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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *ACALYPHA INDICA* AND ITS ANTIMICROBIAL ACTIVITY

D. KUMARASAMYRAJA*[1] AND N. S. JEGANATHAN [2]

[1]Department of pharmacy, Annamalai University, Annamalai Nagar, Chidambaram - 608002.
[2]Periyar College of Pharmaceutical Sciences, Trichy – 620021

ABSTRACT

In the present study, bio synthesis of silver Nanoparticles using aqueous extract of *Acalypha indica* and its antimicrobial activity against different micro organisms were investigated. About 10 ml of aqueous extract of *A. indica* added with 90 ml of $AgNo_3$ (1mM) solution, the resulting mixture was incubated at 37°C under static condition. The development of yellowish brown color indicated the formation of Ag-Np's. The Ag-Np's monitored with the help of UV-visible spectrophotometer at the wavelength of 200– 800 nm. The observed absorbance peak at 400 nm indicated the formation of Ag -Np's. The particle size of Ag-Np's was determined by using particle analyzer and the results showed that average size range was found to be 0.516 nm.TEM technique was employed to visualize the size and shape of Ag-Np's. The antibacterial activity of *A. indica* Ag-Np's was evaluated against both Gram positive and Gram negative pathogenic microorganism by disc diffusion method. The diameter of inhibition zone of *A. indica* Ag-Np's was analyzed at different concentrations ranging from 100 to 300µg/ml. The maximum zone of inhibition was observed with *P.aeruginosa* (16 mm), followed by *E.coli* (14 mm), *B.subtilis*((13 mm) and *S.aureus* (13 mm) when compared with standard drug Amikacin. The antifungal activity of *A. indica* Ag-Np's at 300 µg/ml concentration was found to be 23mm and12 mm for *C. albicans* & *A. niger* respectively when compared with the standard antifungal drug ketokonazole. It is observed from the results that biologically synthesized Ag-Np's from *A. indica* aqueous extract showed effective antimicrobial and antifungal activity against selected microorganisms which are comparable with standard.

KEYWORDS: Ag-Np's, Green synthesis, UV-Vis spectrometry, TEM, Antibacterial activity

D. KUMARASAMYRAJA
Department of pharmacy, Annamalai University,
Annamalai Nagar, Chidambaram - 608002.

1. INTRODUCTION

Nanotechnology is now creating a growing sense of excitement in the life sciences especially biomedical devices and Biotechnology [1]. Nanoparticles are a special group of materials with unique features and extensive applications in diverse fields [2]. It is being utilized as therapeutic tools in infections, against microbes thus understanding the properties of nanoparticles and their effect on microbes is essential for clinical applications. Among noble metal nanoparticles, silver nanoparticles (Ag-Np's) have received considerable attention due to their attractive physicochemical properties. Ag-Np's have already been tested in various fields of biological science, drug delivery and water treatment. The antimicrobial activity of Ag-Np's tested against different organisms by various researchers. The Ag-Np's are influenced by the dimensions of the particles the smaller the particles, the greater antimicrobial effect. The mechanism of Ag-Np's is not well known, it may attach to the cell wall, thus disturbing cell-wall permeability and cellular respiration. The nanoparticles may also penetrate inside the cell causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein. Another possible contribution to the bactericidal properties of Ag-Np's is the release of silver ions from particles [3]. The Ag-Np's have been synthesized using different techniques: electrochemical methods, laser ablation, microwave irradiation, thermal decomposition and sono-chemical synthesis [4]. Although chemical and physical methods have been reported in literature, most of the methods are extremely expensive and also toxic and potentially dangerous to the environment. The Biological methods of synthesis of nanoparticles such as microorganism, enzymes, fungus and plant or plant extracts have been suggested as possible eco friendly alternative methods for chemical and physical methods. Sometime Nanoparticles synthesized from plant or plant parts can prove advantages over other biological methods [5].

