

Research Article

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Catalytic performance of the biosynthesized AgNps from *Bistorta amplexicaule*: antifungal, bactericidal, and reduction of carcinogenic 4-nitrophenol

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Abstract: This study was focused to synthesize silver nanoparticles (AgNps) from the aqueous extract of *Bistorta amplexicaule* and to evaluate their antimicrobial, antifungal, and 4-nitrophenol (4-NP) degradation potential. The AgNps from *B. amplexicaule* were characterized by field emission scanning electron microscopy, energy dispersive X-ray spectrometry, X-ray diffraction, and X-ray photoelectron spectroscopy studies. The biological activity of the AgNps was checked against the three bacterial and two fungal strains. The inhibition activities of the synthesized nanoparticles on pathogenic bacteria and fungi were equally studied using the colony-forming unit method. The AgNps synthesized showed excellent bactericidal and fungicidal activities against pathogenic *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans*, and *Candida tropicalis*.

The removal of nitrophenols is one of the most demanding tasks, due to their injurious impact on the health of the living organisms. The AgNps showed superior performance compared with the pure plant extract. The AgNps also displayed efficient catalytic ability in reducing toxic 4-NP into harmless 4-aminophenol in the presence of NaBH₄ solution. Hence, the synthesized AgNps can be effectively used against bacterial and fungal infections and in the decontamination of 4-NP polluted water.

Keywords: *Bistorta amplexicaule*, AgNps, biosynthesis, bactericidal, pollutants reduction

1 Introduction

Nanotechnology is an important domain of modern research, which deals with the strategy, synthesis, and manipulation of the structure of the particles ranging in size from about 1 to 100 nm. All the properties of these particles change in a fundamental way of both individual atoms/molecules and their corresponding bulk. The new or enhanced properties of these nanoparticles (Nps) and nanomaterial are dependent on their size, distribution, and morphology, which are responsible for their vast and rapidly growing applications in diverse fields such as food and feed industry, biomedical, cosmetics, health care, drug/gene delivery, health, and environment [1,2]. Due to the widespread distribution of antimicrobial resistance, there is a huge demand to develop novel antimicrobial agents that can kill resistant microorganisms. Advances in the field of nanoscience enabled the researchers to tailor nanomaterials with the desired properties. Due to the large surface area and perspective dimensions, these Nps possess excellent antimicrobial properties [3]. Among the Nps of the noble metals, silver nanoparticles (AgNPs) are an excellent product in the field of nanotechnology, which has gained great interest

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of researchers. Some of the unique properties of these nanoparticles are good conductivity, chemical stability, and catalytic efficiency and most importantly their antifungal, anti-viral, anti-inflammatory, anti-angiogenic, and anti-cancer activities with less toxicity and enhanced bioavailability [4–7]. In addition to the above-mentioned applications, AgNps are also being successfully used for the diagnosis and treatment of cancer [8,9].

Various techniques have been developed and are being used for the synthesis of AgNps, such as physical, chemical, and green synthesis. In all these methods, reducing agents are used to produce stabilized Nps by the reduction of silver ions to elemental form, followed by the nucleation and growing processes [10]. Using physical and chemical methods, synthesis of Nps with desired properties is quite easy, but the expensive and non eco-friendly procedures make them unpromising [11]. Although different pathways are available for the synthesis of nanoparticles, the development of cost-effective, non-toxic, and environmentally friendly procedures are the need of the time. Hence, the popularity and importance of the biological approach for the synthesis of Nps are rapidly increasing. Biomolecules have some special qualities, which are responsible for their highly controlled and hierarchical assemblies, thus making them more suitable for developing reliable and eco-friendly techniques for the synthesis of metal nanoparticles [12]. The route of green syntheses is the preferred one over the physical and chemical methods as it is eco-friendly and cost-effective and can be scaled up easily for the synthesis of Nps on large scale. Furthermore, this procedure does not require high input of energy, high temperature and pressure, and harmful chemicals [13]. According to the literature, different organisms such as bacteria, fungi, and plants are used for the synthesis of AgNps. The antioxidants or reducing agents present in these organisms are responsible for the reduction of metallic compounds in their respective Nps. A variety of metabolites are found in plants such as phenolic acids, terpenoids, alkaloids, polyphenols, sugars and proteins that play a crucial role in the bioreduction of Ag^{1+} to AgNps [14]. One of the most important species of *Bistorta* is *amplexicaule*, which is an herbaceous perennial plant. The plant is widely distributed in the northern areas of Pakistan and locally known as “maslon.” Literature survey revealed, that, the plant is used as a traditional medicine for the treatment of inflammations, fractures, dysentery, pain, hemorrhage, as diuretic and for promoting blood circulation [15]. It is well known that *B. amplexicaule* contains higher concentrations of antioxidants [16]. The plant is

