Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [2] 2023: 044-047

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Synthesis of Silver Nanoparticles using Cashew Nutshell and Mango Peels Extracts and their Antibacterial Activity

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ABSTRACT
Silver nanoparticles were synthesized using cashew nutshell liquid (CNSL) and mango peels (MP). The synthesized silver nanoparticles were characterized by UV-Vis spectroscopy and have maximum absorption at 400 nm, indicating a reduction of $AgNO_3$. MP showed maximum absorption at 300 - 400 nm, indicating a reduction of $AgNO_3$. The antibacterial activity of AgNPs of the extract of CNSL & MP showed significant activity against lab pathogens used. The results revealed that the AgNPs of extract of MP showed the highest inhibitory activity at a concentration of 100 mg/mL against various pathogens by showing a zone of inhibition of (18 mm) for E. coli. In the case of CNSL AgNPs of extract showed the highest inhibitory activity against P. aeruginosa (14 mm). From the findings, it was revealed that the synthesized nanoparticles can be effectively used against lab pathogens.
Key words: *Silver nanoparticles, UV-Vis spectra, Cashew nutshell liquid, Mango peels, Antibacterial activity*

Received 20.04.2023 Revised 20.05.2023 Accepted 16.06. 2023

INTRODUCTION

Nanoparticles (NPs) display completely new or improved properties as a result of specific qualities, such as size (1–100 nm), shape, and structure [1]. Silver nano-particles (AgNPs) considered as a commercialized nanomaterial, which is extensively used for medical antimicrobial and personal care products, construction materials, water filtration, medical instruments, food packaging, animal husbandry, electronics, agriculture etc. [2, 3]. There are several approaches for the synthesis of AgNPs including green methods, chemical and physical methods. But, AgNPs green synthesis by using plant and plant extracts have been used widely in agricultural sector [4, 5]. Multiple pieces of research have been conducted to demonstrate the impact of shapes of Ag-NPs on their antibacterial activity [6, 7, 8, 9].

MATERIAL AND METHODS

a. Sample Collection

Cashew nuts were collected from Mahalaxmi Cashew nut industry (Kandgaon, Kolhapur) to extract the cashew nut shell liquid and mango peels were collected from local market.

b. Peel Extract CSNL and Mango Preparation

The shells (50 g) of cashew nuts were washed thoroughly dried and ground using a stone grinder. Twenty grams of cashew nut shell were boiled in a 500 mL beaker, using 250 mL of Milli-Q Ultrapure water, for 15 min at 100°C. The beaker was covered with aluminium foil in order to prevent excess evaporation. The contents of the beaker were cooled, mixed thoroughly and filtered through Whatman No. 1 filter paper to obtain the extract. Thisextract was prepared freshly for each experiment. The fresh fruits peels of mango were washed several times with distilled water to remove the dust.

The fruits peels were cut into small pieces. 35 g of properly washed fruit peels were added in 175 mL ultrapure water in a 500 mL Erlenmeyer flask and boiled for 10-15min. Then Whatman filter paper was used for the filtration of boiled material to prepare the aqueous fruit extract, which was used as such for nano particles synthesis.

c. Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) was used for the synthesis of AgNPs. In 100-mL Erlenmeyer flasks, 45 mL of Milli-Q Ultrapure water and 5 mL of CNSL was added to separate AgNPs. Combination of nanoparticle was performed by the addition of 1 mM $AgNO_3$, for 100mL. To this above mixture mix 5 ml was added and kept 24hrs. After 24hrs AgNPs were confirmed by scanning adsorption maxima of reaction mix between 200 to 800 nm on a UV-Vis spectrophotometer. The readings were taken of 0-hr 24h, 48h. Then mixture

was filtered through Whatmann filter paper and centrifuged at 1200 rpm for 15 minfor AgNPs isolation. The obtained nanoparticles were dried to obtain powder. Aqueous solution (1 mM)of silver nitrate was prepared. For the synthesis of silver nanoparticles 1.8mL of fruit peel extract was mixed to 50mL of prepared silver metal ion solution and stirring continued for 4 min at room temperature. The reduction takes place rapidly as indicated by brown yellow color solution was formed after 30 min which indicating the formation of silver nanoparticles. The effects of reaction conditions such as the silver ion concentration and reaction time were also studied.

d. UV-Vis Spectral Analysis

Synthesized silver nanoparticles was confirmed by sampling the aqueous component of different time intervals and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300-800 nm.

e. In vitro antibacterial activity of extract of mango peels & CNSL

Extract of *M. indica (mango)* were dissolved in a few drops of Dimethyl sulfoxide (DMSO) and made up with distilled water to give a stock solution of 100 mg/mL separately. From this stock solution 100 mg/mL concentrations were prepared. The stock solutions were kept at 4-8°C. Standard bacterial organisms *S. aureus, E. coli and P. aeruginosa*, *B. subtilis* and *P. vulgaris* were used. The organisms were first isolated on nutrient broth for 24 h and then diluted to 1:1000 with the sterile nutrient dextrose broth. The dilutions formed were used as bacterial stock solutions for the agar-well diffusion assay and same procedure was done for CNSL.

