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ACHYRANTHES ASPERA MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES

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Abstract

Bio-inspired AgNPs were rapidly synthesized at room temperature using fresh aqueous leaf extract of Achyranthes aspera. A green and low cost synthesis was effective in the formation of stable crystalline NPs in the solution. Carboxylic, ketone and aldehyde groups present in the A. aspera leaf extract functioned as reducing as well as stabilizing agent to produce shape controlled AgNPs. SPR confirmed the formation of AgNPs in UV-Visible spectra at 450 nm. The XRD result also showed the presence of elemental Ag^+ as a crystalline nature and the FT-IR analysis was carried out to identify and study the functional groups responsible for the bioreduction of Ag^+. TEM and SEM with EDX image showed spherical crystalline AgNPs. Thermogravimetric analysis was used to measure the weight loss of AgNPs as a function of temperature under a controlled atmosphere. Hence, the plant-based bio AgNPs could be used in biomedical applications.

Keywords: *Achyranthes aspera, Biosynthesis, AgNPs*

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INTRODUCTION:

Nanomedicine is an emerging field expanding rapidly because of the development and incorporation of new nano composites into a range of products and technologies. In recent years, the application of nanoparticles (NPs) in medicine has increased and expanded to the fields of molecular imaging [1], drug delivery [2], diagnosis and treatment of cardiovascular diseases [3], wound healing [4] and development of materials and medical devices with antimicrobial properties [5].

New applications of NPs and nanomaterials are emerging rapidly in biomedical sciences [6]. This decade has witnessed the inception of new significant technological products particularly based on nanotechnology; NPs synthesis is being widely explored, since they exhibit unique size and shape dependent properties for applications in optics, electronics, catalytic systems, magnetic and biomedical fileds such as HIV inhibition, cancer cell cytotoxicity and genotoxicity [7]. Apart from this, recently the anti-tumor effect of AgNPs has been reported against different cancerous cell lines [8]. NPs with the size range between 1 and 1000 nm are mainly explored for the diagnosis and treatment of human cancers, which led to the new discipline of nano-oncology [9].

There are number of methods used for the synthesis of silver nanoparticles (AgNPs) including physical and chemical methods [10-13], electrochemical reduction [14-15], photochemical reduction [16] and thermal evaporation [17-18]. However, rapid and green synthesis method using plant extract has developed enormous interest in AgNPs synthesis due to green chemistry approach. Moreover, it is simple, cost effective, eco-friendly, easily scaled up for large scale synthesis, without using toxic and redundant chemicals in solid, liquid and gaseous form [19]. Indeed, a number of bacteria [20], fungi and yeast have been well-known for synthesis of non-toxic noble NPs [21]. However the microbial-mediated synthesis of NPs is not industrially feasible as it requires expensive medium and maintenance of highly aseptic conditions [22].

In this context, plant-mediated NPs synthesis seems to be a cost-effective as well as eco-friendly method. Moreover, NP synthesis from plants with medicinal properties proves to be beneficial in treating various ailments in a better and easy way. On such plant is *Achyranthes aspera* L. (Amaranthaceae), which is distributed as weed throughout India, tropical Asia and other parts of the world. Ayurvedic, Yunani practitioners and Kabirajes use different parts of this plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, gonorrhea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria, toothache *etc*. The plant is a popular folk remedy in traditional system of medicine throughout the tropical Asian and African countries. The plant is reported to be used as antimicrobial, larvicidal, antifertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, antianasacra, analgesic, antipyretic, antinoiceptive, prothyrodic, antispasmodic and hepatoprotective.

Phytochemical investigations were carried out on this plant by several authors, which revealed the presence of sterols, alkaloids, saponins, sapogenins, cardiac glycosides, ecdysterone *etc.*, from different parts of this plant. Some other species of the genus *Achyranthes viz. A. fauriei, A. bidentata, A. japonica, A. ferruginea etc.* have also been investigated for their active constituents and pharmacological potential [23-33]. Survey of literature revealed that NPs synthesis from this plant is scanty. In view of this, the present study was designed to biosynthesize NPs from *Achyranthes aspera* leaves to study the reducing Ag^+ ions and stabilizing the particles and confirm AgNP synthesis by using various spectroscopy and microscopic methods.

MATERIALS AND METHODS:

Collection and identification

Fresh leaves of *Achyranthes aspera* were collected from Presidency College, Chennai, Tamil Nadu, India, and were authentically identified by Central Council for Research in Ayuveda and Siddha, Chennai, India, as *Achyranthes aspera*. (Amaranthaceae) with voucher specimen no: 16475.

Preparation of leaf extract and synthesis of AgNPs

Twenty grams of fresh leaves were washed thoroughly in tap water and distilled water for 30 min. in order to remove the debris. The aqueous extract was prepared by taking 25 g of washed and finely chopped leaves in 100 ml conical flask along with 100 ml of distilled water and the mixture was boiled at 45˚C for 30 min. This aqueous extract was filtered through Whatmann No. 1 filter paper and was used for synthesis of AgNPs. 10 ml of this aqueous leaf extract was added to 90 ml of 1mM aqueous silver nitrate solution for the synthesis of AgNPs. A control setup silver nitrate was also maintained without *A. aspera* extract.