A. indica is an annual erect herb commonly called as "Kuppai meni" in Siddha literature. It belongs to the family Euphorbiaceae. It is a common shrub in Indian gardens, backyards of houses and waste places throughout the plains of India. The root, stem and leaf of *A.indica* possess herbal activity [6]. Very little research was reported on antimicrobial activity of *A. indica*, in general and *A. indica* Ag-Np's in particular. Hence, the aim of the present study was to develop a novel approach for the green synthesis of Ag-Np's using aqueous extract of *A. indica* and exploring its antimicrobial activity against some selected Gram positive and Gram negative organisms.

2. MATERIALS AND METHODS

2.1Materials

Ag No_3 and Nutrient Agar broth were purchased from Hi-Media, India. Entire plants with roots of *A. indica* were collected from the Herbal garden, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu. The collected plant was authenticated by the Head, Department of Botany, Annamalai University, Annamalai nagar, Tamil Nadu and a voucher specimen (No.1958) was kept in the Pharmacognosy Lab, Department of Pharmacy, Annamalai University for future reference. The microorganism used in this experiment were *Bacillus subtilis* (10877), *Staphylococcus aureus* (29838), *Pseudomonas aeruginosa* (27854), *Escherichia coli* (1130) and fungus culture *Candida albicans* and *Aspergillus niger* They were obtained from Boss Laboratories, Madurai, India.

2.2 Methods

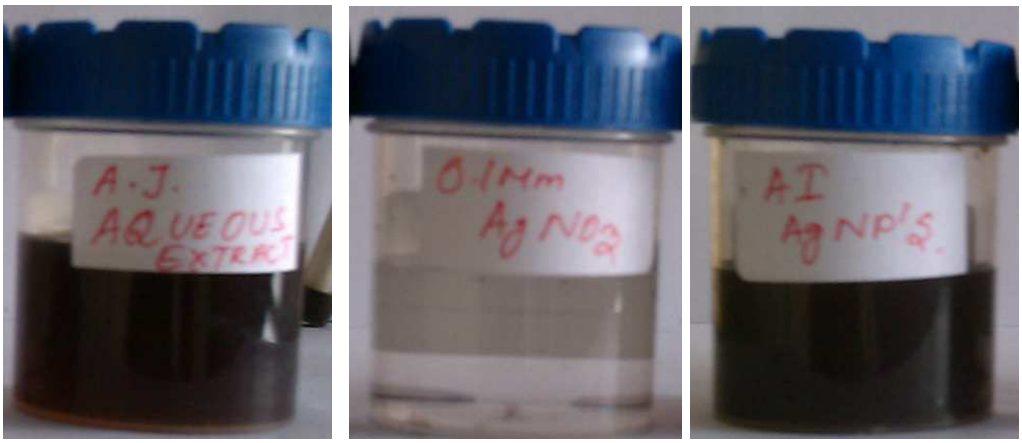
2.2.1 Preparation of aqueous extract of A. indica

The entire plant of *A. indica* was washed thoroughly three times with purified water and once with distilled water. The plant materials were air dried under shade and powdered by using a disintegrator to get a coarse powder. The

powdered samples were kept in sealed containers for extraction purposes. About 10gm of the sample was placed with double sterilized distilled water in 100ml Erlenmeyer flask and then boiling the mixture for 5 min. The extract was cooled and filtered through Whatman no 1 filter paper. The resultant filtrate was kept in refrigerator [7].

2.2.2 Synthesis of silver nanoparticles (AgNPs)

In a typical reaction procedure, 10 ml of refrigerated filtrate was treated with 90ml of $AgNo_3$ (1mM) solution. The resulting solution was incubated in dark (to minimize the photo activation of silver nitrate), at 37°C under static condition. The observed color change from watery to yellowish brown color solution indicated the formation of *A. indica* -Ag-Np's [8]. The colored Ag-Np's solution was centrifuged at 10,000 rpm for 10 min, the supernatant liquid was decanted. The resulting suspension was re dispersed in 10 ml sterile distilled water and centrifugation process was repeated for three times. Thereafter, the purified suspension was used for characterization of Ag-Np's [9].



