GREEN SYNTHESIS OF SILVER NANO PARTICLES BY PLANT LEAF EXTRACT

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Abstract

In the present work total reducing strength or phenolic compounds in leaf extract of *Conocarpus erectus* and *Nerium indicum* were determined and then Silver nanoparticles (Ag NPs) were synthesized by using only methanol extract of *Conocarpus erectus* leaves as reducing agent because of its higher values of total phenolic compounds (296 ± 9 µg/g) in comparison to *Nerium indicum* (185 ± 6 µg/g). Characterization of the green synthesized Ag NPs was performed by SEM (Scanning Electron Microscope) and XRD (X-Ray Diffractometer) techniques. The size of Ag NPs was estimated in the range of 35-55 nm. The usage of plant extract for the preparation of Ag NPs makes the process cost effective, non-toxic and green method.

Introduction

Conocarpus erectus is the species family Combretaceae. It is an evergreen tree and grows on coastal areas of hot regions of the world (Bailey, 1976). *Nerium indicum* is also an evergreen shrub or small tree of the genus *Nerium* and family *Apocynaceae*. It also grows everywhere in tropical regions (Vinayagam and Sudha, 2011). Though extract of both compounds have been reported rich in phenolic compounds but *Conocarpus* leaf extract was reported a large variety of these compounds (Vinayagam and Sudha, 2011; El-Sayed *et al.* 2012).

Phenolic compounds like tannins, flavonoids and phenolic acids are considered to be involved in redox activities so they are key performer to the reducing or antioxidant activity of medicinal plants, fruits or vegetables. The phenolic compounds because of their redox activities behave as hydrogen donators, reducing agents, singlet oxygen quenchers and also metal chelating agent (Rice-Evans, 2004). In plant extracts there are numerous types of phenolic compounds. They are highly reactive compounds and get involved in redox reactions. The presence of total phenolic substances within the plant extract could be liable for metal ions reduction and creation of the respective metal's nanoparticles (Nasrollahzadeh and Sajadi, 2015).

Nanoparticles are those particulate materials which have at least one dimension of less than 100 nm (Vidya *et al*., 2013). All the special Characteristics of NPs (nanoparticles) is due to their small size and large surface which make it highly reactive then the other larger particles. Noble metal NPs like platinum, gold and silver are highly recognized to have multiple applications in magnetic, electronics, information storage and Optoelectronics (Esumi *et al*., 1990; Sun *et al.,* 2001). Ag NPs (Silver Nanoparticles) are widely used in soaps, shampoos, detergents, toothpastes, cosmetics, medicines, drugs and pharmacy products; hence they are directly attached with human life (Pileni, 2000).

Various methods are available for the preparation of Ag NPs; it includes radiation (Henglein, 2001), electrochemical (Henglein, 1998), chemical (Henglein,1993), Langmuir-Blodgett (Chang and Yen, 1995) photochemical (Shiraishi and Toshima, 2000) and biological methods (Matejka *et al*.,1992; Armendariz *et al*.,2002; Chandran *et al.,2006;* Sharverdi *et al*.,2007). The synthesis of Ag NPs using plant-Extract as reducing agent is considered as a broadly acceptable technique for early production of Ag NPs (Klaus *et al.,* 1999) and successfully defeating the excessive demand and current market need and resulting in a decline in the labor force or production of hazardous materials to human life and the related environment (Nair and Pradeep, 2002; Willner *et al*., 2006).

The aim of present work is to synthesize Ag NPs by using leaf extracts of plant (*Conocarpus erectus* or *Nerium indicum*) due to higher values of total phenolic compounds because to the best of our knowledge through literature it was the first time to use plant leaf extract (*Conocarpus erectus* or *Nerium indicum*) for the green synthesis of silver nanoparticles.