Qualitative phytochemical analysis

Preliminary phytochemical analysis of aqueous extract was carried out by method of Harborne (1973) and Parekh and Chanda (2007) [34-35].

Characterization of silver NPs

UV-Visible spectra were recorded as a function of the reaction time on PG Instruments spectroscopy. The studies on size, morphology and composition of the NPs were performed by means of TEM (PHILIPS TECNAI 10) and SEM with EDX (Carl Zesis). The purified AgNPs were examined for the presence of biomolecules using FTIR analysis. Briefly, the spectrum obtained from the dried sample was recorded on FT-IR spectrum (Perkin-Elmer, USA) in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Particle size analyzer was done dynamic light scattering (Malvern, MAL 1062727, UK). Crystalline AgNPs were determined by XRD. Briefly, the biosynthesized AgNPs were laid onto glass substrates on Phillips PW 1830 instrument operating at a voltage of 40 kV and current of 30 mA with Cu Kα1 radiation. Thermal stability of NPs was done by TG-DTA (NETZSCH STA 409 PC/PG).

RESULTS AND DISCUSSION:

The present study was aimed to identify the phytocompounds present in *Achyranthes aspera* and to synthesize AgNPs from the aqueous extract of *A. aspera* leaves which is distributed as weed throughout India. Phytochemical study of *A. aspera* leaf extract shows the positive results for carbohydrates, tannins, phenols, flavonoids and triterpenoids. On the other hand, acids, alkaloids, anthocyanins and betacyanins, cardiac glycosides, coumarins, glycosides, proteins, quinones, saponins, starch and steroids were absent in the aqueous extract (Table 1). The bioactive compounds such as polyphenol, carbohydrates, vitamin and trace elements presnt in the leaf extract plays an important role as an antioxidant, anticancer, antitumor, anti-inflammatory, anti-obesity, anti-helminthic, analgesic, anti-pyretic, anti-ociceptive, anti-hepatitis, hepatoprotective, cardiac and Diuretic agent [36-40].

Table 1: Qualitative phytochemical analysis of *A. Aspera* aqueous extract

S.No.	Phytochemicals	Aqueous extract
1.	Acids	-
2.	Alkaloids	-
3.	Anthocyanins and Betacyanins	-
4.	Carbohydrates	+++
5.	Cardiac Glycosides	-
6.	Coumarins	-
7.	Flavonoids	+++
8.	Glycosides	-
9.	Phenols	+++
10.	Proteins	-
11.	Quinones	-
12.	Saponins	-
13.	Starch	-
14.	Steroids	-
15.	Tannins	+++
16.	Terpenoids	++
17.	Triterpenoids	+++

+++ Strongly present, ++ Mildly present
+ Present - Absent

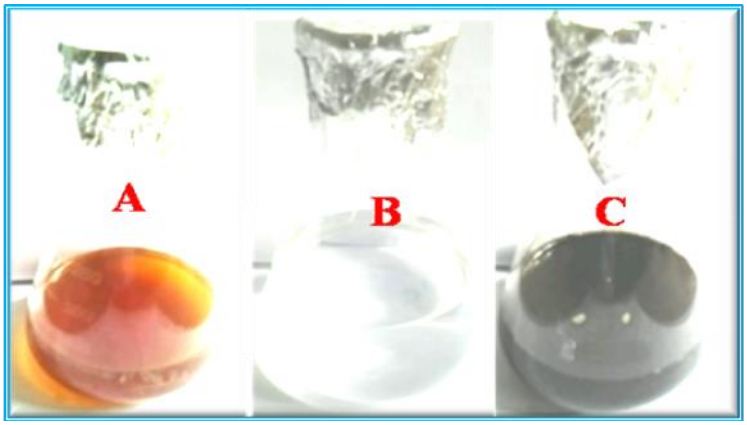

Fig. 1: A. Aqueous extract of *A. aspera* (pale yellowish brown colour)

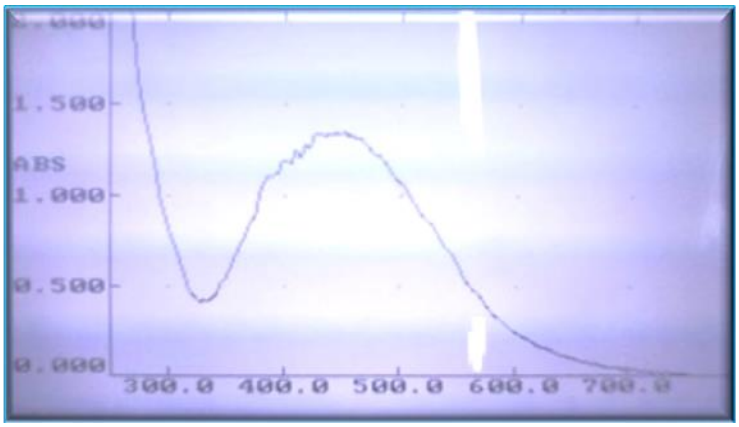

Fig. 2: UV-Vis spectral image of aqueous extract-based synthesized AgNPs.

