Research Article

FTIR Spectral Analysis and Comparative Antioxidant Activity of Chemical and Biological Silver Nanoparticles

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ABSTRACT

Silver nanoparticles are gaining importance in research due to its variety of applications. The electronic configuration of silver nanoparticles is ready to accept or donate an electron to quench free radicals. The capping agent attached to silver nanoparticles during process of synthesis may involve in an antioxidant property. Thus this study designed to focus on comparison of chemical and biological silver nanoparticles in relation to their capping agent and antioxidant properties. *Aegle marmelos* leaf extract and citric acid were used as reducing agents for synthesizing biological and chemical silver nanoparticles respectively. FTIR analysis of reducing agents and silver nanoparticles was carried out in the range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1}. DPPH, nitric oxide, hydrogen peroxide, super oxide and reducing power assay were carried out to study the antioxidant activity of silver nanoparticles using 50-1000 µg/mL concentrations. The FTIR patterns of both silver nanoparticles shows various peaks which correspond to functional groups from their reducing agents. Chemical silver nanoparticles showed negligible antioxidant activity while biological silver nanoparticles have shown good radical scavenging activity of DPPH (IC_{50}-72.24µg/mL), nitric oxide (IC_{50}-446.2µg/mL), hydrogen peroxide (IC_{50}-557.35µg/mL), super oxide (IC_{50}-173.47µg/mL) and reducing power (IC_{50}-91.226µg/mL).Biological silver nanoparticles have moderate antioxidant activity as compared to ascorbic acid and chemically synthesized silver nanoparticles. This efficacy of biological silver nanoparticles may be due to its capping agent acquired from plant extract. The antioxidant activity of silver nanoparticles varies with method and reducing agent used for synthesis.

Keywords: Chemical silver nanoparticle; biological silver nanoparticle; *A. marmelos*; FTIR analysis; antioxidant activity.

INTRODUCTION

Free radicals are highly reactive atoms it always strives to form stable bond, due to gaining or losing of an unpaired electron.[1] These free radicals can cause chronic complications like cardiovascular diseases, cancer, and neurodegenerative diseases.[2]

Till today antioxidant activity of many plant materials and extracts have been studied.[3,4] Recent studies showed that the silver nanoparticles (SNPs) synthesized from plant extract also exhibit the antioxidant property.[5] SNPs electron configuration is ready to accept or donate an electron to quench radicals. The radical quenching activity of silver nitrate is lesser than SNPs due to its less stability, less reactivity and also lesser electron donating ability of salt.[6]

Biological synthesis of SNPs using *A. marmelos* leaf extract is easy, economic and eco-friendly method. *A. marmelos* leaf extract considered to be reach in antioxidant property[7]and has essential components required for synthesizing and capping the SNPs. Citric acid is well known antioxidant agent used for food preservation[8] and it is commonly used mediator in chemical SNPs synthesis. So citric acid is used in chemical method of SNPs synthesis, it acts as both reducing and capping agent.[9]

SNPs synthesized from *Conthium comandelicum* leaves[10], *Dillenia indica*[11], *Ceropegia thwaitesii*[12] and *Morinda pubescens*[13] have shown both antioxidant and antibacterial activities. With these references, this study was designed to assess the comparative analysis of FTIR pattern and antioxidant activity of chemical silver nanoparticles (CSNPs) and biological silver nanoparticles (ASNPs), using ascorbic acid as a standard.

MATERIALS AND METHODS

Silver nanoparticle synthesis

CSNPs were synthesized using silver nitrate as a precursor and citric acid as a reducing agent using the method described by Silekaite et al.[14] ASNPs were synthesized using silver nitrate as a precursor and *A. marmelos* leaf extract as a reducing agent.[15] Both chemical and biological nanoparticles were isolated by centrifugation, dried and stored in well closed, light resistant container, and used for further antioxidant activity study.

FTIR analysis

To determine functional groups attached to SNPs, FTIR analysis of *A. marmelos,* citric acid, ASNPs and CSNPs was carried out in the range of 4000–600 cm^{-1} at a resolution of 4 cm^{-1} by using Shimadzu FTIR spectrophotometer.

Antioxidant activity

To study antioxidant activity different concentrations of CSNPs, ASNPs and ascorbic acid were prepared in a range between 50-1000 µg/mL. The 1,1-diphenyl-2-picryl

hydrazyl (DPPH) radical scavenging activity was determined according to method described by Malterud et al.[16] The hydrogen peroxide assay was carried out by using the method of Gocer et al.[17] Oyaizu's method[18] was used for analyzing the reducing power of ASNPs and CSNPs. In nitric oxide scavenging assay sodium nitroprusside was used for nitric oxide generation by using method described by Nabavi et al.[19] Superoxide scavenging activity was determined by the nitro-blue tetrazolium reduction assay using Nishikimi et al. method of synthesis.[20]The IC_{50} values of all scavenging assays were calculated by using graph pad prism software.