Materials and Methods

All reagents such as Silver Nitrate and Methanol which were used throughout the research work were of analytical grade supplied by Merck (Germany) and Sigma-Aldrich (USA). Equipment were Analytical balance (Sartorius, Germany), SEM analyzer (Hitachi S4160, Japan) XRD analyzer (Karaltay, DX-2700 MIN), Magnetic stirrer/Hot plate (MS-H-Pro+), vacuum filtration assembly (Thomas 4595D45), Thermostat/incubator (Seimens), Spectrophotometer (Tomos), grinder (West point).

Preparation of extract of samples: Samples (*Conocarpus erectus* and *Nerium indicum*) were obtained from the Main Campus of NED University Karachi and they were shad-dried for one week and then they were grinded.

100 g of shade-dried leaves were grinded to form powder then it was added to 500 mL methanol, ethanol and distillated/deionized water in 1L flask and mixed vigorously. The preparation of plant extract was performed by using hot plate/magnetic stirrer at 50° C for 1 h. The acquired plant extract was filtered by vacuum filtration assembly.

Determination of total reducing strength or total phenolic compounds: Total reducing strength or total phenolic compounds in all plant extracts were determined as described by singleton *et al*., (1999) with the help of spectrophotometer. Briefly 0.5 mL of extract added with 10 % Folin-Ciocalteu's reagent (2.5 mL in equal voume of 7.5 % $NaHCO_3$). Blank was prepared by adding methanol (0.5 mL), 10 % Folin-Ciocalteu's reagent (It was dissolved in water and 7.5 % $NaHCO_3$ in equal volumes of 2.5 mL). The reaction mixtures were incubated at a temperature of 45° C for 45 minutes in an incubator/thermostat. The absorbance of solution was noted at 765 nm wavelength using spectrophotometer. Standard solution of gallic acid was used as standard.

Green synthesis of silver nanoparticles: The silver nanoparticles were prepared in a 250 mL conical flask in which 50 mL cent molar solution of silver nitrate was mixed with 10 mL of the plant extract (100 g of dried leaves powder of was added to 500 mL methanol, ethanol and deionized/distillated water in 1L flask) along with vigorous shaking on a hot plate till the appearance of yellowish brown colour.

Characterization of Ag NPs Characterization: The external appearance and size of produced Ag NPs were characterized by using SEM (Scanning Electron Microscope) and X-Ray Diffractometer (XRD).

Results and Discussions

Total phenolic compounds: Leaves extracts (water, ethanol and methanol) of *Conocarpus erectus* and *Nerium indicum* were investigated for total phenolic compounds and results are presented in table 1. It can be seen that the total phenolic compounds were found higher in methanol extracts (*Conocarpus erectus*, 296 ± 9 µg/g; *Nerium indicum*, 185 ± 6 µg/g) lower in water extract (*Conocarpus erectus*, 59 ± 8 µg/g; *Nerium indicum*, 27 ± 2 µg/g). As methanol leaf extract of *Conocarpus erectus* contained higher values of phenolics so in the present study it was used for the green synthesis of Ag NPs.

El-Sayed *et al.* (2012) have reported that the total phenolic compound were higher in ethyl acetate fraction of fruits and flowers (303.45 and 301.15 mg/g GAE respectively) whereas they were lower (186.21 and 181.61 mg/g GAE) in leaves and stem. On the contrary our results shows the methanol extract of leaves contain higher values which might be due to environmental effect and choice of solvent (Ahmed *et al*., 2015). Vinayagam and Sudha, (2011) has reported in scientific literature that the total reducing strength or total phenolic compounds was found to be higher in *Nerium indicum* flower extract (449 mg/100g), as compared to leaves 227mg/100g. Our selection of leaves extract as raw material for the green preparation of zinc nanoparticles is only due to the abundance and availability of leaves throughout the year.

Table 1. Total phenolic compounds in leaves extract of *Conocarpus erectus* and *Nerium indicum*.