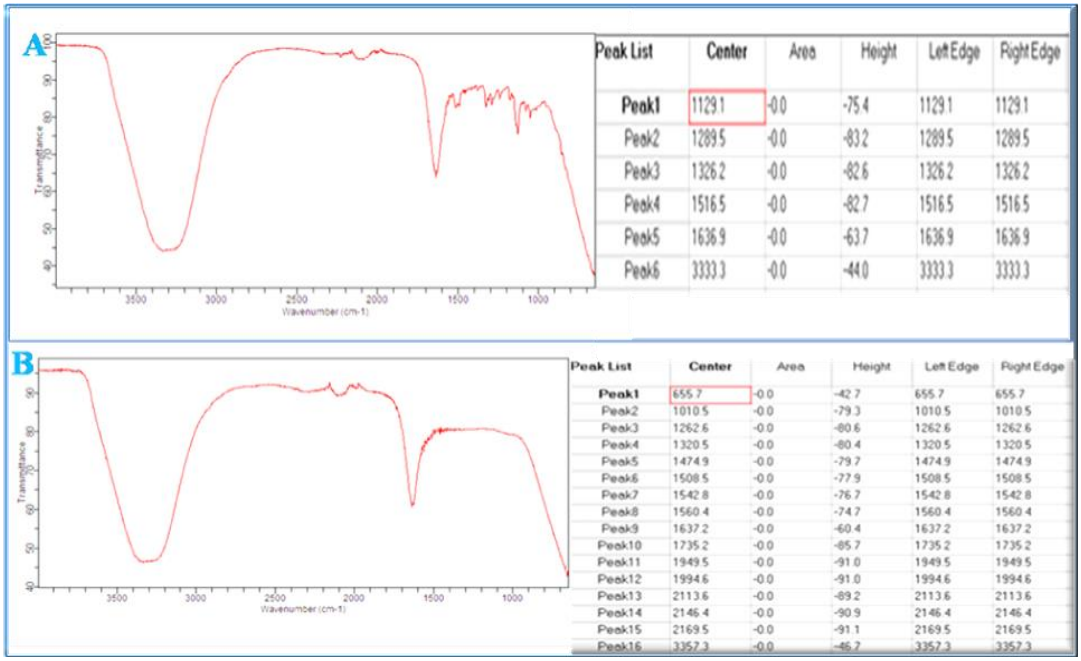

Fig. 3: FT-IR spectral image of various functional groups (1000 to 3500 cm-1) A- *A. aspera* aqueous extract and B- Aqueous extract-based synthesized AgNPs

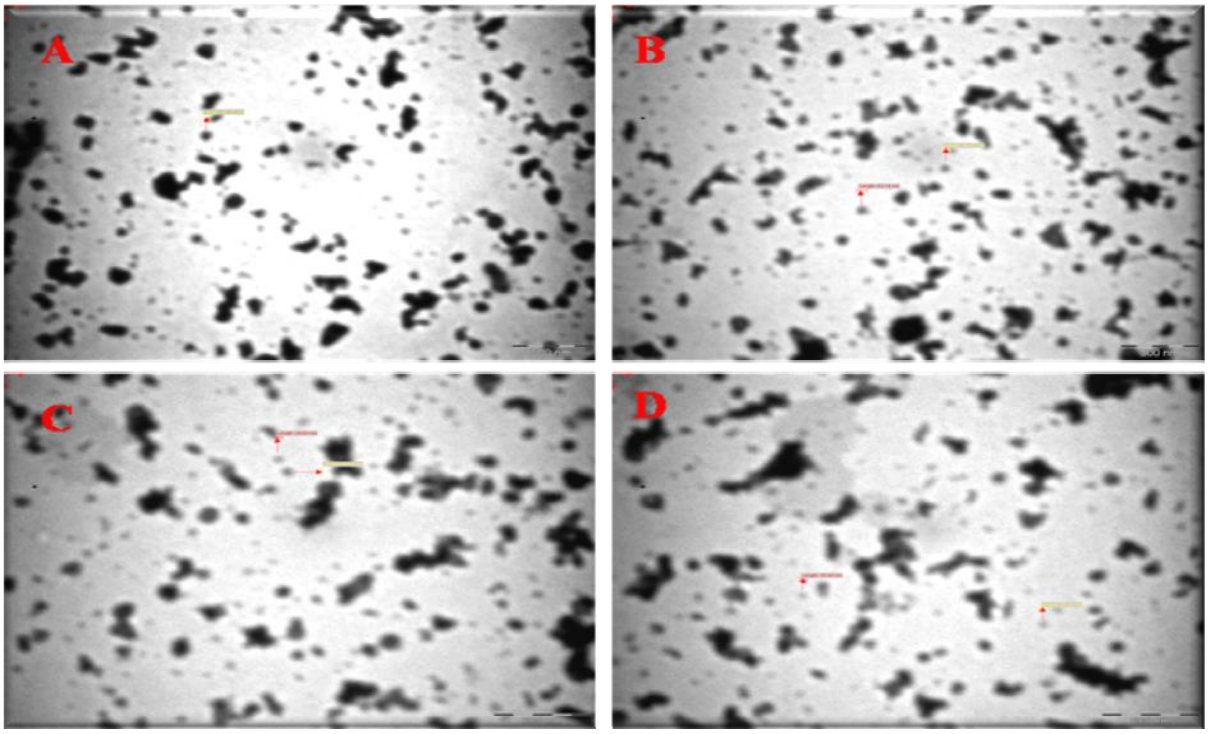

Fig. 4: TEM images of AgNPs formed by reduction of silver nitrate using *A. aspera* (A-D) 500 nm