used as herbal tea for the treatment of ulcers, leucorrhoea, dysentery, fever, and various heart-related problems [17]. Considering the ease of availability, phytochemical constituents, and ethnobotanical applications of *B. amplexicaule*, the plant has been selected for the synthesis of AgNps that may have potential applications.

2 Materials and methods

2.1 Sample collection and preparation of rhizome aqueous extract

The fresh rhizome samples of *B. amplexicaule* were selected and purchased, without any physical damage, from a local grocery shop at Swat Valley, Khyber Pakhtunkhwa, Pakistan. The samples were transported in a clean polythene bag to the laboratory before processing. The plant rhizome samples were identified, and the identity was authenticated using the standard procedure of the Department of Botany, Government Post Graduate Jahanzeb College, Swat Valley, Khyber Pakhtunkhwa, Pakistan. The unprocessed rhizomes were washed under tap water, and the adhered particles were removed. The clean rhizomes were then rinsed with enough quantity of Millipore Milli-Q water and allowed to dry under shade for 1 week at room temperature. The dried rhizomes were ground to a coarse powder with a household blender. The powdered rhizome (15 g) was soaked in 200 mL of Millipore Milli-Q water in 500 mL capacity Erlenmeyer flask. The flask containing the mixture was properly covered with aluminum foil and incubated on a shaker at 200 rpm for 24 h. The mixture was then boiled on water bath at 80°C for 30 min, after which it was allowed to cool down at room temperature. The mixture was then filtered through a 20 mesh cloth, and the filtrate was finally centrifuged. The supernatant was refiltered with Whatman No. 1 filter paper, and the filtrate was stored as rhizome aqueous extract (RAE) at 4°C before use.

2.2 Biosynthesis of AgNps

The synthesis of the AgNps was optimized using different volumes of the RAE, 10, 20, and 30 mL, and 1 mM AgNO_3 solution. After the optimization, 25 mL of RAE was added to 400 mL of 1 mM AgNO_3 , and the final volume of the mixture was made to 500 mL (5:95) with

Millipore Milli-Q water. The reaction mixture was stirred continuously in a magnetic stirrer until it completely changed to dark brown color, signifying the completion of reduction of Ag^+ to Ag^0 by the biomolecules in the RAE. After reduction, the reaction mixture was centrifuged at 10,000 rpm for 20 min, and the synthesized AgNps were recovered as pellets. The uncoordinated biomolecules and the excess of AgNO_3 were removed by washing with Millipore Milli-Q water, followed by centrifugation, and the uncontaminated pellet was obtained. The washing procedure was carried out three times, and the clean AgNps obtained were transferred and dried on a watch glass at room temperature. The crust obtained after drying was scratched and stored for further studies.

2.3 Characterization of the biosynthesized AgNps

The techniques used in this experiment and their models are as follows: field emission scanning electron microscopy (FESEM; model JSM-7600F; JEOL, Japan), transmission electron microscopy (TEM; model Philips FEI Tecnai 12, UK), energy dispersive X-ray spectrometry (EDS; Oxford-EDS system), and UV-vis spectroscopy (Thermo Scientific Evolution-300; USA). The elemental composition was recorded by using X-ray photoelectron spectroscopy (XPS; ESCALAB 250Xi; Thermo VG Scientific, East Grinstead, UK). X-ray diffraction (XRD) was performed with the X-ray diffractometer (Thermo Scientific) and Fourier transform infrared spectroscopy (FTIR) was performed with the FTIR spectrometer (Thermo Scientific).

