A Study on Antimicrobial Activity of Prepared Silver Nanoparticles using *Ixora coccinea* L. Leaf Extract

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Abstract:

The synthesis of silver nanoparticles was prepared from (1mM) silver nitrate solution using various volume ratio of *Ixora coccinea* L. leaf extract as a reducing and stabilizing agent. This research investigated the preliminary phytochemical screening of *Ixora coccinea* L. leaf. The prepared silver nanoparticles were characterized by using UV-Vis Spectrophotometer, XRD, FT IR and SEM techniques. The XRD analysis showed that the average crystallize sizes of silver nanoparticles were increased with volume of extract were increased. The diffraction peaks data obtained were in accordance with the reports of a cubic structure from JCPDF file-89-3722>silver Ag.The SEM microphotograph showed the smaller spherical shaped particles aggregate into larger conglomerates. The silver nanoparticles obtained were subjected to biological evaluation and tested against Gram-positive and Gram-negative bacterial strains as well as fungal strains. It was evident that the silver nanoparticles of *Ixora coccinea* L. leaf displayed antimicrobial property. Due to smaller nanoparticle size of silver nanoparticles it may achieve the higher efficiency of antimicrobial activity.

***Keywords*:** *Ixora coccinea* L. leaf, silver nanoparticles, green synthesis, antimicrobial activity

1. Introduction

Nanotechnology and nanotools have gained much attention due to their wide range of applications in physics, chemistry, biology, material science, and medicine. Metal nanoparticles like silver, gold, and copper have been used for diagnosis and treatment of disease because of their catalytic, optical, electronic, antimicrobial, and magnetic properties. The prefix nano is derived from Greek word nanos meaning "dwarf" or extremely small. Nano-sized materials, known as nanoparticles, possess unique and improved properties. Nanoparticles can be broadly grouped into two, namely, organic nanoparticles and inorganic nanoparticles which include noble metal nanoparticles (like silver and gold), semi-conductor nanoparticles (like titanium oxide and zinc oxide) (Mitiku and Yilma, 2017). Green synthesis (biological method) is a plant mediated synthesis of metal nanoparticles; it is simple, easily available, fast and eco-friendly among all the synthesis methods (Iqbal *et al*., 2016). The *Ixora* ornamental plants (*Ixora coccinea*) related to the family Rubiaceae is considered as an important plant used in outdoor gardens, for its flower colors and its potential for other uses such as hedge plant or single plant in different designs (Eldeen and Elgimabi, 2008).*Ixora* is a popularflowering plant in gardens. Red *Ixora* flowers are commonly used in Hinduworship, as well as in Indian folk medicine.*Ixora* is said to be native to Asia and whose name derives from an Indian deity. There are about 400 species spread from Africa to India to Southern Asia. Members of *Ixora* prefer acidic soil, and are suitable choices for bonsai.In tropical climates they flower year-round. This plant which blooms throughout the year is easy to grow (Dontha *et al*., 2015). Figure 1 show the photograph of *Ixora coccinea* L. plant.

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Figure 1. Photograph of *Ixora coccinea* L. plant.

2. Materials and Method

In this research, all the chemicals used were obtained from reagent (BDH). Silver nanoparticles were prepared using *Ixora coccinea* L. leaf extract and silver nitrate solution by biological method.Fresh leaf of *Ixoracoccinea*L. were collected from Yangon University Campus and identified by authorized botanists at Botany Department, University of Yangon.The freshly collected leaf of *Ixoracoccinea*L. were thoroughly washed with running tap water followed by deionized water to remove the fifth. The cleaned leafwere taken in an Erlenmeyer flask and boiled (5 mins) with 150 mL of deionized water. The leaf extract was filtered through filter paper.Silver nanoparticles (Ag NPs) were formed by the reduction of silver nitrated by using *Ixoracoccinea* L. Each of leaf extracts (1-4 mL) was added to 10 mL of 0.001M silver nitrate aqueous solution. After few minutes, the colourless reaction mixture changed to dark brown colour which indicates the formation of silver nanoparticles. The green synthesized Ag NPs were centrifuged at 5000 rpm for 15 min to obtain the residue. These residues were washed with deionized water and dried in oven at 60 ºC for 20 h. The preliminary phytochemical tests of *Ixora coccinea* L. leaf were investigated by test tube method. The antimicrobial activity was determined by agar diffusion method.

