Biocomponents loaded silver nanoparticles of *Volkameria inermis* L. flowers and fruits as alternative antibacterial and antioxidant agents

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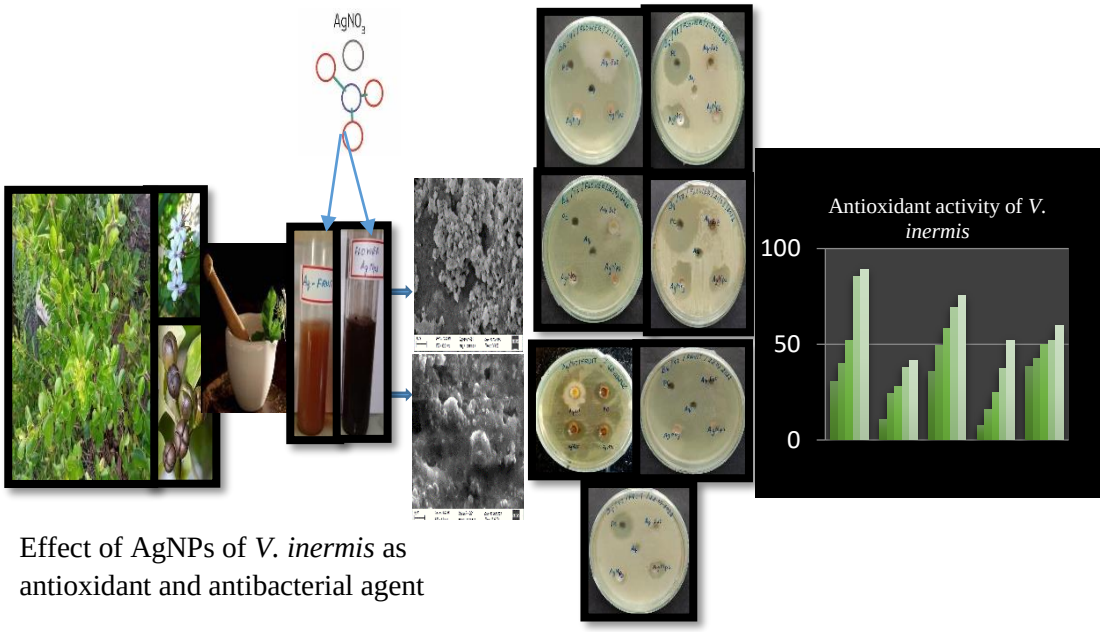
Abstract

The potential biological features of green synthesized silver nanoparticles have made them popular worldwide in a variety of sectors. The present study aimed to synthesize silver nanoparticles using flower and fruit extracts of V. inermis to evaluate their antibacterial and antioxidant properties. 9 mL of 0.1M concentration of silver nitrate solution and 1 mL of fresh aqueous plant extracts were used for the biosynthesis of silver nanoparticles. The silver nanoparticles (SNPs) were monitored by UV-vis spectroscopy. Functional groups of compounds that are responsible for the bio-reduction of silver ions into silver nanoparticles, were determined through FT-IR spectrometry. The size and shape of the nanoparticles were determined through SEM analysis and the crystalline phase was assessed through X-ray diffraction.

The UV-vis spectroscopy showed the maximum absorbance of V. inermis flower SNPs with single Surface Plasmon Resonance (SPR) at 457.10nm and fruit SNPs solutions with two SPR bands at 398.75 and 433.50 nm, indicating the spherical and square shape morphology. FT-IR spectroscopic study of synthesized flower and fruit detected 15 and 23 major peaks respectively which represent the functional compounds involved in the reduction and stabilization of SNPs. The size of flower and fruit SNPs was determined as 10nm and 9nm by SEM analysis. XRD inferred the average crystalline size of SNPs as 30.044 and 28.11nm and observed visible diffraction rings corresponding to (210), (111), (200) and (231) sets of planes for flowers, three diffraction rings with respect to (210), (111) and (231) set of planes for the fruits which are attributed to face-centered cubic metallic silver. Green synthesized SNPs revealed potential antibacterial activity against Gram-negative Klebsiella pneumoniae, Gram-positive Micrococcus luteus, Mycobacterium smegmatis and Rhodococcus rhodochrous. They showed strong antioxidant efficacy (DPPH) with the IC_{50} of 117.071 µg/mL and 100.818µg/mL. The study concludes that the green synthesized SNPs are facile and nontoxic and can be used as an alternate source of antibacterial and antioxidant agents.

Keywords: Aqueous extracts, phytochemicals, reducing agents, silver nanoparticles, antibacterial and antioxidant agents.