2.4 Media preparation and culturing of bacterial cells

The antibacterial activity of the biosynthesized AgNps was assessed on *Staphylococcus aureus*, *Escherichia coli* (O157:H7), and MRSA cells. These bacterial cells were used as the model organisms and received from the King Fahad Medical Research Center, Jeddah. The nutrient agar was used for the bacterial growth. The nutrient agar contains peptones (5 g/L), meat extract (1 g/L), sodium chloride (2 g/L), yeast extract (2 g/L), and agar (15 g/L) in distilled water. After sterilization, the medium was poured into the sterilized Petri plates, and the plates containing the media were subsequently used for the growth of bacteria.

2.4.1 Bactericidal assay

The bactericidal activities of the RAE and the biosynthesized AgNps were assessed on *S. aureus*, *E. coli* (O157:H7), and MRSA. The colony-forming unit (CFU) count method was used for this assay. Five milliliters of the nutrient broth was mixed with 100 $\mu\text{g}/\text{mL}$ of the sample in different test tubes. To each tube, 100 μL of MRSA and *E. coli* (O157:H7) and Nps were added, and the mixture was incubated at 37°C for 24 h. The samples were successively diluted and spread over the nutrient agar. The nutrient agar plates were incubated for 24 h to check the number of colonies grown on the plates. The number of colonies were counted using a colony counter, and the experiment was repeated three times.

2.5 Media preparation and culturing of fungal cells

The antifungal activities of the biosynthesized AgNps were assessed on *Candida albicans* and *Candida tropicalis*. The two fungal strains were used as the model organisms in this study and were obtained from the King Fahad Medical Research Center, Jeddah. They were grown on the yeast extract agar medium (HiMedia) containing casein hydrolysate (5 g/L), yeast extract (3 g/L), and agar (15 g/L) in distilled water. The yeast extract agar medium was prepared and sterilized and poured into the sterilized Petri dishes after cooling. After solidification of the media, the plates were used for the growth of these fungal strains.

2.5.1 Antifungal assay

The fungicidal activities of the RAE and the biosynthesized AgNps were assessed through the CFU count methods. The AgNps (100 $\mu\text{g}/\text{mL}$) were added to each of the test tubes containing 5 mL of the liquid medium (yeast extract). Then, 100 μL of *C. albicans* and *C. tropicalis* culture was added to each tube containing 5 mL of the liquid medium and AgNps. The mixture was then incubated at 37°C for 24 h. The samples were serially diluted and spread on the yeast extract agar and then incubated for 24 h to check the number of colonies. The number of colonies were counted using a colony counter, and the experiment was repeated three times.

2.6 AgNp-based reduction of 4-nitrophenol

Exactly 2.5 mL of an aqueous solution of 0.1 mM 4-nitrophenol (4-NP), 0.5 mL of 1 M NaBH_4 , and 10 mg

of the prepared AgNps were taken in a quartz cell. The addition of NaBH_4 leads to the conversion of 4-NP to phenolate anions, and the variation in the concentration of the phenolate anions with time was measured continuously with a spectrophotometer at 400 nm.

3 Results and discussion

One of the most important species of *Bistorta* is *B. amplexicaule*, an herbaceous perennial plant, which is commonly called maslon. The plant is widely distributed in the northern part of Pakistan (Figure S1). The plant contains higher concentrations of antioxidants [16] and is commonly used in the treatment of inflammation, fractures, dysentery, pain, hemorrhage [15], ulcers, leucorrhoea, fever, and various heart diseases and to promote blood circulation and acts as a diuretic. The sap of the plant rhizomes are used to treat fresh wounds in the eyes [17]. The higher content of antioxidants in the rhizomes attracted their use in the reductive synthesis of AgNps.

