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CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITIES OF SILVER NANOPARTICLE USING *WATTAKAKA VOLUBILIS* LINN.F.

WASEEM IQBAL KHANDAY[1], EYINI MUTHUKUMARASAMY[2] AND BALAJI PAULRAJ[1*]

[1]PG and Research Centre in Biotechnology, M.G.R College, Hosur-635 109. Tamil Nadu, India.

ABSTRACT

The dried leaves of *Wattakaka volubilis* was used for the synthesis of silver nanoparticles and evaluation of their antibacterial and antioxidant biological activities. The silver nanoparticles were characterized by UV-visible and Fourier transform infra-red spectroscopy (FTIR). The antibacterial activity of 1mM concentration of the silver nanoparticles against *Escherchia coli* produces 18mm zone of inhibition and against *Bacillus subtilis* and *Staphylococuss aureus* produces 17mm zone of inhibition at 50µg/ml concentration. Among the different concentration,1mM silver nanoparticles showed the highest anti-oxidant activity of 92.98% at 100µg/ml and thus being a good electron donor silver nanoparticles showed highest reducing power activity with optical density value of 0.144 at 100µg/ml.

KEYWORDS: **Wattakaka volubilis, DPPH, MIC, AFM, DLS**

BALAJI PAULRAJ
PG and Research Centre in Biotechnology, M.G.R College, Hosur-635 109.
Tamil Nadu, India.

INTRODUCTION

Nanoparticles have characteristic of physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties[1, 2] . Therefore, nanoparticles are considered as building blocks of the next generation of optoelectronics, electronics, and various chemical and biochemical sensors[3]. Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics antimicrobials and therapeutics[4,5.] catalysis and micro-electronics. Silver nanoparticles was reported to possess antifungal [6] anti-inflammatory, anti-angiogenesis[7] and anti-platelet activity[8,9]. Silver nanoparticles have been synthesized using various plant extracts such as *Cinnamon camphora*[10,] *Cinnamon zeylanicum*[11], *Geranium*[12,] *Neem* leaf broth[13], *Aloe vera* plant extracts [14], *Tamarind* leaf extract[15], *Phyllostachys sp* leaves extract[16] and *Acalypha indica*[17]. The leaves *Wattakaka volublis* Linn.f. are used in skin diseases ,diabetes, cough, jaundice, poison bites and purifying blood. [28]. The present study, focuses on the effective, and environmental safe method for synthesis of silver nanoparticles using leaf extract of *Wattakaka volublis* Linn.f. and evaluation of their antibacterial and antioxidant activity.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Wattakaka volubilis* Linn. f. where collected from Kalvarayan Hills, Villupuram district,Tamilnadu ,India, in June 2013. The plant was identified by Dr. G. Surendiran (Taxonomist) University of Madras, Tamilnadu, India. The voucher specimen (WV 01) has been deposited in herbarium, PG and Research Centre in Biotechnology, M.G.R College, TamilNadu, India, for future references.

Organisms used

Organisms used for the present study were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Citrobacter*, *Citrobacter koseri*, *Escherichia harmanni*, *Proteus mirrabules*, *Klebsiella rihnoscleroma*, *Klebsiella oxytoca*, *Klebsiella pneumonia.*

Preparation of leaf extracts of Wattakaka Volubili Linn. f.

The fresh leaves were dried in the laboratory under shade and were pulverized to a coarse powdered and grinded material was used for the extraction process. Five grams of powder (pulverized) material was soaked in 95 ml triple distilled deionized waterb(demineralised water) . It was boiled for one hour at 100 °C and the final volume was reduced to 70 ml.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles was followed by the previous publication[9]. In a typical reaction procedure, five ml of *Wattakaka volubilis* Linn. f. Leaf extract was added in 95ml of aqueous solution of 1mM silver nitrate for reduction into silver ion (Ag^+). This aqueous solution was placed in 250 ml of Erlenmeyer flask and heated on water bath for one hour at 100 ° C. The fully reduced solution were centrifuged at 5000 rpm for 30 minutes. Reduction of silver nitrate to silver ions was confirmed by the colour change from colourless to brown.

