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SYNTHESIS AND CHARACTERIZATION OF A NOVEL NANOCOMPOSITE USING SILVER NANOPARTICLES, SODIUM ALGINATE AND PAPAYA LEAF EXTRACT AND ITS ANTIOXIDANT POTENTIAL

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ABSTRACT:

Introduction: The biomedical field has witnessed a surge in research focusing on tailored nanomaterials, owing to the remarkable versatility of nanotechnology. These materials find applications across a spectrum, from disease treatment to enhancing product performance. Among them, silver nanoparticles (AgNPs) have gained prominence as effective antioxidant and antibacterial agents, thanks to their unique physicochemical properties. The goal of this research is to examine the antioxidant potential of silver nanocomposite containing sodium alginate and Carica papaya leaf extract.
Materials and Methods: The research involved using silver nitrate, Mueller Hinton Agar, and Carica papaya leaves obtained from Tamil Nadu, India. Extracts from Carica papaya leaves were prepared, and silver nanoparticles (AgNPs) were synthesised using eco-friendly methods. Sodium alginate was added to create a silver sodium alginate nanocomposite. The characterization process included UV-Vis spectroscopy, and antioxidant properties were checked using the DPPH assay (1,1-diphenyl-2-picrylhydrazyl).
Results: Carica papaya-mediated silver nanoparticles (AgNPs) and sodium alginate nanocomposite were successfully synthesised. Visual examination confirmed colour changes, validating AgNP formation. UV-Vis spectroscopy revealed a peak at 420 nm, indicating AgNP presence and nanocomposite formation. The DPPH assay demonstrated antioxidant activity of AgNPs, comparable to ascorbic acid, with no significant differences observed in absorbance between AgNPs and controls ($p > 0.005$).
Conclusion: This research underscores the promising potential of Carica papaya-mediated silver nanocomposites, showcasing their comparable antioxidant activity to the control. The environmentally friendly synthesis approach holds significance for biomedical applications, paving the way for innovative developments in nanotechnology.

Keywords: Nanocomposite, silver, papaya, nanoparticles, alginate, antioxidant.

1. INTRODUCTION

Nanotechnology has garnered considerable attention recently due to its diverse applications in the biomedical domain[1]. This trend has prompted numerous researchers to concentrate on crafting different nanomaterials customised for specific purposes, including disease treatment and boosting performance in healthcare products, cosmetics, and household goods. The precise nanoscale dimensions of these particles play a pivotal role in their efficacy within living cells, particularly in addressing bacteria linked to oral health, and their ability to scavenge oxygen[2,3].

Silver nanoparticles (AgNPs) are effective antibacterial and antioxidant agents in various research studies[4]. Silver nanoparticles are widely used in treating burns and injuries, effectively reducing the risk of wound infections by preventing contamination [5].They stand out as highly sought-after nanomaterials, prized for their exceptional physicochemical properties, including a remarkable specific surface area and oxygen scavenging characteristics. The rapid progression in the synthesis of nanosized metal and metal oxide particles, especially in the medical domain, stems from the extraordinary attributes these nanoparticles exhibit[6,7]. While various methods, such as physical, chemical, and organic approaches, can be employed to synthesise metallic nanoparticles, the biological method takes precedence due to its cost-effectiveness. The plant-mediated synthesis of nanocomposites and nanoparticles is gaining significance, thanks to its eco-friendly nature[8,9,10].

Several polymers like sodium alginate have displayed promising wound healing properties when combined with silver nanoparticles (AgNPs). Additionally, AgNPs loaded with pectin extracted from blackberries have exhibited cardioprotective effects. It's noteworthy that AgNPs synthesis through chemical methods may pose potential harm in animal models, whereas those produced using bacteria, plant components, fungi, and algae show significant potential for biomedical applications[11,12]. Sodium alginate, a biopolymer, has been employed in numerous studies to enhance the antioxidant properties of metallic nanocomposites[13]. In the current research, sodium alginate was integrated into papaya leaf extract-mediated silver nanoparticles (AgNPs) to produce a nanocomposite, with the objective of enhancing the antioxidant capabilities of AgNPs. Carica papaya, commonly referred to as papaya, belongs to genus Carica and the family Caricaceae. Phytochemical analysis of Carica papaya signified that the plant has various bioactive compounds such as tannins and antioxidants.
The focus of this study revolves around creating a nanocomposite by combining silver nanoparticles and sodium alginate with the use of Carica papaya extract. The null hypothesis posits that there is no difference in the antioxidant activity of the nanocomposite formed using Silver NPs and sodium alginate with Carica papaya extract compared to the standard antioxidant, Ascorbic Acid.