Effect of AgNPs of *V. inermis* as antioxidant and antibacterial agent

Graphical Abstract

Introduction

Synthesis of silver nanomaterials (SNPs) using green plants is an emerging field of science popularized worldwide for their antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anti-angiogenesis, antitumor, antidiabetic and anticancer activities[7]. The unique properties of silver nanomaterials synthesized using various parts of green plants are considered safe, biocompatible and non-toxic to research activities in the field of science. Secondary metabolites that are present in plant substances including terpenoids, flavonoids, ketones, aldehydes, amides and carboxylic acids are directly involved in the reduction of silver ions and the formation of silver nanoparticles. It is a bottom-up approach where a reduction or oxidation reaction takes place while synthesizing the silver nanoparticles[2].

In the production of nanoparticles, concentration and the nature of plant extract, the concentration of metal salt, pH, temperature and time of interactions directly influence the speed, amount and properties of synthesized nanoparticles[14]. Silver metal was recognized as a very potent antibacterial agent, which can overcome numerous types of microorganisms causing infectious diseases[9]. The transformation of silver ions into silver nanomaterials through biological and biomimetic methods of synthesis constantly reduces toxicity and enhances their antibacterial properties enormously. Due to this specific nature, SNPs act as wonderful weapons for the clinical management of bacterial diseases[13,22]. Silver nanoparticles were also employed in textiles, home water purification systems, medical devices, cosmetics, electronics and household appliances[11,24].

Besides biological methods, various strategies are applied for the synthesis of SNPs. They can be synthesized by the reduction in solution[8], microwave-assisted synthesis[23], thermal decomposition of silver compounds[19] and laser-mediated synthesis[26]. The former method is the most preferred way for the synthesis of nanoparticles as it offers an eco-friendly way of synthesis of nanoparticles. *Volkameria inermis* L., an ethnomedicinal scrambling shrub belongs to the family Lamiaceae. The plant is native to coastal India, Sri Lanka, Burma, Malaya, tropical Australia, Polynesia and the Philippine Islands. Traditionally the plant is used in the treatment of fever, cough, scrofulous infection, venereal infection and skin diseases in folklore medicines[10].

It possesses many pharmacological activities such as anti-inflammatory, analgesic, antipyretic antimicrobial, antidiabetic, antioxidant, antiparasitic, insecticidal, anti-allergic and anticancer properties[5].

Based on ethnomedicinal properties and the worldwide application of SNPs, the present investigation aimed to study the bioactivity of green synthesized silver nanoparticles (SNPs) using aqueous flower and fruit extracts of *V. inermis*. We describe a simple, efficient and economically viable method that furnishes stabilized SNPs and has been characterized and evaluated for its antibacterial and antioxidant properties.

Material and Methods

Chemicals used: Silver nitrate $AgNO_3$ (99.99%), DPPH (99%), Ascorbic acid ($C_6H_8O_6$, 99%), MHA (Muller Hinton Agar) medium and Streptomycin (antibiotic) were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Double distilled water and sterilization kits were used throughout the experiment and were purchased from Ponmani & Co. Pvt. Ltd., Tamil Nadu, India.

Collection of Experimental Plants and Extracts Preparation: Fresh and healthy plant of *Volkameria inermis* L. were collected from Tiruchirappalli District, Tamil Nadu, India. and were authenticated at Rapinat Herbarium (Accession No. 2991), St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. Flowers and fruits of *V. inermis* were removed from the collected plant and washed with tap water as well as with distilled water to remove the adherent particles. Twenty grams of finely chopped fresh flowers and fruits were boiled with 100 ml of distilled water in separate beakers for 30 minutes at 100˚C. The aqueous mixture was cooled down and was centrifuged at 10000 rpm for 10 minutes and the filtered supernatant was stored for further analysis.

Biosynthesis of Silver nanoparticle: The biosynthesis of silver nanoparticles was carried out by following the procedure of Yousaf et al[25] with slight modification. The filtered 1 mL of plant extract was mixed with 9 mL of silver nitrate (0.1M) solution (1:9 ratio) and allowed to react in direct sunlight for the reduction of silver ions. The reduction of Ag^+ ions into Ag^0 and formation of SNPs in flower and fruit solutions were evaluated by visual observation of the yellow color solutions turning brown. Further, the biosynthesized SNPs were assessed using various analytical characterization methods.

Characterization of silver nanoparticles: The optical absorbance of biosynthesized SNps was measured by UV–visible absorption spectrophotometer (Shimadzu 1800) for the surface plasmon resonance (SPR) peak in the wavelength range from 200 to 1000 nm. The biomolecules (functional groups) of plant extract acting as a capping agent, responsible for the reduction and stabilization of SNps, were analyzed using Perkin Elmer FT-IR spectrometry. After the initial assessment, the reaction solution was centrifuged at 1000 rpm for 20 minutes to obtain purified SNPs in dried form and used for further characterization.

