Comparative studies of Silver Nano particles synthesis and its *in-vitro* activities in *Ixora coccinea*

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Abstract:

The present investigation was carried out to study the synthesis, characterization of silver nanoparticles and to evaluate the phytochemical screening and In-vitro activities of the Ixora coccinea from its flower extract and the study revealed the presence of significant amount of phytochemicals and silver nanoparticles in vitro activities in the extracts of Ixora coccinea.

***Keywords*:** Ixora coccinea, secondary metabolites, nanoparticle synthesis, anti inflammatory.

Introduction

Ixora coccinea Linn. belongs to the family *Rubiaceae* and Order Gentianales. It is a common flowering shrub native to Southern India, Bangladesh, and Sri Lanka. *Ixora coccinea* Linn. (*Rubiaceae*) commonly known as *rathmal* in Sinhalese and *vedchi* in Tamil. The *Rubiaceae* are a family of flowering plants, commonly known as the coffee, madder, or bedstraw family. It consists of terrestrial trees, shrubs, lianas, or herbs that are recognizable by simple, opposite leaves with interpetiolar stipules. Different plant parts of *I. coccinea* are used for treatment of various disease conditions some of which are associated with inflammation. A decoction of the flowers is given for haemoptysis, acute bronchitis and dysmenorrhoea. Further, the flowers and bark are used on reddened eyes and eruptions in children. A decoction of the root is given for dysentery, loss of appetite, fever, and gonorrhoea, and as a sedative for hiccoughs and nausea. The leaves are used for dermatological disorders in traditional systems of medicine.
The *Rubiaceae* are a family of flowering plants, commonly known as the coffee, madder, or bedstraw family. The family contains about 13,500 species in 611 genera, which makes it the fourth-largest angiosperm family(Gerard J Tortora,*et.al.*,2016). *Rubiaceae* has a cosmopolitan distribution; however, the largest species diversity is concentrated in the (sub)tropics. Economically important species include *Coffea*, the source of coffee, *Cinchona*, the source of the antimalarial alkaloid quinine, some dye plants (e.g. *Rubia*), and ornamental cultivars (e.g. *Gardenia*, *Ixora*, *Pentas*).Woody stems, Tubular, 4-petaled, bright red flowers bloom in coyrmbose cymes. Primary bloom is in summer, but sporadic bloom occurs throughout the year. Flowers are followed by round dark purple/black fruits.

Materials and methods

Sample preparation:

The flowers of *Ixora coccinea* were collected from Coimbatore district and they are cleaned and shade dried for two weeks and was made into fine powder and stored in a air tight container. Then 5gm of shade dried sample was taken in conical flask, then added 50ml of distilled water

and left for 24hours for the extraction in a mechanical shaker by covering with cotton in order to avoid contamination, It was shaken vigorously. After 24 hours the extraction was collected, filtered and dried on water bath. The residue obtained was stored in refrigerator for further analysis.

Phytochemical analysis:

Test for steroids (Salkowski reaction): To the sample solution, chloroform was added followed by concentrated sulphuric acid along the sides of the test tubes. A red- brown colouration the presence of steroids.

Test for alkaloids (Wagner's test): One milliliter of Wagner's reagent was added to the sample solution. Formation of reddish brown precipitate indicated the presence of alkaloids.

Test for tannins (Lead acetate test): Small quantity of the sample solutions was dissolved in distilled water and 10% lead acetate solution was added to them , a white precipitate indicated the presence of phenolics and tannins.

Test for flavonoids (Alkaline reagent test): To the sample solution, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour, which turned colourless after addition of few drops of diluted hydrochloric acid indicated the presence of flavonoids.

Test for carbohydrates (Molisch's test): The solution to be tested are mixed with small amount of Molisch's reagent in a test tube and mixed well. A small amount of concentrated sulphuric was slowly added down the sides of the sloping test tube. Appearance of purple ring at the junction indicates the presence of carbohydrates.

Test for saponin glycosides (Froth formation test): A small quantity of the sample was diluted with 20ml of distilled water and shaken vigorously. Formation of 1cm layer of foam which is stable for 10 mins indicated the presence of saponins.

Test for amino acids (Ninhydrin test): A solution of ninhydrin in ethanol is added to the sample solution. Appearance of a purple colour indicated the presence of amino acids.

Test for cardiac glycosides (Keller-Killiani test): Glacial acetic acid and few drops of 5% ferric chloride solution are added to the sample solutions. Concentrated sulphuric acid is added along the side of the test tube carefully. The formation of blue colour in the acetic acid layer confirmed the presence of cardiac glycosides.

Anthraquinone glycosides (Hydroxyanthraquinone test): To the 1ml of sample, few drops of 10% potassium hydroxide solution were added. Formation of a red colour confirmed the presence of anthraquinone glycosides.

