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RAPID GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM *FICUS RACEMOSA* FRUIT EXTRACT

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ABSTRACT

An eco-friendly approach for the preparation of silver nanoparticles (AgNPs) from silver nitrate solution using ethanolic Ficus racemosa fruit extract was investigated. The reduction of silver ions in solution was monitored using UV–visible absorption spectroscopy, and the surface plasmon resonance of AgNPs at 435 nm was observed. The proper condition to biosynthesize AgNPs using Ficus racemosa fruit extract was optimized by UV–visible absorption spectroscopy. The biosynthesised nanoparticles were characterised using scaning electron microscopy (SEM), The prepared AgNPs were spherical in shape, and their average particle size determined by SEM was about 75.6 to 145 nm. Furthermore, the AgNPs were found to exhibit effective antibacterial activities against Escherichia coli and Staphylococcus aureus.

KEYWORDS: Ficus racemosa, silver nanoparticles, green synthesis, antibacterial activity.

1. INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants and plant products. From centuries till date, these medicinal plants have been extensively utilized in Ayurveda. Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs).[1]

Nanoparticles have unique properties as a consequence of their size, distribution and morphology and, therefore, are a very important component in the rapidly developing field of nanotechonology. Silver has been known, for more than 2000 years, as a metal that exhibits good medical properties, silverbased compounds being used in numerous antimicrobial applications. Silver ions are highly toxic for microorganisms and, therefore, have multiple roles in the medical field.[2]

AgNPs are a very important part of nanotechnology mainly because they do not induce modification on living cells and, so, are unable to cause microbial resistance. Recent studies revealed that AgNPs have the ability to attach to cell walls and alter cellular respiration. AgNPs are widely used in biology and medicine especially because of their attractive and unique physiochemical properties. Researches carried out in the late 1970s used silver particles for the treatment of orthopedic diseases caused by different infections with microorganisms and a faster bone recovery was noticed. Many other applications can be attributed to AgNPs, for example: catalysts in chemical reactions, bio-labeling, spectral selective coatings for the absorption of solar energy, food additives, textile industry etc. Different applications of silver nanoparticles AgNPs can be obtained by using conventional or unconventional methods, using two different approaches: "topdown" and "bottom-up". Although there are numerous conventional methods used to obtain AgNPs (e.g.: solution, chemical / photochemical reactions in reverse micelles, thermal decomposition of different silver compounds, electrochemical, sonochemical, radiation and microwave-assisted routes) they usually involve hazardous chemicals, low compound conversions, high energy requirements and wasteful purifications.[3,4]

In recent years green chemistry and biosynthetic methods have become more attractive ways to obtain AgNPs. These unconventional methods use either biological microorganisms (e.g.: bacteria, fungi, marine algae, yeasts) or different alcoholic or aqueous plant extracts. Green synthesis has multiple advantages over classical routes: it is cost effective, eco – friendly and does not require high pressure, energy, temperature or the use of toxic chemical reagents.[5]
In comparison to microorganisms, the application of plantextracts for the synthesis of AgNPs is more advantageous in terms of resource availability, security, reaction rate and convenience, and feasibility of large scale synthesis.[6, 7] It has been proved that many plant extracts are suitable for the phytosynthesis of AgNPs.[8, 9, 6–8, 7–14] Several factors including pH, dosage of plant extract, dosage of silver ions, reaction temperature and time affect the

phytosynthesis of AgNPs.[6, 8, 7, 10–13] The reduction rate of silver ions is associated with the species of plants and the key active components of plant extracts.[15]

Ficus racemosaa sacred tree of Hindus and Buddhists and belongs to the familyMoraceae. This is native to Australia, SouthEast Asia and the Indian subcontinent. It is unusual in this plant that its figs grow on or close to the tree trunk. It is one of the herbs mentioned in all ancient scriptures of Ayurveda. It has various synonyms like yajnanga, yajniya, yajnayoga, yajnyasara etc. suggesting its use in ritual sacrifice. The plant grows all over India in many forests and hills. It is frequently found around the water streams and is also cultivated.[16]

It has long been used in Indian medicinal practice as astringent, carminative, stomachic, vermicide, etc. It is believed to be a good remedy for visceral obstructions.

