBC cell lines showed AgNPs to be significantly higher in the constancy of plant extracts and NPs to confirm natural antioxidants compared to synthetic extract. Natural sources are also more capable and safer for pharmaceutical, food, agriculture industries, and biodegradation studies.

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CRediT authorship contribution statement

Aafreen: Writing – original draft, Software, Methodology, Formal analysis, Data curation. Pavithra Thiraviyam: Writing – original draft, Software, Methodology, Formal analysis. Kamala Kannan: Writing – review & editing, Validation, Software, Formal analysis, Conceptualization. Dhanraj Ganapathy: Review & editing, Validation, Project administration Sivaperumal Pitchiah: Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Disclosure statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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