3. Results & Discussion

In this research, the synthesis of silver nanoparticles was prepared from (0.001M) silver nitrate solution using various volume ratio of *Ixora coccinea* L. leaf extract.In the preliminary phytochemical test of *Ixora coccinea* L. leaf, the presence of alkaloids, amino acid, carbohydrates, flavonoids, phenolic compounds, sponins, starch, steroids and tannins. The important compounds in the plant extract are hydroxyl and carbonyl groups. Both functional groups allowed plant extract to act as reducing agent. Functional groups present in the leaves extract of *Ixora coccinea* L. such as phenolic compounds and alkaloids are responsible for capping thereby stabilization of nanoparticles reduced. The formation of silver nanoparticles was observed by the change of color from coloreless to dark brown by the addition of leaves extract to silver nitrate solution showed in Figure 1. The existence of silver nanoparticles in colloidal solution was confirmed by Tyndall effect showed in Figure 2. The prepared silver nanoparticles were characterized by using UV-Vis Spectrophotometer, XRD, FT IR and SEM techniques.

(a) 15 mins after addition**(b)** 30 mins after addition

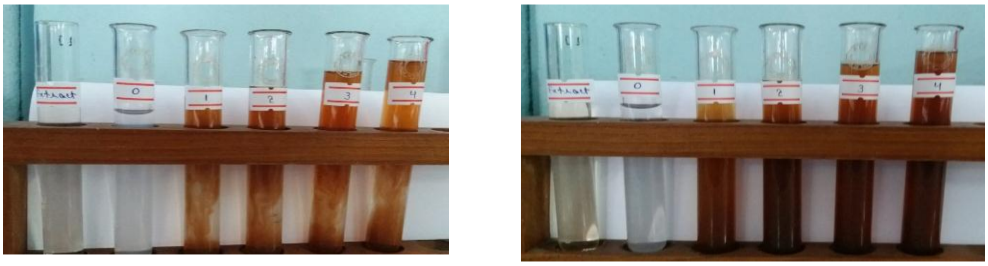

Figure 2. Before and after addition of *Ixora coccinea* L. leaf extract into $AgNO_3$ solution
(a) $AgNO_3$ solution **(b)** *Ixora coccinea* L.-Ag NPs solution

Figure 3. Photograph of confirmation test for presence of nano colloids by Tyndall effect

3.1 Characterization of Silver Nanoparticles

3.1.1 UV-Visible

The UV-vis spectroscopy is one of the most widely used simple technique for the analysis of silver nanoparticles synthesis. Absorbance in the range of 410-450 nm has been used as an indicator to confirm the reduction of Ag^+ to metallic Ag. The maximum wavelength of silver nanoparticles was found to be at 414-420nm, this indicating the presence of silver nanoparticles. Absorbance values of silver nanoparticles at different volume ratios of leaves extract show in Table 1.

Table 1. Absorbance Values of Silver Nanoparticles at Different Volume Ratio of Leaf Extract

$AgNO_3$: Leaves Extract Ratio(mL)	Maximum λ (nm)	Maximum Absorbances
10 : 1	414	0.495
10 : 2	415	0.497
10 : 3	416	0.498
10 : 4	420	0.498

3.1.2 XRD (X-ray diffraction)analysis

XRD is a popular analytical technique which has been used for the analysis of both molecular and crystal structures, qualitative identification of various compounds, quantitative resolution of chemical species, measuring the degree of crystallinity, isomorphous substitutions, particles size, etc. In this research, the crystalline nature of silver nanoparticles was confirmed by XRD analysis Figure 3showed XRD spectrum of silver nanoparticles (10:1, 10:2, 10:3. 10:4 ratios of $AgNO_3$ and leaves extract). The peak values at 37.79, 43.89 and 64.14 which correspond to lattice planes at (111),

(200) and (220) have face centered cubic crystal structure of silver nanoparticles. The XRD pattern results are corroborated with the database of JCPDS file no. 89-3722 > silver Ag. Few unassigned peaks were recorded that might be due to the crystallization of bioorganic phases present in *Ixora coccinea* L. extract on the surface of silver nanoparticles.The crystalline sizes were determined from XRD using Scherrer equation as

$$t = \frac{0.9\lambda}{B\cos\theta}$$

where t is the average crystalline size, λ is wavelength (Å) as nm, B is observed FWHM (in radians) and θ is diffraction angle of the peak under consideration at FWHM (°). The X-ray diffraction analyses were carried out to determine the known phase and crystalline size of silver nanoparticles. The XRD analysis showed that the average crystallize sizes of silver nanoparticles were 15.45,19.58, 22.17 and, 30.41 respectively. The amount of plant extract was increased (1-4 mL), the size of silver nanoparticles was increased (15.45 to 30.41 nm) showed in Table 2.