Statistical analysis

The results were expressed as the mean ± standard deviation of three independent tests performed under identical experimental condition. A one way analysis of variance (ANOVA) was applied to confirm the significance using Bonferroni multiple comparison test. SPSS/13 software was used for statistical analysis. p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

FTIR analysis

For investigating the functional groups responsible for capping and stabilization of SNPs, a FT-IR analysis of citric acid, CSNPS *A. marmelos* leaf extract and ASNPs were carried out separately and the spectra are shown in Fig.1 and 2.

FT-IR specra of CSNPs was shown the peaks at 609 cm^{-1} for –C-H stretch, 1078 cm^{-1} for –C=O stretch for carboxylic acid and 2372 –C-OH stretch assigned for carboxylic acid are attributed from tri-sodium citrate a capping agent.

A. marmelos constitutes Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol and Marmesinine.[21] The functional groups associated with these chemical constituents were involved in reducing Ag^{+} to Ag^{o}. The ASNPs absorption peaks at 999,1066, 1246, 2360 and 3156 are associated with –C–C bending, C–O, C–N, C–OH and O–H stretching of functional groups. These functional groups correspond to the various chemical compositions of *A. marmelos* leaf extract. It confirms the association of citric acid as well as *A. marmelos* biomolecules with the CSNPs and ASNPs.

DPPH radical scavenging activity

DPPH in its radical form showed absorbance at 517 nm, and its absorbance decreases with reduction by an antioxidant.[22] Both SNPs have shown remarkable scavenging activity when compared with standard ascorbic acid (IC_{50} 68.33 µg/mL).

The radical scavenging activity of SNPs was increased in dose dependent manner. DPPH scavenging activity ASNPs (IC_{50} 72.24 µg/mL) is significantly higher than CSNPs (IC_{50} 115 µg/mL) as shown in Fig.3. The antioxidant potential of ASNPs may be due to functional groups adhered to SNPs from leaf extract and tri-sodium citrate. As the *A. marmelos* leaves are good source of several antioxidant components such as euginol, marmesinin, glutathione, β-carotene, α-tocopherol, ascorbic acid, total polyphenols and total flavonoids.[21] The DPPH scavenging activity of ASNPs is as efficient as previous reports.[23, 24]

