Journal of Applied Biological Sciences
E-ISSN: 2146-0108 15(1): 64-74, 2021
Research Article

BUTEA MONOSPERMA BARK EXTRACT TO ITS GREEN SYNTHESIS OF SILVER NANOPARTICLES AND THEIR ANTIOXIDANT, TOTAL FLAVONOID CONTENT AND ANTIMICROBIAL ACTIVITIES

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(Received 31st August 2020; accepted 22nd December2020)

ABSTRACT. Biological methodologies utilizing microorganisms and plants or plant extracts for metal nanoparticles union have been recommended as important options in contrast to concoction strategies. The utilization of plant materials for the preparation of nanoparticles could be more worthwhile, in light of the fact that it doesn't require expound procedures, for example, intracellular amalgamation and numerous purging advances or the maintenance of microbial cell societies. An endeavor has been made to build up a straightforward quick method for bioreduction of silver nanoparticles (AgNPs) using bark concentrate of *Butea monosperma* and to assess the antimicrobial action of prepared silver nanoparticles against different microorganisms. Characterization was determined by using UV-VIS spectroscopy and the antimicrobial activities of the produced Ag nanoparticles were resolute using the disc diffusion assay method. UV-Vis range of the aqueous medium holding silver nanoparticles revealed an absorption peak at 478 nm. The formed silver nanoparticles revealed profound antimicrobial drive against different microorganisms like Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Streptococcus pneumonia. Present examination shows *Butea monosperma* has solid potential for the synthesis of silver nanoparticles by quick reduction of silver particles (Ag^+ to Ag^o). Present investigation suggests strong proof for basic, fast and prudent course to integrate AgNPs using aqueous bark concentrate of *Butea monosperma*. The formed AgNPs showed strong antimicrobial potential. The results were compared with the effect of antibiotics. And were found to be more potent than antibiotics. It was observed that the growth rate was strongly inhibited by the presence of small concentration of nanoparticles.

Keywords: *Antioxidant activity, Antimicrobial activity, Butea monosperma, Silver nanoparticles,*

INTRODUCTION

Nanotechnology concept is in the use since ancient times without actually defining the science. But nowadays nanotechnology is expected to be the basis of many technological innovations in the 21st century. Nowadays, noble metal nanoparticles have been the subject of centered exploration because of their novel optical, electronic, mechanical, magnetic and chemical properties that are fundamentally not quite the same as those of mass materials [1]. These special and unique properties could be attributed to their small sizes and large surface areas. For these reasons, metallic nanoparticles have found uses in many applications in different fields, such as catalysis, photonics, and electronics.

Preparation of silver nanoparticles has fascinated particularly substantial attention due to their various properties and uses like electrical conductivity [2] antimicrobial and antibacterial activities [3,4], DNA sequencing [5] and Surface-Enhanced Raman Scattering (SERS) [6]. Numerous strategies of integrating silver nanoparticles, for example, chemical reduction of silver particles in aqueous arrangements with or without balancing agents [7], thermal deterioration in organic solvents [8] chemical photoreduction and reduction in turn around micelles [9,10] and radiation chemical reduction[11,12] has been recorded previously. Majority of these techniques are very costly and, furthermore, utilizes poisonous, risky synthetic concoctions, indicating natural and biological dangers. Since noble metal nanoparticles are broadly applied to zones of human contact [13] there is a growing need to develop environmentally responsive procedures for nanoparticle amalgamation that are not destructive to the nature. Biological strategies for nanoparticle union utilizing microorganisms [14 -16], chemicals [17], growth [18] and plants or plant have been proposed as conceivable eco-friendly options in contrast to substance and physical strategies. Late exploration announced that silver nanoparticles prepared utilizing different natural products like green tea Camellia sinensis [19], Azadirachta indica leaf broth [20] natural rubber [21], Aloe vera plant extract [22] Latex of Jatropha cureas [23]etc. *Butea monosperma* (Lam) Taub (Butea frondosa) commonly known as Palas in Sanskrit belonging to family Fabaceae is a traditionally used medicinal plant. Seeds, leaves bark, flowers all have medicinal properties. Besides it has been reported to have antibacterial, antifungal properties also. In the present examination, we have reported the union of silver nanoparticles, reducing the silver ions present in the arrangement of silver nitrate by the fluid bark concentrate of *B. monosperma.* Further these green synthesized silver particles were explored for their potential against different pathogenic bacteria.