3.1 Biosynthesis of AgNps

The reduction of silver ions in the synthesis of AgNps was confirmed by the color change of the reaction mixture from light brown to reddish brown and finally dark brown, as indicated in Figure S1a–c. The kinetics of the formation of AgNps was studied at room temperature, using a spectrophotometer with a resolution of 1 nm, and the UV-vis spectra of the reaction mixture were measured. During the reduction of silver ions by the RAE of *B. amplexicaule*, 3 mL of the reaction mixture was taken and the spectrum was recorded between 200 and 800 nm at $t = 0$. Then, the reaction was started and after every 2 min, the reaction system was analyzed for the progression of the reaction by recording spectrum of the mixture. For background correction of the experiment, distilled water was used as a blank reference.

3.2 Characterization of the AgNps

The UV-vis graph was recorded immediately after mixing the RAE and AgNO_3 solutions at $t = 0$ by taking 3 mL of the reaction mixture, which showed a flat line, confirming only the mixing of two solutions without any instant

reaction. Slight increases in the Plasmon resonance of the AgNps were observed after 2 min, suggesting the start of the formation of AgNps. Another reading was taken after 4 min, which exhibited again a much better increase in the Plasmon resonance of the Nps.

The Plasmon resonances of AgNps are gradually increased at 430 nm with time, as indicated in Figure S2a. This indicates that RAE contains more polar compounds and responsible for the reduction and stabilization of Ag^{1+} to Ag^0 Nps.

The XRD analysis shows that RAE AgNps were crystalline in nature, as indicated in Figure S2b. The four distinct peaks at 2θ values of 38.5, 44.0, 64.8, and 77.4 are indexed to (101), (111), (200), and (220) reflection planes of the face centered cubic structure of silver [18]. These peaks also matched with the indexed reflection values reported in the literature and also in line with the previously reported results [9,18,19].

The FESEM analysis of the RAE AgNps showed the presence of white spots at the surface, which confirm that the AgNps formed have uniform spherical shape morphology. The results also show that the AgNps were clustered into a sort of beautiful flower-like structure with spherical morphologies (Figure 1a and b). The elemental constituents of the RAE AgNps were found to be Ag, O, C, and Cl atoms, as indicated by the EDS analysis results. The percentage of Ag, O, C, and Cl atoms were found to be 40.41%, 18.88%, 34.02%, and 6.69%, respectively. Of these, Ag atom contribute the higher percentage due to the formation of the AgNps, but other elements were from the active plant components from RAE, as indicated in Figure 1c and d.

The elemental composition and binding energy were further confirmed through XPS. The XPS analysis indicated the high purity of the synthesized AgNps with the formation of zero-valent AgNps. The XPS analysis shows that the AgNps were in the zero state and no oxide of the Ag was appeared in the XPS spectrum. The main spectrum of the XPS is indicated in the inset of Figure 2a, where different peaks for Ag, O, and C appeared. The C 1s appeared at 285 eV (Figure 2b), and metallic Ag $3d_{(5/2)}$ and Ag $3d_{(3/2)}$ at 368 and 373 eV (Figure 2c) respectively, while the Ag 2p appeared at 573 eV (Figure 2d).

3.3 Degradation of toxic 4-NP

The compound 4-NP is one of the common organic pollutants mostly found in wastewater, which poses a