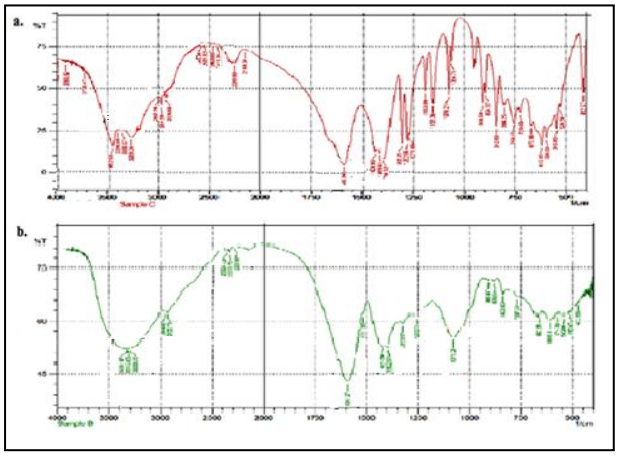

Figure 1: FT-IR spectra of a. Tri-sodium citrate b. CSNPs

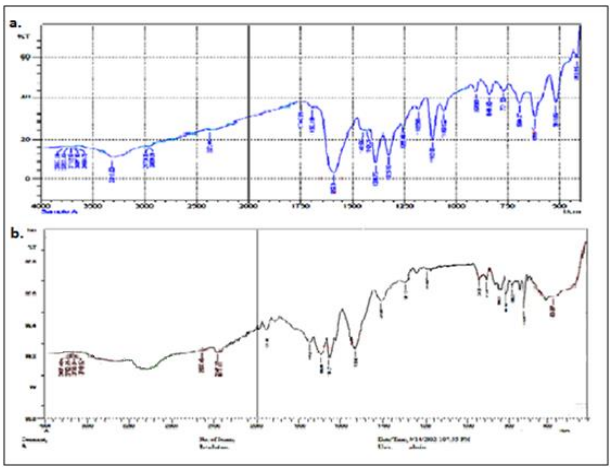

Figure 2: FT-IR spectra of a. *A. marmelos* leaf extract b. ASNPs

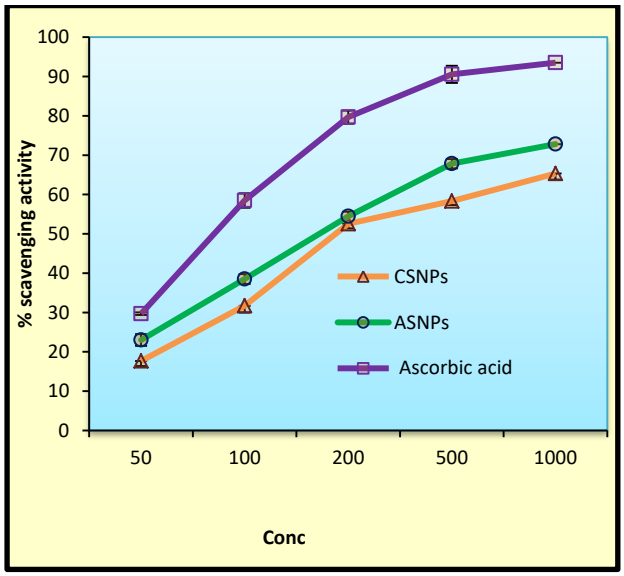

Figure 3: Graphical representation of DPPH scavenging activity of CSNPs, ASNPs and ascorbic acid.

Hydrogen peroxide assay

Hydroxyl radical scavenging activity of SNPs is shown in Fig. 4. CSNPs have lowest hydroxyl radical scavenging effect as compared to ASNPs and standard ascorbic acid. ASNPs have shown moderate hydroxyl radical scavenging activity. The IC_{50} values of CSNPs, ASNPs and ascorbic acid are 965 µg/mL, 557.35 µg/mL and 345.623 µg/mL respectively. The activity of NPs was increased in dose dependent manner.

Hydroxyl radicals are highly reactive free radicals formed in biological system and has been reported as a highly damaging radical, capable to affect almost every molecule and induce severe injury to adjacent bio-molecules.[25] Green synthesized SNPs are good scavengers of hydroxyl radicals and can be employed in such conditions. The phenolic compounds are major compounds responsible for antioxidant activity,[26] these are present in leaf extract of *A. marmelos* as the FTIR spectra of ASNPs had shown the presence of–OH peak at 2360.

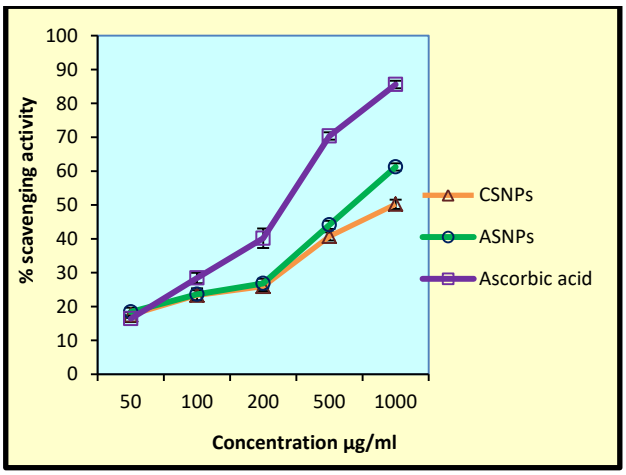

Figure 4: Graphical representation of hydrogen peroxide radical scavenging activity of CSNPs, ASNPs and ascorbic acid.

Reducing power assay

Reducing power of SNPs was evaluated by the transformation of Fe^{3+} to Fe^{2+}.[27]The ASNPs showed increased reducing power in dose dependent manner as shown in Fig. 5. The IC_{50} value of ASNPs is 91.226 µg/mL, it showed nearly same efficacy as that of standard ascorbic acid IC_{50} 90.105 µg/mL. Surprisingly with lower concentration SNPs showed greater reducing efficacy than standard. CSNPs were shown lesser reducing power as compared to ASNPs and standard ascorbic acid. These results implied that ASNPs have significant ability to react with free radicals to convert them into more stable nonreactive species and to terminate radical chain reaction.

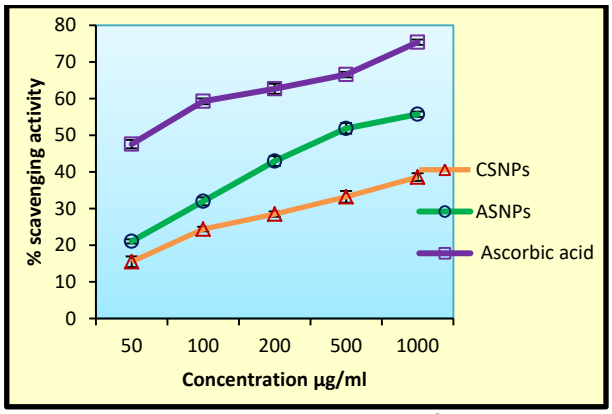

Figure 5: Graphical representation of reducing power assay CSNPs, ASNPs with standard ascorbic acid.