The surface morphology of the synthesized SNPs was determined by Scanning Electron Microscopy. X-ray Diffraction was also carried out to reveal the crystallographic nature of the biosynthesized SNPs using XRD-7000, X-ray Diffractometer (Cu Kα radiation wavelength of 1.5406Å; scanning angle 2θ from 10º to 90˚).

Antibacterial Activity: Antibacterial activity of synthesized SNPs and the fresh aqueous extracts from the flower and fruit of *V. inermis* was performed according to Cheesbrough[4] against the clinical pathogen's Gram-negative *Klebsiella pneumoniae*, Gram-positive *Mycobacterium smegmatis*, *Micrococcus luteus* and *Rhodococcus rhodochrous*. The bactericidal effect was evaluated using the standard agar well diffusion method. Wells were made using sterile stainless steel cork borer on a culture plate containing nutrient agar medium seeded with 100 µL of pathogenic bacteria. Wells were filled with 100 µL of SNPs and crude aqueous solution. $AgNO_3$ solution as well as distilled water were used as the negative control and Streptomycin acts as positive control. The cultured plates were refrigerated for 2 h and then incubated at 37ºC for 24 h. Finally, the diameter of the inhibition zone was measured.

2,2- diphenyl -1- picrylhydrazyl (DPPH) method: The antioxidant activity (AA %) of SNPs synthesized from the flower and fruit of *V. inermis* was assessed by DPPH free radical assay following the modified method of Brand-Williams et al[3]. Diverse concentrations of 20, 40, 60, 80 and 100 µg/ml of SNPs and ascorbic acid (positive control) were prepared in separate test tubes. About 1mL of DPPH (0.3mM) reagent was added to the SNP samples and was vortexed thoroughly. Finally, the solution was incubated at room temperature in the dark for 30 minutes. When DPPH reacts with antioxidant compounds present in the SNP solutions (which can donate hydrogen), it reduces the DPPH and changes its color from deep violet to light yellow.

After thirty minutes, the absorbance was recorded at 517nm in UV-visible spectrophotometry and the percentage of radical scavenging activity i.e. anti-oxidant activity was calculated by following standard formulae. Control reading was taken by adding one milliliter of solvent with two milliliters of DPPH reagent. Linear regression plots calculated the IC50 values.

$$\% \text{ of DPPH Scavenging} = \frac{\text{Ab of control - Ab of test}}{\text{Ab of control}} \times 100$$

Ab of control = Control Absorbance; Ab of test = Test solution Absorbance.

Results

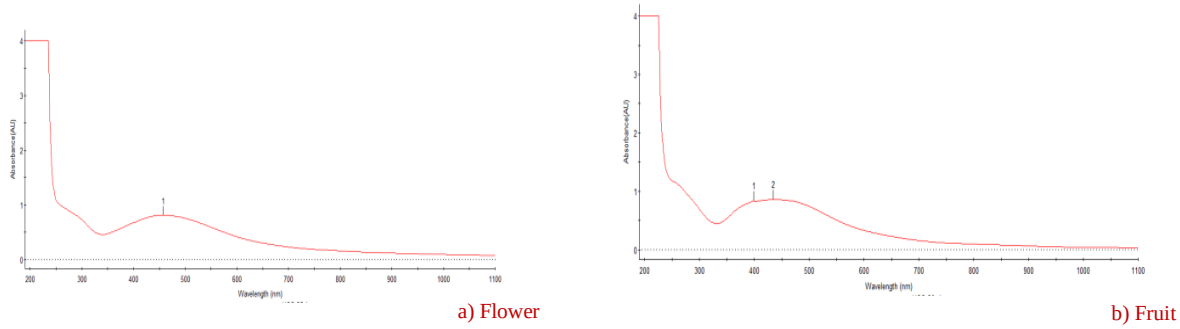
In the present study, green synthesis of silver nanoparticles from flower and fruit extracts of *Volkameria inermis* was carried out and was characterized using UV-vis, FT-IR, SEM and XRD analysis. The synthesized particles were evaluated for their potential antibacterial and antioxidant efficacy.

Biosynthesis of Silver nanoparticles: Fresh aqueous extracts of *V. inermis* flowers and fruits were used for the biosynthesis of nanoparticles. The aqueous extracts were mixed up with $AgNO_3$ in a ratio of 1:9 and the mixture was kept in sunlight for 30 minutes to enhance the production of silver nanoparticles. It was observed that the colorless $AgNO_3$ solution slowly turned dark brown with the addition of plant extracts. $AgNO_3$ without plant extracts did not show any changes. The colour change in the reaction mixture gets deep brown and was taken as primary evidence for the biosynthesis of SNPs. The results are shown in figure 1.

Characterization of synthesized silver nanoparticle: The green synthesized silver nanoparticles are subjected to various characterizations using UV-vis, FT-IR, SEM and XRD analysis to determine their size, shape and crystalline nature.

