Original Article

SYNTHESIS OF ECOFRIENDLY SILVER NANOPARTICLES USING MANGROVE ASSOCIATE PLANT *PEMPHIS ACIDULA* J.R.FORST & FORST EXTRACT: ANTIOXIDANT AND ANTIINFLAMMATORY STUDIES

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Abstract

The current study is to advance an easy and eco-friendly method for the synthesis of AgNPs using aqueous whole plant extract of mangrove associate plant *Pemphis acidula*. This study also evaluated its antioxidant and antiinflammatory activity. The presence of flavonoids, phenols, terpenoids, saponins, tannins, glycosides and xanthoproteins were settled by the phytochemical analysis of the plant aqueous extract. These chemicals can be used as reducing, stabilizing and capping agents. The PA-AgNPs were characterized by UV-Vis spectrophotometry, XRD, FTIR, SEM and AFM. AgNPs formation was checked by UV-Vis spectra at 428.5 nm. FTIR had shown that the phytochemicals were accountable for the reduction and capping material of silver nanoparticles. The size and shape of the AgNPs were regulated using SEM. The XRD results disclosed a crystalline nature of AgNPs with an average size of 30.24nm. The surface morphology was approved by AFM techniques. The PA-AgNPs had revealed strong antioxidant (DPPH, H_2O_2 scavenging) action. The PA – AgNPs displayed strong antiinflammatory (albumin denaturation, proteinase inhibitory) activity. To our best knowledge, this is the first effort on the synthesis of AgNPs by mangrove associate plant *Pemphis acidula* whole extract. Lastly, it can be accomplished that PA-AgNPs from *Pemphis acidula* whole plant extract showed distinguishing antioxidant and antiinflammatory action.

Key words: Mangrove associate plant, AgNPs, Characterization, Antioxidant, Antiinflammatory.

Introduction

Nanotechnology is an evolving field of scientific research that associates the chemistry, physics, biology and material sciences and catches extensive applications in biotechnology, pharmaceuticals and nano-medicine (Keshari *et al*., 2021). The nanoparticles have the aspects in the range of 1-100 nm. For the reason of their unique properties and the enormous variety of applications, silver nanoparticles (AgNPs) have been extensively studied for decades. The use of microorganisms or plant extracts to manufacture AgNPs has emerged as a worthwhile alternative. They are tranquil to use, cost-operative, provide prodigious yields and are eco-friendly (Vincy *et al*.,

2017; Burange *et al*., 2021). Microorgnaism based AgNPs synthesis is also not preferred since most of the microbes are pathogenic (Bindhani and Panigrahi, 2015) numerous works already reported that plants extracts used for the mixture of AgNPs for example *Cestrum nocturnum* (Keshari *et al*., 2020), *Catharanthus roseus* (Keshari *et al*., 2021), *Aloe vera*, *Thuja orientalis* (Burange *et al*., 2021), *Oldenlandia auricularia* (Manivasan *et al*., 2022), *Lawsonia inermis*, *Olea europaea* (Sabeeh *et al*., 2022), *Citrullus colocynthis* (Rasool *et al*., 2022) and *Passiflora foetida* (Lade and Patil 2022).

Pemphis acidula J.R. Forst & Forst fit into the family Lythraceae, normally known as bantigue or mentigi. It is a mangrove associate plant found during most of the tropical Indo-pacific on rocky shores. An infusion of the bank has been consumed as an abortifacient. The bark holds 19-43% tannins. Bark extracts launches to have antioxidant, antibacterial and topoisomerase Iinhibitor activity (Sivagama Sundari *et al*., 2022). Due to the above said reasons the current work is designed to use *Pemphis acidula* (PA) whole plant extract for the synthesis of AgNPs from silver nitrate and test its antioxidant and antiinflammatory properties was evaluated.

Materials and Methods

Collection of mangrove associate plant

The whole plant of *Pemphis acidula* J.R. Forst & G. Forst (PA) was gathered from Harbour estate, Thoothukudi coast, Gulf Mannar Biosphere Reserve. The gathered mangrove associate plant was recognized with the aid of local flora. The voucher specimen (EPH 366) was acclaimed in the Ethnopharmacology unit, P.G. Research Department of Botany, V.O. Chidambaram College, Thoothukudi.

