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Encapsulated enhanced silver nanoparticles biosynthesis by modified new route for nano-biocatalytic activity

N. Sripriya[a], S. Vasantharaj[b], U. Mani[c], M. Shanmugavel[a,*], R. Jayasree[d], A. Gnanamani[a]

[a] Biological Material Laboratory, CSIR-Central Leather Research Institute (CLRI), Chennai, 600020, TN, India
[b] Dept. of Biotechnology, Hindusthan College of Arts and Science, Coimbatore, 641028, TN, India
[c] Biotechnology Division, CSIR-Central Leather Research Institute (CLRI), Chennai, 600020, TN, India
[d] Department of Biotechnology, Rajalakshmi Engineering College, Chennai, TN, India

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ABSTRACT

The study aims to investigate the green synthesis of silver nanoparticles (AgNPs) by *Phyllanthus acidus* extract (leaf and twig) as reducing and capping agents and evaluation of their bionanocatalytic activities for the first time. The synthesized AgNPs were characterized by UV-Visible spectrophotometry, Fourier Transform Infrared spectroscopy (FT-IR), Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS) and Differential Scanning Calorimetry (DSC). The antioxidant ability of plant extract and AgNPs was analyzed using DPPH*, NO* and OH*scavenging assays. SEM and DLS analysis confirmed the morphology of the nanoparticles to be spherical and the average size to be 48.36 and 164.30 nm. The most efficient scavenger was LAgNPs with EC_{50} of 58.83 ± 1.65 μg/ml in DPPH* and TAgNPs in NO* (EC_{50} 60.75 ± 1.59 μg/ml) and OH* (EC_{50} 43.07 ± 1.62 μg/ml) scavenging assays. Further, they have enhanced the enzyme activity of α-amylase, cellulase, and xylanase over 2–6 folds. The results suggest that the Phytofabricated AgNPs from *P. acidus* can be exploited for industrial and biomedical applications.

1. Introduction

Nanoparticles have significant role in pharmaceutical, industrial and biotechnological applications. In recent years, nanoparticles and nanomaterials are used for different purposes, which includes targeted drug delivery systems, tumor imaging, diagnosis, cosmetics and biosensor (Mubarak Ali et al., 2011; Jeyaraj et al., 2013; Gopinath et al., 2016). Physical, chemical and biological approaches are employed for the synthesis of metal nanoparticles. The synthesis of nanoparticles through chemical and physical methods requires high pressure, energy, temperature and toxic chemicals. In this regard, green synthesis of metallic nanoparticles is of interest, primarily due to their ease of synthesis, eco-friendly approach and cost-effectiveness. In recent years, synthesis of AgNPs facilitated by phytocompounds is gaining significance due to their availability and eco-friendliness (Rout, 2012). Biosynthesis of AgNPs using plant sources such as *Eucalyptus chapmaniana* (Sulaiman et al., 2013), *Momordica charantia* (Ajitha et al., 2015), *Terminalia bellirica* (Patil et al., 2017), *Cochlospermum religiosum* (Sasikala et al., 2015) and many more have been reported. Silver nanoparticles (AgNPs) are proven to have potential antioxidant, antibacterial, antifungal, antiplasmodial and larvicidal properties (Abdel Aziz et al., 2014; Bharathi et al., 2018a; Saxena et al., 2010; Elumalai et al., 2010). Antimicrobial activity of green synthesized AgNPs allows them to use in water filtration process, textiles and food industries (Van Dong et al., 2012; Balashanmugam and Kalaichelvan 2015; Sathiyavimal et al., 2018; Vasantharaj et al., 2019). AgNPs as amperometric biosensor using fungal enzyme can be one of the application for healthcare applications (Subrahmanyam et al., 2001). On the other hand, enhanced catalytic activity of NPs is already in use in different fields, such as urea sensing (Manikandan et al., 2017), glucose sensing, biodiesel production (Xie and Ma., 2010) and controlling blood coagulation (Sanfins et al., 2014). In healthcare, AgNPs can act as potential drug delivery systems as such (Kolanthai et al., 2017) or with appropriate polymeric nano carriers (Ranganathan et al., 2018) and for the treatment of cancer (Khargonekar et al., 2017; Nandagopal et al., 2016).

In the present study, for the biosynthesis of AgNPs, the leaves and twigs of *Phyllanthus acidus* (*P.acidus*) belonging to the family of Euphorbiaceae is used. The plant is abundantly found in Asian countries and their leaves are used to treat psoriasis (Burkill and Hanif, 2002), cough, asthma, bronchitis, soles (Caius, 1986), rheumatism, skin disorders (Morton and Miami 1987), hypertension, respiratory illness

* Corresponding author.
E-mail addresses: shanmugavel_m_2001@yahoo.com, shanmugavel@clri.res.in (M. Shanmugavel).

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(Sousa et al., 2007), diabetics (Banik et al., 2010) and for hepatoprotection (Lee et al., 2006). In traditional medicine, the leaves, bark and the root of the tree are used to treat fever (Hadi and Bremner, 2001; Jain and Singhai, 2011), as antimicrobial (Jagessar et al., 2008) and nephroprotective (Vidya et al., 2013) agents. However, no reports exist related to synthesis of AgNPs from different parts of *P. acidus*. We are reporting for the first time on the biosynthesis of AgNPs from leaf and twig extract of *P. acidus*. We have demonstrated the phytochemical screening of aqueous leaf and twig extract, biosynthesis of AgNPs, characterization of the NPs and studied their free radical scavenging and bionanocatalytic activities.