UV -visible spectroscopy analysis of flower and fruit of *V. inermis*: The bio-reduction of silver ions in the flower and fruit extracts was further accomplished by UV-visible spectrum after 24 hours of reaction. Observation of peculiar absorption bands in the visible regions of 390 -500 nm confirms the existence of surface plasmon resonance (SPR) of AgNPs. In the present spectroscopic study, a single sharp absorption band at 457.10 nm in flower and two absorption bands at 398.75 and 433.50 nm in fruit SNPs confirms the presence of silver nanoparticles. The number of SPR bands in the synthesized nanoparticles also helps to predict the shape of the synthesized nanoparticles. From the results (Figure 2), flower SNPs with a single SPR band and fruit SNPs with two SPR bands reveal spherical and rectangular shapes respectively.

Figure 1: Biosynthesis of silver nanoparticles. a) Formation of *V. inermis* flower SNPs b) Formation of *V. inermis* fruit SNPs



a) Flower b) Fruit

Figure 2: UV vis absorption spectrum images after 24 hours. a) *V. inermis flower* with single SPR band b). *V. inermis* fruit with double SPR bands

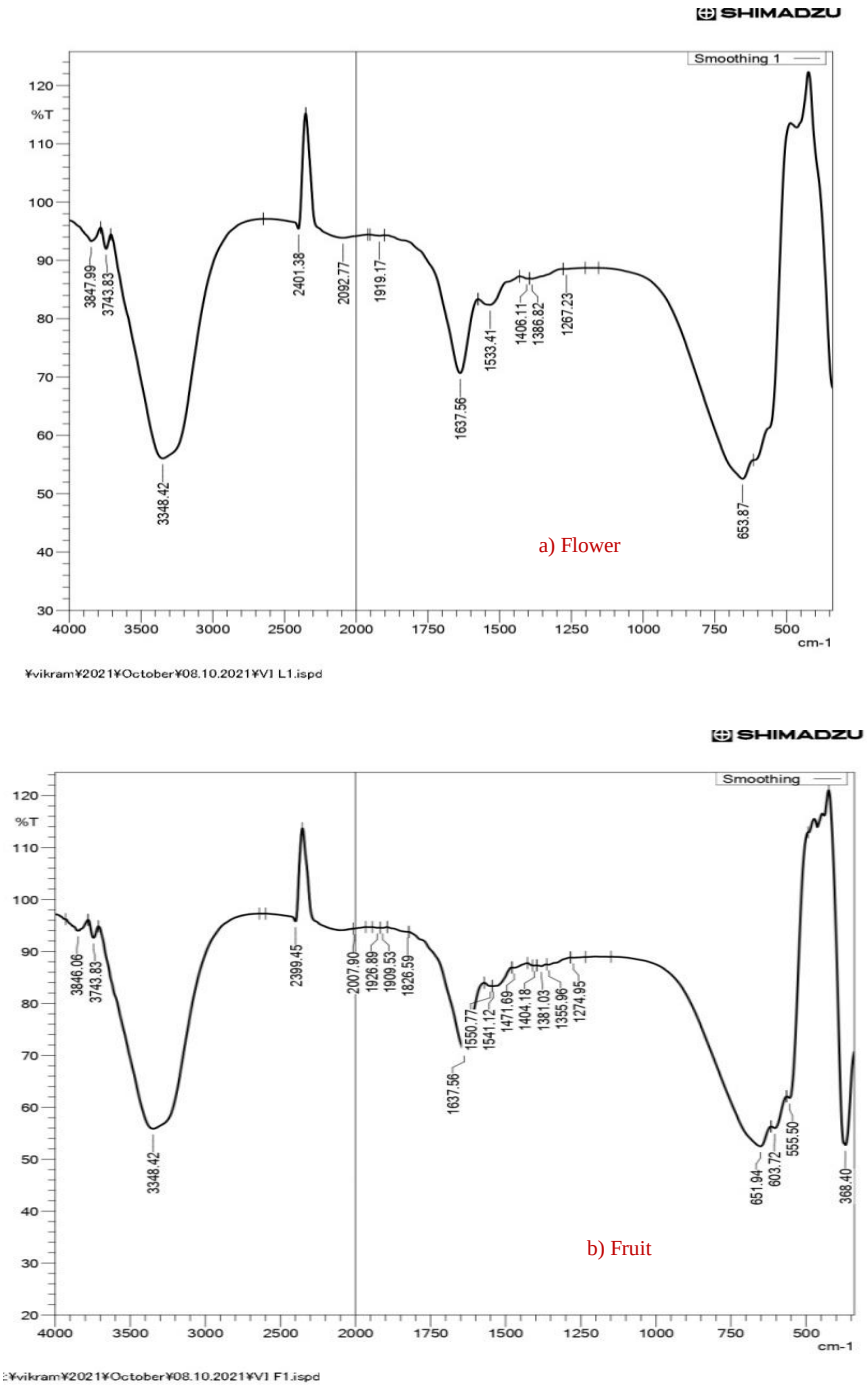


Figure 3: FTIR Images of SNPs synthesized from a) *V. inermis* flower b) *V. inermis* fruit