The collected plant samples were cut into minute fragments. This is shade dried in anticipation of the fracture being uniform and smooth. The dried material was granulated or powdered using blender. Further it is sieved to get uniform powder. The same powder is utilized for the extraction of active constituents of the plant material.

Preparation of Extract for Phytochemical Screening (Cold Maceration Method)

Needed quantity of powder was weighed and transferred to stoppard flask. This is treated with aqueous separately until the powder is fully immersed. The flask was shaken every hour for the first six hours. Then the extract was filtered through Whatman No.1 filter paper. The extract was subjected to qualitative tests for the identification of numerous phytochemical constituents as per standard procedures (Brinda *et al*., 1981; Lala, 1993).

Green Synthesis of Nanoparticles

Preparation of Whole Plant Extract (Reducing Agent)

Newly collected whole plant was washed methodically with double distilled water and cut into fine pieces. For 20 minutes in a glass beaker, twenty gram of fine pieces of whole plant was boiled in 100 ml double distilled water. Succeeding to boiling, the extract was filtered by means of Whatman No. 1.

Preparation of Precursor

Precursors for silver nanoparticle ($AgNO_3$) was purchased from Hi-media chemicals, India and prepared newly. Precursor for preparing silver nanoparticle was 1 mM of silver nitrate by means of double distilled water.

Synthesis of Silver Nanoparticles

Under continuous stirring, ten ml aqueous solution of whole plant extract was slowly added into 20 ml of 1 mM solution of silver nitrate for 20 mins. The solution was set aside warm for 24 hrs at room temperature. Colourless solution is converted into pale yellow colour primarily and after 24 hours colour turns from pale yellow to reddish brown which stipulates the formation of silver nanoparticles. It is displayed that aqueous silver ions could be diminished by aqueous extract of whole plant to generate tremendously stable silver nanoparticles in water. The colloidal solution is then centrifuged at 9000 rpm, supernatant was collected and protected for additional analysis.

Characterization of the Synthesized Silver Nanoparticles

UV – Vis Spectroscopy

Ultraviolet-visible spectroscopy (UV-Vis) means absorption of spectroscopy absorbed in the UV-visible spectral region. The silver nanoparticles were depicted in a Shimadzu V 650 UV- Vis spectrophotometer. The scanning sequences for the samples were 300-700 nm. The double distilled water was employed as a blank reference.

Fourier Transform Infrared Spectroscopy (FTIR)

The nanoparticles were distinguished by means of a Fourier Transform Infrared Spectrophotometer (FTIR Thermo-scientific iS5). Two milligrams of the sample was blended with 100 mg Potassium bromide (KBr). Then, condensed to make a salt disc roughly 3mm in diameter and the disc were unswervingly kept in the sample holder. FTIR spectra were confirmed in the absorption range between 400 and 4000 cm^{-1}.

Scanning Electron Microscope (SEM) Analysis

Morphological analysis of the synthesized AgNPs was carried out by means of scanning electron microscope (SEM) (Carl zeiss Microscopy GmbH, German Model EVO18) prepared with 15kv acceleration voltage.

X-Ray Diffraction (XRD) Analysis

The particle size and nature of the silver nanoparticle were found out by means of XRD. This action was done out by Shimadzu XRD – 6000/6100 model with 30 kv, 30 mA with Cukα radians at 2θ angle. X-ray powder diffraction is a rapid analytical technique mostly used for phase classification of a crystalline material. This can supply information on unit cell dimensions. The analyzed material is excellently ground, and the mean bulk composition is found out. The particle or grain dimension of the particles on the silver nanoparticles was founded by means of Debye Sherrer's equation.

$$D = 0.94 \lambda / B \cos \theta$$

AFM Analysis

Surface topology of the synthesized silver nanoparticles were studied using 1µm x 1µm Atomic Force Microscopy (AFM Nanosurf 2) analysis, 0.01 g synthesized nanoparticles were blended with 20 ml of acetone and sonicated for 5-10 minutes by means of ultrasonicator. The solution was discharged on a clean glass slide. This was allowed to dry till all the acetone gets evaporated. Now this glass slide is studied by means of the Atomic Force Microscopy in a noncontact mode and the captured image was processed by XEI software.