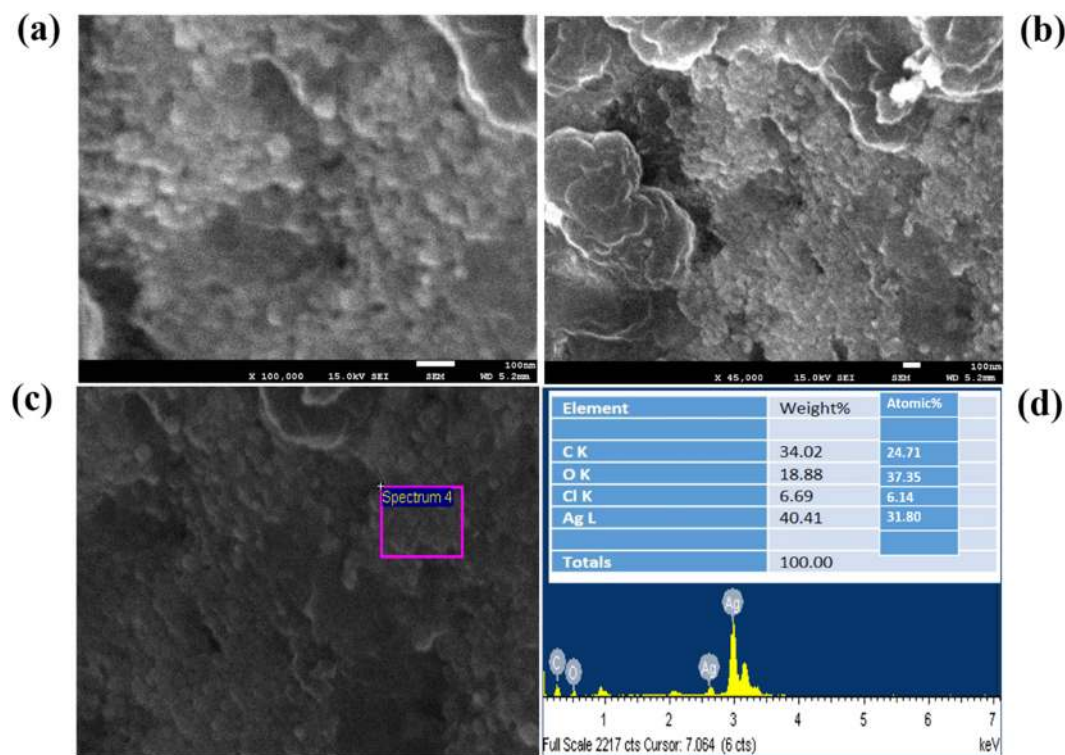


Figure 1: (a and b) FESEM spectrum window from where the area was selected for (c) EDS spectrum, and (d) AgNps synthesized from RAE of *B. amplexicaule*.

risk to the human and animal health and the environment [20]. The spectroscopic peaks for the pure 4-NP were found at 318 nm, but after addition of NaBH_4 to it, a red shift was observed at $\lambda_{\text{max}} = 400$ nm as a result of the formation of phenolate anions [21,22]. The dried RAE-based AgNps from *B. amplexicaule* were employed against the reduction of phenolate anions were studied.

The reduction of 4-NP in the presence of AgNps and NaBH_4 is shown in Figure 3a. 0.5 mL of 1 M NaBH_4 solution was mixed with 2.5 mL of 0.1 mM 4-NP, which leads to the conversion of 4-NP its phenolate anions. Then, 10 mg of the dried RAE-based AgNps was added to the cuvette; the conversion to 4-NP to 4-aminophenol (4-AP) by the AgNps is shown by a decrease in the intensity of phenolate anion peak at 400 nm and an increase in the 4-AP peak at 290 nm, as shown in Figure 3a. Also, similar experiments were repeated but without AgNps (4-NP + NaBH_4); the results of these experiments clearly indicate a negligible decrease in the intensities after the same time periods, as indicated in Figure 3b. Figure 3c shows a decrease in the phenolate anion concentration at 400 nm and the effect of the AgNps (catalyst) on the overall reduction of phenolate anions compared with the uncatalyzed reaction, as shown in Figure 3c. The percentage reduction rates of the phenolate anions are depicted in Figure 3d, which shows that approximately 80% of the phenolate anions were

reduced to 4-AP in 6 min, but no change was observed in the uncatalyzed (without AgNps) mixture.