FT-IR spectroscopic analysis of flower and fruit of *V. inermis*: The capped biomolecules which are responsible for the reduction and stabilization of SNPs were detected using FT-IR spectroscopy with the range of 400 cm^{-1} to 4000cm^{-1}. The peaks observed in the spectrum were used to separate the functional classes of biomolecular components. The FT-IR spectrum of *V. inermis* flower SNPs revealed 15 peaks between ranges of 368.40 and 3846.06 cm^{-1} represents various C, H, O, N, Br stretching, bending with single and double bonds constituting aliphatic (N-H stretching) and aromatic amine (C-N stretching), isothiocyanate (N-C-S stretching) alkene (C=C=C), nitro compounds (N-O stretching), carboxylic acid (O-H bending) and halo compound with C-Br stretching (Figure 3a).

The FT-IR spectrum of fruit SNPs revealed 23 peaks between ranges of 358.76 and 3741.90 cm^{-1} representing the functional compounds of aliphatic primer amine (N-H stretching), isothiocyanate (N=C=S stretching), allene (C=C=C stretching), alkene (C=C), alcohol (O-H bending), nitro compound (N-O stretching), sulfonic acid (S=O stretching), alkyl aryl ether (C-O stretching) and halogen compound with C-I stretching (Figure 3b). The presence of maximum IR peaks in the IR spectrum indicates the role of plant components in the reduction and stabilization of SNPs.

SEM and XRD analysis of the flower and fruit of *V. inermis*: The particle morphology and surface area of the synthesized SNPs were investigated with a Scanning Electron Microscope (SEM). The resulting SEM images of *V. inermis* flower and fruit SNPs revealed spherical and rectangular shapes with particle sizes of 10mm and 9mm respectively (Figure 4a and 4b).

The XRD patterns confirmed the phase-centred crystalline structure, size and purity of the prepared SNPs. In flower and fruit SNPs of *V. inermis*, the highest peak intensity of plane (111) with narrow full width at half maximum (FWHM) proves the crystalline nature of synthesized SNPs. XRD pattern of the flower showed four prominent diffraction peaks at 2Θ = 27.75º, 32.42º, 38.02º and 46.16º which correspond to the diffraction planes of (210), (111), (200) and (231) respectively (Figure 5a). Three strong peaks were observed at 2Θ = 27.77º, 32.18º, 46.19º in the XRD pattern of fruit which could be attributed to diffraction planes of (210), (111) and (231) respectively (Figure 5b). The additional peaks present in the XRD pattern might be due to the presence of organic molecules in the flower and fruit extracts of *V. inermis*.