Extractof the fruit is used in leprosy, diarrhoea, circulatory and respiratory disorders and menorrhagia.[16,17,18]

2. EXPERIMENTAL

2.1. Materials

Silver nitrate was purchased from Sinopharm Chemical Reagent Co. Ltd., China. Eriobotrya japonica leaf was collected from the orchard in the suburb of Suzhou, China. Suzhou is one of the important and famous localities of E. japonica in China.

2.2. Preparation of Ficus racemosa fruit extract

Ficus racemosa fruit was thoroughly washed with tap water, dried in the oven at 60 °C, and then grounded into powder for storage. To prepare the ethanolicFicus racemosa fruit extract, 5g of the fruit powder was mixed with 150ml of ethanol in the 250ml Erlenmeyer flask. The mixture was then heated in the oscillator at 60 °C for 90min. Afterwards, the extract ehanolic solution was centrifuged at 4000rpm for 20min to remove impurities. Subsequently, the supernatant was collected, and finally stored at −4 °C for further experiments.

2.3. Synthesis of AgNPs

For the typical reduction of silver ions to AgNPs, 2ml of the freshly prepared extract was added to 48ml of the aqueous silver nitrate solution (1mM) and then the mixture was incubated in the oscillator at 80 °C under moderate stirring for a period of time. As the reaction proceeded, the color change from colorless to yellowish brown was observed and recognized for the formation of AgNPs. The effect of reaction time was studied by incubating

the reaction mixture (2ml Ficus racemosafruit extract, 1mM AgNO3, 80 °C) for different time intervals (5, 10, 20, 30, 60 and 80min). To evaluate the effect of reaction temperature on the biosynthesis of AgNPs, the reaction mixtures containing 2ml extract and 1mM AgNO3 were incubated at different temperatures (25, 40, 60 and 80 °C) for 60min. The effect of initial silver ions on the green synthesis of AgNPs was investigated by varying the silver nitrate concentration from 0.5 to 2.0mM.

2.4. Characterisation of AgNPs

The bio-reduction process of silver ions in solution was monitored using UV–visible absorption spectroscopy. At regular time intervals, the reaction mixture was sampled and diluted. The UV–visible absorption spectra were recorded on the UV-1800 UV–visible absorption spectrophotometer (Shimadzu Co., Japan) from 200 to 800nm at a resolution of 1nm. The particle size of the prepared AgNPs colloid were determined by the SEM technique The SEM measurement at a 3kV accelerated voltage conducted on the S-4800 scanning electron microscope (Hitachi High Technologies America Inc., Japan) equipped with energy dispersive spectrometer.

For the purification of the synthesised AgNPs, the mixture after reaction was subjected to centrifugation at 10000rpm for 10min and then the obtained precipitation was washed with deionized water. This procedure was repeated three times to eliminate the uncoordinated bio-molecules on the surface of AgNPs. The final product was collected and dried in the vacuum oven.

2.5. Antibacterial activity evaluation of AgNPs

The antibacterial activity of the biosynthesised AgNPs was determined using the disc diffusion method. Two different bacterial strains E. coli and S. aureus were inoculated into the nutrient broth and kept in the rotatory shaker for incubation at 37 °C for 24h. 100 µl of the overnight incubated bacterial cultures after a series of dilution were uniformly spread on the surface of the freshly prepared agar medium with the help of a sterilized triangle glass rod. Subsequently, the sterilized circular filter papers with a diameter of 25mm which were impregnated with the prepared AgNPs of different concentrations were gently put down on the surface of inoculated nutrient agar plates by means of a sterilized tweezer. Eventually, the zone of inhibition in millimeter was measured after incubation at 37 °C for 24h.

