SYNTHESIS OF SILVER NANOPARTICLES FROM *Dodonaea angustifolia LEAF* EXTRACT AND EVALUATION OF ITS ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticles. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its wide range of applications for many sectors of life and Industry. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM $AgNO_3$ solution through the extract of Dodonaea angustifolia leaf as it is acts as a reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy and SEM analysis showed the average particle size range10-63nm with higher density polydispersed spherical in shape. The synthesized silver nanoparticles exhibited potential anti-inflammatory activity.

Key words: *Dodonaea angustifolia, Silver nanoparticles, SEM, Anti-inflammatory activity.*

INTRODUCTION

Nanotechnology can be defined as the manipulation of atom by atom from the material world by the combination of engineering, chemical and biological approaches. In the past decade, considerable attention has been paid for the development of novel strategies for the synthesis of different kind of nano-objects. Most of the current strategies are usually worked by the use of physical or chemical principles to develop a variety of nano-objects with multiple applications. Main fields of nanotechnology applications range from catalysis, micro- and nano-electronics (semiconductors, single electrons transistors), non-linear optic devices, photo-electrochemistry to biomedicine, diagnostics, foods and environment, chemical analysis and others (Contescu and Putyera, 2009).

Metal nanoparticles can be prepared by physical, chemical and biological routes; the first one is a physical approach that utilizes several methods such as evaporation/condensation and laser ablation. The second one is a chemical approach in which the metal ions in solution are reduced in conditions favoring the subsequent formation of small metal clusters or aggregates (Khomutov and Gubin, 2002). Various metals like copper, titanium, gold, silver and iron were used for the synthesis of nanoparticles. Among the noble metals, silver nanoparticles have become the focus of intensive research due to its wide range of applications for many sectors of life and industry (El-Kheshen and Gad El-Rab, 2012). Recently, biosynthetic methods employing naturally occurring reducing agents such as polysaccharides, biological microorganism such as bacteria and fungus or plants extract, i.e. green chemistry, have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain AgNP's (Amanullah and Yu, 2005).

In the recent decades, increased development of green synthesis of nanoparticles is inevitable because of its incredible applications in all fields of science. There were numerous work have been produced based on the plant and its extract mediated synthesis of nanoparticles. AgNP's has been synthesized by using the plant broth from a wide variety of plants such as *Catharanthus roseus* (Mukunthan *et al.*, 2011) and *Bacopa monnieri* (Krishnaraj *et al.*, 2012). Keeping in view, in the present study aimed to explore the novel approaches for the biosynthesis of silver nanoparticles using *Dodonaea angustifolia* leaf and also evaluate the anti-inflammatory activity of the synthesised AgNP's.

Materials and Methods

Chemicals

All the experiments were conducted at room temperature. Materials used for the synthesis of silver nanoparticles were AR grade silver nitrate ($AgNO_3$) purchased from Merck, India.

Collection of *Dodonaea angustifolia* leaves

The *Dodonaea angustifolia* leaves were collected in January 2017 from Tamil University campus, Thanjavur, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, Director, Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (NR001) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of leaves extract

The dried leaves were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed with 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of Ag nanoparticles using leaf extracts

For the Ag nanoparticles synthesis, 5 ml of *Dodonaea angustifolia* leaf extract was added to 45 ml of 1mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in dark 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without leaf extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freezed and dried for using SEM analysis (Arunachalama *et al.*, 2012).

UV-Vis and FTIR Spectra analysis

The reduction of pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium for 5 hours after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu). The resulting pellet is dissolved in deionized water and filtered through whatman filter paper No: 42. An aliquot of this filtrate containing silver nanoparticles were used for Fourier transmission Infrared spectroscopy (FTIR).

SEM analysis of silver nanoparticles

Scanning electron microscopic (SEM) analysis was done using ZEISS machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by placing it under a mercury lamp for 5 min.

Anti-inflammatory activity

Anti-inflammatory activity of the *Dodonaea angustifolia* leaves extract and $AgNO_3$ was evaluated by protein denaturation method as described by Padmanabhan and Jangle (2012).

Results and Discussion

Synthesis of silver nanoparticles

The synthesis of silver nanoparticles through leaf extracts were carried out. Leaf extract is used as reducing agent for its distinctive properties with catalytic and chemical stability. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials). The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies from plant to plant. The phytochemicals present in the leaf extract were considered responsible for the reduction of silver ions. It is also well known that silver nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmoo vibrations in silver nanoparticles The appearance of yellowish-brown colour (Plate.1) in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Thirumurgan *et al.* 2010).