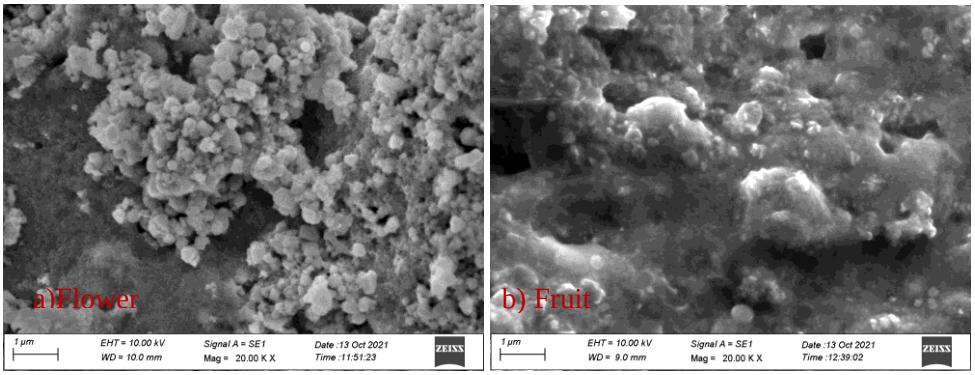


Figure 4: SEM images of SNPs from a) *V. inermis* flower b) *V. inermis* fruit

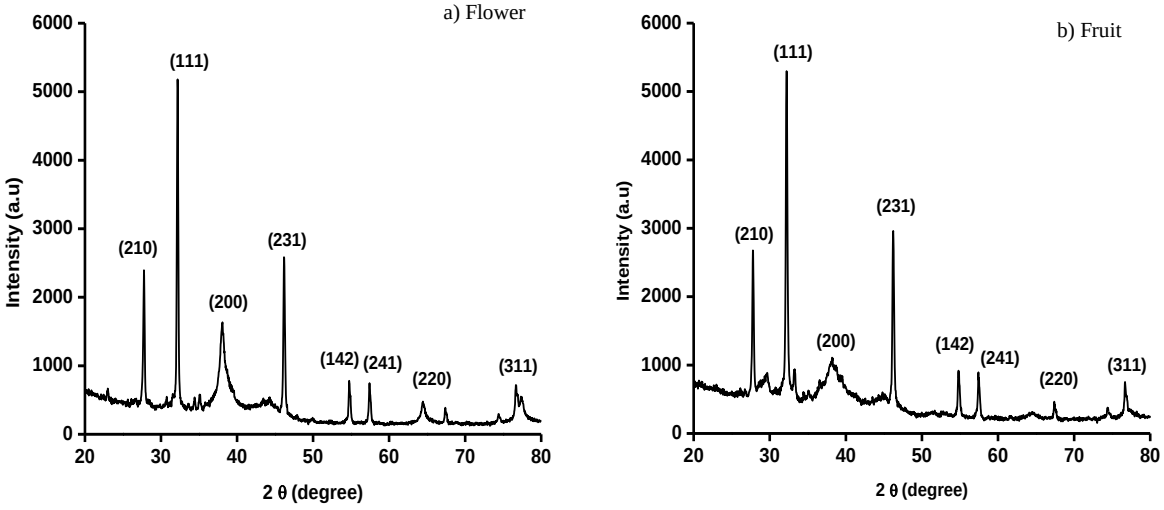


Figure 5: X-ray diffraction pattern images from a) *V. inermis* flower b) *V. inermis* fruit

Antibacterial studies of biosynthesized SNPs: Biosynthesized SNPs of *V. inermis* flower and fruit extracts and their aqueous solution were tested against Gram negative *Klebsiella pneumoniae*, Gram positive *Micrococcus luteus*, *Mycobacterium smegmatis* and *Rhodococcus rhodochrous* for their bactericidal activity. Inhibition zones formed around the wells revealed the bacterial sensitivity with the concentration of 100µL per well which was measured after 24 h of incubation time at 37ºC.

From the study, it was observed that the SNPs of fruit extracts exhibits remarkable bactericidal activity followed by flower SNPs. The aqueous flower and fruit extracts showed mild effect against the clinical organisms and confirms the bactericidal potency of silver nanoparticles. The SNPs of *V. inermis* fruit extracts showed strong inhibitory effect against the Gram-negative bacteria *K. pneumoniae* with the ZOI of 4.63 ± 0.25mm followed by Gram positive *M. luteus* and *M. smegmatis* with the inhibition zone of 3.16±0.25mm and 3.23±0.28mm respectively. The ZOI for the aqueous fruit extracts were observed as 1.46±0.25mm, 1.33±0.25mm and 1.13±0.25mm against *K. pneumoniae, M. luteus* and *M. smegmatis* whereas the bacterium *R. rhodochrous* was active and resistant against to the fruit extracts.

SNPs obtained from the flower extracts recorded bactericidal effects against *K. pneumoniae* with the ZOI of 4.33±0.15mm followed by *M. luteus, M. smegmatis* and *R. rhodochrous* with inhibition zone of 2.63±0.25mm, 3.20±0.20mm and 2.43±0.15mm. The aqueous flower solution shows 1.66±0.25mm of inhibition against *K. pneumoniae*, 1.36±0.20mm, 1.16±0.15mm and 1.23±0.20mm of inhibition zone against *M. luteus, M. smegmatis* and *R. rhodochrous* respectively and observed results were presented in table 1.

Antioxidant studies of biosynthesized SNPs: The antioxidant activity of SNPs synthesized from the aqueous extracts of *V. inermis* flower and fruit exhibited profound free-radical scavenging activity against DPPH molecule in dose dependent manner. Ascorbic acid was used as a reference substance to compare the antioxidant efficiency.

Table 1
Antibacterial Activity of Aqueous and SNPs of *Volkameria inermis* L. (Flower and Fruit)

MEASUREMENT OF ZONE OF INHIBITION (mm)										
	FLOWER					FRUIT				
Name of the Organisms	**PC**	**Aq-Ext**	**Aq**	**SNPs**	**$AgNO_3$**	**PC**	**Aq -Ext**	**Aq**	**SNPs**	**$AgNO_3$**
Klebsiella pneumoniae	7.26±0.25	1.66±0.25	-	4.33±0.15	2.16±0.15	7.26±0.25	1.46±0.25	-	4.63 ± 0.25	2.16±0.15
Micrococcus luteus	9.23±0.25	1.36±0.20	-	2.63±0.25	1.63±0.15	9.23±0.25	1.33±0.25	-	3.16±0.25	1.63±0.15
Mycobacterium smegmatis	9.16±0.15	1.16±0.15	-	3.20±0.20	2.26±0.25	9.16±0.15	1.13±0.25	-	3.23±0.28	2.26±0.25
Rhodococcus rhodochrous	9.56±0.15	1.23±0.20	-	2.43±0.15	2.70±0.20	9.56±0.15	-	-	-	-
PC- Positive control; Aq- Ext- Aqueous extract; Aq- Aqueous; SNPs- Silver nanoparticles										