2. Materials and methods

2.1. Collection of plant materials

The leaves and twigs of *P. acidus* were collected from CSIR-Central Leather Research Institute, Chennai, Tamil Nadu, India. The collected samples were washed with double distilled water to remove dust particles and air dried under shade condition.

2.2. Preparation of aqueous extract

5 g of powdered leaves was extracted with 100 ml of water at 60–70 °C using Soxhlet extractor, to obtain the aqueous extract of *P. acidus* (leaves and twigs) until the extract was clear. The extract was further filtered using WhatmanNo.1, and the filtrate was refrigerated (4 °C) until further use.

2.3. Qualitative phytochemical screening

Phytochemical screening of leaves and twigs extracts was performed for the detection of different phytocompounds such as tannins, flavonoids, saponins, alkaloids, quinones, coumarins, terpenoids and cardiac glycosides. All tests were performed according to the standard methods described by Al-Owaisi et al. (2014) and Sheel et al. (2014).

2.4. Synthesis of AgNPs

To synthesize AgNPs from *P. acidus*, 10 ml of aqueous extract was added to 90 ml of 1 mM $AgNO_3$ solution at room temperature and maintained in the dark. The silver nanoparticles using leaf extract (LAgNPs) and the twig extract (TAgNPs) were primarily checked for their plasmon resonance by UV-Visible spectrophotometer (Shimadzu UV 2450).

2.5. Antioxidant activity

The antioxidant activity of plant extracts and AgNPs was evaluated by 2,2-Diphenyl-1-picryl hydrazyl (DPPH) (Udaya Prakash et al., 2014), nitric oxide (NO) (Jagetia et al., 2004) and hydroxyl (OH) radical scavenging assays (Saeed et al., 2012). Different concentrations (50–250 µg/ml) of the NPs were studied against the radicals. The percent inhibition and effective concentration-50 (EC_{50}) were recorded.

2.6. Characterization of the synthesized AgNPs

The phytofabricated AgNPs were characterized by different analytical methods, viz., Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) (Phenom Pro), (JASCO FT-IR 4700), Dynamic light scattering analysis (DLS) (Malvern Instruments Ltd., Malvern, UK) and Differential scanning calorimetry (DSC, TA Instruments, model Q200).

2.7. Bio-nanocatalysis

The bionanocatalytic activity of α-amylase [EC 3.2.1.1], cellulase [EC 3.2.1.4] and xylanase [EC 253-439-7] was studied by DNS method using the substrates starch, cellulose and xylan respectively (Dastjerdi et al., 2015; Worthington and Worthington. 2011). Briefly, to 200 µg of the enzyme, equal concentration of the plant extract and the NPs were added. The substrate concentration was varied from 100 to 500 µg/ml. The substrate, enzyme and the NPs were allowed to react for 10 min at room temperature, after which 25 µl of DNS reagent was added and maintained at 40 °C for 10 min. The volume was made up to 300 ìl using distilled water and the absorbance was measured at 540 nm. The enzyme activity was measured in Units/µg from the formula:

$$\text{Units}/\mu g = \frac{\text{Monosaccharide released } (\mu g)}{\text{Enzyme added } (\mu g) \times \text{Incubation time}}$$

3. Results and discussion

3.1. Qualitative phytochemical screening

The experimental outcomes of the qualitative phytochemical screening of leaf and twig extracts of *P. acidus* are shown in Table 1. Phytochemical screening showed the presence of tannins, flavonoids, saponins, alkaloids, coumarins, terpenoids and cardiac glycosides in leaf extracts and flavonoids and alkaloids in twig extracts. Plant phenolics are known to possess high reducing potential (Pietta, 2000), which can be utilized for the reduction process in synthesis of AgNPs (Martinez-Castanon et al., 2008).

3.2. Biosynthesis of AgNPs

The aqueous extract of *P. acidus* (leaf and twig) was added to the $AgNO_3$ solution, and the color of the reaction mixture turned from colorless to dark brown which indicated the formation of AgNPs (Vanaja et al., 2013). The formation of AgNPs was confirmed by the surface plasmon resonance and the peaks were observed at 433 and 465 nm respectively for the AgNPs synthesized from the leaf extract (LAgNPs) and twig extract (TAgNPs) (Fig. 1). The mechanism of synthesis of AgNPs is attributed to the presence of the phytoconstituents, which is known to play a significant role in the formation, capping, and stabilization of AgNPs (Bharathi et al., 2018b).

3.3. Materials characterization

The synthesized AgNPs showed several peaks at 3440 cm^{-1}, 1637 cm^{-1}, 1370 cm^{-1} and 598 cm^{-1} were assigned to stretch vibrations of O—H of alcohol, N—H of primary amines, C—H of alkanes and C—Br of alkylhalides which indicates the complex nature of the phytochemicals present in the plant extract (Venugopal et al., 2017a,b) (Fig. 2). The shape and morphology of the AgNPs were analyzed by

Table 1
Phytochemical screening of *P. acidus* leaf and twig extract.

Phytochemical	*P. acidus*	
	Leaves	Twigs
Tannins	+	–
Flavonoids	+	+
Saponins	+	–
Alkaloids	+	+
Quinones	–	–
Coumarins	+	–
Terpenoids	+	–
Cardiac glycosides	+	–

+ Presence - Absence.