$AgNO_3$ = 1 mM $AgNO_3$ without *Dodonaea angustifolia* extract.
AgNPs= 1 mM $AgNO_3$ with *Dodonaea angustifolia* leaf extract after 5 hrs of incubation (Brown colour)

Plate.1: Formation of brown colour after addition of $AgNO_3$ indicate synthesis of AgNPs in the process of reduction of Ag^+ to Ag nanoparticles and control ($AgNO_3$)

UV-Vis and FTIR Spectra analysis

It is generally recognized that UV–Vis spectroscopy could be used to examine the size and shape-controlled nanoparticles in aqueous suspensions**.** Fig.1 shows the UV-Vis spectra recorded for the reaction medium after 5 hours. The UV–Vis spectra of the reaction mixture of silver nitrate solution with *Dodonaea angustifolia* leaf extract shoed peaks at 422 nm indicate the presence of silver nanoparticles which is synthesized by *Dodonaea angustifolia* extract; The peak was raised due to the effect of surface plasmon resonance of electrons in the reaction mixture and the broadening of peak indicated that the particles are polydispersed. Appearance of this peak assigned to a surface plasmon, is well-documented for various metal nanoparticles with size ranging from 2 nm to 100 nm (Henglein, 1993).

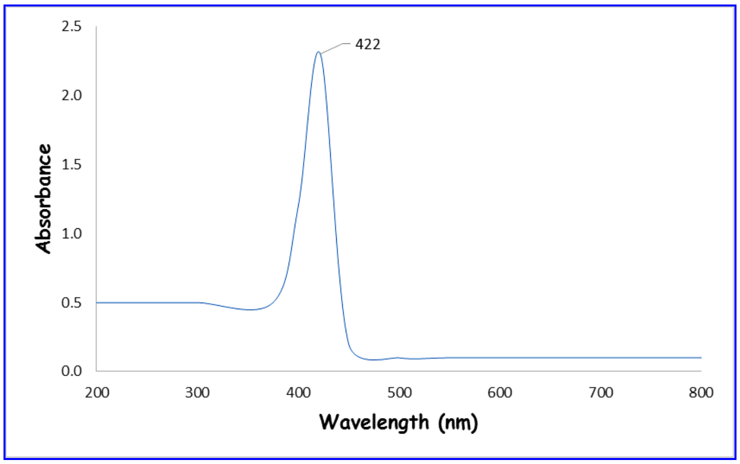

Fig.1: UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Dodonaea angustifolia leaf* extract after 5 hrs.

FTIR is an important tool which enables us to understand the involvement of functional groups in the interactions between metal particles and biomolecules. In the present work, FTIR spectra was used for the identification of biomolecules responsible for capping and stabilizing the silver nanoparticles. The FTIR spectra of the *Dodonaea angustifolia* is given in the Fig 2. FTIR spectrum of *Dodonaea angustifolia* extract shows peak at 3304, 2775, 1651, 1405, 1313 and 674. The band appeared at about 1405 cm^{-1} can be assigned the aromatic rings. The strong broad band appearing at 3304 cm^{-1}can be associated to the stretching vibrations of alcoholic and phenolic O–H. At peak at 2775cm^{-1} that could be assigned aldehyde to H–C=O: C–H Stretch group. Therefore, from the results of FTIR analyses of extract mediated synthesised of silver nanoparticles it can be concluded that some of the biological molecules of leaf extract such as alkaloids, phenols, flavonoids, amino acids, glycosides, and tannins are responsible for the biotransformation of silver ions to silver nanoparticles and its stabilization in aqueous medium. This results were good agreement with the earlier reports (Manimegalai and Velavan, 2015; Amargeetha and Velavan, 2017).