(a) **(b)** **(c)**

Figure 1
The colour change of plant extract after adding $AgNo_3$ (1mM) solution (a) A.indica extract (b) $AgNo_3$ solution (c) Ag-Np's

2.2.3 Characterization of silver nanoparticles (Ag-Np's)

The biosynthesis of the *A. indica* Ag-Np's was monitored with the help of UV-visible spectrophotometer UV-2450 (Shimadzu) at the wavelength of 200– 800 nm. The distilled water was used as a blank. The particle size range of the Ag-Np's was determined by using particle size analyzer, Mastsizer 2000. The particle size was determined based on the Brownian motion of the nanoparticles. Transmission electron microscopy (TEM) technique was employed to visualize the size and shape of Ag-Np's. TEM selected area images were taken on Philips model CM 200 instrument operated at an accelerating voltage at 200 kV [10].

2.2.4 Antimicrobial activity of AgNPs

The antibacterial activity of *A. indica* -Ag-Np's was evaluated against both Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative (*Pseudomonas aeruginosa, Escherichia coli*) pathogenic microorganisms by disc diffusion method. The organisms were sub-cultured on Mueller Hinton Agar (MHA) medium, incubated at 37°C for 24 hrs and stored at 4°C in the refrigerator to maintain stock culture. Petri plates were prepared with 20 ml of sterile MHA medium. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of 100,200 and 300 µg /ml respectively of *A. indica* -Ag-Np's suspension prepared from the extract. The loaded discs

were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Amikacin (50 µg/ml) was used as positive control. The plates were incubated for 24 h at 37°C and the zone of inhibition was recorded in millimeters [11].

2.2.5 Anti fungal screening

The anti fungal activity was performed according to the standard reference method. Fungus culture *Candida albicans* and *Aspergillus niger* were used for this study. The initial concentration of aqueous extract of *A. indica* Ag-Np's suspension was 100µg/ml. The initial test concentration was serially diluted twofold. Each one was inoculated with 50µl of suspension containing 104 spore/ml of fungi. The anti fungal agent ketokonazole was included in the assay as positive control. The plates were incubated between 24 hrs and 72 hrs at 27 °C and the zone of inhibition was recorded in millimeters [12].

3. RESULTS AND DISCUSSION

3.1. UV–Vis spectral studies

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The plant extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish brown due to reduction of silver ion, which may be the indication of formation Ag-NP's [13].The UV- spectrum of *A. indica* Ag-Np's was recorded from the reaction medium. The results showed maximum absorption peak ranging between 390 – 410 nm.