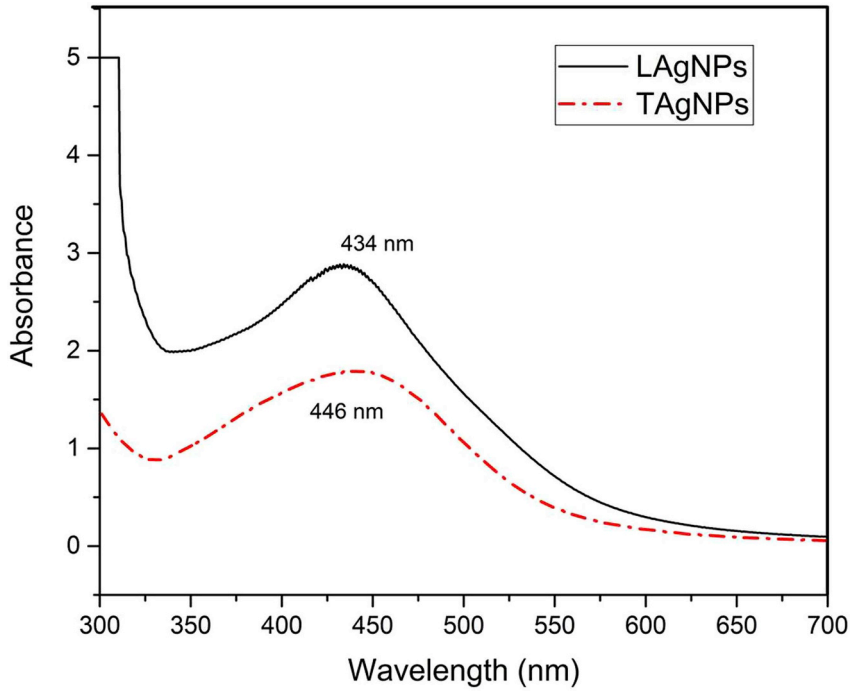

Fig. 1. UV–Visible spectrum of AgNPs synthesized using leaf and twig extracts of *P. acidus*.

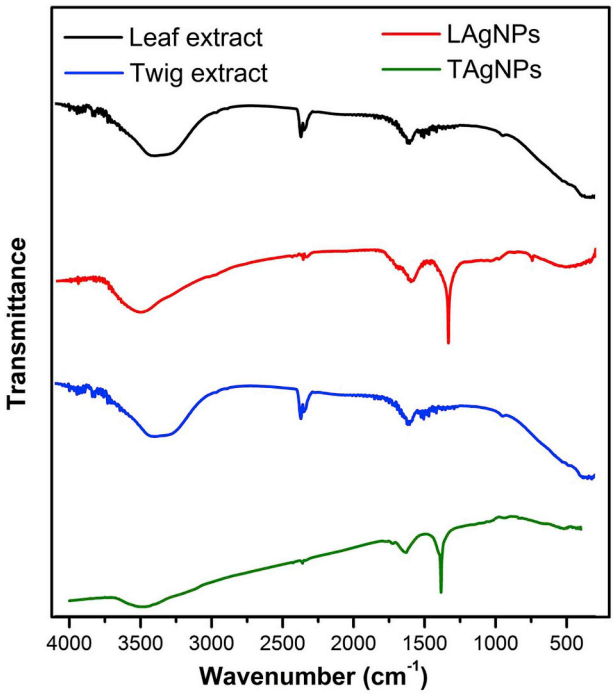

Fig. 2. FTIR spectrum of AgNPs synthesized using leaf and twig extracts of *P. acidus*.

SEM and the particles are in the shape of disc like spheres in the range of 75 and 112 nm in size (Fig. 3). The average size and distribution of AgNPs using DLS analysis were 48.36 and 164.30 nm (Fig. 4) with a negative charge of zeta potential as −7.97 and −22.4 respectively (Fig. 5). According to the DSC curve, the AgNPs synthesized showed an endothermic peak at 101.53 and 104.94 °C (Fig. 6). This suggests that phytocompounds responsible for the reduction of nanoparticles possess low thermal stability. Nanoparticles synthesized using the aqueous extract of *R. tuberosa* has also shown similar results (Vasantharaj et al., 2018).

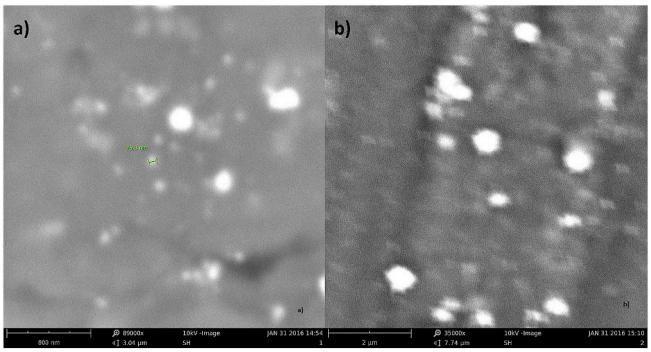

Fig. 3. SEM images of AgNPs synthesized using. (a) Leaf and (b) twig extracts of *P. acidus*.

3.4. Antioxidant activity

In the present study, the free radical scavenging ability of the synthesized NPs was better than the extracts as assessed by DPPH, NO and OH radical scavenging assays. The most efficient scavenger was LAgNPs with EC_{50} of 58.83 ± 1.65 µg/ml in DPPH and TAgNPs in NO (EC_{50} 60.75 ± 1.59 µg/ml) and OH (EC_{50} 43.07 ± 1.62 µg/ml) radical scavenging assays. The EC_{50} values recorded for the DPPH, NO and OH radical scavenging assays are represented in Table 2. Compounds capable of inhibiting radicals are known to have beneficial effects on some aspect of inflammation and tissue damage. The NPs are found to possess high activity in scavenging DPPH, NO and OH radicals which might be due to the presence of antioxidant ligands of the plant extracts, on the NPs.