MATERIALS AND METHODS

All chemicals used in the experiment were of maximum purity and gained from Merk and Hi-media laboratories Pvt Ltd Mumbai, India. The bacterial culture of Streptococcus pneumonia, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis were bought from National Center for cell science (NCCS) Pune. Anti-infection agents (Vancomycin and Erythromycin) were bought from Hi-media Mumbai, India. Bark of *B. monosperma* was collected locally from Gondia City, Maharashtra.

Collection of specimens

The bark for the current investigation were gathered in the early daytime throughout the late spring season in March 2020 from a *B. monosperma* tree close to Mundipar forest region, Dist.- Gondia, Maharashtra. The 1 kg of bark were collected and dried under shade and pummeled utilizing a mechanical processor and coming about force was put away in an impenetrable holder for the further examination.

Preparation of Bark Extract

Bark extract was prepared by mixing 10 g dried powder with 100 mL sterile distilled water and filtered through Whatman filter paper No. 1 (pore size 0.45 µm) and was further filtered through 0.22 µm filters. The extract was boiled for 10 min and stored at 4°C for

further experiments.

Synthesis of Silver Nanoparticles

The aqueous solution of 0.1 mM silver nitrate ($AgNO_3$) was arranged and utilized for the union of silver nanoparticles. Ten milliliters of *B. mon*osperma bark extract was mixed to 90 mL of 0.1 mM of $AgNO_3$ and then heated at 80°C for 15 min for the reduction of Ag^+ ions. A shading change from yellowish earthy colored to rosy earthy colored was observed.

Procedure for total flavonoid contain

The 2 mL of plant extract of difference concentrated solution was taken in a test tube to each test tube 2 ml aluminium chloride (2 %) and 3 mL of sodium acetate (5 %) was added to get 1000 µg/mL (1mg/mL) concentrated extract solution. The incubation of test solution was done at room temperature for 150 min. Blank consists of all the reagent, except for the extract solution is substituted with 2 mL of methanol. The stock solution was prepared by dissolving 1 mg quercetin in 1 mL distilled water, so the concentration of the solution is 1 mg/mL (1000 µg/mL). Then serial dilution was performed in order to prepare different concentrated solution 10 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL. Then the absorbance of each test tube measured at 440 nm using spectrophotometer (Shimadzu UV-2450, Japan) against blank.

Procedure for antioxidant activity

The 2 mL of methanol solution of plant extract at different concentration was taken in a test tube to that 3 mL methanol solution of DPPH was added. The incubation was conducted for 30 min at room temperature in dark place so as to complete the reaction. Then the absorbance of the solution was measured at 517 nm using spectrophotometer against blank. Then % inhibition wear plotted against concentration and from the graph IC50 was calculated [24]**.**

RESULTS AND DISCUSSION

The response blend, bark concentrate of *B. monosperma* with watery arrangement of the silver nitrate, began to change its shading from yellowish earthy colored to rosy earthy colored (Fig. 1). Which demonstrated the formation of AgNPs with the reduction of silver particles. The characteristic surface plasmon absorption bands were observed at 478 nm. UV spectra of silver nano particle synthesis from $AgNO_3$ were shown in Fig. 3. Biosynthesized silver nanoparticles were studied for antimicrobial activity (Fig.5) against pathogenic microorganisms by using standard zone of inhibition. The effect of different concentrations such as 0.2 mM, 0.4 mM and 0.8 mM of silver nanoparticles on bacteria were performed. A clear inhibition zone treated with silver nanoparticles was observed. (Table 1.) The standard antibiotics like vancomycin, erythromycin shows smaller zone of inhibition as compared to the nanoparticles treated discs. (Plate. 1)

A-Before **B- After**

Fig. 1. *Colour changes before (A) and after (B) the process of reduction of Ag^+ to Ag nanoparticles.*