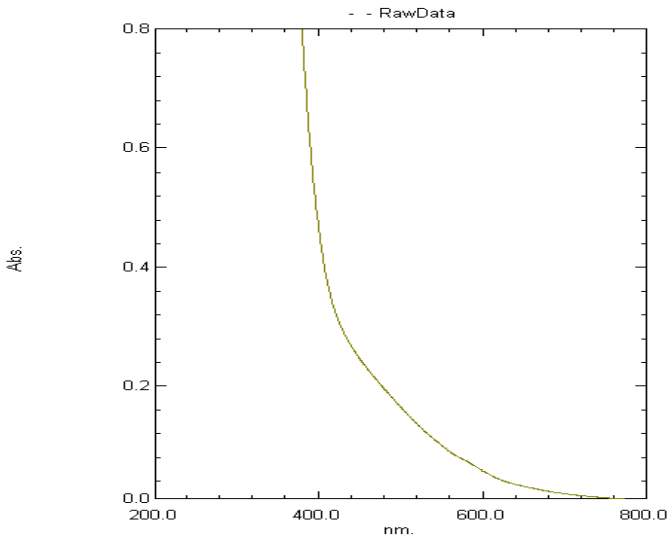
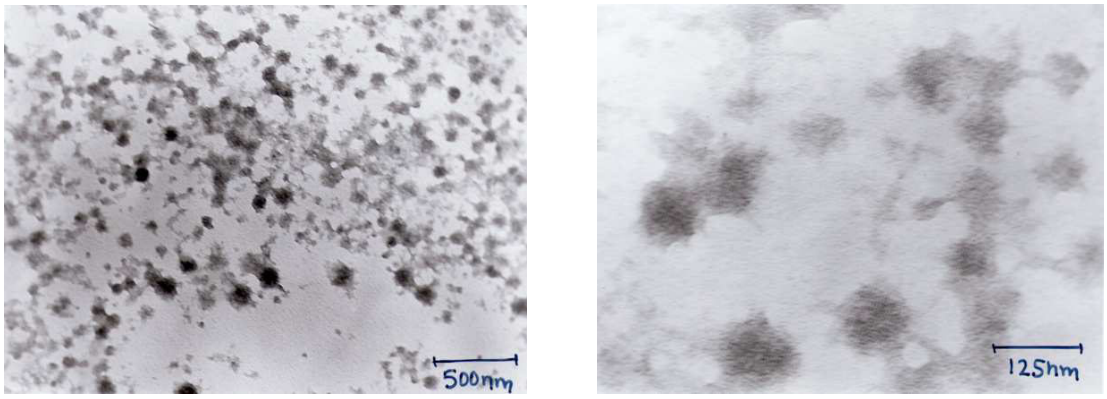


Figure 2
UV-Vis spectra of Ag-Np's biosynthesized from aqueous extract of A. indica

3.2. Transmission electron microscopy (TEM)

The morphology and size of the *A. indica* Ag-Np's were investigated by TEM analysis. Pictures below obtained by TEM showed the Ag-Np's formed in aqueous extract of *A. indica*. It was observed that the Ag-Np's were predominantly spherical. The overall morphology of the Ag-Np's produced by reduction of Ag+ ions with 1mM $AgNO_3$ was composed of almost uniform Nanoparticles [14]. The typical TEM image of the biosynthesized Ag-Np's from aqueous extract of *A. indica* shown in Fig 3.



(a) (b)

Figure 3
TEM images of Ag-Np's synthesized by using aqueous extract of A. indica

3.3. Particle size distribution

The particle size range of Ag-Np's synthesized from *A. indica* monitored by using particle size analyzer Mastsizer 2000. The result showed that Ag-Np's average size range was found to be 0.516 µm.