3.5. Bio-nanocatalytic activity

The phytofabricated AgNPs considerably enhanced the activity of α-amylase, cellulase and xylanase (Table 3). Among the three enzymes studied, the combination of α-amylase with AgNPs showed enhanced activity (1.0 U/µg LAgNPs, 0.992 U/µg TAgNPs and enzyme alone 0.18 U/µg) (Fig. 7). The dramatic increase of enzyme activity may be due to

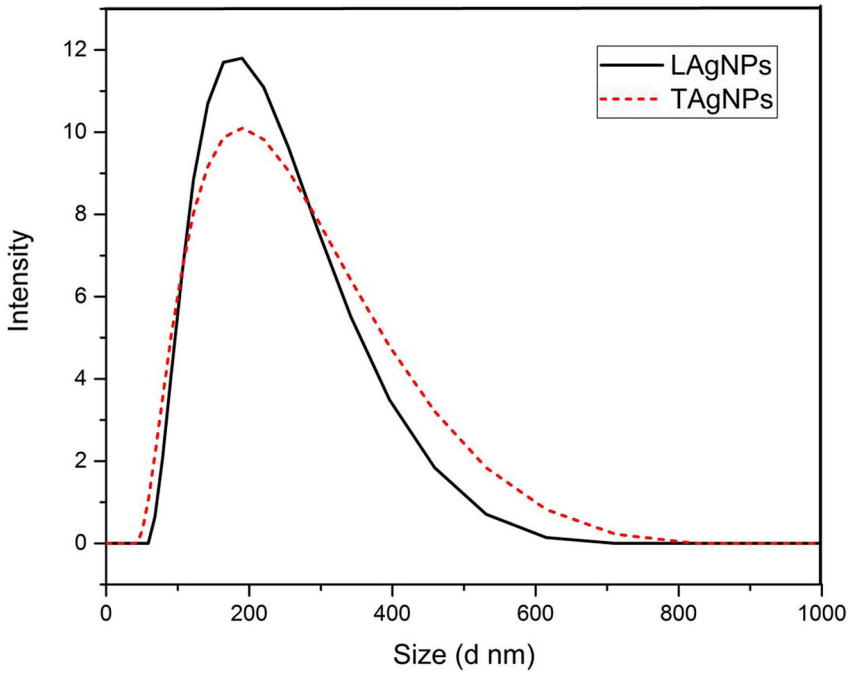

Fig. 4. Particle size distribution AgNPs synthesized using leaf and twig extracts of *P. acidus*.

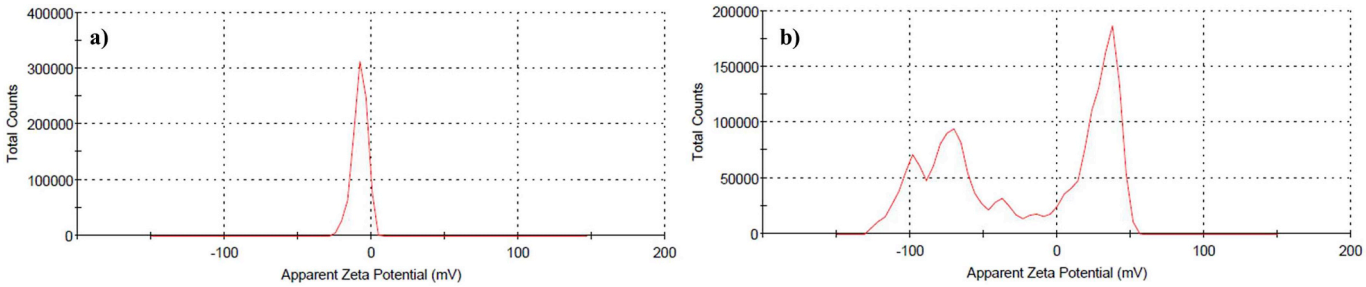

Fig. 5. Zeta potential of AgNPs synthesized using (a) leaf and (b) twig extracts of *P. acidus*.

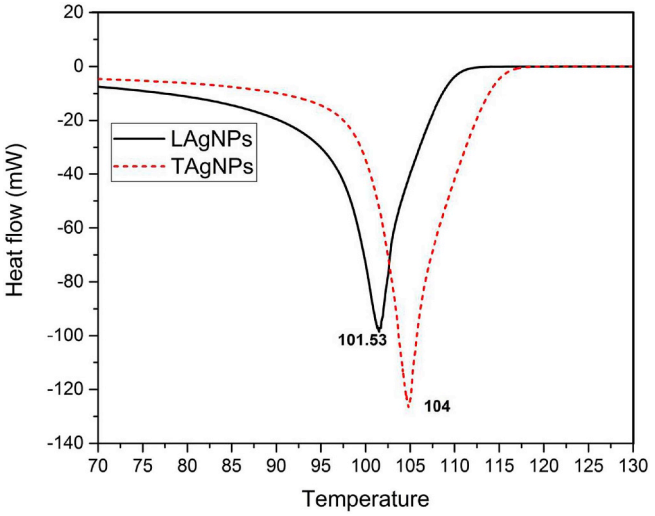

Fig. 6. DSC curve of AgNPs synthesized using leaf and twig extracts of *P. acidus*.

the conjugation of multiple sites of the enzymes with the NPs. Previously, the enhanced biocatalytic activity was observed in protease, α-amylase and lipase using various nano-composites (Jin et al., 2012; Wang et al., 2013; Jia et al., 2003; Wu et al., 2008, 2011; Dumri and Hung Anh, 2014). The enhanced activity of enzyme-NP conjugate in the

Table 2
Antioxidant activity of plant extract and AgNPs.