Characterization

UV-Vis spectral analysis was done using Jasco v- 530 spectrophotometer. UV-visible absorption spectrophotometer with a resolution of 1 nM between 400 and 900 nM possessing a scanning speed of 300 nM/min was used. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after diluting a small aliquot of the sample into de ionized water. One milliliter of the sample was pipette into a test tube and diluted with 4 ml of de ionized water and subsequently analyzed at room temperature[9] . Infra red spectra of the compounds were recorded for 400-4000 cm^{-1} in potassium bromide (KBr) pellet using a FT-IR spectrophotometer (Shimadzu, Japan). The particle size and distribution were measured in aqueous suspension, using light scattering equipment (ALV-5000E, ALV). The intensity data of DLS was taken. The particles size and morphology was determined by atomic force microscopy (AFM).

Determination of antibacterial activity by disc-diffusion method

The silver nanoparticles (AgNP's) synthesized from *Wattakaka volubili*s Linn. f. Leaf extract was tested for antimicrobial activity by conventional disc-diffusion method against clinical pathogens. Disc of 6-mm diameter were made on Muller–Hinton agar plate. Each strain were spreaded uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, various concentrations (10 µg - 50 µg) of the sample of nanoparticles solution were poured onto each disc. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured[29]..

Antioxidant activity

The antioxidant activity of silver nanoparticles of *Wattakaka.volubilis* leaves were measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH method[18] and as modified[19]. A methanolic solution (0.1ml) of sample at various concentrations was added to 3.9ml (0.025gL^{-1}) of DPPH solution. The decrease in absorbance at 515nM was determined continuously recorded in a spectrophotometer for 16 minutes. The decrease in the absorbance depends on the concentration of the antioxidant and the radical, the molecular structure of the antioxidant, and its kinetic behavior. The scavenging effect (decrease of absorbance at 515nM) was plotted against the time and percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration by the formula below.

% DPPH activity = control OD- sample OD/ control OD X100

Determination of Reducing Power

The reducing power of silver nanoparticles was determined according to the suitable method[20,21] as Lyophilized extract (1mg) in 1ml of methanol was mixed ith a phosphate buffer (5ml, 0.2 M, pH 6.6) and potassium ferric cyanide (5ml, 1.0%); the mixture was incubated at 50^{o}C for 20min. A portion (5ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 650g for 10 min. The upper layer of the solution (5ml) was mixed with distilled water (5ml) and ferric chloride (1ml, 0.1%), and then the absorbance was read spectrophotometrically at 700nm. A higher absorbance of the reaction mixture indicated greater reducing power.

% increase in Reducing Power= A test/A blank-1x100

RESULTS AND DISCUSSION

Synthesis and characterization of silver nanoparticles

The formation of silver nanoparticles from acetone leaf extracts of *Wattakaka volubilis Linn.f.* confirmed by the reduction of silver nitrate to silver ions by the colour change from colourless to brown in 1mM aqueous silver nitrate solution[22].

UV visible spectrum

UV visible spectra were recorded from the reaction medium after heating the solution at 75c for 60 minutes. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance speak at 430 nM indicating that the particles are reduced (Fig.3).

Atomic Force Microscopy

AFM images of nanoparticles prepared with the plant extracts that the most of the particles were spherical. Particle size of 100 – 150 nm diameter were observed. The AFM image of the nano film has low roughness as well as molecular clusters randomly distributed. Typically 100 – 150 nm in size and possibly constituted of the same materials as the overall surface (Fig.6).

Dynamic Light Scattering

The particle size and distribution were measured in aqueous suspension, using light scattering equipment (ALV-5000E, ALV). The samples were prepared by dilution of the formulation in ultra-pure water without filtration. The scattered light was measured at different angles in the range of 60°–120°. The temperature was set to 25°±0.1 °C. The primary information given by DLS data are intensity distribution whereby the relative amount of each particle size is measured by the intensity scattered by all the particles of the considered size. Particle size of 100 – 150 nm diameter were observed. The particle size was measured by Dynamic Light Scattering (Fig.5).

FTIR analysis

FTIR analysis of the acetone extracts, absorbance bands were observed for various functional groups present in the silver nanoparticles. The large absorbance peak at 3751-3874 cm^{-1} was found to be C-H stretching vibration of alkanes and N-H stretching was observed at the range of 3431-3450 cm^{-1} .C-H stretching peaks of methyl group (alkanes) was between 2929-2930. Corbonyl absorption bands in addition to ammonium bands was observed between 2345-2370cm^{-1} The absorption peak at 1653-1686 was N=0 stretching trans isomer of nitrite. Ring stretching of heteroaromatic compounds was observed between 16000-1300,and peaks for C-0 stretching vibrations to the alcohol group was observed at 1089-1092cm^{-1} .The organic halogen group was observed between 670.2-675 (Fig.4).