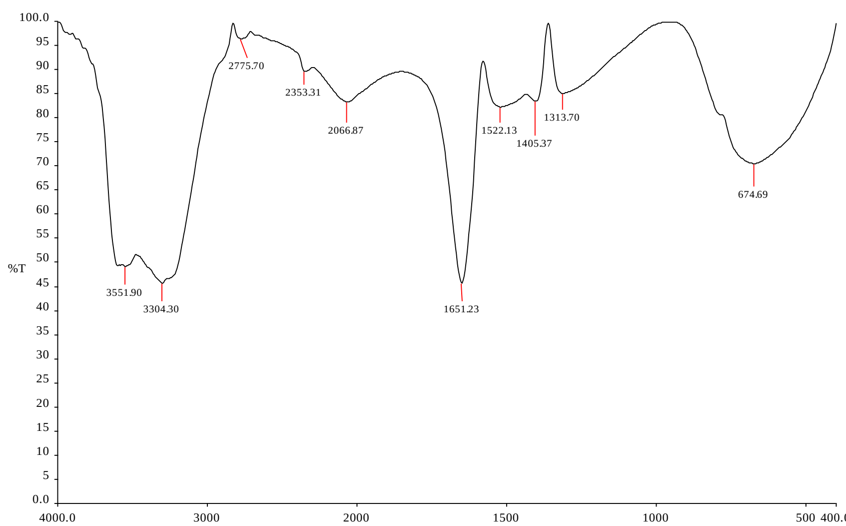

Fig.2: FTIR analysis of silver nanoparticles synthesized by treating 1mM aqueous $AgNO_3$ solution with *Dodonaea angustifolia* extract.

Scanning Electron Microscope (SEM)

The surface morphology, size and shape of the silver nanoparticles were analyzed by Scanning Electron Microscope. Plate 2 shows the SEM image of silver nanoparticles synthesized from leaf extract. The SEM images show individual silver nanoparticles which are of higher density polydispersed spherical in shape as well as number of aggregates with no defined morphology. The presences of biomolecules in the leaf extract has resulted in the synthesis of spherical silver nanoparticles and the aggregation may be due to the presence of secondary metabolites in the leaf extract. The SEM image shows the size of the silver nanoparticles ranging from 10 to 63 nm. Similar result of the silver nanoparticles size was reported by Gnanasundaram and Balakrishnan (2017) study using *Cissus vitiginea* leaf extract.

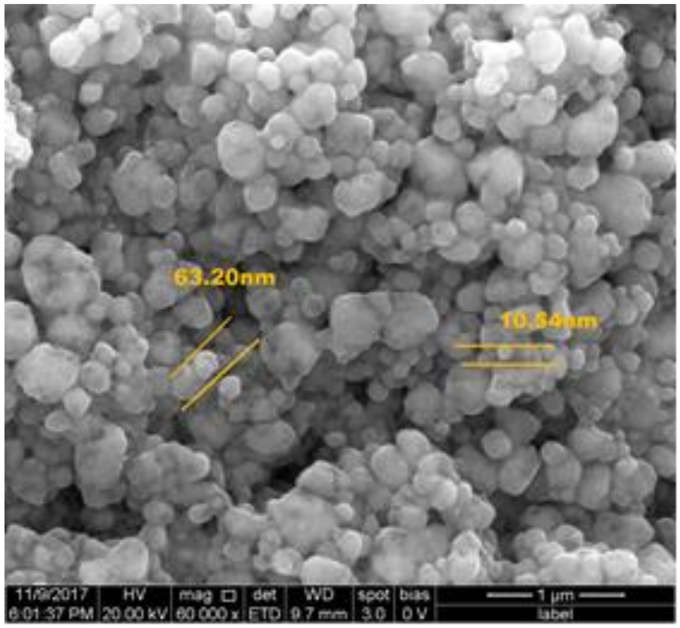

Plate.2: High resolution scanning electron microscopic (SEM) image of silver nanoparticles (AgNPs). Polydispersed (Cluster) AgNPs ranged between 13–63nm.

Anti-inflammatory activity

The use of nano-herbal-technology to synthesize compounds with improved anti-inflammatory properties is an area of current research by many scientists. In our study, we report the non-toxic, practical and environmentally benevolent approach for the synthesis of silver nanoparticles using the aqueous leaf extract of *Dodonaea angustifolia* with potent anti-inflammatory activity. The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein denaturation by *Dodonaea angustifolia* leaf extract, AgNPs and reference drug diclofenac sodium. The present findings exhibited a concentration dependent inhibition of protein denaturation by the *Dodonaea angustifolia* leaf extract and AgNPs. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard diclofenac sodium. The half inhibition concentration (IC_{50}) of *Dodonaea angustifolia* leaf extract, AgNPs and diclofenac sodium were 328.54, 312.45 and 290.45µg/ml^{-1} respectively. From the present study it can be concluded that AgNPs showed marked *in vi*tro anti-inflammatory effect against the denaturation of protein (Table 1 and Fig 3). Our results was in good agreement with the earlier reports (Aparna Mani *et al*., 2015; Giridharan *et al*., 2014).