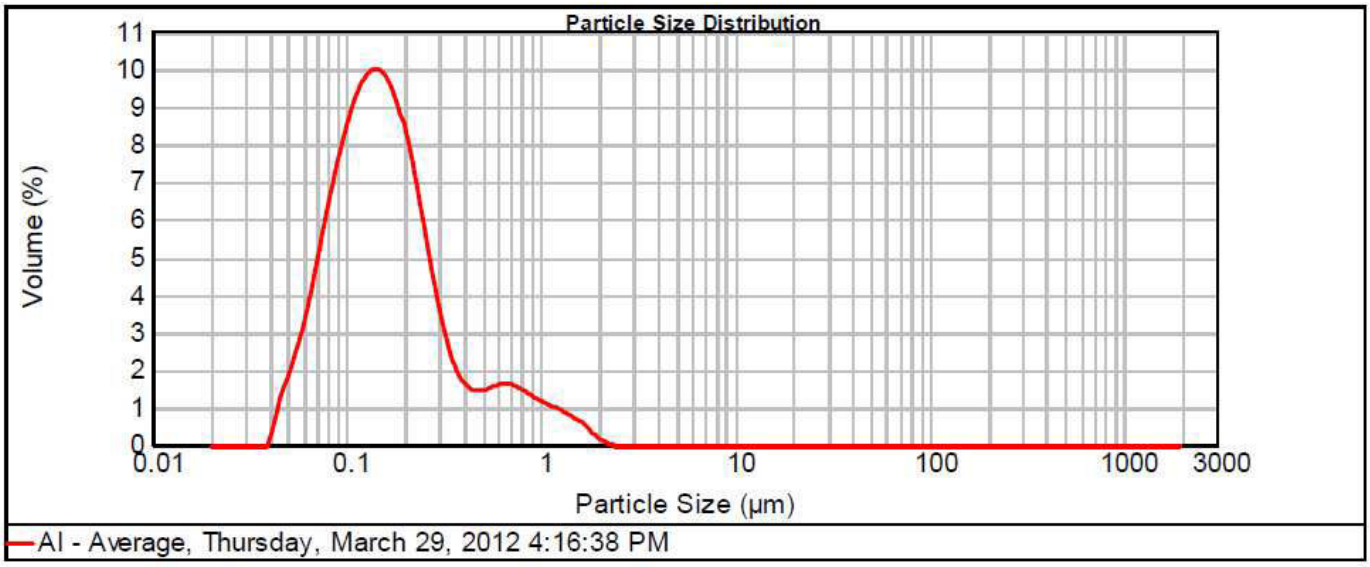


Figure 4
Particle size distribution of A. indica -Ag-Np's

3.4. Evaluation of antimicrobial activity of Ag-Np's synthesized from A. indica

The antimicrobial activity of Ag-Np's prepared from aqueous extract of *A. indica* has been investigated against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Escherichia coli* microorganisms and fungus cultures *Candida albicans* and *Aspergillus niger* by disc diffusion method on Mueller-Hinton broth.

Table 1
In vitro antibacterial potential of Ag-Np's synthesized from A. indica

S.No	Drug	Con: µg/ml	Zone of inhibition (mm)			
			B. subtilis	*S.aureus*	*P.aeruginosa*	*E.coli*
1	*A. indica* Ag-Np's	100	10	12	15	13
2		200	13	13	15	13
3		300	13	13	16	14
4	Standard		20	17	17	17

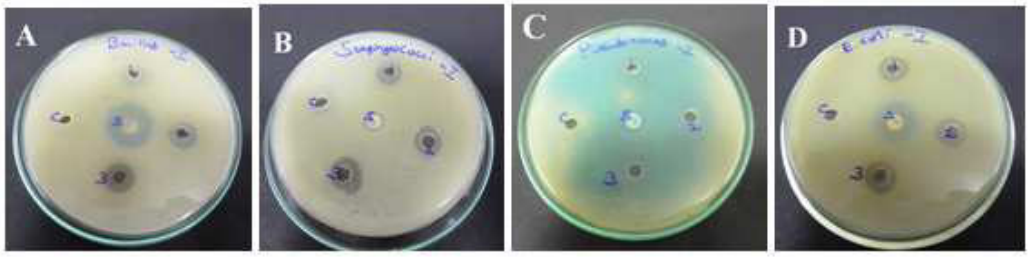


Figure 5
Inhibition of bacterial growth by Ag-Np's synthesized from aqueous extract of A. indica by Disc diffusion method A- Bacillus subtilis, B-Staphylococcus aureus, C-Pseudomonas aeruginosa, D- Escherichia coli '1','2' and '3' represents zone of inhibition of A. indica Ag-Np's at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antibacterial agent (Amikacin) at the concentration of 50 µg/ml and 'C represents zone of control.

The antibacterial activity of aqueous extract *A. indica* Ag-Np's showed sensitive response against all investigated microorganisms. The diameter of inhibition zone was analyzed with different concentrations ranging from 100 to 300µg/ ml. The maximum zone of inhibition was observed with the *P.aeruginosa* (16 mm), followed by *E.coli* (14 mm) and *Bacillus subtilis* & *Staphylococcus aureus* (13 mm). The results clearly indicated that *A. indica* Ag-Np's have potential antimicrobial activity against Gram positive and Gram negative bacteria which are comparable with standard drugs, Amikacin.