Figure 3. XRD spectrum of silver nanoparticles (10:1, 10:2, 10:3. 10:4 ratios of $AgNO_3$ and leaf extract)

Table 2. Average Crystalize Size of Silver Nanoparticles by using Different Volume Ratios of *Ixora coccinea* L. Leaf Extract

$AgNO_3$: Leaf Extract Ratio(mL)	Average Crystalize size (nm)
10 : 1	15.45
10 : 2	19.58
10 : 3	22.17
10 : 4	30.41

3.1.3 FT IR (Fourier Transformer Infrared Spectroscopy) analysis

The functional groups in *Ixora coccinea* L. leaf extract responsible for the reduction of Ag^+ ions were identified by FT IR analysis. The FT IR spectra of silver nanoparticles using *Ixora coccinea* L. leaf showed sharp absorbance between 400 and 4000 cm^{-1} as shown in Figure 4. The spectra showed absorption bands at 3404, 3207,1626,1384 and 455 cm^{-1}. The strong peaks at 3404 and 3207 cm^{-1} corresponded to O-H stretching due to phenolic compounds present in the *Ixora coccinea* L. leaf extract. The peak at 1626 cm^{-1} corresponded to stretching vibration of amide group and 1384 cm^{-1} was corresponded to stretching vibration of C-N in aromatic amine group. Another band at 455 cm^{-1} was related to AgNPs banding with oxygen from hydroxyl group.

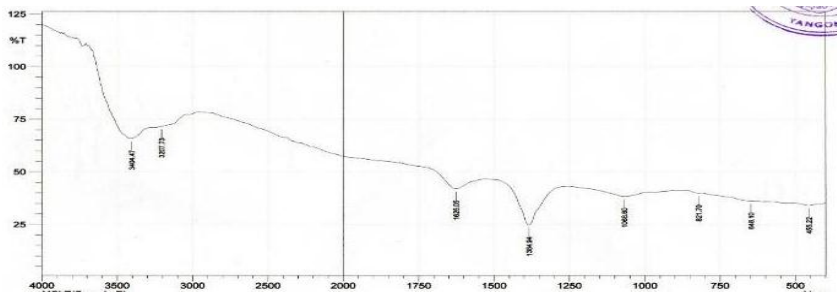

Figure 4. FT IR spectrum of silver nanoparticles (10:1 ratio of $AgNO_3$ and leaf extract)

3.1.4 SEM (Scanning Electron Microscopy) analysis

The SEM microphotograph of biosynthesized silver nanoparticles (Figure 5) showed the smaller spherical shaped particles aggregate into larger conglomerates, this may due to the stabilizer acquired from plant extract which was responsible for sample loading effect during attempts of receiving higher magnification.

Figure 5. SEM microphotograph of biosynthesized silver nanoparticles

3.2 Antimicrobial Activity

Silver ions can inhibit bacterial DNA replication, damage bacterial cytoplasm membranes, depleting levels of intracellular adenosine triphosphate (ATP) and finally cause cell death.The ability of silver nanoparticles to release silver ions is a key to their antimicrobial activity. Stabilization is achieved using capping agents that bind to the nanoparticles surface and improve stability and water solubility. Silver nanoparticles resistance of bacteria to bactericides and antibiotics has increased due to the development of resistant strains. In this research, the antibacterial and antifungal activities of prepared *Ixora coccinea* L.-silver nanoparticleswas studied against Gram-positive bacteria (*B.Sub*, *B.pumilus* and *S. aureus*), Gram-negative bacteria(*P.aeruginosa*and *E. coli*) and fungal strain (*C.albican*) using the agar well diffusion method. The result indicated that prepared 10:1 and 10:2 ratio of $AgNO_3$ and leaves extracthave good antibacterial and antifungal activities but prepared 10:3 and 10:4 ratio of $AgNO_3$ and leaves extract have moderated activities in all tested microorganisms show in Figure 6 & 7 and the zone of inhibition was tabulated as shown in Table 3. The 10:1 ratio of $AgNO_3$ and leaves extract (smallest nanoparticles size) showed maximum zone of inhibition against microorganisms. As the amount of plant extract was increased (1-4 mL), the size of silver nanoparticles was increased (15.45 to 30.41 nm).It was evident that the silvernanoparticles displayed good antimicrobial properties. It was also observed that the antimicrobial efficiency varied with the amount of plant extract and size of nanoparticles. The silver nanoparticles attached to the surface of the cell membrane of microorganisms, leading to the disturbance ofits functions like permeability and respiration. The binding of particles to the microorganism depends on the surface area available for intraction. Small nanoparticles have a larger surface area for intraction with bacteria, as compared to that of largerparticles and since the easily penetrate into the cell. Therefore, the smaller nanoparticle size of silver nanoparticles (15.45 nm) showed higher antimicrobial activity than the larger particle sizes of silver nanoparticles (30.41 nm).