3. RESULTS AND DISCUSSION

3.1. UV–visible absorption spectroscopy

It is well known that AgNPs exhibit strong absorption band and generate specific color in solution due to the surface plasmon resonance (SPR). The formation of AgNPs was monitored by color change observation and UV–vis absorption spectroscopy. The reduction of silver ions into AgNPs during exposure to Ficus racemosa fruit extract was evidenced by the change of color from colorless to yellowish brown and a characteristic, well-defined SPR band at around 435 nm, both of which are attributed to the excitation of the SPR of AgNPs.[16,17]

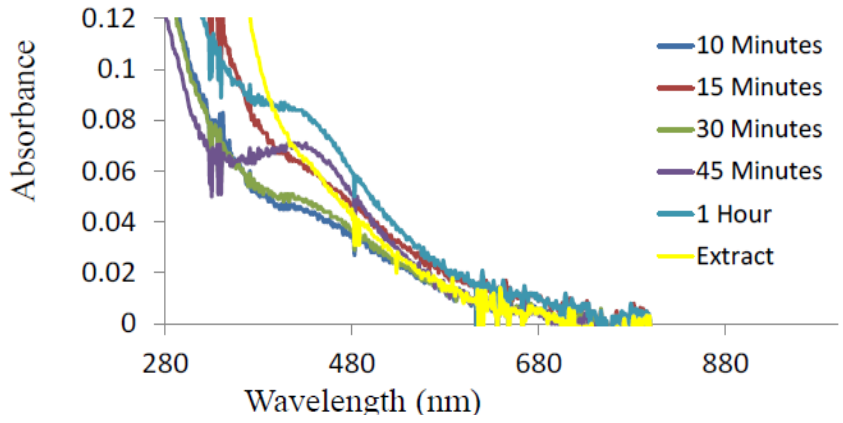
Table 2: Bioreduction parameters for the syntheses of silver and its hybrid nanoparticles using the extract of Ficus racemosafruit at 70^{O}C.

NPs	Maximum intensity							
	0.5 mM		1.0 mM		2.0 mM		3.0 mM	
	Abs	λmax	Abs	λmax	Abs	Λmax	Abs	λmax
Ag	0.118	Broad peak	0.312	416	0.222	Broad peak	0.323	Broad peak

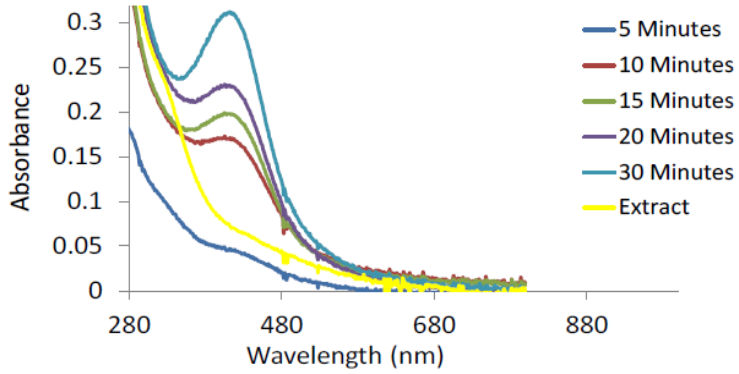
3.2. Conditions of the AgNPs synthesis

3.2.1. Reaction time

The effect of reaction time on the biosynthesis of AgNPs was studied by using UV–visible absorption spectroscopy. It can be seen that AgNPs began to generate within 5min after the addition of silver nitrate to the Ficus racemosa fruit extract solution. As the reaction time was prolonged, the intensity of the SPR peak at 435nm increased gradually, indicating an increasing number of AgNPs formed in the mixture. The UV–visible absorption spectrum recorded after 60min showed no obvious increase in absorbance (inserted figure), indicating the AgNPs synthesis reaction tended towards equilibrium. Thus, the optimum time for the completion of reaction in our study was 60min. The rapid generation of AgNPs is attributed to the great reducibility of the chemical components in the Ficus racemosa fruit extract, which also play a significant role in stabilizing nanoparticles formed in the medium.