Table 2
In vitro antifungal potential of A. indica Ag-Np's

S.NO	Drug	Con:µg/ml	Zone of inhibition (mm)	
			C.albicans	*A.niger*
1	*A. indica* Ag-Np's	100	22	10
2		200	23	12
3		300	23	12
4	Standard		18	20

Figure 6
Inhibition of fungal growth by A. indica Ag-Np's by Disc diffusion method E-Candida albicans, F-Aspergillus niger '1','2' and '3' represents zone of inhibition of biosynthesized A. indica Ag-Np's at the concentration of 100, 200 and 300 µg/ml respectively. The centre zone 'S' represents zone of inhibition of standard antifungal agent (ketokonazole) at the concentration of 50 µg/ml and 'C' represents zone of control.

The antifungal activity of *A. indica* -Ag-Np's *was* investigated against *C. albicans* and *A. niger* by using disc diffusion method. The average diameter of the inhibition zone surrounding the disc was measured with a ruler, the maximum zone of inhibition range of Ag-Np's was found to be 23 &12 mm for *C. albicans* and *A. niger* respectively at 300 µg/ml concentration. It is observed that biologically synthesized *A. indica* Ag-Np's *extract* showed effective antifungal activity against *C. albicans* and *A. niger* when compared with standard drug, ketokonazole.

4. DISCUSSION

Recently several approaches have been reported to synthesize Ag-Np's in smarter way which includes both chemical and biological approaches. Nowadays using bio-based matter in synthesizing materials is of great interest to the scientific community [15]. According to green chemistry principles, bio synthesis of Nanoparticles has many advantages such as, ease with which the process can be scaled up apart from economic viability [16]. In this project an attempt has been made to develop a fast, eco-friendly and convenient method for the green synthesis of Ag-Np's using *A. indica* extract with an average particle size range of 0.516 µm. Reduction of silver ion into silver Nanoparticles during exposure to the plant extracts could be followed by color change. Silver Nanoparticles exhibited dark reddish-brown color in aqueous solution due to the surface Plasmon resonance phenomenon [8]. The entire plant extract of *A. indica* mixed with aqueous solution of silver nitrate primarily responsible for the color change from watery to yellowish brown color. This color change indicated the formation of Ag-Np's. The formation of Ag-Np's is generally recognized by UV-Vis spectroscopy and could be used to examine size- and shape controlled Nanoparticles in aqueous suspensions [17]. The morphology and size of the *A. indica* -Ag-Np's were investigated by TEM analysis. The results showed that average size range of *A. indica* -Ag-Np's was found to be 0.516 µm. *A. indica* -Ag-Np's have shown promising antimicrobial activities on dose dependent manner which are comparable with the standard drugs.

Though the anti bacterial mechanism of *A. indica* Ag-NP's is not well known, the reports available from the literature indicated that the inhibitory action of Ag-Np's on microorganisms showed that the Nanoparticles got attached to the cell membrane and penetrate inside the bacteria[18]. The bacterial membrane which contains sulfur containing proteins interacts with these Nanoparticles in the cell as well as with the phosphorus containing compounds like DNA. When Ag-Np's enter the bacterial cell it forms a low molecular weight region in the center of the bacteria to which the bacteria conglomerates thus, protecting the DNA from the silver ions. The nanoparticles preferably attack the respiratory chain, cell division and finally leading to cell death. The nanoparticles release silver ions in the bacterial cells, which enhance their bactericidal activity [19].

5. CONCLUSION

In the present study, it was disclosed that entire aqueous extract of the entire plant of *A. indica* can be converted into Ag-Np's by green synthesis and the resulting *A. indica*- Ag-Np's showed potential antimicrobial efficacy against pathogenic microorganisms. The biologically synthesized *A. indica*- Ag-Np's can be used in the medical field for their efficient antimicrobial activity after undertaking proper clinical trial.

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