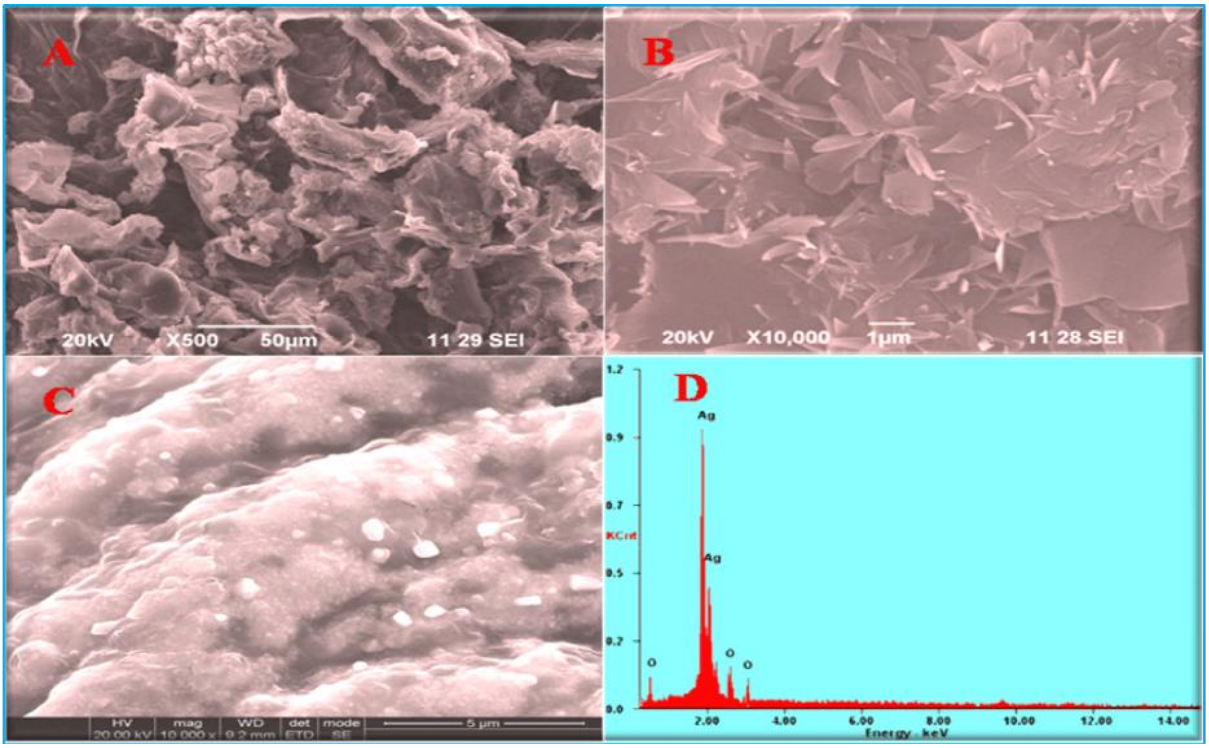

Fig. 5: A. SEM micrograph of *A. aspera* aqueous extract, B. SEM micrograph of 1 mM silver nitrate solution, C. SEM micrograph of *A. aspera* aqueous extract-based synthesized AgNPs and D. EDX analysis of AgNPs synthesized from the aqueous extract of *A. aspera*