Determination of antioxidant activity

Free radical scavenging ability on 2, 2-diphenyl-2-picrylhydroxyl (DPPH)

To assess the scavenging ability on DPPH, dissimilar concentration of PA-AgNPs / aqueous plant extract / ascorbic acid (12.5, 25, 50, 100 and 200 µg/ml) in water was mixed with 1ml of methanol solution encompassing DPPH radicals (0.2mM). The mixture was shaken dynamically and left to stand for 30 min in the dark previously measuring the absorbance at 517nm against a blank (Braca *et al.,* 2001). Then the scavenging capability was calculated by means of the following equation (1) as agreed below

$$\text{Percent inhibition} = (\text{A blank} - \text{A sample} / \text{A blank}) \times 100 \quad \text{----- (1)}$$

here, 'A blank' is the absorbance of the control reaction (containing all reagents except the test compound) and 'A sample' is the absorbance of test compound.

Hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) scavenging capacity of the synthesized PA-AgNPs / aqueous plant extract / ascorbic acid was defined according to the method (Revathi *et al.,* 2014). A solution of H_2O_2 (40mM) was formulated in phosphate buffer (pH 7.4). Dissimilar concentrations (12.5, 25, 50, 100 and 200 µg/ml) of PA-AgNPs / aqueous plant extract / ascorbic acid in 3.4 ml phosphate buffer were supplemented to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230nm. The percentage of H_2O_2 scavenging activity was computed by means of equation (1) as remarked above.

***In vitro* antiinflammatory activity of PA-AgNPs**

Inhibition of albumin denaturation

The reaction mixture (5ml) consisting of 0.2ml of 1% bovin albumin, 4.78ml of phosphate buffer saline (pH 6.4) and 0.2ml of varying concentration of PA-AgNPs (50, 100, 200, 300, 400, 500µg/ml) was mixed and incubated in a water bath (37°C) for 15 min. Then the reaction mixture was heated at 51^0C for 5 min. After cooling, the turbidity was measured at 660 nm (using a Genesys 10s UV-Vis spectrophotometer). Phosphate buffer solution was employed as the control. Aspirin (Standard drug) was recycled as reference drug as treated as such for the purpose of absorbance. The experiment was performed in triplicate (Sakat *et al.,* 2010; Gunathilake *et al.,* 2018). The percentage inhibition of protein denaturation was computed by using the subsequent equation (2) as given below.

$$\% \text{ inhibition of dentauration} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad \text{---------- (2)}$$

here 'A control' is the absorbance of solution without PA-AgNPs, 'A sample' is the absorbance of solution with PA-AgNPs / standard.

Proteinase inhibitory activity

Proteinase inhibitory activity of the synthesized PA-AgNPs was completed affording to the method of Saket *et al*. (2010), which is adapted by Gunathilake *et al*. (2018). Momentarily the reaction mixture (2ml) consisted of 0.06 mg trypsin, 1 ml of 20mM Tris –HCL buffer (pH7.4) and 1 ml of fluctuating concentration of PA-AgNPs 100, 200, 300, 400 and 500 µg/ml. The mixture was protected (37° C for 5 min) and then 1 ml of 0.8 % (w/v) carein was improved. The mixture was incubated for a supplementary 20 min, 2 ml of 70 % perchloric acid was added to terminate the reaction. Cloudy suspension was separated and the absorbance of supernatant was read at 210 nm contrary to buffer as the blank. Aspirin was the standard drug used. The experiment was repeated thrice. The percentage of inhibition of proteinase inhibitory activity was calculated by means of equation (2) as indicated above.

Results and Discussion

Phytochemical analyzers

The presence of saponins, tannins, terpenoids, flavonoids, phenols, glycosides and xanthoproteins was found through the phytochemical analysis of PA aqueous extract (Table 1). These compounds are assumed to perform as stabilizing and capping agents for green synthesis of PA- AgNPs.