Table.1: Effect of *Dodonaea angustifolia* , AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

Concentrations	% of inhibition		
	Dodonaea angustifolia	**AgNPs**	**Diclofenac sodium (Standard)**
100µg/ml	15.63 ± 0.95	16.25 ± 1.52	20.88 ± 1.82
200µg/ml	25.36 ± 1.37	30.74 ± 1.99	32.45 ± 2.44
300µg/ml	40.65 ± 2.47	52.14 ± 3.71	54.55 ± 3.63
400µg/ml	53.62 ± 3.39	63.52 ± 4.56	67.52 ± 4.75
500µg/ml	63.77 ± 4.08	78.65 ± 4.44	81.23 ± 5.76
IC_{50} (µg/ml)	328.54	312.45	290.45

Values are expressed as Mean ± SD for triplicates

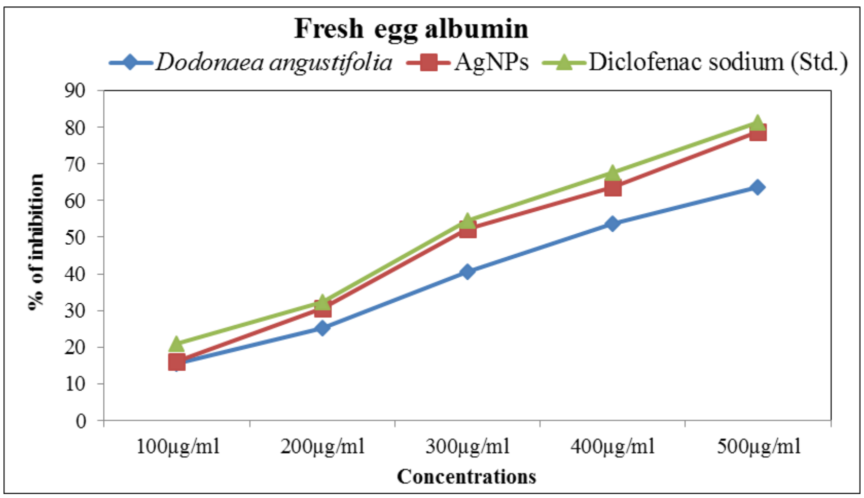

Fig.3: Effect of *Dodonaea angustifolia* , AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

The present findings exhibited a concentration dependent inhibition of protein (Bovine serum albumin) denaturation by the *Dodonaea angustifolia* leaf extract and AgNPs. The half inhibition concentration (IC_{50}) of *Dodonaea angustifolia* leaf extract, AgNPs and ascorbic acid were 302.45, 263.23 and 251.50µg/ml^{-1} respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard diclofenac sodium. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 2 and Fig 4). Our result agrees with the earlier report (Aparna Mani *et al.*, 2015; Giridharan *et al.*, 2014)

Table.2: Effect of *Dodonaea angustifolia,* AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

Concentrations	% of inhibition		
	Dodonaea angustifolia	AgNPs	Diclofenac sodium(Standard)
100µg/ml	22.42 ± 1.56	25.57 ± 1.78	26.31 ± 1.84
200µg/ml	35.68 ± 2.49	40.10 ± 2.80	48.84 ± 3.41
300µg/ml	46.31 ± 3.24	52.42 ± 3.66	59.26 ± 4.14
400µg/ml	53.36 ± 3.73	61.05 ± 4.27	66.63 ± 4.66
500µg/ml	65.15 ± 4.56	72.31 ± 5.06	78.73 ± 5.51
IC_{50} (µg/ml)	302.45	263.23	251.50