	DPPH*	NO*	OH*
L extract	128.09 ± 1.87	86.03 ± 1.87	188.65 ± 1.97
LAgNPs	58.83 ± 1.65	81.59 ± 1.65	80.66 ± 1.71
T extract	127.2 ± 1.59	76.43 ± 1.5	79.94 ± 1.81
TAgNPs	82.6 ± 1.5	60.75 ± 1.59	43.07 ± 1.62
Standard	BHT	Ascorbic acid	BHA
	107.72 ± 1.62	127.34 ± 1.54	74.87 ± 1.79

Table 3
Effect of nanoparticles on α-amylase, cellulase and xylanase activity.

Substrate concentration (μg/ml)	100	200	300	400	500
α-amylase (200 μg/ml)	0.045	0.074	0.095	0.103	0.180
LAgNPs	0.449	0.599	0.777	0.872	1.005
TAgNPs	0.397	0.517	0.622	0.743	0.992
Cellulase (200 μg/ml)	0.046	0.05	0.067	0.098	0.125
LAgNPs	0.177	0.209	0.271	0.296	0.316
TAgNPs	0.176	0.222	0.297	0.359	0.440
Xylanase (200 μg/ml)	0.070	0.081	0.098	0.119	0.141
LAgNPs	0.459	0.527	0.549	0.588	0.641
TAgNPs	0.514	0.539	0.566	0.610	0.710

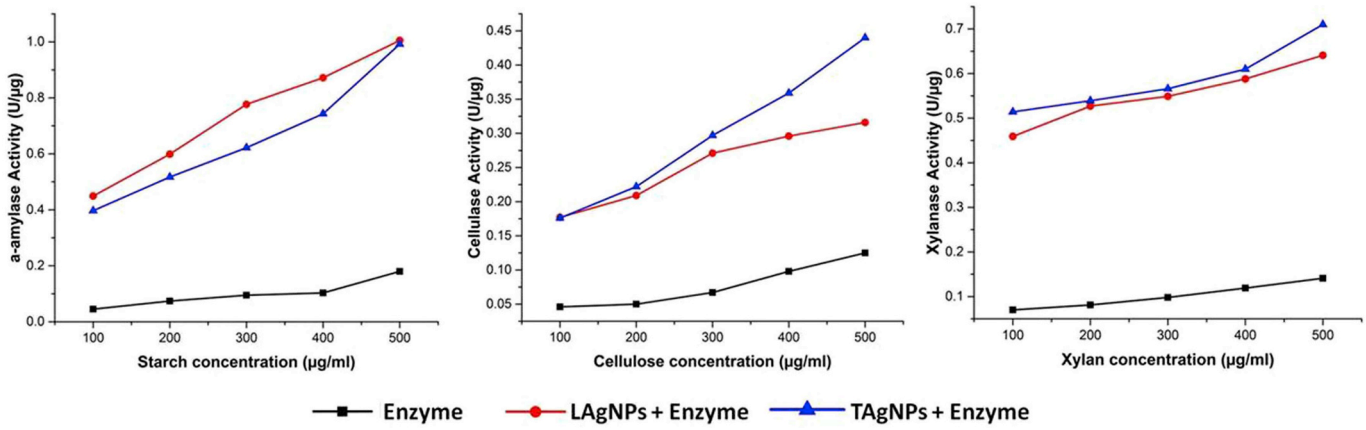

Fig. 7. Bio-nanocatalytic activity of AgNPs on α-amylase, cellulase and xylanase.

present study is still not fully evaluated, but specific characteristics of enzyme density, mass transport, NP morphology, NP surface chemistry, enzyme orientation (Venugopal et al., 2017a,b; Ardao et al., 2012; Ding et al., 2015) and trapping the ions on the cell surface (Zhang et al., 2011) are associated with the enhanced activity. Enzymes such as amylase, lipase and xylanase are widely employed in industries (Fujinami and Fujisawa., 2010). Most of the previous reports deal with the immobilization of enzymes to enhance enzyme activity (Talbert and Goddard., 2012). The present study suggests the use of nanoparticles to enhance the enzyme activity in various industrial sectors.

4. Conclusion

In summary, the study reveals the use of phytocompounds from the leaves and twigs of *P. acidus* as efficient capping and reducing agents for the synthesis of AgNPs. The most efficient scavenger was LAgNPs against DPPH* and TAgNPs against NO* and OH*, with their respective EC_{50} values being 58.83 ± 1.65, 60.75 ± 1.59 and 43.07 ± 1.62 µg/ml. The biosynthesized AgNPs have shown excellent bio-nanocatalytic activities on α-amylase, cellulase and xylanase, as evidenced by an increase of enzyme activity by 2–6 folds when compared with the pure enzymes. The results indicate that the synthesized AgNPs has the potential to act as enhancers for various enzymes in biomedical and industrial applications.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101045.