2. MATERIALS AND METHODS

Chemicals

Silver nitrate, used as the precursor chemical was provided by Sigma Aldrich chemicals Pvt. Ltd. (India). Carica papaya plant leaves were sourced from Vellore, Tamil Nadu, India.

Carica papaya leaf extract preparation

Carica papaya leaves were meticulously cleaned with tap water followed by double distilled water, and then dried for five days. The dried leaves were converted to a powder form and stored in vacuum. Subsequently, 100 mL of distilled water was mixed with 1 g of the powdered material. The solution was heated on a heating mantle at 70°C for 30 minutes to extract all phytochemical compounds in the Carica papaya plant. Afterward, the solution was filtered with Whatman filter paper no. 1 and refrigerated for further experimental usage.

Synthesis of nanoparticles

In 90 mL of deionized water, 1 mM of silver nitrate ($AgNO3$) was dissolved. Subsequently, 10 mL of filtered papaya solution was added to the silver nitrate solution. The resultant solution was stirred using a magnetic stirrer at 600-700 rpm for 48 hours. The synthesis of AgNPs was monitored using a UV-Vis double beam spectrophotometer, starting from an initial wavelength of 360 nm and scanning up to 500 nm. After synthesis, the biosynthesized AgNPs were centrifuged at 8,000 rpm for ten minutes to segregate the pellet from the aqueous reaction mixture. The resulting supernatant was disposed of, and the pellet was washed three times with ethyl alcohol and then dried in a hot air oven at 70°C for two hours. Additionally, the powdered AgNP was stored in an Eppendorf tube for subsequent experiments.

Nanocomposites formation

To dissolve 0.5 g of sodium alginate, 1 mL of 1% glacial acetic acid mixed with 49 mL of deionized water was used. The sodium alginate solution was then mixed with AgNPs and subjected to magnetic stirring for 3-4 hours. Subsequently, Carica papaya-mediated AgNPs solution was added, leading to the formation of a dark brown nanocomposite gel. The mixture underwent further magnetic stirring for forty-eight hours. UV-Vis spectroscopy, within the wavelength range of 360-500 nm, was employed to monitor the formation of silver nanocomposites. The silver nanocomposite was then centrifuged at 10,000 rpm for ten minutes. The resulting pellet was washed with deionized water before being centrifuged again and subjected to lyophilization. After lyophilization, the silver sodium alginate nanocomposite was reconstituted in distilled water.

AgNPs and nanocomposites characterization

The UV-double beam spectrophotometer (UV-2450, Shimadzu) was used to characterise the Carica papaya-mediated AgNPs and nanocomposites in the range of 360-500 nm wavelength.

Antioxidant activity of nanocomposite

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was employed in this study to evaluate the antioxidant activity of silver nanoparticles produced using green synthesis methods. [14] Incorporating 0.78 mg of DPPH into 10 ml of methanol constituted the DPPH solution. Varied concentrations of extracts (20 µl, 40 µl, 60 µl, 80 µl, and 100 µl) were introduced to 2 ml of this solution. The standard antioxidant, ascorbic acid, served as reference. After a 30-minute incubation period at room temperature in darkness, the colour of the reaction mixture changed from violet to yellow, indicating the reduction of DPPH by silver nanoparticles through hydrogen atom donation. The absorbance of the reaction mixture at 517 nm was measured using a UV-Vis spectrophotometer. The percentage of DPPH inhibition was calculated using the formula:

$$\% \text{ of scavenging activity} = \frac{(A\ of\ control - A\ of\ test)}{A\ of\ control} X100$$

A: Absorbance

Statistical Analysis

The normality of data was evaluated through the application of the Kolmogorov-Smirnov test, while statistical analysis of absorbance at different concentrations was executed using an unpaired student t-test. The data analysis was done utilising the SPSS software (Version 26.0; SPSS, Inc., Chicago, IL, USA), and significance was ascertained at $P<0.05$.