Nitric oxide scavenging activity

The Nitric oxide (NO) scavenging activity of the SNPs is determined by its ability to inhibit the formation of nitrite through direct competition with oxygen and oxides of nitrogen in a reaction mixture.[28] ASNPs (446.2 µg/mL) were shown superior NO scavenging activity than CSNPs (Fig. 6) but, lesser as compared to ascorbic acid IC_{50} 91.90 µg/mL.

Present study showed that the nitric oxide scavenging activity both chemical and biological SNPs is increased in dose dependent manner. In comparative study, NO scavenging activity of *Solanum torvum* mediated gold nanoparticles, SNPs and their salts, showed that SNPs have greater NO scavenging capacity.[6]

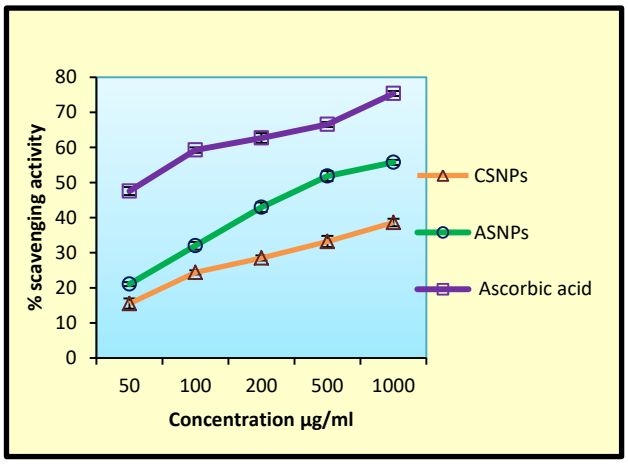

Figure 6: Graphical representation of nitric oxide scavenging activity CSNPs, ASNPs with standard ascorbic acid.

Superoxide radical scavenging activity

Superoxide radical scavenging ability of SNPs was quantified spectrophotometrically. The consumption of superoxide anion by antioxidants was indicated by the decrease in absorbance at 560 nm. The ASNPs have greater superoxide radical scavenging activity as that of the CSNPs (299.09µg/mL IC_{50}) as shown in Fig. 7. ASNPs have shown IC_{50} at 173.47 µg/mL and standard ascorbic acid have shown 73.23 µg/mL IC_{50}. As compared to the

CSNPs and ASNPs, ascorbic has the potent radical scavenging activity.

CSNPs have the lowest radical scavenging activity between 19-57%. ASNPs have shown significant scavenging activity which may contributed by the capping agent flavonoids an effective antioxidants having superoxide anion scavenging activity.[29]

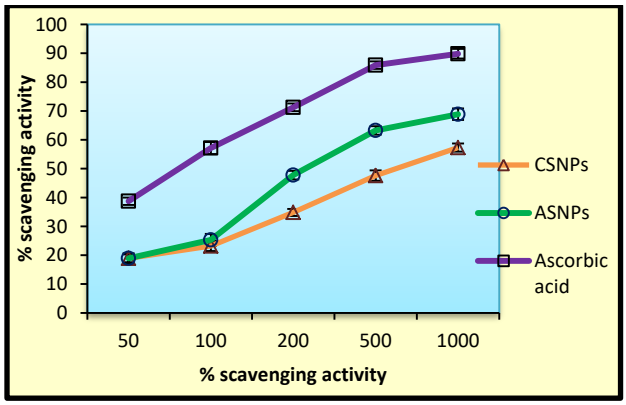

Figure 7: Graphical representation of superoxide radical scavenging activity CSNPs, ASNPs with standard ascorbic acid.

Biologically synthesized ASNPs have moderate activity, while CSNPs have very less antioxidant activity as compared to ascorbic acid. The differences between IC_{50} values of both silver nanoparticles clearly indicates that method of synthesis, capping agents have considerable contribution in antioxidant activity of SNPs.

CONCLUSION

Citric acid and *A. marmelos are* precursors used for SNPs synthesis. Some molecules from reducing agents were attributed as capping agents of CSNPs and ASNPs which proved by FTIR spectral peaks. These functional groups may also have contribution in the antioxidant properties of SNPs. From this study, it is clear that ASNPs have shown the moderate antioxidant activity as that of standard ascorbic acid and CSNPs have shown insignificant antioxidant activity. This efficacy of ASNPs is due to their capping agent acquired from plant extract. The radical scavenging activity depends on the surface charge and chemical properties of SNPs. So the method of synthesis, reducing agent and capping agent play an important role in antioxidant activity of silver nanoparticles. The exact components and factors responsible for antioxidant activity are needed to find out.

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