References

Abdel-Aziz, M.S., Shaheen, M.S., El-Nekeety, A.A., Abdel-Wahhab, M.A., 2014. Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. J. Saudi Chem. Soc. https://doi.org/10.1016/j.jscs.2013.09.011.

Ajitha, B., Reddy, Y.A.K., Reddy, P.S., 2015. Biosynthesis of silver nanoparticles using *Momordica charantia* leaf broth: Evaluation of their innate antimicrobial and catalytic activities. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2015.02.017.

Al-Owaisi, M., Al-Hadiwi, N., Khan, S.A., 2014. GC-MS analysis, determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregrina* (Forssk.) Fiori leaves. Asian Pac. J. Trop. Biomed. https://doi.org/10.12980/APJTB.4.201414B295.

Ardao, I., Comenge, J., Benaiges, M.D., Álvaro, G., Puntes, V.F., 2012. Rational nano-conjugation improves biocatalytic performance of enzymes: Aldol addition catalyzed by immobilized rhamnulose-1-phosphate aldolase. Langmuir. https://doi.org/10.1021/la3003993.

Balashanmugam, P., Kalaichelvan, P.T., 2015. Biosynthesis characterization of silver nanoparticles using *Cassia roxburghii* DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity. Int. J. Nanomed. https://doi.org/10.2147/IJN.S79984.

Banik, G., Bawari, M., Choudhury, M.D., Choudhury, S., Sharma, G.D., 2010. Some Anti-Diabetic Plants of Southern Assam. Assam Univ. J. Sci. Technol. 5 (1), 114–119.

Bharathi, D., Vasantharaj, S., Bhuvaneshwari, V., 2018a. Green synthesis of silver nanoparticles using *Cordia dichotoma* fruit extract and its enhanced antibacterial, anti-biofilm and photo catalytic activity. Mater. Res. Express. https://doi.org/10.1088/2053-1591/aac2ef.

Bharathi, D., Josebin, M.D., Vasantharaj, S., Bhuvaneshwari, V., 2018b. Biosynthesis of silver nanoparticles using stem bark extracts of *Diospyros montana* and their anti-oxidant and antibacterial activities. J. Nanostructure. Chem. 8 (1), 83–92. https://doi.org/10.1007/s40097-018-0256-7.

Burkill, I.H., Hanif, M., 2002. A Botanical and Linguistic Update of Malaya Village.

Caius, J.F., 1986. The medicinal and poisonous plants of India.

Dastjerdi, Z.M., Namjoyan, F., Ebrahim Azemi, M., 2015. Alpha Amylase Inhibition Activity of Some Plants Extract of *Teucrium* Species. Eur. J. Biol. Sci. https://doi.org/10.5829/idosi.ejbs.2015.7.01.91127.

Ding, S., Cargill, A.A., Medintz, I.L., Claussen, J.C., 2015. Increasing the activity of immobilized enzymes with nanoparticle conjugation. Curr. Opin. Biotechnol. https://doi.org/10.1016/j.copbio.2015.04.005.

Dumri, K., Hung Anh, D., 2014. Immobilization of Lipase on Silver Nanoparticles via Adhesive Polydopamine for Biodiesel Production. Enzym. Res. https://doi.org/10.1155/2014/389739.

Elumalai, E.K., Prasad, T.N.V.K.V., Hemachandran, J., Viviyan Therasa, S., Thirumalai, T., David, E., 2010. Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. J. Pharmaceut. Sci. Res. https://doi.org/10.1080/1091581029016990.

Fujinami, S., Fujisawa, M., 2010. Industrial applications of alkaliphiles and their enzymes - Past, present and future. Environ. Technol. https://doi.org/10.1080/09593331003762807.

Gopinath, V., Priyadarshini, S., MubarakAli, D., Mun Fai Loke Thajuddin, N., Alharbi, Naiyf S., Yadavalli, Tejabhiram, Alagiri, M., Vadivelu, J., 2016. Anti-*Helicobacter pylori*, cytotoxicity and catalytic activity of biosynthesized gold nanoparticles: Multifaceted application. Arabian Journal of Chemistry. https://doi.org/10.1016/j.arabjc.2016.02.005.

Hadi, S., Bremner, J.B., 2001. Initial studies on alkaloids from Lombok medicinal plants. In: Molecules, . https://doi.org/10.3390/60100117.

Jagessar, R.C., Mars, A., Gomes, G., 2008. Selective Antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. Nat. Sci. 6 (2), 24–38.

Jagetia, G.C., Rao, S.K., Baliga, M.S., Babu, K.S., 2004. The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: A preliminary study. Phyther. Res. https://doi.org/10.1002/ptr.1494.

Jain, N.K., Singhai, A.K., 2011. Protective effects of Phyllanthus acidus (L.) Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pac. J. Trop. Med. https://doi.org/10.1016/S1995-7645(11)60128-4.

Jeyaraj, M., Rajesh, M., Arun, R., MubarakAli, D., Sathish kumar, G., Sivanandhan, G., KapilDev, G., Manickavasagam, M., Premkumar, K., Thajuddin, N., Gnanapathi, A., 2013. An investigation on the cytotoxicity and caspase-mediated apoptotic effect of biologically synthesized silver nanoparticles using *Podophyllum hexandrum* on human cervical carcinoma cells. Colloids Surfaces B Biointerfaces. https://doi.org/10.1016/j.colsurfb.2012.09.042.

Jia, H., Zhu, G., Wang, P., 2003. Catalytic Behaviors of Enzymes Attached to Nanoparticles: The Effect of Particle Mobility. Biotechnol. Bioeng. https://doi.org/10.1002/bit.10781.