Values are expressed as Mean ± SD for triplicates

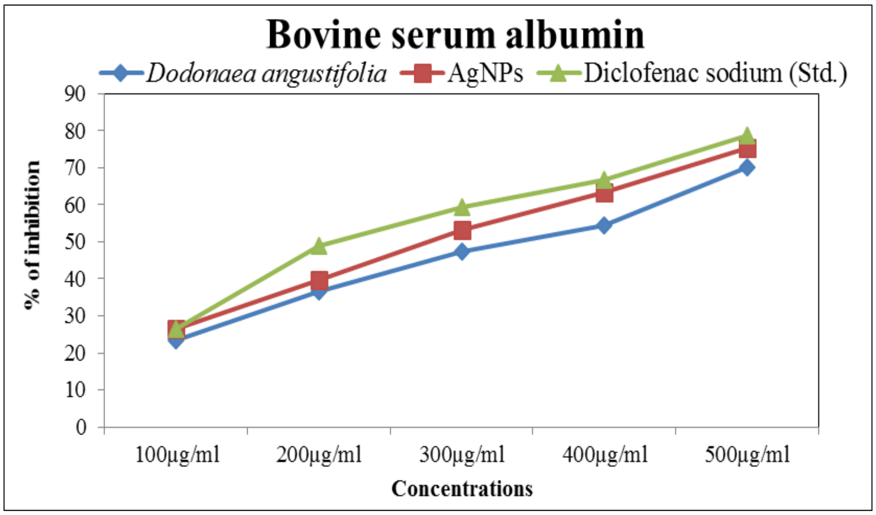

Fig.4: Effect of *Dodonaea angustifolia* , AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

Conclusion

The UV-visible spectroscopy, FTIR and SEM studies of the synthesized silver nanoparticles elucidated that the silver nanoparticles were crystalline in nature, spherical in shape with size ranging between 10 and 63nm and stable. The synthesized silver nanoparticles exhibited antimicrobial activity. This finding suggests that the synthesised AgNPs using *Dodonaea angustifolia* leaf extract could be a good source for developing green nano-medicine for the inflammatory diseases.

References

Amanullah, M., & Yu, L. Green synthesis of silver Naoparticles J Petrol Sci Eng. 48 (2005) 199.

Amargeetha, A., & Velavan, S. Green synthesis of silver nanoparticle using Erythrina indica flower extract, J. Nanosci. Tech. 3(4) (2017) 293–295.

Aparna Mani KM, Seethalakshmi S, Gopal V. Evaluation of In-vitro Anti-Inflammatory Activity of Silver Nanoparticles Synthesised using Piper Nigrum Extract. J Nanomed Nanotechnol 6 (2015) 268; 1-5.

Arunachalama, R., Dhanasingha, S., Kalimuthua, B., Uthirappana, M., Chellan Rosea., & Mandal, A.B. Phytosynthesis of silver nanoparticles using Coccinia grandis leaf extract and its application in the photocatalytic degradation, Colloids and Surfaces B: Biointerfaces. 94 (2012) 226– 230.

Contescu, C.I., & Putyera, K. Dekker encyclopedia of nanoscience and nanotechnology, 1-8 (2009) 256-300.

El-Kheshen, A.A., & Gad El-Rab, S.F. Effect of reducing and protecting agents on size of silver nanoparticles and their anti-bacterial activity, Der Pharma Chemica, 4 (1) (2012) 53-65.

Giridharan T., Chandran Masi, S. Sindhu and P. Arumugam. Studies on Green Synthesis, Characterization and Anti-proliferative Potential of Silver Nano Particle using Dodonaea viscosa and Capparis decidua. Biosci., Biotech. Res. Asia, 11(2), 665-673 (2014).

Henglein, A. 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 (1993) 5457.

Gnanasundaram I., and Balakrishnan K. Synthesis and evaluation of anti-inflammatory activity of silver nanoparticles from Cissus vitiginea leaf extract, J. Nanosci. Tech. 3(3) (2017) 266–269.

Khomutov, G., & Gubin, S. Antimicrobial activity of Silver Nanoparticles synthesized by using Medicinal Plants Mater. Sci. Eng. 22 (2002) 141.

Krishnaraj, C., Jagan, E.G., Ramachandran, R., Abirami, S.M., Mohan, N., & Kalaichelvan, P.T. Effect of biologically synthesized silver nanoparticles on Bacopa monnieri (Linn.) Wettst. Plant growth metabolism. Process Biochemistry, 47 (2012) 651–6581.

Manimegalai, B., & Velavan, S. Green Synthesis of Silver Nanoparticles Using Azima tetracantha leaf extract and evaluation of their antibacterial and in vitro antioxidant activity. nanoscience and nanotechnology: An International Journal. 5(2) (2015) 9-16.

Mukunthan, K.S., Elumalai, E.K., Trupti, N.P., & Ramachandra Murty, V. Catharanthus roseus: a natural source for the synthesis of silver nanoparticles. Asian Pacific Journal of Tropical Biomedicine, (2011) 270-274.

Padmanabhan P and Jangle SN. Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four herbal plants. Int J App Basic Med Sci. 2(1) (2012) 109-116.

Thirumurgan, A., Tomy, N.A., Jai Ganesh, R., & Gobikrishnan, S. Biological reduction of silver nanoparticles using plant leaf extracts and its effect an increased antimicrobial activity against clinically isolated organism., De. Phar. Chem. 2 (2010) 279-284.