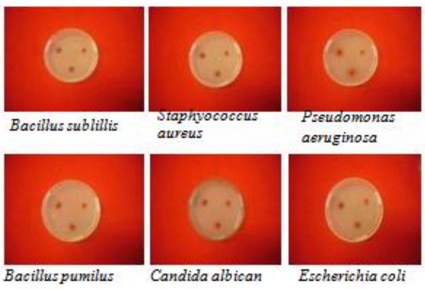

Figure 6. Antimicrobial activity of silver nanoparticles (10:1& 10:2ratio of $AgNO_3$ and leaf extract)

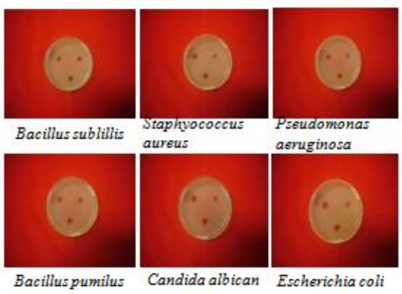

Figure 7. Antimicrobial activity of silver nanoparticles (10:3& 10:4 ratio of $AgNO_3$ and leaf extract)

Table 3. Inhibitiion Zone (mm) of Antimicrobial Activity for Silver Nanoparticles

AgNO$_3$: Leaf Extract Ratio(mL)	Zone of Inhibition (mm)					
	Gram-positive bacteria			**Gram-negative bacteria**		**Fungi**
	B.Sub	**S.aureus**	**B.pumiks**	**Pseudomonas**	**E.coli**	**Candida**
10 : 1	13 (+)	15 (++)	15 (++)	20 (+++)	15 (++)	16 (++)
10 : 2	12 (+)	13 (+)	13 (+)	17 (++)	13 (+)	14 (+)
10 : 3	-	13 (+)	13 (+)	-	14 (+)	13 (+)
10 : 4	-	14 (+)	13 (+)	-	14 (+)	14 (+)

Agar well – 10mm *Organisms*
10mm ~ 14mm (+) *Bacillus sublilis* (N.C.T.C-8236), *Bacillus pumilus*(N.C.I.B-8982)
15mm ~ 19mm (++) *Staphylococcus aureus* (N.C.P.C-6371), *Candida albicans*
20mm above (+++) *Pseudomonas aeruginosa* (6749) , *E-coli* (N.C.I.C -8134)
(-)no activity

4. Conclusion

The green synthesis of silver nanoparticles has been performed using the bio-reducing agent *Ixora coccinea* L. leaf extract. This process is an economic and eco-friendly simple one step method. In this work, silver nitrate has been used as the metal precursor and *Ixora coccinea* L. leaf extract as a reducing agent. In the preliminary phytochemical tests of *Ixora coccinea* L. leaf indicated the presence of amino acid, carbohydrate, sponins, starch, terpenoids, tannins, steroids, alkaloids, flavonoids, phenolic compounds and glycosides. The prepared silver nanoparticleswere confirmed in the UV-visible absorption spectra, which showed the SPR band characteristics of silver nanoparticles in the range of 414-420 nm. The XRD results confirmed that the silver nanoparticles possessed a cupic crystal structure from JCPDFfile-89-3722>silver Ag.The functional groups in *Ixora coccinea* L. leaf extract responsible for the reduction of Ag^+ ions were identified by FT IR analysis showed the absorbance between 400 and 4000 cm^{-1}. The SEM microphotograph showed the smaller spherical shaped particles aggregate into larger conglomerate. This study indicates that silver nanopartices can be used as effective antimicrobial agent which can endanger human beings. Antimicrobial efficiency varied with the amount ofplant extract and size of nanoparticles. The smaller nanoparticles size of silver nanoparticles showed higher antimicrobial activity than the larger particles size.

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