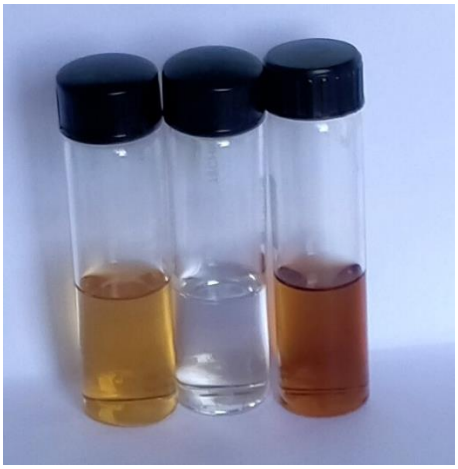
Table 1: Phytochemical screening of aqueous extract of *P. acidula* (PA)

Name of the Phytochemical	**Results**
Alkaloids	-
Anthraquinones	-
Catechins	-
Coumarins	-
Flavonoids	+
Phenols	+
Quinones	-
Saponins	+
Steroids	-
Tannins	+
Terpenoids	+
Sugars	-
Glycosides	+
Xanthoproteis	+

Characteristics of PA-AgNPs

The colour change is an indispensable factor that authorizes the synthesis of AgNPs. When the PA extract was added to the $AgNO_3$, the colour changed from pale yellow to reddish brown (Fig. 1). The existence of active phytochemicals in the extract of PA is responsible in reducing silver metal to AgNPs. This is due to excitation of surface plasmon resonance of synthesized PA-AgNPs. Other studies have reported comparable colour changes from yellow to brown, as a result of surface plasmon vibrations. This confirm the formatting AgNPs (Tanase *et al.*, 2019; Keshari *et al*., 2021).

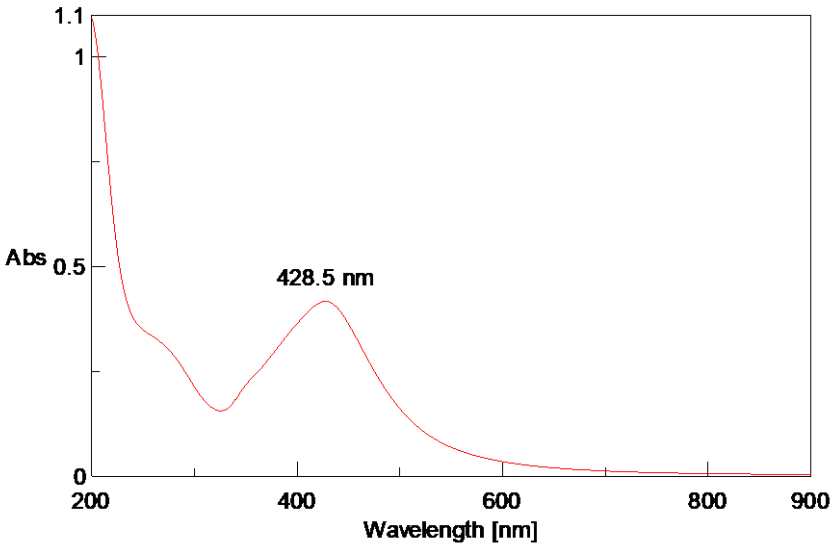
Fig. 1: Synthesis of AgNPs from *P. acidula*



UU-Vis spectrophotometer analysis

Formation of PA-AgNPs was once more confirmed using UV-visible spectral analysis. The characteristic surface plasmon resonance absorption band of biosynthesized PA-AgNPs was attained at 428.5 nm (Fig. 2). Similar observation has been reported in preceding studies showing the absorption maxima of AgNPs at 400, 420, 430, 443 and 441 nm using stem and root extracts of *Lysiloma acapulcensis* (Garibo *et al*., 2020) leaf extract of *Avicennia marina* (Tian *et al*., 2020) flower extracts of *Abelmoschus esculentus* (Devanesan and Al Salhi, 2020), *Aerva lanata* (Palithya *et al*., 2021) and seed extract of *Abrus precatorius* (Santhiya Selvam *et al*., 2022) correspondingly.