(a)



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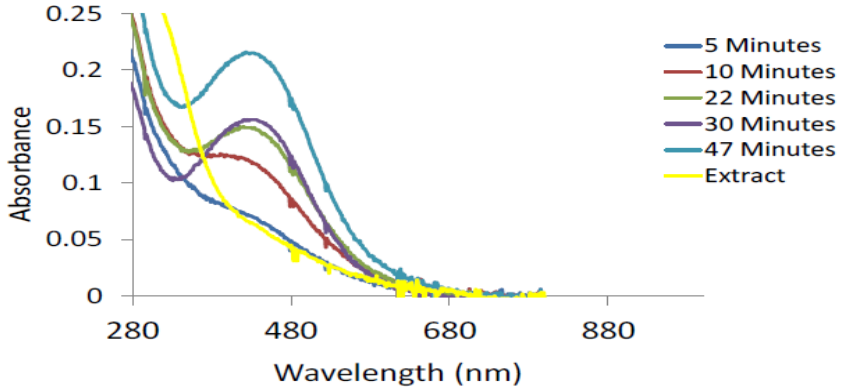


(c)

Figure 6: UV-Vis spectra of Ag NPs prepared by reducing (a) 0.5 mM (b) 1.0 mM (c) 2.0 mM precursor solutions using the extract of Ficus racemosa.

3.2.2. Temperature

The reaction temperature has great impact on the transformation of silver ions to AgNPs. The effect of temperature on the biosynthesis of AgNPs was studied by performing reactions at different temperatures (25, 40, 60 and 80 °C). At a relatively low temperature, the intensity of SPR peak was a little weak and the width of the SPR band was broad, implying that few

nanoparticles was formed in number and a distribution in particle size was wide. However, as the reaction temperature was elevated from 25 to 80 °C, the significant increase in the intensity of the SPR peak was observed due to the enhanced formation rate of AgNPs. In addition, there was a blue shift in the SPR peak location from 451 nm to 435 nm, These results reveal that higher temperature is in favor of the formation of AgNPs with higher yields, smaller sizes and uniform size distribution, which is in accordance with previous reports[1819] In sum, the temperature chosen in our study to fabricate AgNPs was 80 °C.