Plant	Total Phenolic Compounds (µg/g)		
	Water	**Ethanol**	**Methanol**
Conocarpus erectus	59 ± 8	158 ± 5	296 ± 9
Nerium indicum	27 ± 2	149 ± 5	185 ± 6

Green synthesis of silver nanoparticles: In the present work our main focus is on the synthesis of Ag NPs using reducing properties of total phenolic compounds inside the plant leaves according to the scheme/mechanism (Fig. 1). Of course, the effect of other phytochemicals inside the plant is also possible (Nasrollahzadeh and Sajadi, 2015).

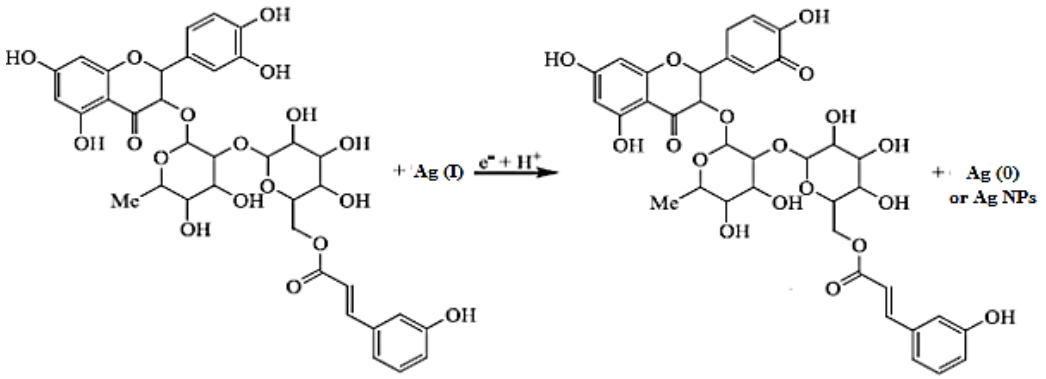

Fig. 1. Scheme/Mechanism for the formation of Zn NPs by phenolic compound.

SEM analysis: The SEM analysis is helpful in determining the structure of the Nanoparticles (or reaction products) that were fashioned. The SEM image (Fig. 2) disclosed a number of discrete Ag NPs as well as larger groups. The SEM image of Ag NPs also revealed that irregular and to the some extent spherical shaped nanoparticles were fashioned with the diameter range 30-80 nm whereas grouped particles were fashioned above the range 100 nm. Similar types of images for Ag NPs were also reported by various researchers (Henglein 1998; Sun *et al.* 2001; Pileni, 2000; Chandan *et al*., 2006; Sharverdi *et al*., 2007)

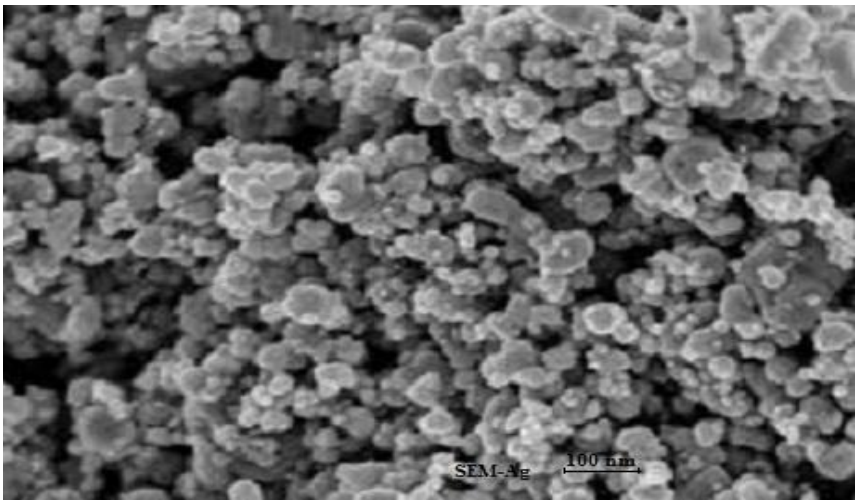

Fig.2. Scanning Electron Microscope Image of Green synthesized Ag NPs by *Conocarpus erectus.*