Fig. 2: UV-Visible spectra of AgNPs of *P. acidula*



Fourier transform infrared (FTIR) spectroscopy analysis

FTIR measurements were carried out to recognize the possible biomolecules responsible for the reduction of the Ag+ ions and capping of the bioreduced. AgNPs synthesized by PA extract. Figure 3a displays the FTIR spectrum of whole plant powder of PA. The PA exhibits the distinctive peak at 3417 cm^{-1} corresponds to the O-H stretching of alcohols or phenols, peak at 2922 cm^{-1} symbolize C-H stretching of carboxylic acid, peak at 2364 cm^{-1} assigned as NH^{+} stretching of tertiary amines salt, peak at 1618 cm^{-1} approve with >N H bend of secondary amine, peak at 1442 cm^{-1} resembles to the C-C stretching of aromatics, peak at 1383 cm^{-1} assigned as C-H rock of alkanes, peak at 1021cm^{-1} characterize C-N stretching of aliphatic amines, peak at 874 cm^{-1} decide with P-O-C stretching of aromatic phosphates, peak at 788 cm^{-1} characterize C-H "oop" of aromatics and peaks at 620 and 470 cm^{-1} assigned as C-Br stretching of alkyl halides. Figure 3b displays the FTIR spectrum of biosynthesized PA-AgNPs which obviously indicates the characteristic peak at 3142 cm^{-1} characterize the O-H stretching of carboxylic acid, peak at 1607 cm^{-1} corresponds so the > N-H bend of secondary amine, peak at 1400 cm^{-1} decide with C-C stretching of aromatic, peaks at 1197 and 1102 cm^{-1} assigned as C-O stretching esters, ethers, peaks at 820 and 751 cm^{-1} resembles to the C-H "oop" of aromatics and peaks at 661, 616 and 436 cm^{-1} represent the C-Br stretching of alkyl halides. The FTIR results approved the numerous functional groups which are existing on the surface of bioactive compounds. This functional group is accountable for the capping of silver nanoparticles and stable its nano size (Keshari *et al.,* 2020). The slight disparity in the peak for capped PA-AgNPs observed (Table 2) shows the collaboration of biomolecules with the citation in the surface of formulated samples (Tudu *et al.*, 2020).

Fig. 3a: FTIR spectra of *P. acidula*

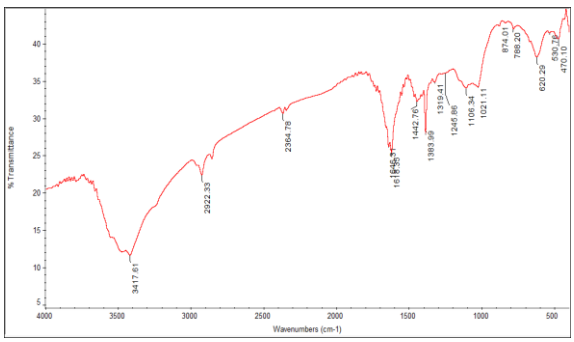


Fig. 3b: FTIR spectra of AgNPs

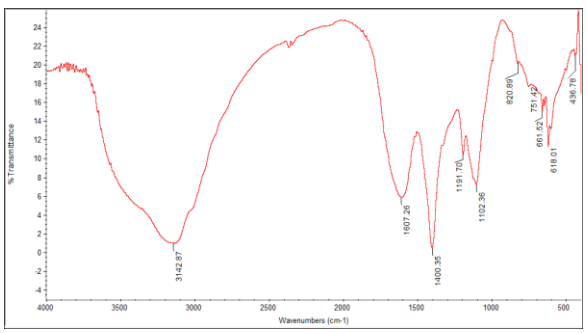


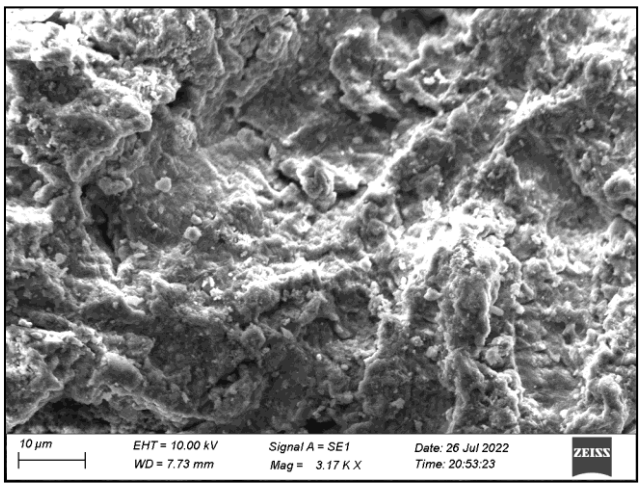
Table 2: FTIR analysis report with probable functional groups in *P. acidula* whole plant powder and synthesized AgNPs