3.4 Antibacterial activity of the AgNps

These plant-based AgNps from RAE of *B. amplexicaule* were found to display good activity, inhibiting the growth and killing of the pathogenic *E. coli*, *S. aureus*, and MRSA. The bactericidal effects of these Nps are of great concern due to their pharmacological and environmental uses. The prepared AgNps were found to kill *E. coli*, *S. aureus*, and MRSA by 100% after 12 and 24 h incubation, as shown in Figure S3 and Table 1, respectively. The efficient bactericidal effect exhibited by the Nps may be as a result of their specific charge interaction with peptidoglycan of the bacterial cell wall, which caused *S. aureus* membrane disruption [23].

The binding of the AgNps may lead to cellular leakage due to the induced process of pit formation [24]. It was also reported that Nps binding may cause cell death through the inhibition of respiratory chain dehydrogenase enzyme. Some membrane proteins and phospholipids interact specifically with AgNps by the virtue of their opposite charges, which also contributes

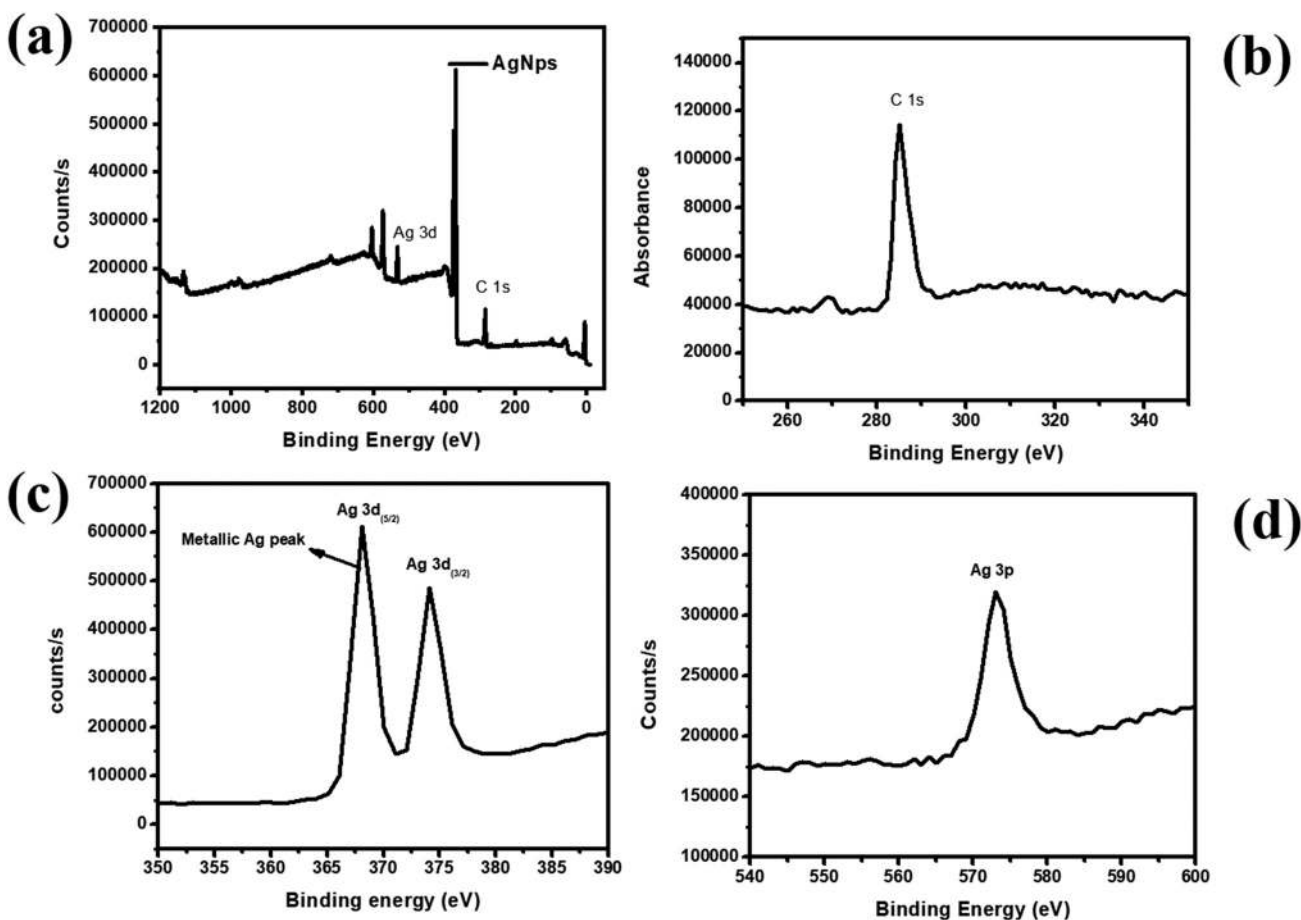


Figure 2: XPS survey scan spectrum of the synthesized AgNps (a), C 1s (b), Ag 3d (c), and Ag 3p (d).

to the eventual cell death due to membrane disruption and decomposition [23].

3.5 Antifungal activity of the REA-based AgNps