Figure 1: Formation of Silver Nanocomposite

Figure 1 shows the Formation of Silver Nanocomposite

3. RESULTS

Visual examination

Upon visual inspection, the intensity of color in the solution mixture containing silver nanoparticles (AgNPs) synthesised through Carica papaya exhibited a gradual escalation over time. Furthermore, the transition of silver nitrate to elemental silver facilitated by papaya extract, functioning as a reducing agent, manifested as a shift in colour from a pale-yellow hue to a distinct brown shade. This alteration was substantiated through spectrophotometric analysis. The development of nanoparticles from papaya leaves is indicated by the brown hue observed in the synthesis of AgNPs, while the incorporation of sodium alginate in this process confirms the formation of a nanocomposite through color changes in the AgNPs[15].

UV-Vis spectrophotometer optical analysis

UV-Vis spectroscopy was employed to evaluate the formation of silver nanoparticles [16]. The primary peak of absorption of AgNPs was detected at 420 nm. Within the range of 380 to 460 nm, a peak was observed confirming the existence of the nanocomposite (Figure 2). The minor variations in absorbance observed in UV-Vis spectroscopy validate the presence and stability of AgNPs. The signal detected at 400-440 nm indicates the presence of AgNPs synthesised using Carica papaya leaf extracts [17].

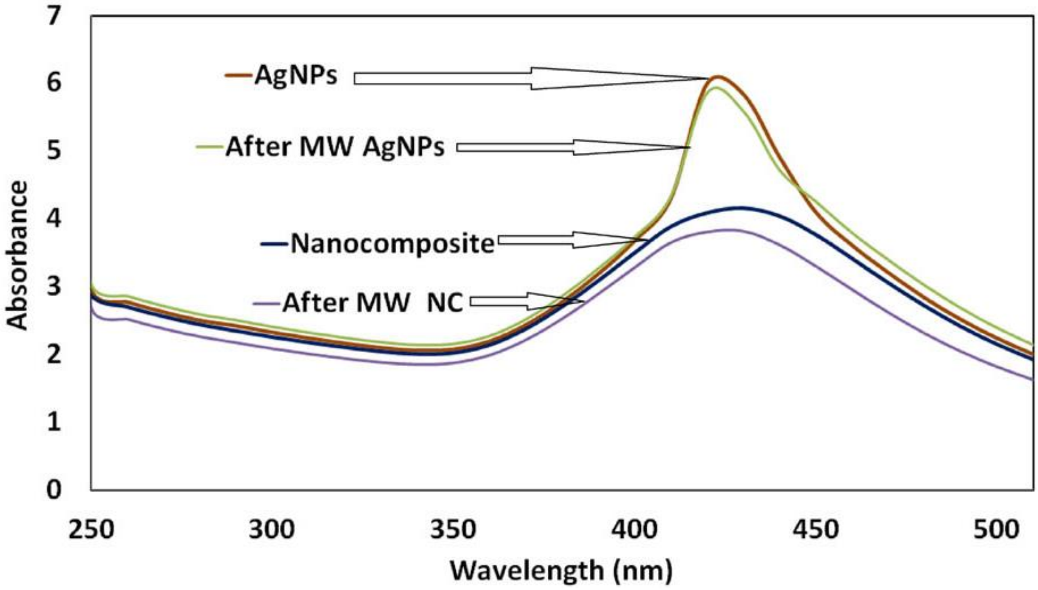

Figure 2: UV-Vis spectrophotometer analysis

Figure 2 shows the UV-Vis spectrophotometer analysis

Antioxidant activity of nanocomposite

The evaluation of antioxidant efficacy for silver nanoparticles (Ag NPs) synthesised through a biosynthetic process was executed utilising the DPPH assay, with Ascorbic acid as the reference. The findings from the investigation demonstrated that the synthesised Ag NPs displayed a level of free radical scavenging activity comparable to that of the established standard. Figure 3 illustrates the mean absorbance values for both the nanocomposite and the control. The unpaired t-test yielded no statistical significant results for absorbance across different concentrations as compared to control ($p > 0.005$). (Table 1) Notably, the maximum absorbance was observed at a concentration of 100 ul, while the minimum was recorded at 20 ul concentration.