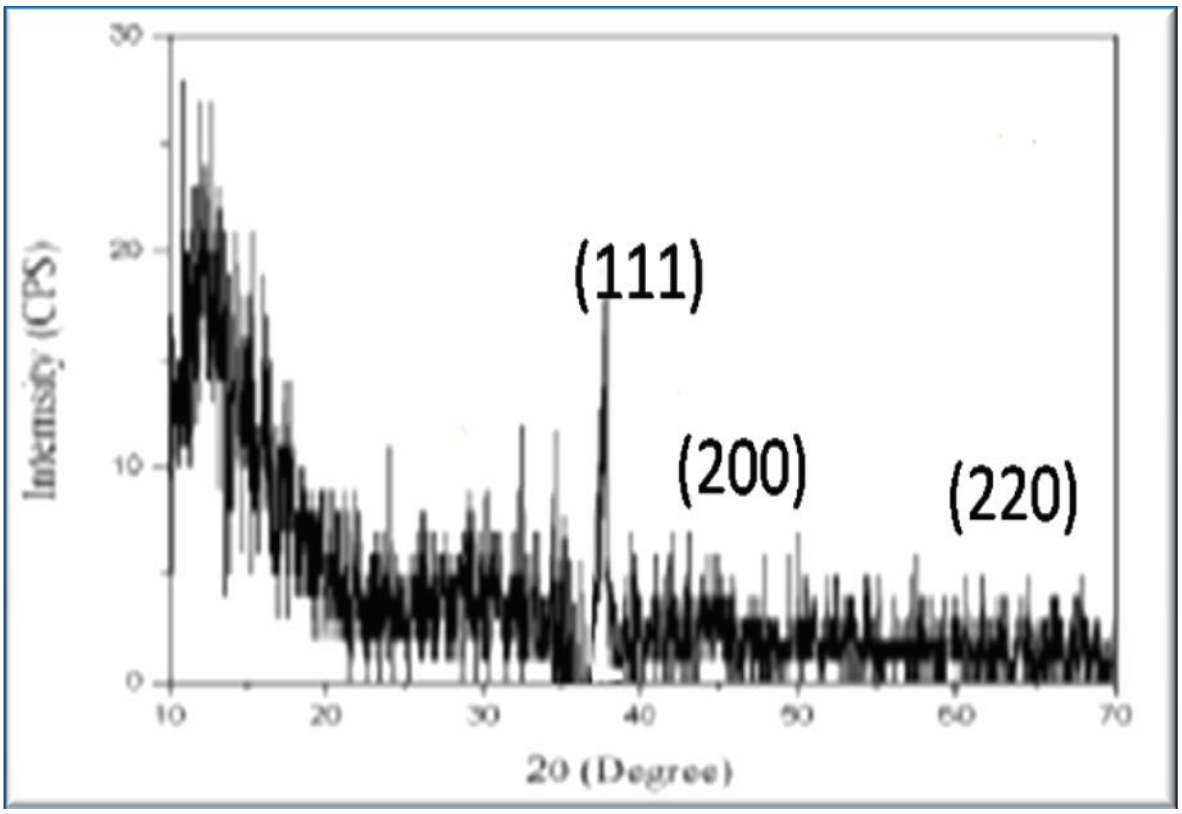

Fig. 6: XRD pattern for *A. aspera* mediated AgNPs

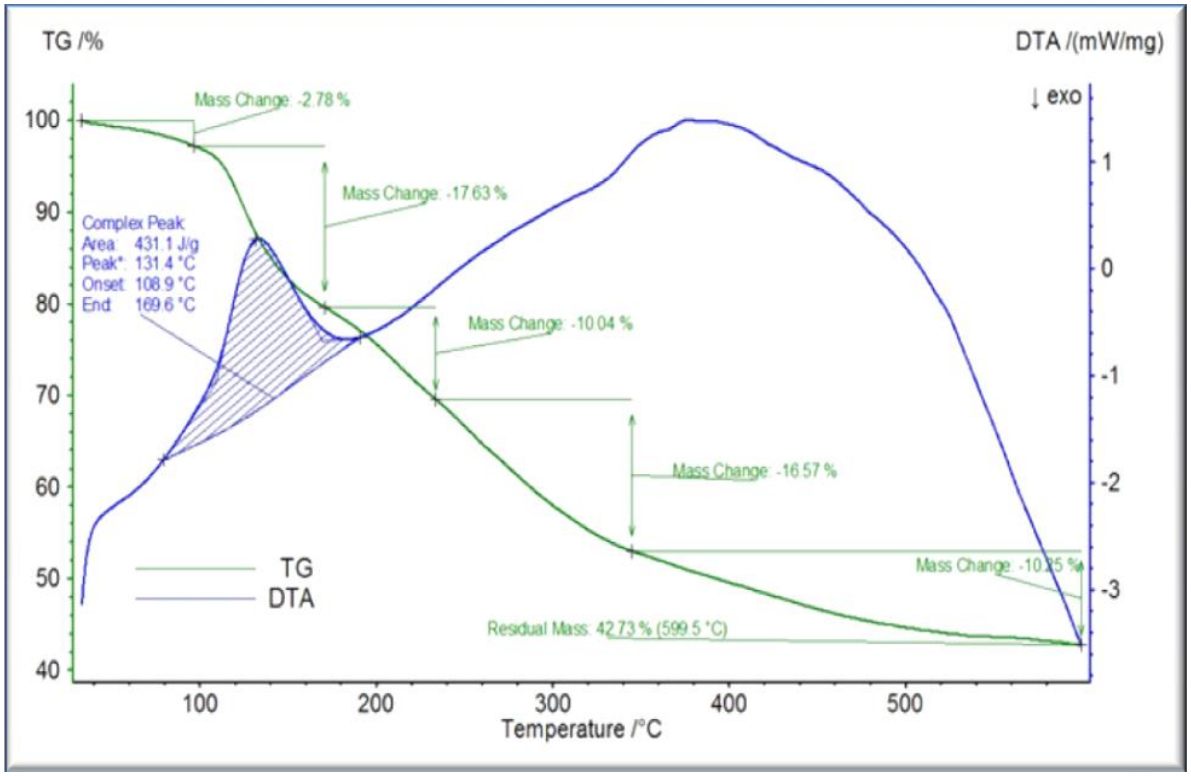

Fig. 7: TG-DTA analysis of *A. aspera* mediated AgNPs

During AgNP synthesis, the colour formation occurred within 15 min., with appearance of dark brownish black colour from pale yellowish brown colour solution. This might be due to the reduction of Ag+, indicating the formation of AgNPs (Fig. 1). The aqueous extract-based AgNPs showed prominent peak around λ max 450 nm within 15 min. (Fig. 2) with the elevated dark brownish black colour formation. Based on colour change and UV-Vis spectral analysis, aqueous extract-based synthesized *A. aspera* AgNPs were taken for further analysis. Similarly, AgNPs synthesized by using aqueous extract of *Sargassum polycystum* showed absorbance at 430 nm [41], *Sargassum longifolium* showed absorbance peak at 460 nm [42], *Eucalyptus hybrid* [43], *Acalypha indica* [44], *Solanum tarvum* [45], *Helianthus annus* [46], and *Cassia auriculata* [47], the absorbance peaks were between 400 and 450 nm. When compared with these plants and seaweeds, AgNPs synthesized from aqueous extract of *A. aspera* were active at relatively lower wavelength.