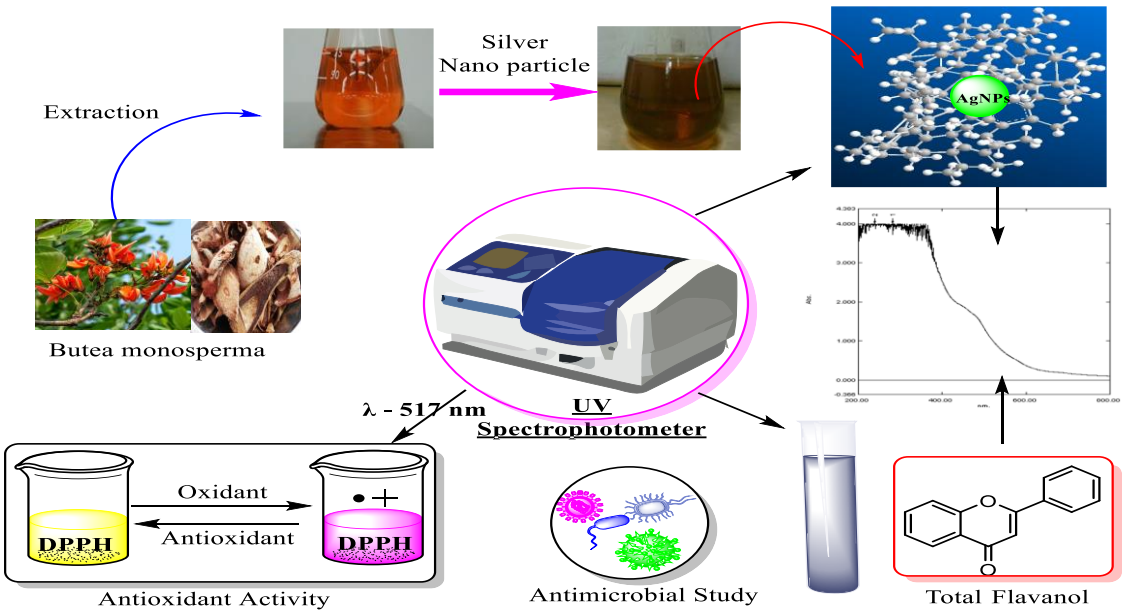


Fig. 2. *Butea monosperma extraction, analysis and testing.*

UV-VIS spectra analysis

The UV-VIS unearthly examination was carried out by utilizing UV-VIS spectrophotometer (Shimadzu UV-2450, Japan). The decrease of unadulterated Ag^+ particles was observed by estimating the UV-VIS range of the response medium at room temperature worked at a goal of 1 nm. The reduction of silver particles was affirmed by subjective testing of supernatant got after centrifugation with a spot of NaCl.

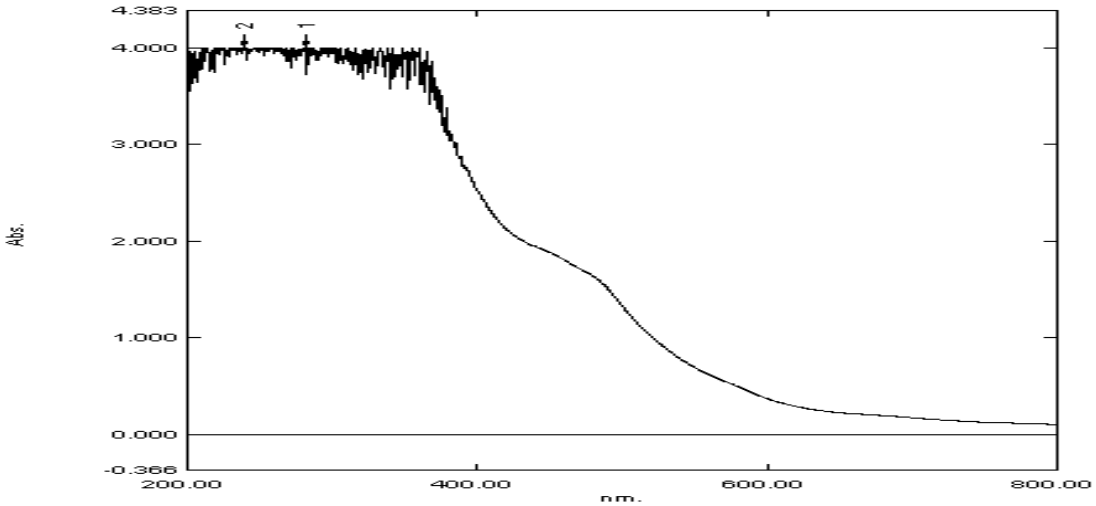


Fig. 3. *UV-VIS absorption spectra of silver nanoparticles (478 nm) synthesized from B. Monosperma*

Total flavonoid content

The flavonoids content of *Butea monosperma* was determined according to the Aluminium chloride method performed by Kumaran and Karunakaran [25]. Flavonols are a class of flavanoids that have 3-hydroxy flavanone. Flavonols are present in the wide variety of fruits and vegetables. Flavanoids shows strong oxidative ability in laboratory studies. In the study, flavonols content of the *Butea monosperma* extract was measured using 5% $AlCl_3$. Table 1 shows the total flavonoid content of sample tested quercetin equivalent by reference of a standard curve Y=25.5 x, R^2 = 0.9812, x is the observance, Y is the quercetin equivalent. The total flavonols content of in *Butea monosperma* was 69.144±0.14 mg quercetin equivalence/g of extract which was shown in the table 1.