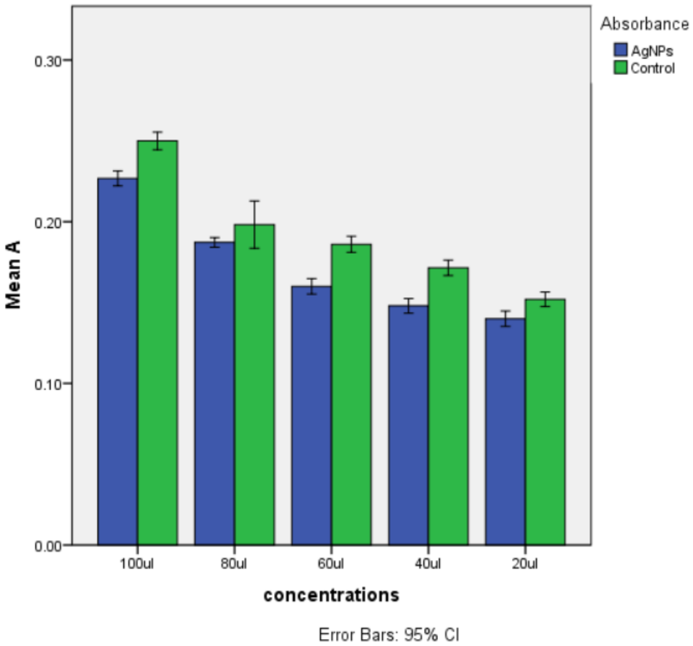

Figure 3: Mean Absorbance of AgNP NC and control at different compositions

Figure 3 shows the Mean Absorbance of AgNP NC and control at different compositions

Groups	N	Mean± S.D	Std. error	95% CI		F value	P value
				lower	upper		
AgNPs	50	0.172±0.032	0.005	-0.031	-0.005	0.167	0.684
Control	50	0.191±0.034	0.005				

Table 1: Unpaired t test to compare antioxidant activity of AgNPs with control(p>0.05)

Table 1 shows the Unpaired t test to compare antioxidant activity of AgNPs with control(p>0.05)

4. DISCUSSION

In the current study, no statistical difference was found in the antioxidant activity of silver nanocomposite and Control, thus the null hypothesis is accepted.
The use of heterocyclic compounds for synthesising nanoparticles and nanocomposites is becoming increasingly favoured because of its eco-friendly nature[18]. In earlier research, it was noted that various plants, including lemon grass , Mangosteen, Velvet bean, and sweet flag can synthesise AgNPs [19,20]. In the current investigation, silver ions in an aqueous solution were transformed into AgNPs by combining them with Carica papaya extract, followed by an incubation period. This process resulted in a colour shift from yellow to reddish-brown, a phenomenon previously documented by different researchers [21]. These scholars proposed that the colour alteration is because of the surface plasma resonance of the deposited AgNPs. The elevated total phenolic content in Carica papaya extract plays a pivotal role in reducing silver ions to nano-sized particles, facilitated by the electron-donating capabilities of these phenolic compounds. Additionally, the quinoid compound formed through the oxidation of phenol groups in phenols has the potential to adhere to the nanoparticle surface, playing a role in stabilising their suspension [22,23]. The documented literature underscores the direct contribution of phenolic compounds to antioxidative effects [24]. The antioxidative activity associated with plant phenolic contents is likely linked to their redox properties, enabling them to function as reducing agents, hydrogen donors, and quenchers of oxygen free radicals [25,26].

The antioxidant of environmentally-friendly silver nanoparticles was evaluated through visual inspection, evident in a colour shift from violet to yellow. This alteration indicates DPPH reduction via hydrogen atom donation, reflecting potent antioxidant capabilities. Carica papaya leaf extract, abundant in polyphenols and flavonoids, is linked to these nanoparticles. These bioactive compounds may potentially augment the antioxidant prowess of silver nanoparticles, opening new avenues for advancing traditional medicine and addressing various incurable diseases [9].

Nanoparticles find potential utility in modifying vascular function, particularly in cases of endothelial dysfunction linked to oxidative stress. This circumstance may result in decreased bioavailability of nitric oxide (NO), influencing the regulation of vascular tone and contributing to the initial stage of cardiovascular disease development [27]. Therefore, nanoparticles with antioxidant characteristics produced in this study could be utilised to ameliorate vascular dysfunction in various medical and dental conditions.

Despite valuable insights provided, this study has limitations. The focus on antioxidant activity is specific, and broader physiological effects remain unexplored. Additionally, the study lacks in vivo experimentation, warranting further research to validate the potential applications of Carica papaya-mediated silver nanocomposites in real-life medical scenarios.

5. CONCLUSION

This research underscores the promising potential of Carica papaya-mediated silver nanocomposites, showcasing their comparable antioxidant activity to the control. The environmentally friendly synthesis approach holds significance for biomedical applications, paving the way for innovative developments in nanotechnology.

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