Fig. 3 shows the FT-IR spectra of aqueous leaf extract of *A. aspera* in which the interaction of biomolecules had intensive peak at 1000 and 3500 cm^{-1} (carboxylic groups) and it indicates the hydrogen bonded (O-H) stretch and the purified AgNPs at different hours shows peak shift of 3373 and 2925 of carboxylic groups indicates the O-H stretch. Earlier reports on the plant derived compounds *viz*., polyphenols like tannic acids have emphasized their efficacy as reducing agent in the synthesis of AgNPs [48, 49]. In the present study, the band at 3524 cm^{-1} is assigned for O-H stretching vibration of alcohol and phenol compounds and band observed at 1627.3 cm^{-1} corresponds to N-H groups of primary amines. The band at 1034.2 cm^{-1} shows that the C-O stretching vibrations of alcohols and carboxylic groups. Proteins present in the extract can bind to AgNPs through either free amino/carboxylic group in the proteins (Fig. 3A and Fig. 3B). Particularly, the peak at 1034.2cm^{-1} of the extract changed is to 1037cm^{-1} after synthesis confirming the reduction of Ag^{+} to AgNPs.

The morphology and size of the particles were determined by TEM and SEM. Fig.4 shows that the particles are spherical and triangular in shape well dispersed in nature and overall particle size ranges from 35.40 nm to 50.89 nm in 500 nm scale as depicted in TEM image. SEM was also used to investigate the morphology and size of the AgNPs. SEM image was recorded at different magnification and SEM image showed high density of AgNPs synthesized by *A. aspera* with the spherical and ovoid morphology (Fig. 5C). In contrary, aqueous extract and aqueous 1mM silver nitrate solution showed aggregated morphology (Fig. 5A and Fig. 5B) and SEM image has clearly proved the bioreduction of Ag^{+} to Ag^{0} by the formation of spherical ovoid morphology. Overall the size and morphology distribution of AgNPs was found to be below 100 nm.

Elemental Ag can be seen in the graph presented by the EDX analysis in support of SEM results, which indicated the reduction of Ag^{+} to elemental silver (Fig. 5 D). The recent study of Khalifa *et al*. [50] stated that particle size distribution obtained by DLS was found to be mono-dispersed, or poly-dispersed ranging from 10 nm to 100 nm. XRD analysis of the NPs showed intense peak corresponding to (111), (200) and (220) Bragg's reflection based on the face centered cubic structure of AgNPs (Fig. 6). The peak corresponding to (111) plane is more intense than the other planes, suggesting that the (111) plane is in the predominant orientation. Similar results were reported by [50-51], these author's biosynthesized AgNPs using *Cinnanonum camphora* and marine algae (*Sargassum polycystum* and *Sargassum longifolium*) extract compound respectively.

TG-DTA analyses for biosynthesized AgNPs have been recorded. A ceramic crucible used for heating the sample and the analysis were carried in an atmosphere of N_2 at the heating rate of 20 ºC per minute and the temperature ranges from 30 ºC to 600 ºC. The TG-DTA curve of biosynthesized silver NPs is illustrated in Fig. 7. The initial mass of the material subjected to analysis was 2 mg and final mass left out after the experiment was only 42.73% (599.5ºC) of the initial mass at the temperature of about 600ºC indicating that bulk decomposition occurred in the sample. When the biosynthesized AgNPs reached 90ºC, the weight loss was up to 2.78-10.25%, which is basically due to vapor or water being released. The TG-DTA analysis of AgNPs revealed that NPs was thermally stable at 90ºC in a nitrogen atmosphere. According to Ankamwar *et al.* [52], the thermogravimetric analysis of tamarind leaf extract reduced gold nanotriangle powder showed an initial weight loss at 125ºC, which is due to the presence of water molecules in the tamarind leaf extract and also there was a steady weight loss until 600 ºC. The weight loss may due to desorption of 22 % of calcium and other bioorganic compounds present in the NPs.

CONCLUSION:

In this paper, we have reported cost-effective and eco-friendly bio reducing method for synthesizing silver NPs using fresh leaves aqueous extract. These biologically synthesized silver NPs play a crucial role

in protecting our environment as green. UV-Vis spectroscopy revealed the surface plasmon property, while TEM and SEM with EDX image revealed the nano nature of the prepared sample. The structural analysis by XRD strongly suggests the formation of elemental silver NPs instead of their oxides in biosynthesized NPs. The XRD structural analysis of AgNPs showed that they were crystalline in nature, which might be due to the presence of bioreduction of AgNPs. The thermal stability of AgNPs was proved by TG-DTA, where AgNPs were found to be thermally stable up to 90ºC and the residual mass (599.5ºC), in sample was 42.73%, indicating the small amount of organic contents in synthesized sample. Therefore, the use of natural antioxidants for the synthesis of AgNPs seems to be a good alternative which could be attributed to its benign composition. The plant material responsible for the reduction and stabilization of nanoparticle needs further study including extraction and identification of the bioactive compounds presented in the extract.