The fungicidal effects of the RAE and its AgNps of *B. amplexicaule* were studied on the fungal strains *C. albicans* and *C. tropicalis* using the CFU method. The management of fungal infections in humans and animals is of great concern globally, and numerous efforts are put in place for the formulation or development of effective or efficient and safe antifungal agents [25]. The results of these work are depicted in Figure S4 and Table 2, which clearly reveal that the RAE-based AgNps were very effective and efficient in retarding the growth and killing of the pathogenic *C. albicans* and *C. tropicalis*. Since after incubation of the fungi with the Nps for 12 and 24 h, 100% of the organisms were killed. These results were

in line with the fact that silver ions bind to the DNA of the organisms and alter their replication capabilities [26], which is due to the inactivation of the expression of the ribosomal subunits of proteins and other cellular proteins in addition to the enzymes that are essential for ATP synthesis [26].

The results are also supported by the fact that the positive charge of Ag^+ is attracted by the membrane and creates a pit, which alters the function of membrane-bound enzymes that are central to respiration [27]. This shows that the effective antifungal activities of the AgNps synthesized in this study was probably through the destruction of the membrane integrity.

4 Conclusion

In conclusion, the AgNps synthesized from RAE of *B. amplexicaule* were characterized physically with FESEM, EDS, XRD, and XPS studies and biologically assessed on pathogenic bacteria and fungi of clinical