XRD analysis: The powdered sample was used for XRD Analysis in order to confirm the presence of Ag NPs. Relative intensities and Peak positions (Five peaks at 2θ values of 38.1, 44.5, 64.3 and 75.9 and 81.7 degrees corresponding to (111), (200), (220), (311) and (222) planes of Silver) for green synthesized Ag NPs (Fig. 3) were matched to values from JCPDS (Joint Committee on Powder Diffraction Standards) card for Ag file # 87–0720. The careful observations of peaks in the graph revealed that they are fairly agreed with the literature report (JCPDS silver file # 87–0720).

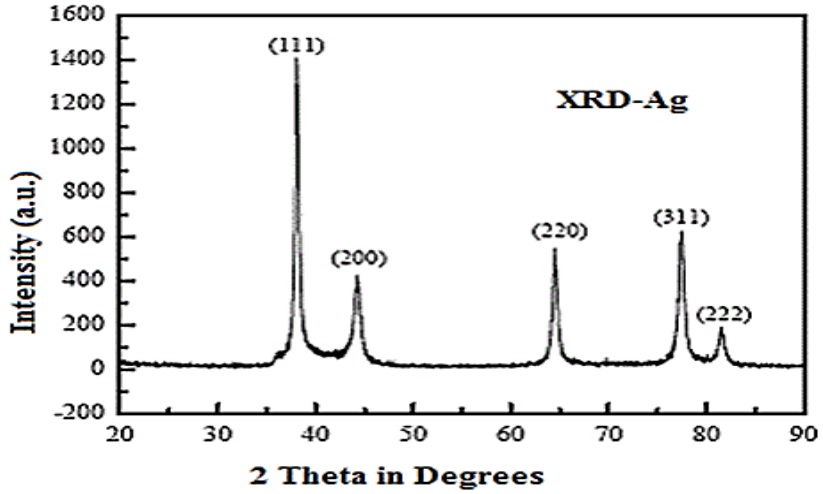

Fig. 3. XRD of Green synthesized Ag NPs by leaf extract of *Conocarpus erectus.*

Conclusions

It was concluded that methanol extract of *Conocarpus erectus* leaves contained higher value of total phenolic compounds (296 ± 9 µg/g) than *Nerium indicum* (185 ± 6 µg/g). It is also concluded that extract of *Conocarpus erectus* leaves can be utilized as a good reductant for the non-toxic or green synthesis of metal (Ag) nanoparticles.

References

Armendariz V, Gardea-Torresdey J. L., Jose Yacaman M., Gonzalez J, Herrera I, Parsons J. G. (2002). Gold nanoparticle formation by oat and wheat biomasses. *Proceedings of Conference on Application of Waste Remediation Technologies to Agricultural Contamination of Water Resources.*

Ahmed K., S. Munawar, T. Mahmood and I. Mahmood. (2015). Biochemical Analysis of Some Species of Seaweeds from Karachi Coastal Area. *FUUAST J. Biol*., 5(1): 43-45.

Bailey L. H. (1976). Hortus Third: *A Concise Dictionary of Plants Cultivated in the United States and Canada.* New York, MacMillan Collier MacMillan, p.306.

Chang L. T. and Yen, C. C. (1995). Studies on the preparation and properties of conductive polymers. Use of heat treatment to prepare metallized films from silver chelate of PVA and PAN. *J Appl Polym Sci*. 55(2): 371-374.

Chandran, S. P., Chaudhary M, Pasricha R, Ahmad A and Sastry M. (2006). Synthesis of gold Nano triangles and silver nanoparticles using *Aloe Vera* plant extract. *Biotechnol Prog*. 22: 577-583

El-Sayed, S. Abdel-Hameed, A. S. Bazaid, M. M. Shohayeb, M. M. Sayed and E. A. El-Wakil. (2012). Phytochemical Studies and Evaluation of Antioxidant, Anticancer and Antimicrobial Properties of Conocarpus erectus L. Growing in Taif, Saudi Arabia. *European Journal of Medicinal Plants.* 2(2): 93-112.