Test for proteins (Biuret test): To 2ml of the sample solutions,5 drops of 1% copper sulphate solution are added followed by 2ml of 10% NaOH. The contents are mixed thoroughly. Formation of a purple or violet colour confirmed the presence of proteins.

Synthesis of Silver Nanoparticles

Preparation of silver: Take 0.169 gm of silver nitrate (AgNO3) is dissolved with 100ml of distilled water.

Synthesis of silver Nanoparticles: Add 90 ml of silver nitrate solution and 10ml of aqueous extract in conical flask cover tightly keep it dark place . After some period of incubation ,the colour of the mixture solution changed from pink to brown colour. This colour changes indicates the formation of silver nanoparticle.

Characterization of Silver Nanoparticles: An ultraviolet-Vis spectroscopy was used to conduct optical measurement. UV-Vis spectroscopy was operated in the range from 200-800nm. The silver nanoparticles formation was monitored upto 48hr in UV- spectrophotometer measurements. FTIR measurements were obtained on a Nexus 670 FTIR instrument with the sample in the range of 800 to 4000 cm. X-ray diffraction measurement was used to study the phase and purity of the sample.

In-vitro Anti-inflammatory activity

Determination of Anti-inflammatory activity by Mizhusima method: The anti-inflammatory activity of *Ixora coccinea* was studied by using inhibition of albumin denaturation technique. The anti-inflammatory activity in extracts of flowers of plant were measured by bovine serum albumin (BSA) was determined by the spectrophotometrically. When BSA reacts with an anti-inflammatory compound. The changes in colour were measured at 660nm on a spectrophotometer.

Reagents:
BSA reagent: 1mg of BSA solution was weighed and dissolved in 100ml of distilled water for the further use.

Phosphate buffer: Prepare 400 mL of distilled water in a suitable container. Add 10.107 g of Na_2HPO_4-$7H_2O$ to the solution. Add 1.697 g of $NaH_2PO_4H_2O$ to the solution. Adjust solution to final desired pH using HCl or NaOH. Add distilled water until volume is 500ml.

Method: The reaction mixture was consists of test extracts(10,20,30,40,50ul) and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ^oC for 20 min and then heated to 51 ^{o} C for 20 min, after cooling the samples the turbidity was measured at 660nm. The Percentage inhibition of protein denaturation was calculated as follows:

The Percentage inhibition = 100- (Abs Control –Abs Sample) X 100

Results

Preliminary phytochemical screening of *Ixora coccinea* was carried out and revealed the presence of alkaloids, steroids, glycosides and flavonoids in aqueous extract. Some

phytoconstituents were observed in aqueous extract such as steroids, glycosides and flavonoids and the data was represented in table Table 1.

Table 1. Preliminary Phytochemical analysis

PHYTOCHEMICALS	RESULT
Steroids	+++
Alkaloids	+++
Tannins	-
Flavonoids	++
Carbohydrates	+
Saponin glycosides	+++
Amino acids	-
Cardiac glycosides	++
Anthraquinone glycosides	-
Proteins	-

(+) = Presence of phytochemicals
(++) = moderately present
(+++) = highly present
(-) = Absence of phytochemicals

Table 2. Characterization of Silver Nano particles

S. No	Wave length	Initial stage	24hrs inhibition
1.	200	0.0411	0.0760
2	240	0.0216	0.0616
3.	280	0.0398	0.0826
4.	320	1.8848	2.0506
5.	360	1.9868	2.1590
6.	400	2.0790	2.6271
7.	440	2.0468	2.6805
8.	480	2.0568	2.7729
9.	520	2.0670	2.9329
10.	560	2.0015	2.9393
11.	600	1.9021	1.7623
12.	640	1.8213	1.1788
13.	680	1.7398	0.9520
14.	720	1.6626	0.8264
15.	760	1.5877	0.7331
16.	800	1.5248	0.6616

UV-Vis spectroscopy: Concentration of the presence of metal nanoparticles were analysed by using UV-Vis spectral analysis. UV visible absorption spectrum was noted at 520 nm and a

broadening of the peak indicated that the particles were polydispersed. An ultraviolet-Vis spectroscopy was used to conduct optical measurement. UV-Vis spectroscopy was operated in the range from 200-800nm.