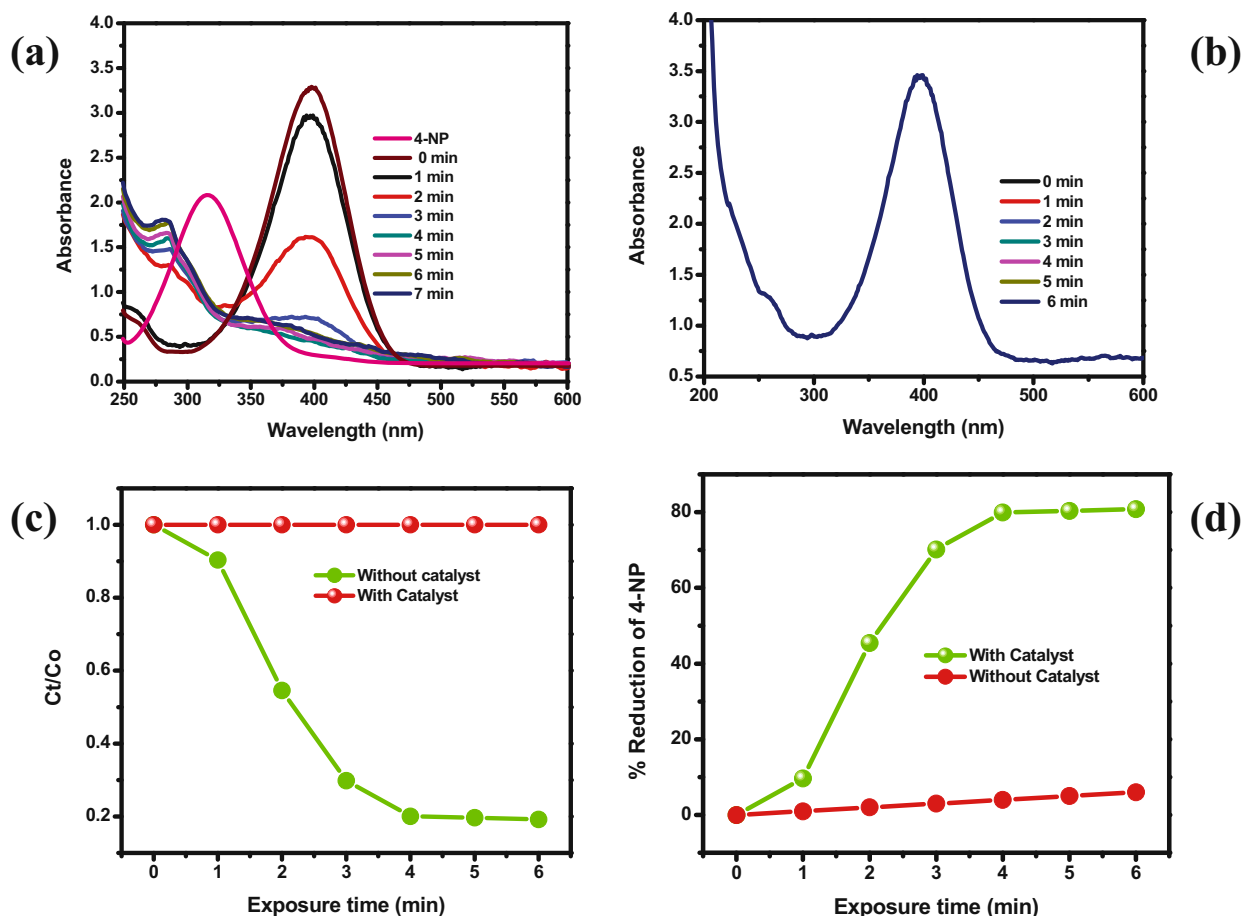


Figure 3: The UV-vis graphs for the reduction of 4-NP (a) with AgNps and (b) without AgNps. (c) C_t/C_0 kinetics for the reduction of 4-NP and (d) % reduction for 4-NP.

Table 1: Growth condition and percentage inhibition of *E. coli*, *S. aureus*, and MRSA treated with RAE and AgNps

S. no.	Sample	Incubation time (h)	<i>E. coli</i>		<i>S. aureus</i>		MRSA	
			Cfu/mL	% reduction	Cfu/mL	% reduction	Cfu/mL	% reduction
1	RAE	12	90×10^6	0	95×10^6	0	88×10^6	0
		24	155×10^6	0	170×10^6	0	160×10^6	0
2	AgNps	12	0×10^6	100	0×10^6	100	0×10^6	100
		24	0×10^6	100	0×10^6	100	0×10^6	100

Table 2: Growth condition and percentage inhibition of *C. albicans* and *C. tropicalis* treated with RAE and AgNps

S. no.	Sample	Incubation time (h)	<i>C. albicans</i>		<i>C. tropicalis</i>	
			Cfu/mL	% reduction	Cfu/mL	% reduction
1	RAE	12	65×10^6	0	70×10^6	0
		24	155×10^6	0	135×10^6	0
2	AgNps	12	0×10^6	100	0×10^6	100
		24	0×10^6	100	0×10^6	100

Cfu = colony forming unit; RAE = rhizome aqueous extract.

importance. The results of this study confirmed that AgNps were grown in stable form because the XPS and XRD data indicated no oxide peak formation. This stable form possibly due to the inherent secondary metabolites. This material not only removed the toxic carcinogenic pollutants but also inhibited the bacterial and fungal growth. Therefore, the RAE-based AgNps synthesized may have potential clinical application in the treatment of some fungal and bacterial infections and in industries for treatment and purification of municipal water.

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Conflicts of interest: The authors confirm that they have no conflicts of interest in this work.

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