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REFERENCES:

1. Kohl Y, Kaiser C, Bost W, Stracke F, Fournelle M, Wischke C. Preparation and biological evaluation of multifunctional PLGANPs designed for photoacoustic imaging. Nanomed, 2011;7(2):228-37.
2. Meng H, Liong M, Xia T, Li Z, Ji Z. Engineered design of mesoporous silica NPs to deliver doxorubicin and P-glycoprotein siRNA to overcome drug resistance in a cancer cell line. ACS Nano., 2010;4(8):4539-50.
3. Godin B, Sakamoto JH, Serda RE, Grattoni A, Bouamrani A, Ferrari M. Emerging applications of nanomedicine for the diagnosis and treatment of cardiovascular diseases. Trends Pharmacol. Sci., 2010;31(5):199-205.
4. Tian J, Wong KK, Ho CM, Lok CN, Yu WY, Che CM. Topical delivery of silver NPs promotes wound healing. Chem. Med. Chem., 2007;2 (1):129-36.
5. Rangari VK, Mohammad GM, Jeelani S, Hundley A, Vig K, Singh SR. Synthesis of Ag/CNT hybrid NPs and fabrication of their nylon-6 polymer nanocomposite fibers for antimicrobial applications. Nanotechnology, 2010;21(9):95-102.
6. Yu DG. Formation of colloidal silver NPs stabilized by Na^+–poly(γ-glutamic acid)–silver nitrate complex via chemical reduction process. Colloids Surf. B., 2007;59(2):171-178.
7. Tan Y, Wang Y, Jiang L. Thiosalicylic acid-functionalized silver NPs synthesized in one-phase system. J. Colloid Interface Sci., 2002;249(2):336-345.
8. Petit C, Lixon P, Pileni MP. *In situ* synthesis of silver nanocluster in AOT reverse micelles. Phys. Chem., 1993;97(49):12974-12983.
9. Vorobyova SA, Lesnikovich AI, Sobal NS. Preparation of silver NPs by interphase reduction. Colloids Surf. A., 1999;152(3):375-379.
10. Saha S, Sarkar J, Chattopadhyay D, Patra S, Chakraborty A, Acharya K. Production of silver NPs by a phytopathogenic fungus *Bipolaris nodulosa* and its antimicrobial activity. Dig J Nanomaterials and Biostructures, 2010;5(4):887-895.
11. Sandmann G, Dietz H, Plieth W. Preparation of silver NPs on ITO surfaces by a double-pulse method. J. Electroanal. Chem., 2000;491(5):78-86.
12. Mallick K, Witcombb MJ, Scurrella MS. Self-assembly of silver NPs in a polymer solvent: formation of a nanochain through nanoscale soldering. Mater. Chem. Phys., 2005;90(22):221-224.
13. Bae CH, Nam SH, Park SM. Formation of silver NPs by laser ablation of a silver target in NaCl solution. Appl. Surf. Sci., 2002;197(4):628-634.
14. Smetana AB, Klabunde KJ, Sorensen CM. Synthesis of spherical NPs. J. Colloid Interface Sci., 2005;284(4):521-526.
15. Saxena A, Tripathi RM, Singh RP. Biological synthesis of silver NPs by using onion (*Allium cepa*) extract and their antibacterial activity. Dig. J. Nanomater. Bios., 2010; 5(2):427-432.
16. Priyadarshini S, Gopinath V, Meera Priyadharsshini N, MubarakAli D, Velusamy P. Bio-Inspired Green NPs: Synthesis, Mechanism, and Antibacterial Application. Colloids Surf. B., 2013;102(2):232-237.
17. Fayaz AM, Girilal M, Rahman M, Venkatesan R, Kalaichelvan PT. Biogenic synthesis of silver NPs and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Process Biochem.,

2011;46(6):1958-1962.

18. Laldhas KP, Cheriyan VT, Puliappadamba VT, Bava SV, Unnithan RG, Vijayammal PL, Anto RJ. Green synthesis of silver NPs using herbal plants. J. Cell Mol. Med., 2010; 14(8):636-646.

19. Song JY, Kim BS, Rapid biological synthesis of silver NPs using plant leaf extracts. Bioprocess Biosyst Eng, 2008;32:79-84.

20. Murphy CJ. Sustainability as an emerging design criterion in nanoparticle synthesis and applications. J. Mater Chem., 2008;18:2173-2176.

21. Mubarak Ali D, Thajuddin N, Jeganathan K, Gunasekaran M. Plant-mediated synthesis of silver and gold NPs and its antibacterial activity against clinically isolated pathogen. Colloids Surf B: Biointerfaces, 2011;85(2):360-365.

22. Kumar V, Yadav SK. Plant -mediated synthesis of silver and gold NPs and their applications. J Chem Technol Biotechnol, 2009;84:151-157.

23. Shibeshi W, Makonnen E, Zerihun L, Debella A. Effect of *Achyranthes aspera* L. on fetal abortion, uterine and pituitary weights, serum lipids and hormones. African Health Sciences, 2006;6(2):108-112.

24. Joshi AC. Dedoublement of stamens in *Achyranthes aspera* Linn. J. Indian bot. Soc., 1932;11:335-339.

25. Kajale LB. Embryology of *Achyranthes aspera* Linn. Proceedings: Plant Sciences, 1937;5(5):195-205.

26. Perveen A, Abid R, Fatima R. Stomatal types of some dicots within flora of Karachi, Pakistan. Pak. J. Bot., 2007;39(4):1017-1023.