Sl. No.	***P. acidula* powder wave number**	**Probable functional group & Phytoconstituents**	**AgNPs wave number**	**Probable functional group & Phytoconstituents**
1	3417	O-H stretch alcohols or phenols	-	-
2	2922	O-H stretch carboxylic acid	3142	O-H stretch carboxylic acid
3	2364	NH^+ stretch tertiary amines salt	-	-
4	1618	>N-H bend secondary amine	1607	>N-H bend secondary amine
5	1442	C-C stretch aromatic	1400	C-C stretch aromatic
6	1383	C-H rock alkanes	-	-
7	1021	C-N stretch aliphatic amine	1191, 1102	C-N stretch aliphatic amine
8	874	P-O-C stretch aromatic phosphate	-	-
9	788	C-H “oop” aromatic	820, 751	C-H “oop” aromatic
10	620, 470	C-Br stretch alkyl halides	611, 616, 436	C-Br stretch alkyl halides

Scanning electron microscopy (SEM) analysis

The morphological structure of biosynthesized PA-AgNPs was recognized by SEM image (Fig. 4). The SEM image of PA – AgNPs was flake shape and size in the series of 28–34 nm.

Fig. 4: SEM image of AgNPs

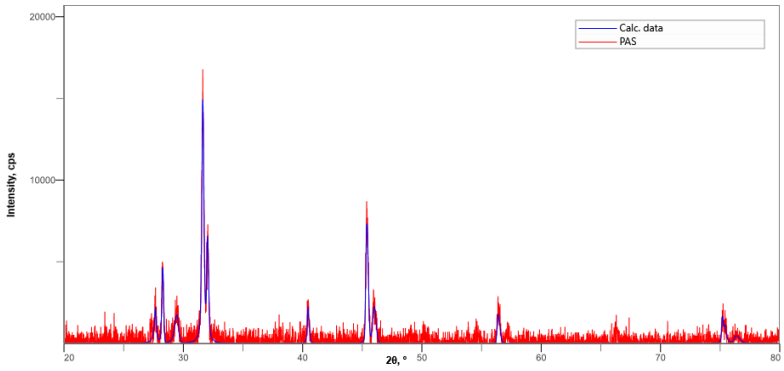


X-ray diffraction (XRD) analysis

XRD spectrum offers an insight into the crystallinity of the nanoparticles represent. The PA-AgNPs synthesized by means of PA extract was authorized by X-ray diffraction analysis. (Fig. 5) The five lattice planes are (110), (111), (200), (222) and (220) was observed for PA-AgNPs as the Bragg’s reflection angles of 28.6, 32.4, 46.3, 57.2 and 67.3° correspondingly. These typical peaks represented that synthesized PA-AgNPs are crystalline. The

XRD pattern of currently synthesized PA-AgNPs is well matched with the data base of Joint Committee on Powder Diffraction Standards (JCPDS file No.04-0783). The size range of the manufactured PA-AgNPs is 30.24 nm.

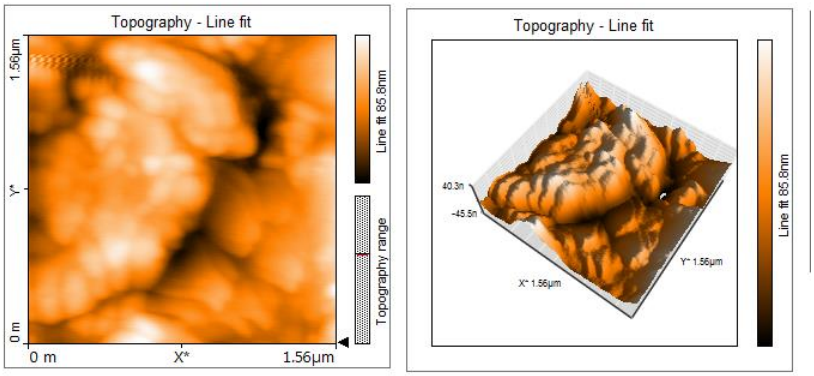
Fig. 5: XRD spectra of AgNPs



Atomic force microscopy (AFM) analysis:

AFM characterization stated about surface morphological structure of green synthesized PA-AgNPs in three dimensional methods. In the present study, the AFM image displayed collective globular like structure for PA-AgNPs (Fig. 6). This method was employed to examine the surface structure and topography roughnees of the manufactured PA-AgNPs.