Antimicrobial activity

The antibacterial activity was performed by disc diffusion method[23] against various micro organisms various concentrations (10 µg - 50 µg). The antibacterial activity of 1mM concentration of the silver nanoparticles against *Escherchia coli* produces 18mM zone of inhibition and against *Bacillus subtilis* and *Staphylococuss aureus* produces 17mM zone of inhibition at 50µg/ml concentration (Fig 1and 2). In comparision to this study the antibacterial activity of this plant a maximum inhibition zone was found to be 15.2mm at 100µl, against *E. coli*, the moderate inhibition zone was found to be 13.3mm at 100µl, against *S. aureus* as[24]. The scavenging activity of 1mM concentration of silver nanoparticles of *Wattakaka volubilis* Linn.f. and nanoparticles of silver nitrate at different concentration was determined by DPPH assay [18,19]. 1mM concentration silver nanoparticles of leaves powder of *Wattakaka volubilis* had a effective radical scavenging activity with a maximum of 92.98% at 100 µg/ml concentration (Table 1 and 2). In this study, the free radical scavenging activity of 1mM concentration of silver nanoparticles of *Wattakaka volubilis* Linn.f. exhibited greater scavenging activity as compared to the results obtained in methanolic extract of *Wattakaka volubilis* (whole plant) which showed only inhibition of 62.29% at 1000µg/ml[25]. The reducing power of 1mM concentration of silver nanoparticles of acetone extract *Wattakaka volubilis* Linn.f.[20,21] The reducing power of the nanoparticles increased with an increasing concentration.1mM concentration of silver nanoparticles appear to be effective on reducing power with optical density value of 0.144 at 100 µg/ml concentration (Table 3) in comprasion with petroleum ether and ethyl acetate extract of *Eichhornia crassipes* (Mart) showed absorbance of 0.12, 034 at 50µg/ml concentration[26].