27. Dastur RH. Origin and course of vascular bundles in *Achyranthes aspera* L. Ann. Bot. 1925;39:539-545.

28. Joshi AC. Contribution to the anatomy of the Chenopodiaceae and Amaranthaceae. II. Primary vascular system of *Achyranthes aspera* L., *Cyathula prostrata* Blume and *Pupalia lappacea* Juss. J. Indian Bot. Soc., 1931;10:265-292.

29. Joshi AC. Variations in the medullary bundles of *Achyranthes aspera* L. and the original home of the species. New Phytologist, 1934;33:53-57.

30. Rajput KS, Rao KS. Secondary growth in the stem of some species of Alternanthera and *Achyranthes aspera* (Amaranthaceae). Iawa Journal, 2000;21:417-424.

31. Bhambie S, Sharma A. Ontogeny of cambium in *Amaranthus caudatus* L. and *Achyranthes aspera* L. Proceedings of the Indian Academy of Sciences, 1985;95:295-301.

32. de Lange PJ, Scofield RP, Greene T. *Achyranthes aspera* (Amaranthaceae), a new indigenous addition to the flora of the Kermadec Islands group. New Zealand J. Bot., 2004;42:167-173.

33. Shafique S, Javaid A, Bajwa R, Shafiqe S. Biological control of *Achyranthes aspera* and *Xanthium strumarium* in Pakistan. Pak. J. Bot., 2007;39(7):2607-2610.

34. Harborne JB. In: "Phytochemical Methods". (Ed), 1st Edn, *Chapman and Hall Ltd*., London, 1973;49-188.

35. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed Res., 2007;10:175-181.

36. Saad A, Siddiqui MMH, Aleem S, Jafri SAH. Effect of Namak Chirchita (*Achyranthes aspera* Linn.) in Zeeq-un-Nafs Sho'bi (Bronchial Asthma). Hamdard Medicus, 2002;45:37-40.

37. Shah GB, Parmar NS. Antiasthmatic property of polyherbal preparation E-721. B, Phytotherapy Research, 2003;17(9):1092–1097.

38. Goyal BR, Mahajan SG. Beneficial effect of *Achyranthes apsera* L. in Toluene-di-isocyanate induced occupational asthma in rats. Global Journal of Pharmacology, 2007; 1(1):06-12.

39. Aswal BS, Goel AK, Kulshrestha DK, Mehrotra BN, Patnaik GK. Screening of Indian plants for biological activity. Ind. J. Exp. Biol., 1996;34:444-467.

40. Gupta SS, Khanijo I. Antagonistic effect of *Achyranthes aspera* on uterine contractility induced by oxytocin. Indian Journal of Physiology and Pharmacology, 1970;14:63.

41. Asha Kanimozhi S, Johnson M, Renisheya Joy Jeba Malar T. Phytochemical composition of *Sargassum polycystum* C. agardh and *Sargassum duplicatum* J. agardh. Int J Pharm Pharm Sci., 2012: 7(8): 393-397.

42. Rajeshkumar S, Malarkodi C, Vanaja M, Paulkumar K. Green-chemical Fabrication of Silver NPs by Marine macro Algae and its Fungicidal Activity. International Research Journal of Pharmaceutical and Biosciences, 2014;1(1):01-07.

43. Dubey M, Bhaduria S, Kushwah BS. Green synthesis of nanosilver particles from extract of *Eucalyptus hybrida (Safeda)* leaf. Digest Journal of Nanomaterials and Biostructures, 2009;4(3):537-543.

44. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf

extracts and its antibacterial activity against water borne pathogens. Colloids and Surfaces B: Biointerfaces, 2010;76: 50-56.

45. Govindaraju K, Tamilselvan S, Kiruthiga V, Singaravelu G. Biogenic silver nanoparticles by *Solanum torvum* and their promising antimicrobial activity. J. Biopesticides, 2010; 3(1):394-399.

46. Leela A, Vivekanandan M. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. Afr. J. Biotechnol, 2008;7:3162-3165.

47. Udayasoorian C, Vinoth Kumar K, Jayabalakrishnan RM. Extracellular synthesis of silver NPs using leaf extract of *Cassia auriculata*. Digest Journal of Nanomaterials and Biostructures, 2011;6(1):279-283.

48. Vivekanandhan S, Misra M, Mohanty AK. Biological synthesis of silver NPs using Glycine max (soybean) leaf extract: an investigation on different soybean varieties. J NanosciNanotechnol, 2009;9:6828-6833.

49. Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Biomedical potential of silver NPs synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad. J. Nanobiotechnology, 2011;9(43):1-8.

50. Khalifa KS, Hamouda RA, Hanafy D, Hamza A. *In vitro* antitumor activity of silver NPs biosynthesized by marine algae. Digest Journal of Nanomaterials and Biostructures, 2016;2(1):213-221.

51. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C. Biosynthesis of silver and gold NPs by novel sun dried *Cinnanonum camphora* leaf. Nanotechnology, 2007;18:105104-105114.

52. Ankamwar B, Damle C, Ahmad A, Sastry M. Biosynthesis of Au and AgNPs using *Emblica officinalis* fruit extract, their phase transfer and transmetallation in an organic solution. J. Nanosci. Nanotechnol, 2005;5(10):1665-1667.