Fig. 6: AFM image of AgNPs



Antioxidant activity

DPPH radical scavenging activity

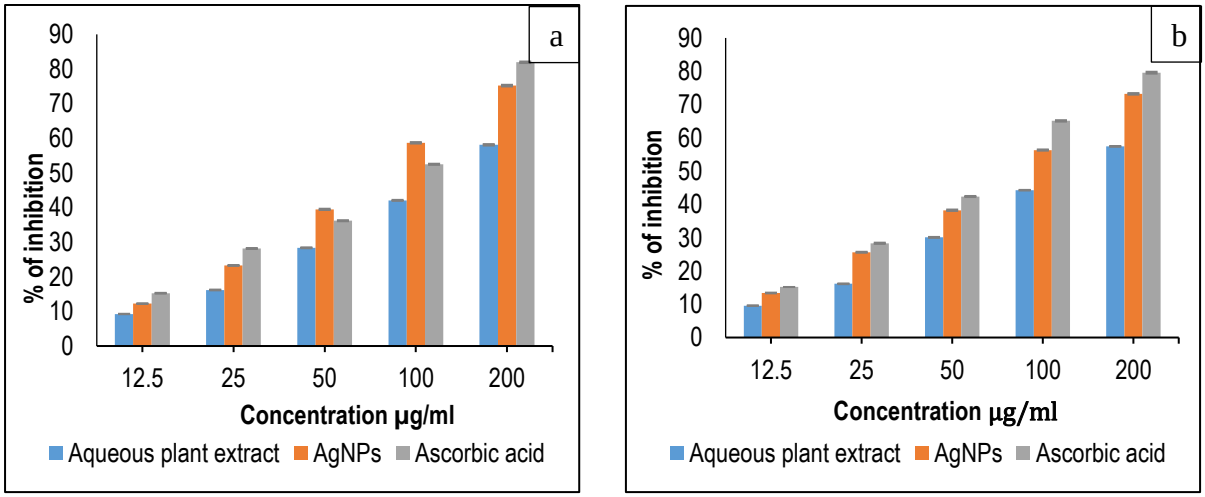
DPPH test, which is based on the capacity of DPPH, a stable free radical to decolorize in the occurrence of antioxidants, is a direct and reliable technique for determining radical scavenging action (Hasan *et al*., 2009). In the current study, DPPH radical scavenging activity of the synthesized PA-AgNPs, aqueous plant extract and standard ascorbic acid were assessed and the results are shown in Fig.7a. The DPPH radical scavenging activity outcomes of synthesized PA-AgNPs at 200 µg/ml. concentration was 75.26% aqueous plant extract was 58.10% and that of the standard ascorbic acid was 81.94%. DPPH radical scavenging activity of currently synthesized PA-AgNPs is found to be higher than formerly studied plants *Chenopodium murale* (Abdel – Aziz *et al.*, 2013); *Andrographis serpyllifolia* (Palithya *et al.*, 2018); *Aerva lanata* (Palithya *et al.*, 2021); *Carissa Caranda* (Singh *et al.*, 2021).

H_2O_2 radical scavenging activity

The accumulation of hydrogen peroxide in the living cells primes to an increase in the reactive oxygen species (ROS) for instance hydroxyl and peroxides that cause plain damage to the cell membrane (Chanderraj and Sundaram, 2021). In the present study, H_2O_2 radical scavenging activity of the synthesized PA-AgNPs, aqueous plant extract and standard ascorbic acid were assessed and the results are showed in the fig. 7b. At 200 µg/ml concentration of biosynthesized PA-AgNPs was created to be 73.20% H_2O_2 radical scavenging action, aqueous plant extract was 57.42% and standard ascorbic acid was 79.56%. The biosynthesized PA-AgNPs exerted developed H_2O_2 radical scavenging activity than formerly studied plants, *Desmostachya bipinnata* (Guntur *et al*., 2018); *Cestrum nocturnum* (Keshari *et al.*, 2020) and *Catharanthus roseus* (Keshari *et al.*, 2021).