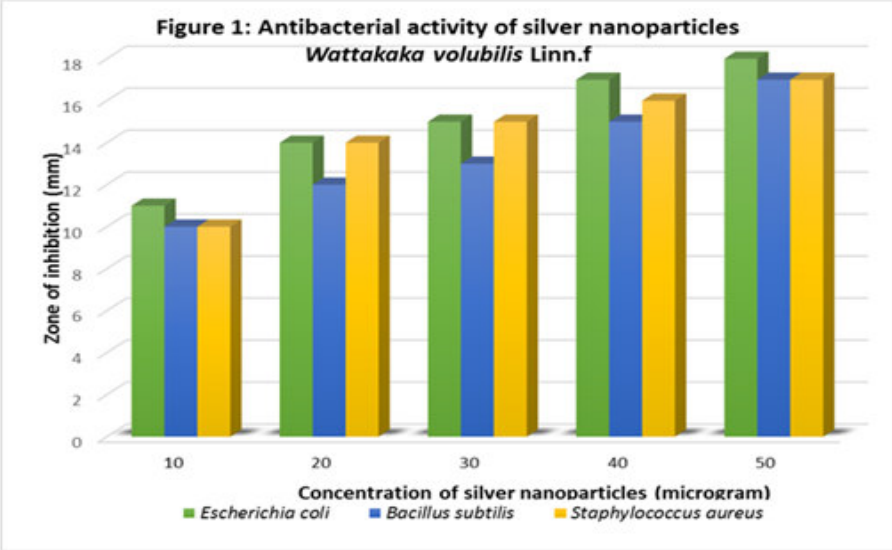

Figure 1

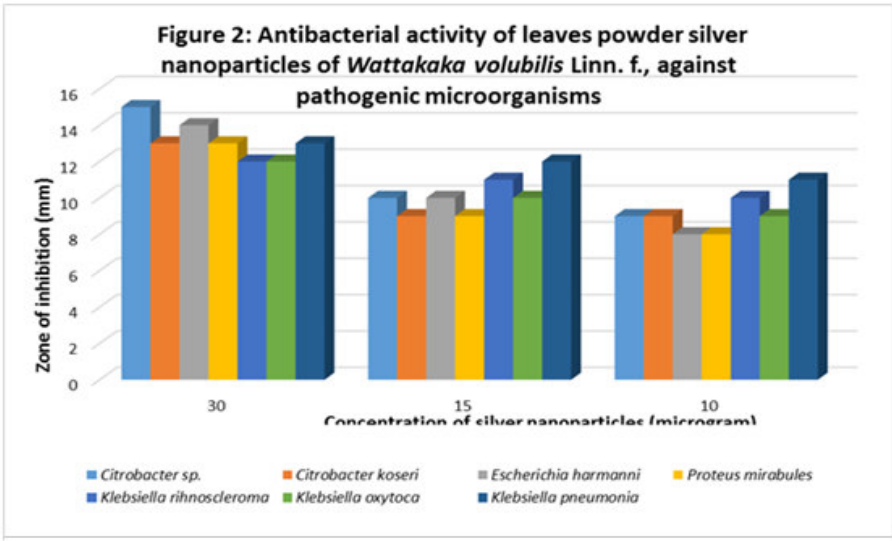

Figure 2

Table 1

1,1 *Diphenyl-2-picryl hydrazyl radical scavenging capacity of ascorbic\ acid and silver nanoparticles of Wattakaka volubilis Linn. f.,*

Silver nanoparticles	OD value at 515_{nm}	DPPH radical scavenging capacity (%)
Negative control (Methanolic DPPH)	0.057	-
Positive control (Ascorbic acid)	0.037	35.08
1mM Concentration	0.035	39.09

Table 2

Antioxidant activity of Silver nanoparticles of Wattakaka Volubilis Linn. F by DPPH assay

Concentration of silver nanoparticles (µg/ ml)	DPPH radical scavenging capacity (%)
	Microgram concentration
10	56.14
20	57.89
30	61.40
40	66.56
50	70.17
60	73.68
70	77.19
80	80.70
90	85.96
100	92.98

Table 3
Reducing power of silver nanoparticles of Wattakaka volubilis Linn. f.

Concentration of silver nanoparticles (µg/ ml)	OD value at 700_{nm}
	Silver nanoparticles of *Wattakaka volubilis* Linn. f.,
	Microgram concentration
	1mM Concentration
10	0.071
20	0.080
30	0.082
40	0.083
50	0.088
60	0.101
70	0.128
80	0.132
90	0.136
100	0.144

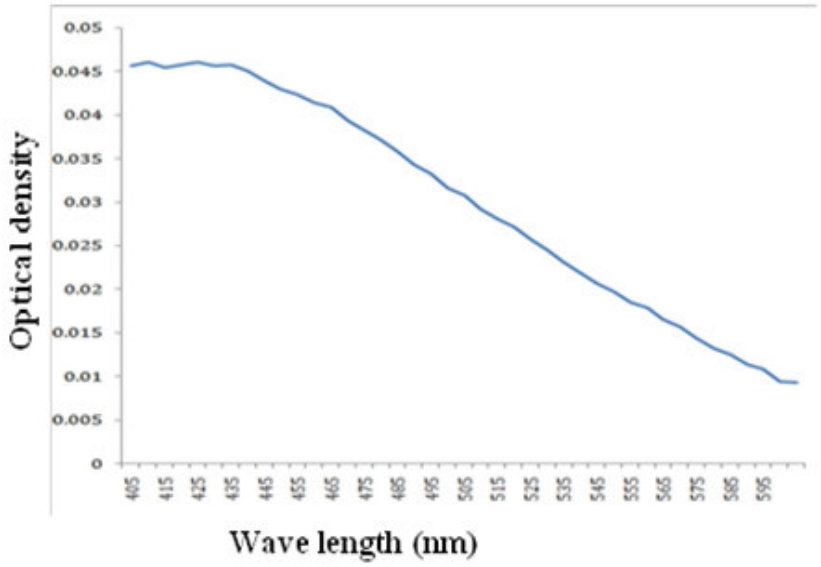

Figure 3
UV-Visisble spectrum of 1mM silver nanoparticles of *Wattakaka volubilis* Linn. f