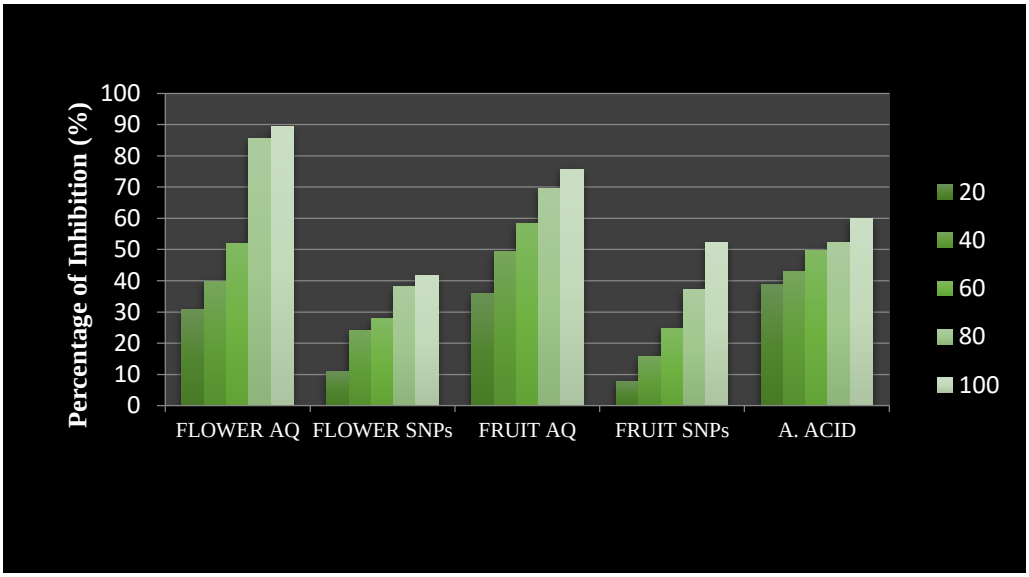


Figure 6: Percentile distribution of free radical scavenging activity of *V. inermis*

The study makes clear that the antioxidant properties of biosynthesized SNPs exhibited the increased scavenging activity with the increasing concentration in the range of 20 µg/mL - 100 µg/mL. The scavenging potential of flower, fruit SNPs and Ascorbic acid at100 µg/mL (maximum tested concentration) are 41.666±0.406, 52.177±0.464 and 59.786±0.616 respectively. The IC_{50} values for the green synthesized SNPs of flower, fruit and Ascorbic acid (Figure 6) were determined respectively as 117. 071µg/mL, 100. 818µg/mL and 65.507µg/ML.

Discussion

Production of plant mediated green synthesized silver nanoparticles is a sustainable method due to low costs, safety and compatibility with environment. Silver nanoparticles are facile, efficient and non-toxic substances. Phytocompounds present in the aqueous extracts act as capping agent and plays a vital role in the reduction and stabilization of silver ions. Addition of plant aqueous extracts with the aqueous based $AgNO_3$ accelerates the excitation of electrons in electronic energy level, which results in reduction of Ag^+ into Ag^0. The present study shows UV-vis spectrum with a sharp absorption band at 457.10nm and 398.75nm in flower and fruit SNPs. This confirms the presence of surface plasma resonance (SPR) and indicates the formation of silver nanoparticles. In corresponds to Muthukrishnan et al[16], the number of SPR band in UV vis spectrum at 300-700 nm predict the shape of nanoparticles.

A single SPR band in UV vis spectrum corresponds to spherical-shaped nanoparticles, whereas the presence of more than one SPR band associates the scattering of SNPs. In the present study, a single SPR band was observed in flower SNPs and two SPR bands were observed in fruit SNPs revealing spherical and rectangular shapes respectively. Similar to our study, aqueous SNPs with single SPR bands (spherical shape), ethanol and methanol SNPs with two and three SPR bands (rectangular and cubic) were observed in *Achillea millefolium*[25].