Jin, L.L., Yang, K., Yao, K., Zhang, S., Tao, H.Q., Lee, S.T., Liu, Z., Peng, R., 2012. Functionalized Graphene Oxide in Enzyme Engineering: A Selective Modulator for Enzyme Activity and Thermostability. ACS Nano. https://doi.org/10.1021/nn300217z.

Khargonekar, P., Sinskey, A., Miller, C., Ranganathan, B., 2017. Convergence Revolution Piloting the Third Scientific Revolution through Start-Ups for Breast Cancer Cure. Cancer Science and Research: Open Access 4 (1), 1–6.

Kolanthai, E., Abinaya Sindu, P., Thanigai Arul, K., Sarath Chandra, V., Manikandan, E.,

Narayana Kalkura, S., 2017. Agarose encapsulated mesoporous carbonated hydroxyapatite nanocomposites powder for drug delivery. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2016.12.005.

Lee, C.-Y., Peng, W.-H., Cheng, H.-Y., Chen, F.-N., Lai, M.-T., Chiu, T.-H., 2006. Hepatoprotective Effect of *Phyllanthus* in Taiwan on Acute Liver Damage Induced by Carbon Tetrachloride. Am. J. Chin. Med. https://doi.org/10.1142/S0192415X06004004.

Manikandan, A., Manikandan, E., Meenatchi, B., Vadivel, S., Jaganathan, S.K., Ladchumananandasivam, R., Henini, M., Maaza, M., Aanand, J.S., 2017. Rare earth element (REE) lanthanum doped zinc oxide (La: ZnO) nanomaterials: Synthesis structural optical and antibacterial studies. J. Alloy. Comp. https://doi.org/10.1016/j.jallcom.2017.06.336.

Martinez-Castanon, G.A., Niño-Martínez, N., Martínez-Gutierrez, F., Martínez-Mendoza, J.R., Ruiz, F., 2008. Synthesis and antibacterial activity of silver nanoparticles with different sizes. J. Nanoparticle Res. https://doi.org/10.1007/s11051-008-9428-6.

Morton, J.F., Miami, F.L., 1987. Otaheite gooseberry.Fruits of warm climates. Julia F. Morton, Miami, pp. 217–219.

MubarakAli, D., Thajuddin, N., Jegananthan, K., Gunasekaran, M., 2011. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens Colloids and Surfaces B: Biointerfaces. https://doi.org/10.1016/j.colsurfb.2011.03.009.

Nandagopal, G.D., Periyathambi, P., Sastry, T.P., 2016. Fabrication of a multifunctional nanocomposite containing INP coated with chitosan coupled with folic acid and loaded with quercetin in diagnosis and its possible treatment of cancer. RSC Adv. https://doi.org/10.1039/c6ra19901a.

Patil, S., Chaudhari, G., Paradeshi, J., Mahajan, R., Chaudhari, B.L., 2017. Instant green synthesis of silver-based herbo-metallic colloidal nanosuspension in Terminalia bellirica fruit aqueous extract for catalytic and antibacterial applications. 3 Biotech. https://doi.org/10.1007/s13205-016-0589-1.

Pietta, P.G., 2000. Flavonoids as antioxidants. J. Nat. Prod. https://doi.org/10.1021/np9904509.

Ranganathan, B., Miller, C., Sinskey, A., 2018. Biocompatible Synthetic and Semi-synthetic Polymers - A Patent Analysis. Pharm. Nanotechnol. https://doi.org/10.2174/2211738505666171023152549.

Rout, Y., 2012. Green synthesis of silver nanoparticles using *Ocimum sanctum* (Tulashi) and study of their antibacterial and antifungal activities. J. Microbiol. Antimicrob. https://doi.org/10.5897/JMA11.060.

Saeed, N., Khan, M.R., Shabbir, M., 2012. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. BMC Complement Altern. Med. https://doi.org/10.1186/1472-6882-12-221.

Sanfins, E., Augustsson, C., Dahlbäck, B., Linse, S., Cedervall, T., 2014. Size-dependent effects of nanoparticles on enzymes in the blood coagulation cascade. Nano Lett. https://doi.org/10.1021/nl501863u.

Sasikala, A., Linga Rao, M., Savithramma, N., Prasad, T.N.V.K.V., 2015. Synthesis of silver nanoparticles from stem bark of *Cochlospermum religiosum* (L.) Alston: an important medicinal plant and evaluation of their antimicrobial efficacy. Appl. Nanosci. https://doi.org/10.1007/s13204-014-0380-8.

Sathiyavimal, S., Vasantharaj, S., Bharathi, D., Saravanan, M., Manikandan, E., Kumar, S.S., Pugazhendhi, A., 2018. Biogenesis of copper oxide nanoparticles (CuONPs) using *Sida acuta* and their incorporation over cotton fabrics to prevent the pathogenicity of Gram negative and Gram positive bacteria. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2018.09.014.

Saxena, A., Tripathi, R.M., Singh, R.P., 2010. Biological synthesis of silver nanoparticles by using onion (*Allium cepa*) extract and their antibacterial activity. Dig. J. Nanomater. Biostructures. https://doi.org/10.5829/idosi.wasj.2015.33.06.9525.

Sheel, D.R., Nisha, K., Kumar, P.J., 2014. Preliminary Phytochemical Screening of Methanolic Extract Of *Clerodendron infortunatum*. IOSR J. Appl. Chem. https://doi.org/10.9790/5736-07121013.