The concentration of quercetin in samples were determined by using an equation that was obtained from standard quercetin graph. The equation is given below.

$$y = 25.5\ x$$

Eqn. 1

Where,
x is the absorbance
C is the Intersection = Zero
m is the slope = 25.5
y is the quercetin concentration µg/mL
Now, the total flavonols content in sample was determined as milligrams of quercetin equivalent by using the following equation 2.

$$A = (C \times v)/m$$

Eqn. 2

Where,
A is a total flavonoid content (mg/g quercetin equivalent)
C is X/1000 = Concentration of quercetin mg/mL
V is volume of extract
m is mass of the extract (g)

Table 1. *Total flavanols content of Butea monosperma silver nanoparticle.*

Sample solution (µg/mL)	Weight of dry extract/mL m(g)	Absorbance (x)	QE conc. 'C'(y = 25.5 x µg/mL)	QE conc. 'C' (mg/mL)	TFLAC as QE, A= (C x v)/m (µg/mL)	Mean ± SEM
1000	0.001	1.361	34.705	0.0347	69.41	
1000	0.001	1.354	34.527	0.0345	69.05	**69.14±0.14**
1000	0.001	1.352	34.476	0.0344	68.95	

Antioxidant Activity

The 0.004 % w/v DPPH solution was prepared by dissolving 4 mg DPPH in 100 mL methanol (95%) in a dark room. The standard stock solution asorbic acid solution (800 µg/mL) was prepared by dissolving 2 mg asobic acid in 2.5 mL distilled water. The further dilution was prepared to get 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL concentrated solution. The 3 mL DPPH solution was used as a negative control. The blank for the solution is ethanol. The absorbance of the test solution was measured at 517 nm using UV spectrophotometer (Shimadzu UV-2450, Japan) against blank [26-28].

The percentage (%) inhibition activity was calculated from the following equation 3.

$$\% I = \{(A_o - A_l)/A_o\} X 100$$

Eqn. 3

Where, A_o is the absorbance of the control, and
A_l is the absorbance of the extract / standard.

Table 2. *IC50 calculations of Butea monosperma silver nanoparticle.*

Concentration µg/mL	Absorbance	% SCV	IC50 (µg/mL)
12.5	0.861	23.125	
25	0.805	28.125	
50	0.712	36.42857143	**112.724296**
100	0.35	68.75	
200	0.251	77.58928571	
400	0.191	82.94642857	
Blank	1.12		