f. Preparation of Stock Cultures of Test Organisms

The laboratory cultures of test organisms *S. aureus, E. coli, P. aeruginosa, B. subtilis* and *P.vulgaris* were transferred onto sterile nutrient agar plates. The test organisms were maintained on nutrient agar slants at 4°C for further use, the extracts of CNSL, and mango peel were prepared in concentrations of 100 mg/mL. the test organisms were spread inoculated on nutrient agar plates and kept at 37°C for 24 h. After incubation agar well diffusion method was used to observe the sensitivity of the extracts.

g. **Agar Well Diffusion Assay**[10]

The agar well diffusion method was used to study the antibacterial activity of the synthesized AgNPs. The bacterial suspension was prepared by growing a single colony overnight in Luria-Bertani broth and by adjusting the turbidity to 0.5 McFarland standards. Mueller- Hinton agar plates were inoculated with each bacterial suspension and 0.1 mg of the AgNps were dissolved in 1 mL deionized water separately. Approximately 50 uL of the resulting solution was added to the center of well with a diameter of 8 mm. Control plates were made using wells containing CNSL only. The plates were incubated at 37^{o}C for 24 h in a bacteriological incubator & zone of inhibition was noted down. Same was done for mango fruit peels.

RESULT AND DISCUSSION

a. Synthesis & Characterization of Silver Nanoparticle was Carried Out by CNSL & Mango Peels

The addition of 1-mM of $AgNO_3$ to the respective reaction mixture resulted in solution with a brown colour indicating the formation of AgNPs. The colour development may be explained by excitation of surface Plasmon vibration in metal nanoparticle.

Results of UV-Vis absorption spectra during formation of silver nanoparticles are shown in Table 1 & Fig. 1. UV-VIS spectroscopy is used to evaluate the production of metal AgNPs in a solution. The solution was scanned under UV-VIS spectroscope, and the maximum absorbance was observed in the range of 300-400 nm. The UV-VIS spectra of the solution were recorded at different time intervals (10, 25, 45, 70 and 90 minutes). The peak increased with the reaction time suggesting the formation of large amount of NP's (Table-2 and Fig.2).

The above results indicate that both the extracts are effective in synthesis of silver nanoparticles. The CNSL showed maximum absorption at 400nm & mango peel extract showed maximum absorption at 350nm.

b. Antibacterial Activity of CNSL AgNPs

The antibacterial activity of the synthesized AgNPs was tested against the pathogens, results were observed after 24h of incubation at 37°c and presented in the Table-3. AgNPs are effective in inhibiting all the bacterial strains.

Two concentrations were used high and low concentration. Except for *E.coli* at low concentration zone of inhibition of AgNPs against bacteria were obtained while no zone of inhibition was observed in control.

c. Antibacterial Activity of Mango Peel AgNPs

The AgNPs were studied for antibacterial activities of using agar well diffusion method. 100 mg/mL concentrations were effective. In the case of *M. indica* all test concentrations were effective (Table-4).

In conclusion, the results of the present study revealed that the AgNPs of selected plants possess antibacterial activity.

The above results indicate that both the extract AgNPs are effective in showing antibacterial activity. The CNSLAgNPs showed maximum zone of inhibition of *P.aeruginosa* (14mm) & mango peel extract AgNPs showed maximum zone of inhibition of *E. coli* (18mm).

Table - 1 – UV-V is Absorption Spectra during Formation of Silver Nanoparticles of CNSL

	CNSL	CNSL	CNSL
O.D	**0 hrs**	**24 hrs**	**48 hrs**
200 nm	4.00	4.00	4.00
300 nm	2.275	4.00	4.00
400 nm	1.811	4.00	4.00
500 nm	0.988	4.00	3.885
600 nm	0.470	3.110	2.055
700 nm	0.235	1.525	0.443
800 nm	0.165	0.886	0.245

Table-2: UV-V is Absorption Spectra during Formation of Silver Nanoparticle of Mango Peels

O.D in nm	0 hrs	24 hrs	48 hrs
200	3.0	3.40	3.0
300	1.45	3.55	2.88
400	1.33	3.60	2.65
500	0.98	2.54	1.50
600	0.74	2.0	1.20
700	0.45	0.90	0.85
800	0.25	0.75	0.10

Table - 3 -Antibacterial Activity of Silver Nanoparticle of CNSL.