Sousa, M., Ousingsawat, J., Seitz, R., Puntheeranurak, S., Regalado, A., Schmidt, A., Grego, T., Jansakul, C., Amaral, M.D., Schreiber, R., Kunzelmann, K., 2007. An Extract from the Medicinal Plant *Phyllanthus acidus* and Its Isolated Compounds Induce Airway Chloride Secretion: A Potential Treatment for Cystic Fibrosis. Mol. Pharmacol. https://doi.org/10.1124/mol.106.025262.

Subrahmanyam, S., Kodandapani, N., Ahamarshan, J.N., Ranganathan, B., Shanmugam, K., Jeyakumar, D., Subramanian, T.V., 2001. Amperometric biochemical characterization of isolated fungal strains. Electroanalysis. https://doi.org/10.1002/1521-4109(200111)13:17<1454::AID-ELAN1454>3.0.CO;2-R.

Sulaiman, G.M., Mohammed, W.H., Marzoog, T.R., Al-Amiery, A.A.A., Kadhum, A.A.H., Mohamad, A.B., 2013. Green synthesis, antimicrobial and cytotoxic effects of silver nanoparticles using *Eucalyptus chapmaniana* leaves extract. Asian Pac. J. Trop. Biomed. https://doi.org/10.1016/S2221-1691(13)60024-6.

Talbert, J.N., Goddard, J.M., 2012. Enzymes on material surfaces. Colloids and Surfaces B: Biointerfaces. https://doi.org/10.1016/j.colsurfb.2012.01.003.

Udaya Prakash, N.K., Bhuvaneswari, S., Sripriya, N., Prameela, L., Bhagya, R., Radhika, B., Balamurugan, A., Arokiyaraj, S., 2014. Antioxidant activity of common plants of Northern Tamil Nadu, India. Int. J. Pharm. Pharm. Sci. 6 (4), 128–132.

Van Dong, P., Ha, C., Binh, L., Kasbohm, J., 2012. Chemical synthesis and antibacterial activity of novel-shaped silver nanoparticles. Int. Nano Lett. https://doi.org/10.1186/2228-5326-2-9.

Vanaja, M., Gnanajobitha, G., Paulkumar, K., Rajeshkumar, S., Malarkodi, C., Annadurai, G., 2013. Phytosynthesis of silver nanoparticles by *Cissus quadrangularis*: influence of physicochemical factors. J. Nanostructure Chem. https://doi.org/10.1186/2193-8865-3-17.

Vasantharaj, S., Sripriya, N., Shanmugavel, M., Manikandan, E., Gnanamani, A., Senthilkumar, P., 2018. Surface active gold nanoparticles biosynthesis by new approach for bionanocatalytic activity. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2018.01.007.

Vasantharaj, S., Sathiyavimal, S., Senthilkumar, P., LewisOscar, F., Pugazhendhi, A., 2019. Biosynthesis of iron oxide nanoparticles using leaf extract of *Ruellia tuberosa*: Antimicrobial properties and their applications in photocatalytic degradation. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2018.12.025.

Venugopal, K., Ahmad, H., Manikandan, E., Thanigai Arul, K., Kavitha, K., Moodley, M.K., Rajagopal, K., Balabhaskar, R., Bhaskar, M., 2017a. The impact of anticancer activity upon Beta vulgaris extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2017.05.031.

Venugopal, K., Rather, H.A., Rajagopal, K., Shanthi, M.P., Sheriff, K., Illiyas, M., Rather, R.A., Manikandan, E., Uvarajan, S., Bhaskar, M., Maaza, M., 2017b. Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of *Syzygium aromaticum*. J. Photochem. Photobiol. B Biol. https://doi.org/10.1016/j.jphotobiol.2016.12.013.

Vidya, S., Ramesh, A., Rajashekar, G., Meghana, D., Nazeer, S.K., 2013. The nephroprotective activity of methanolic extracts of *Phyllanthus acidus* leaves against gentamycin-induced nephrotoxicity in experimental rodents. Int. J. Pharm. Pharm. Sci. 5 (4), 209–213.

Wang, L.B., Wang, Y.C., He, R., Zhuang, A., Wang, X., Zeng, J., Hou, J.G., 2013. A new nanobiocatalytic system based on allosteric effect with dramatically enhanced enzymatic performance. J. Am. Chem. Soc. https://doi.org/10.1021/ja3120136.

Worthington, K., Worthington, V., 2011. Worthington enzyme manual. Worthington Biochemical Corporation.

Wu, C.S., Wu, C.T., Yang, Y.S., Ko, F.H., 2008. An enzymatic kinetics investigation into the significantly enhanced activity of functionalized gold nanoparticles. Chem. Commun. https://doi.org/10.1039/b810889g.

Wu, C.S., Lee, C.C., Wu, C.T., Yang, Y.S., Ko, F.H., 2011. Size-modulated catalytic activity of enzyme-nanoparticle conjugates: A combined kinetic and theoretical study. Chem. Commun. https://doi.org/10.1039/c1cc11020a.

Xie, W., Ma, N., 2010. Enzymatic transesterification of soybean oil by using immobilized lipase on magnetic nano-particles. Biomass and Bioenergy. https://doi.org/10.1016/j.biombioe.2010.01.034.

Zhang, W., Zhang, Z., Zhang, Y., 2011. The application of carbon nanotubes in target drug delivery systems for cancer therapies. Nanoscale Res. Lett. https://doi.org/10.1186/1556-276X-6-555.