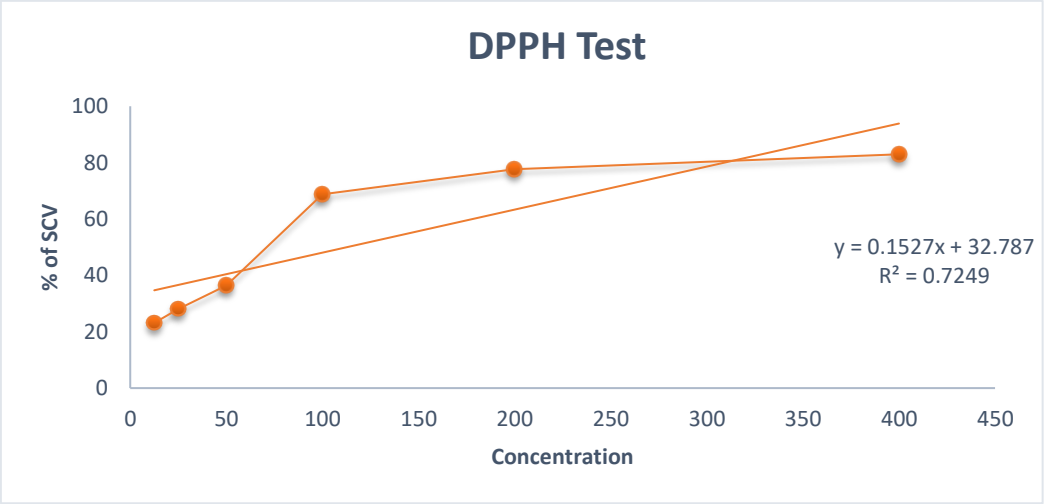


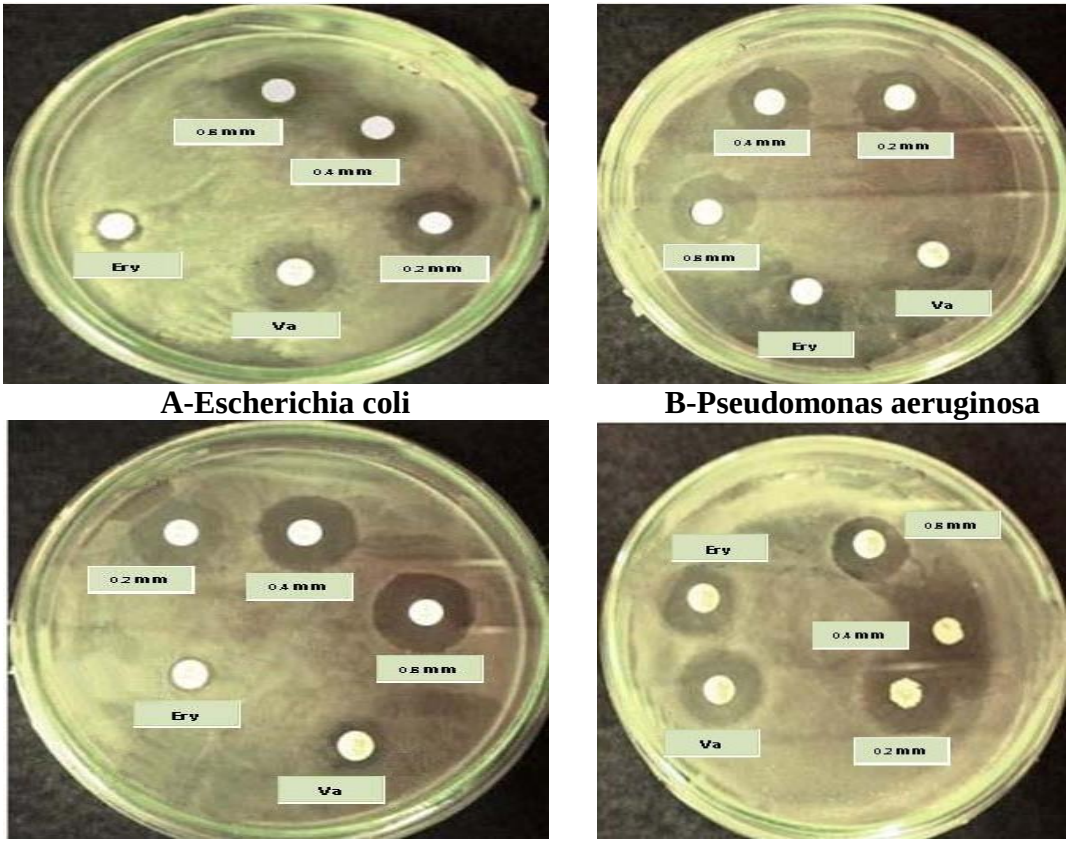
Fig. 4. *IC50 graph for various concentration.*

Antibacterial Activity

The antibacterial test [29] was performed by standard plate dissemination strategy. Supplements were used to cultivate microscopic organisms. The media was autoclaved and cooled. The media was poured in Petri circles and was kept for 30 minutes for hardening. Following 30 minutes, the new short-term societies of inoculum (100 µL) of four distinct living beings were spread on supplement agar plates. Sterile paper discs made of Whatman filter paper, 5 mm diameter dipped in different concentrations of aqueous solution of silver nanoparticles such as 0.2 mM, 0.4 mM, and 0.8 mM along with two standard antibiotics containing discs were placed in each plate. The cultured agar plates were incubated at 37 °C for 24 hr. After 24 hr of incubation, the zone of inhibition was measured in millimeters.