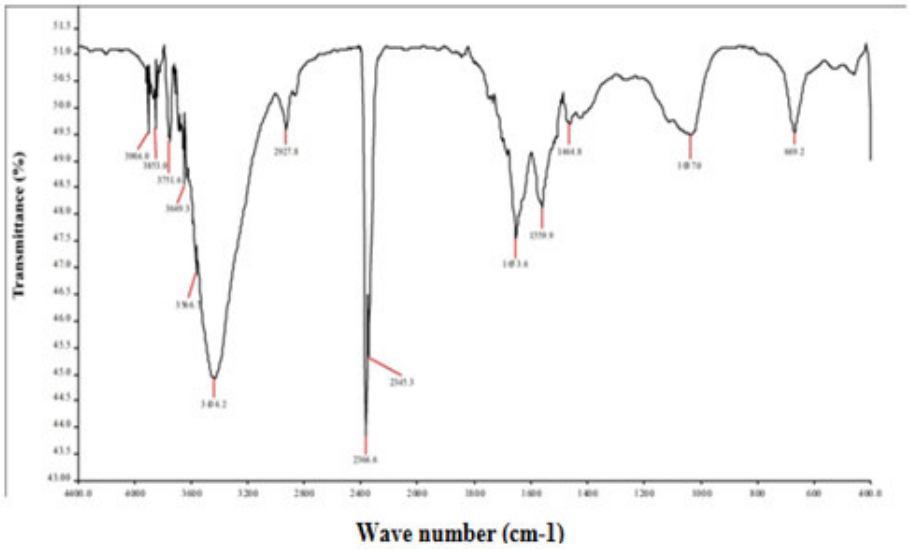

Figure 4
IR- Spectrum of 1mM Concentration of silver nanoparticles of Wattakaka volubilis Linn. fWave number (cm-1)

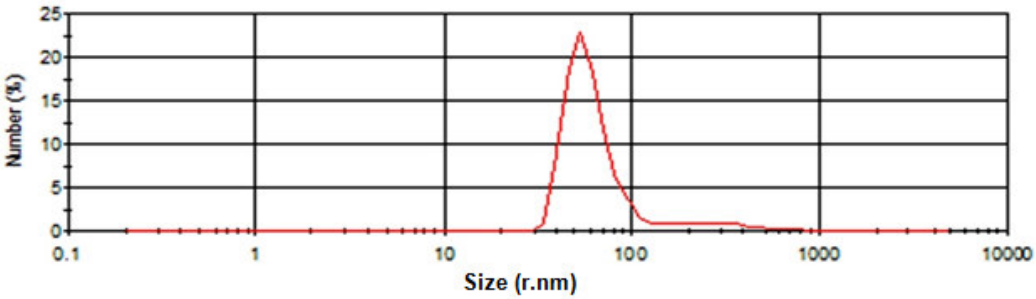

Figure 5
Dynamic light scattering of silver nanoparticles of wattakaka volubilis Linn.f Size Distribution by number (1Mm Concentration of Silver Nanoparticle)

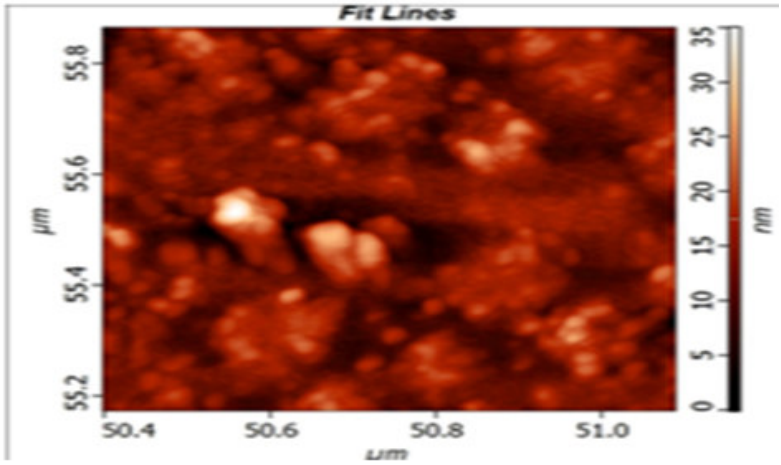

Figure 6
Contact node Atomic Force Microscopic image of 1nM concentraction of Silver nanoparticle Wattakaka volubilis Linn.f

CONCLUSION

Synthesis of silver nanoparticles using leaf extract of *Wattakaka volubilis Linn. F* medicinal plant has been demonstrated in present investigation.. The reduction of Silver ions and their capping was achieved by organic molecules present in the leaf extract .The silver nanoparticles were characterized by uv-visible and fourier transform infra-red spectroscopy (FTIR), Dynamic Light Scattering and Atomic force microscopy (AFM). Silver nanoparticles of of the leaves of *Wattakaka volubilis Linn. f.* exhibited good antibacterial activity against *E. coli,* followed by *Staphylococcus aureus*. the reducing power of silver nanoparticle showed highest activity with optical density value.

CONFLICT OF INTEREST

Conflict of interest declared none.

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