Synthesis of AgNPs by plant extracts displays the highest antioxidant activity as compared to other extracts. These plant extracts encompass certain bioactive compounds for example phenolic and flavonoid compounds that are chief agents for the antioxidant activity, which plays a part in plant defense mechanism (Sriramulu *et al.*, 2017)

Fig. 7: Antioxidant activity biosynthesized AgNPs a) DPPH b) H_2O_2 scavenging



***In vitro* antiinflammatory activity**

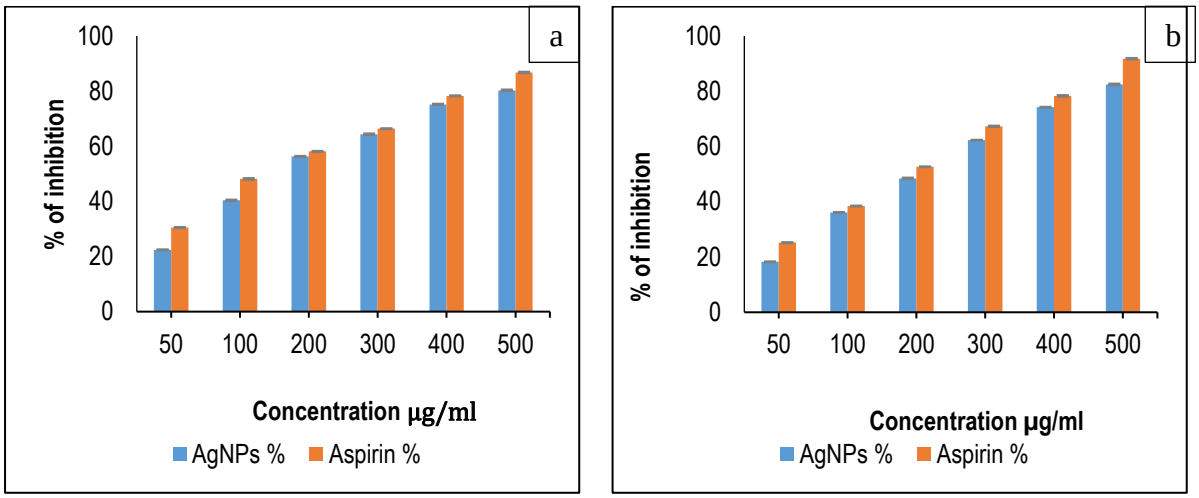
Inhibition of albumin (protein) denaturation

Protein denaturation is the procedure by which proteins lose their tertiary structure and secondary structure. Proteins denaturation is well manuscripted cause of inflammation (Anoop and Bindu 2015). In the current study, the biosynthesized PA-AgNPs was found to be 80.26% inhibition of protein denaturation persuaded by heat at 500 µg / ml. concentration, however standard drug aspirin inhibit 86.76% of protein denaturation at the same concentration (Fig 8a) Comparable observation was also reported by a good number of researchers (Kedi *et al.*, 2018; 2020).

Proteinase inhibitory activity

Neutrophils are recognized to be a rich source of serine proteinase and are restricted a lysosomes. It was formerly reported that leukocytes proteinase play an imperative role in the progress of tissue damage during inflammatory reactions and noteworthy levels of protection was delivered by proteinase inhibitor (Das and Chatterjee, 1995). In the current study biosynthesis PA-AgNPs exhibited proteinase inhibitor activity of 82.46% at 500 µg/ml. concentration; while standard drug aspirin 91.76% proteinase inhibitor activity at the same concentration (Fig. 8b). The current results are authorized with reports of Pretsch *et al*. (2014) and Govindappa *et al*. (2018).

Fig. 8: Invitro anti-inflammatory activity of biosynthesized AgNPs a) inhibition of albumin denaturation, b. proteinase inhibitory activity



Conclusion

Synthesis of nanoparticles using biological agent is eco-friendly. It is of low cost and capable of producing at room temperature. The present study, *Pemphis acidula* whole plant extracts phytocompounds acts as equally reducing and stabilizing agents. The AgNPs were illustrated by UU-vis, FTIR, SEM, XRD and AFM analysis. The UU-Vis spectra authorize the formation of green synthesized AgNPs based on surface plasmon resonance learning. The phytochemicals were accountable for reducing and capping of AgNPs, which was authorized by FTIR spectra. SEM results disclosed flake shaped AgNPs. XRD spectrum shows crystalline nano structured AgNPs and AFM techniques exemplify the aggregate globular like structure. The PA-AgNPs meaningfully showed antioxidant and antiinflammatory activity.

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Conflicts of Interest

The authors declare no conflict of interest.

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