Table 2. *Antimicrobial activity.*

Bioactive agent	**Conc.**	**Zone of inhibition (diameter, mm)**			
		E. coli	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Streptococcus pneumonia***
Ag nanoparticle	0.2 mM	2.5	3.2	3.1	3.2
	0.4 mM	3.4	4.2	3.3	3.4
	0.8 mM	4.5	4.4	4.3	3.8
Erythromycin (10 mcg/disc)		nil	nil	0.6	4.1
Vancomycin (10 mcg/disc)		0.8	nil	0.8	3.8



A-Escherichia coli **B-Pseudomonas aeruginosa**

C- Bacillus subtilis **D- Streptococcus pneumonia**

Fig. 5. *Antibacterial activities of discs of different concentrations of Ag nanoparticles (0.2 mM, 0.4 mM and 0.8 mM, Va-Vancomycin, Ery-Erythromycin) and other antibiotics.*

The union of nanoparticles is in the lime light of present-day nanotechnology scenario. Biosynthesis of nanoparticles by plant removes is now under abuse. The advancement of organically motivated exploratory procedures for the blend of nanoparticles has developed as significant part of nanotechnology. The current investigation manages the amalgamation of silver nanoparticles using bark concentrate of *B. monosperma* and watery Ag^+ particles. Comparative investigations were carried out to examine the pace of bioreduction of silver particles. The methodology described here in the investigation reveals a financially savvy option in contrast to traditional techniques for preparing silver nanoparticles. Arrangement and strength of silver nanoparticles in fluid colloidal arrangement were affirmed utilizing UV-VIS otherworldly investigation. It is notable that silver nanoparticles show yellowish earthy colored shading in fluid arrangement because of excitation of surface plasmon vibration in silver nanoparticles [30,31]. As the *B. monosperma* bark extract was blended in with watery arrangement of the silver nitrate, it began to change the shading from yellowish earthy colored to dim rosy earthy colored due to reduction of silver particles, which showed the development of silver nanoparticles. It is commonly perceived that UV-VIS spectroscopy is utilized to observe size and shape-controlled nanoparticles in watery suspension [32]. Retention spectra of silver nanoparticles framed in the response media has a solid absorbance top at 478 nm and widening of pinnacles demonstrated that the particles are polydispersed.

Silver nitrate which is readily soluble in water has been exploited as an antiseptic agent

for many decades. It is being used as a safe inorganic antibacterial agent since centuries and is capable of killing about 650 microorganisms that causes diseases. Silver has been depicted as being 'oligodynamic' that is, its particles are fit for causing a bacteriostatic (development restraint) or even a bactericidal (antibacterial) sway. i.e., likewise having capacity to apply a bactericidal impact at minute focus. The specific instrument of the counter-bacterial impact of silver particles was somewhat comprehended. Writing overview uncovers that the bactericidal practice of nanoparticles is credited to the nearness of electronic impact that is achieved because of progress in neighborhood electronic structure of the surface because of littler sizes.

These impacts are viewed as contributing towards improvement of reactivity of silver nanoparticles surface. Silver in ionic structure unequivocally associate with thiol gathering of fundamental protein and inactivates them. Shrivastava [33] considered antibacterial action against E. Coli, S. aureus and S. typhi. They have reported that the impact was portioned dependent and was more articulated against gram-negative life forms than gram-positive ones.

They have found that the major mechanisms through which silver nanoparticles manifest antibacterial property was either by anchoring or penetrating the bacterial cell wall or modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues.

The antibacterial efficacy of the biogenic silver nanoparticles revealed in the current investigation might be credited to the system depicted above, however, it despite everything stays to explain the specific impact of the nanoparticles on significant cell digestion like DNA, RNA and protein blend. A basic need in the field of nanotechnology is the improvement of a dependable and eco-accommodating procedure for the amalgamation of silver nanoparticles. We have shown first time the blend of silver nanoparticles utilizing bark concentrate of *B. monosperma* through effective green procedure, evading the nearness of risky and harmful solvents. The green synthesized silver nanoparticles using bark extract of *B. monosperma* shows profound antimicrobial activity in table 2. The present investigation reveals simple, rapid and economical route to synthesize silver nanoparticles.

Acknowledgement. The authors are thankful to the Department of Biotechnology, Shri Shivaji Science College, Nagpur and D.B. Science College, Gondia for providing the facilities to carry out the experimental works. We also extend our thanks to Dr. S.R. Thakare and Sandip Gawande for their valuable guidance.

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