The functional groups of compounds that are capping on the surface which are responsible for the efficient stabilization of silver nanoparticles, are identified with the help of a unique FT-IR spectrum. These results state that phytochemicals are mainly involved in the synthesis of SNPs. The presence of phenolic and carbonyl compounds on the surface of silver nanoparticles might be the reason for the enhanced antioxidant properties. The FTIR spectrum of *V. inermis* leaf extracts showed the presence of alkene, amines and aldehyde groups which are involved in the reduction of silver ions[12]. The FTIR spectrum of silver nanoparticles synthesized from European black elderberry (*Sambucus nigra*) revealed the presence of polyphenols[6]. Our results are following the above studies showing the results of several functional groups in flower and fruit SNPs of *V. inermis*. Scanning Electron Microscope has been widely used to identify the size and morphology of nanoparticles. The present results revealed the size and shape of flower and fruit SNPs. Nadagouda et al[17] reported that the size of synthesized SNPs varies depending upon the nature of plant aqueous extracts as well as the method of preparation. They also stated that the biological activity of SNPs directly correlates to the size of the particles. Similarly, Azarbani and Shiravand[1] reported spherical shape SNPs of *Ferulago macrocarpa* flower extracts with sizes ranging from 14 to 25 nm.

XRD spectrum with the characteristics peaks at scanning angle (2Ɵ) depicts the polycrystalline nature of metallic silver. In flower and fruit SNPs of *V. inermis*, the highest peak intensity of plane (111) with narrow full width at half maximum (FWHM) proves the crystalline nature of synthesized SNPs as observed from the XRD patterns. The peaks observed in the spectrum and their corresponding Bragg's reflections are strongly approved by the Joint Committee on Powder Diffraction Standards (JCPDS, file no. 04-0783). The characteristic peaks of the SNPs indicate their purity. The average of the crystalline particles was calculated using Debye-Scherrer's equation:

$$D = \frac{K\lambda}{\beta\cos\theta}$$

where D is Estimated crystal size in nm, θ is the Bragg's angle in radians, λ is wavelength of X-ray source used (CuK_{α}= 1.5406Å), β is FWHM, 2θ is the Bragg angle (degree) and K is the Scherrer's constant (0.9). The average crystalline size of flower and fruit SNPs was calculated as 28.11nm and 30.04 nm. Similarly, Prakash et al[20] reported four visible diffraction rings corresponding to (111), (200), (202) and (311) sets of planes which are attributed to face-centered cubic metallic silver in aqueous extracts of blue sage; Melkamu and Bitew[15] reported the average crystalline size of *Hagenia abyssinica* leaf NPs as 22 nm. Lavanya et al[12] confirmed the presence and crystalline nature of the SNPs synthesized from *V. inermis* leaf extract and Naika et al[18] found the crystalline size of *Gloriosa superba* L. CuO NPs as 8-17 nm.

The antibacterial efficacy of SNPs synthesized from the flower and fruit extracts of *Volkameria inermis* was tested against clinical isolates using the agar well diffusion method. The SNPs of *V. inermis* flower and fruit were found to be more active against the tested organism than their aqueous extracts. They are remarkably active against the Gram-negative bacteria *K. pneumoniae*. This result was similar to the reports of Liao et al[13]. They stated that SNPs are more active against Gram-negative bacteria than Gram-positive bacteria due to the difference in their cell wall structures. Due to the presence of a single layer of peptidoglycan in the Gram-negative bacteria, SNPs can quickly damage them and prevent their proliferation.

Ronavari et al[21] described the correlation between size and the antibacterial activity of SNPs. From the results, it was concluded that the SNPs of *V. inermis* flower (9nm) and fruit (10nm), show potential antibacterial effects against the tested organisms. The SNPs interfere the cell functions by interacting with amino acids and enzymes causing reactive oxygen species and destroying the bacterial deoxyribonucleic acid (DNA).

The SNPs of *V. inermis* flower and fruit extracts showed reasonable scavenging ability against DPPH molecule when compared with ascorbic acid. SNPs of fruit extract revealed the highest antioxidant potential with the IC_{50} of 100.818 µg/ml, while it was 65.507 µg/ml for ascorbic acid (standard). A similar study was reported in *Hagenia abyssinica*[15] in which, SNPs showed higher antioxidant activity against DPPH molecules. The antioxidant activity of SNPs might be directly proportional to the functional groups as shown in the FTIR spectrum, responsible for the reduction of Ag ions. The present results suggest the possible application of SNPs as an alternative antioxidant source in various treatments of diseases that are caused due to free radicals.

Conclusion

The biosynthesized silver nanoparticles (SNPs) have a wide range of applications. In this present work, SNPs were synthesized successfully using flower and fruit extracts of an ethnomedicinal plant *V. inermis*. Based on UV-vis, FT-IR, SEM and XRD analysis, the synthesized silver nanoparticles were confirmed with the size of 10 nm and 9mm. They were observed with spherical and rectangular shapes along with phytochemical capping over nanoparticles. The potential antibacterial and antioxidant activity signified the synthesized silver nanoparticles as an efficacious bioactive agent.

Furthermore, the synthesized SNPs of *V. inermis* flower and fruit extracts are considered potentially ideal for the human healthcare system and could be used as an alternative source in various fields of biomedicine, biosensor and nanobiotechnology.

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