Isolates of pathogen strains	Zone of inhibition in mm
	Concentration of extract used (0.01 M solution of $AgNO_3$)
E. coli	9
S. aureus	11
P. aeruginosa	14
B.subtilis	10
P.vulgaris	12

Table - 4 -Antibacterial Activity of Silver Nanoparticle of Mango Peel:

Isolates of pathogen strains	Zone of inhibition in mm
	Concentration of extract used (0.01 M solution of $AgNO_3$)
E. coli	18
S. aureus	17
P. aeruginosa	16
B.subtilis	15
P.vulgaris	16

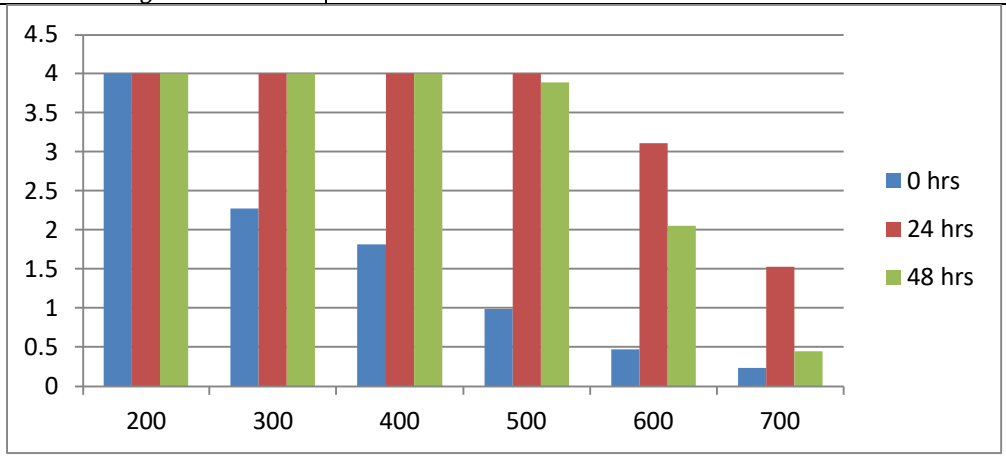

Fig 1 Showing UV-V is Absorption Spectra during Formation of Silver Nanoparticle of CNSL

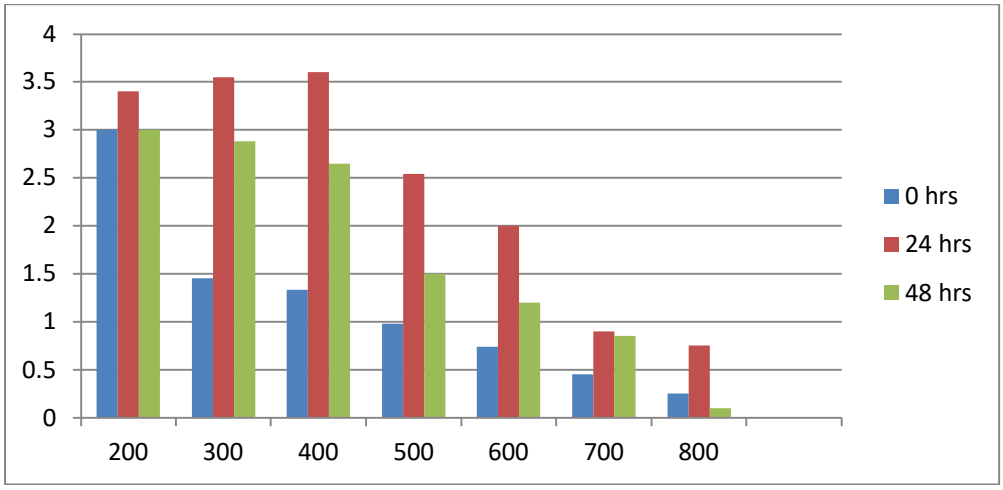

Fig 2: UV-Vis spectra of mango peels at different time intervals.

CONCLUSION

Silver nanoparticles were synthesized using CNSL & mango peels. The synthesized silver nanoparticles were analyzed by observing the UV-Vis spectra at various time intervals. It showed that CNSL has maximum absorption at 400nm, indicating reduction of$AgNO_3$. Mango peels showed maximum absorption at 300- 400mm, indicating reduction of $AgNO_3$. The antibacterial activity of AgNPsof extract of CNSL & mango peels showed significant activity against lab pathogens used. The result revealed that AgNPs of extract of mango peels showed highest inhibitory activity at concentration of 100mg/mL against various pathogens by showing a zone of inhibition of (18mm) for *E.coli*. In case ofCNSL, AgNPs showed highest inhibitory activity against *P. aeruginosa* (14mm).

From the findings it was revealed that the antibacterial activity shown by synthesized nanoparticles can effectively be used against the pathogens. Further studies can give a promising solution in the synthesis of silver nanoparticles and their use in control of pathogens.

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CITATION OF THIS ARTICLE

Prajakta B.Shete, Ajay K. Shinde, Arati P. Gupta, Snehal A. Masurkar and Girish R. Pathade. Synthesis of Silver Nanoparticles Using Cashew Nutshell and Mango Peels Extracts and Their Antibacterial Activity. Bull. Env. Pharmacol. Life Sci., Spl Issue [2]: 2023: 044-047.