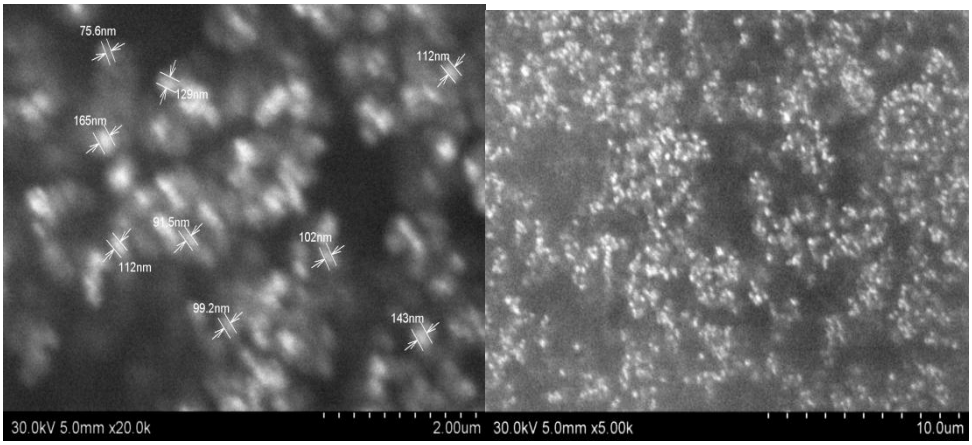
3.2.3. Silver nitrate concentration

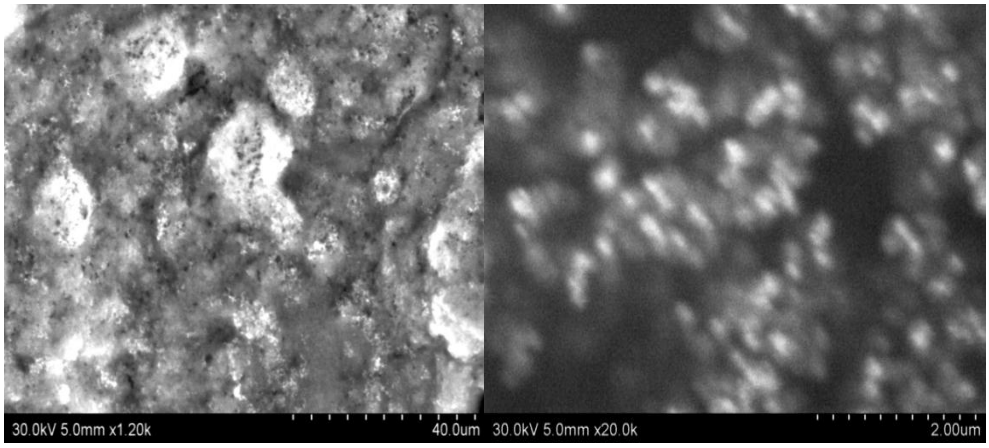
The effect of silver nitrate precursor concentration (0.5, 1.0, 2.0 and 3.0mM) on the formation of AgNPs was evaluated by UV–visible absorption spectroscopy (figure 4(a)). Clearly, the SPR peak of AgNPs became more distinct with increasing initial silver nitrate concentration, indicating that more AgNPs were synthesised as a consequence of abundant silver ions in the medium. Moreover, each of the four SPR bands had a relatively narrow peak with a good symmetry, signifying a narrow and uniform size distribution of the synthesised AgNPs.

3.3. Charaterisation of the biosynthesised AgNPs

SEM Analysis

The morphological features of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of gold was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 30 KV. SEM. The nanoparticles were found have the size ranges from 75.6 to 145 nm.





3.4. Antibacterial activities of the biosynthesised AgNPs

Minimum bactericidal concentration(MBC) is the lowest concentration of antibacterial agentswhich prevents visible microorganism growth after overnightincubation. The observed MBC values for the AgNPs were summarized by Table 3. The MBCs for each particular setof the bacteria show the same concentration, thereforegiving zero standard deviation value (±0.0).

Fig. 8 shows the comparison of plating between the one containing Ag(125 μg/mL) and the one without Ag (control) for *E. coli*.[20] The potent antibacterial properties of AgNPs may be attributed to the released silver ions, which could have an interaction with microorganisms by means of their attaching to the surface of the cell membranes of bacteria, penetrating into the bacterial cells, and affecting the membrane permeability and respiration. In the bacterial cells, AgNPs could even interact with sulfur- and phosphorus-containing compounds like DNA to give rise to the deadly impairment of microorganisms.[21, 22]

Table 3: Minimum bactericidal concentration (MBC) against bacteria.

Bacteria	Minimum bactericidal concentration (MBC) (μg/mL)
E. coli	125 ± 0
S. aureus	1000 ± 0
P. aeruginos	500 ± 0

4. CONCLUSIONS

An eco-friendly and cost-effective protocol for the synthesis of AgNPs by utilizing a renewable natural resource E. japonica leaf was proposed. The proper condition to biosynthesise AgNPs using E. japonica leaf extract was optimized. It was found that the AgNPs synthesis reaction tended towards completion with 60min and higher temperature was

beneficial for the formation of smaller particles. In addition, the initial silver nitrate concentration also had great impact on the size distribution of AgNPs. The biosynthesised AgNPs were characterised by UV–visible absorption spectroscopy, SEM, EDX, TEM, DLS, XRD and FITR. The AgNPs were spherical in shape, and their average particle sizes determined by TEM and DLS were 19.75nm and 54.47 nm, respectively. The biogenic AgNPs exhibited good antibacterial activities against E. coli and S. aureus. Further research on the AgNPs biosynthesised using E. japonica leaf extract could bring a promising application in the fields of medicine and hygiene.

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