Esumi, K., Tano T., Torigoe K. and Meguro K. (1990). Preparation and characterization of biometallic Pd-Cu colloids by thermal decomposition of their acetate compounds in organic solvents. *J Chem Mater*. 2: 564-567.

Henglein, A. (1993). Physicochemical properties of small metal particles in solution: microelectrode' reactions, chemisorption, composite metal particles, and the atom-to-metal transition. *J Phys Chem* B. 97: 5457-5471.

Henglein, A. (1998). Colloidal silver nanoparticles: photochemical preparation and interaction with O_2, CCl_4, and some metal ions. *J Chem Mater*. 10: 444-446.

Henglein, A. (2001). Reduction of Ag (CN)-2 on silver and platinum colloidal nanoparticles. *Langmuir.* 17: 2329-2333.

Joint Committee on Powder Diffraction Standards, Powder Diffraction File No # 87–0720.

Klaus, T., Joerger R., Olsson E. and Granqvist C. G. (1999). Silver-based crystalline nanoparticles, microbially fabricated. *J Proc Natl Acad Sci. USA*. 96: 13611-13614.

Matejka, P, Vlckova B, Vohlidal J, Pancoska P, Baumruk V. (1992). The role of triton X-100 as an adsorbate and a molecular spacer on thesurface of silver colloid: a surface-enhanced Raman scattering study. *J Phys Chem*. 96(3): 1361-1366.

Nair, B and Pradeep T. (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. *Cryst Growth Des*. 2: 293-298.

Nasrollahzadeh, M. and S. M. Sajadi. (2015). Green synthesis of copper nanoparticles using Ginkgo biloba L. leaf extract and their catalytic activity for the Huisgen [3 + 2] cycloaddition of azides and alkynes at room temperature. *J. Colloid Interface Sci.* 457: 141-147

Pileni, M. P. (2000). Fabrication and physical properties of self-organized silver nanocrystals. *Pure Appl Chem*. 72: 53-65.

Rice-Evans, C. (2004). Flavonoids and Isoflavones: absorption, metabolism and bioactivity. *Free Rad. Biol*. 36: 827-828.

Sharverdi A. R., Mianaeian S., Shahverdi H. R., Jamalifar H. and Nohi A. A. (2007). Rapid synthesis of silver nanoparticles using culture supernatants of enterobacteria: a novel biological approach. *Process Biochem*. 42: 919-923.

Singleton V. L., Orthofer R. and Lamuela-raventos R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 299: 152-178.

Shiraishi Y., and Toshima N. (2000). Oxidation of ethylene catalyzed by colloidal dispersions of poly (sodium acrylate)-protected silver nanoclusters. *Colloids Surf A Physicochem Eng Asp*. 169: 59-66.

Sun Y. P., Atorngitjawat P. and Meziani M. J. (2001). Preparation of silver nanoparticles via rapid expansion of water in carbon dioxide microemulsion into reductant solution. *Langmuir*. 17: 5707-5710.

Vidya, C., S. Hirematha, M. N. Chandraprabhab, M. A. L. Antonyraja, I. V. Gopala, A. Jaina and K. Bansal. (2013). Green synthesis of ZnO nanoparticles by Calotropis Gigantea. Proc. of Nat. Conf. on Women in Sci. & Eng. (NCWSE 2013), SDMCET Dharwad. *Int. J. C. Eng. Technol.* 118-123.

Willner I, Baron R. and Willner B. (2006). Growing metal nanoparticles by enzymes. *J Adv Mater*. 18: 1109-1120.

Vinayagam A. and P. N. Sudha. (2011). Antioxidant activity of methanolic extracts of leaves and flowers of *nerium indicum IJPSR*. 2(6): 1548-1553.