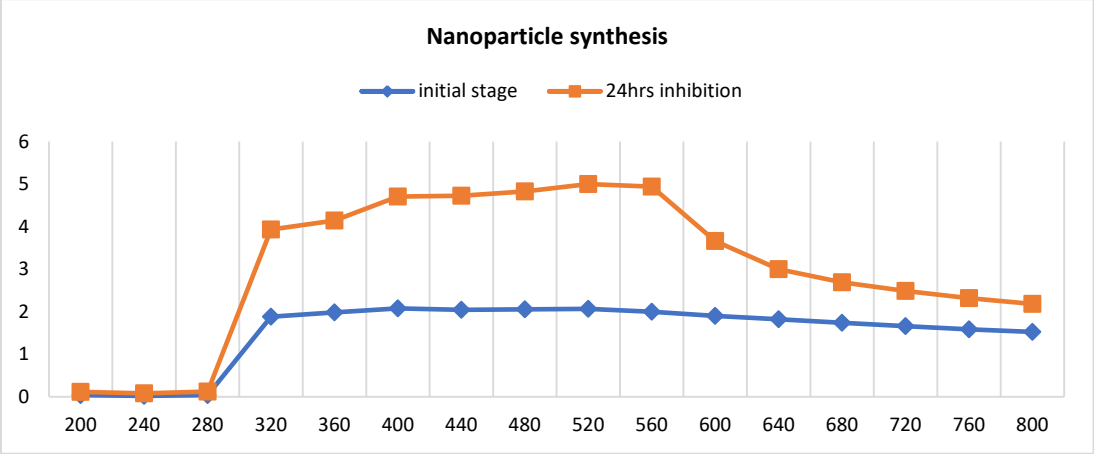

Figure 1: UV-UV-Visible spectroscopy analysis

Fourier-Transform Infrared spectroscopy

Capping and reducing agents for biosynthesis of silver nanoparticles form aqueous flower extract of *I.Coccinea* were analysed by using a FTIR. The infrared bands are observed at 3387.00, 3348.42, 3271.27, 1635.64 and 1219.01 cm-1. This bands represent the presence of aliphatic, carboxylic acid, alkyl aryl ether function groups, N-H stretches, C-O stretching aromatics, O-H stretching and C=C stretching compounds. Analysis of these spectra strongly suggested the presence of flavonoids and phenols, which were mainly responsible for the formation of silver nanoparticles by reducing silver nitrate.

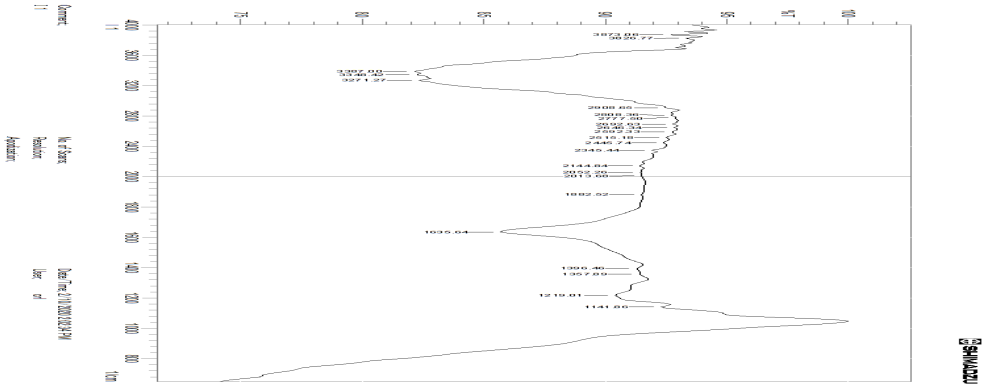

Figure 2: FTIR analysis of Silver nanoparticles

X-ray diffraction:

XRD pattern, which is the primary tool for the characterization of silver nanoparticles. The diffraction peaks at 38.06^{o} , 44.18^{o} , 64.23^{o} and 77.54^{o} were indexed with the planes (111),(200),(220) and (311) for the resultant particles with cubic phase. Plotted XRD pattern indicates the formation high purity of the silver nanoparticles and there are no contamination peaks were detected.

Figure 3. XRD analysis of Silver nanoparticles

Determination of anti-inflammatory activity by Mizhusima method

The anti-inflammatory activity in extracts of flowers of plant were measured by bovine serum albumin (BSA) was determined by the spectrophotometrically. When BSA reacts with an anti-inflammatory compound. The changes in colour were measured on a spectrophotometer. Acetyl salicylic acid was taken as a control. As part of the investigation on the mechanism of the anti-inflammatory activity, extracts were effective in inhibiting process. *Ixora coccinea*(Aqueous extract) were observed as 0.001=29.4%, 0.012=30.5%, 0.027=32%, 0.032=32.5%, 0.040=33.3%. were as in NPs extract 0.002=29.5%, 0.027=32%, 0.035=32.8%, 0.045=33.8%,0.056=34.9% respectively. So NPs extract exposed the effects of inhibition against the aqueous extract.

Table 3. Anti-inflammatory Analysis

		Aqueous extract		NPs extract	
S.No	**concentration**	**Optical values**	**% of inhibition**	**Optical values**	**% of inhibition**
1.	10µl	0.001	29.4%	0.002	29.5%
2.	20µl	0.012	30.5%	0.027	32%
3.	30µl	0.028	32%	0.035	32.8%
4.	40µl	0.032	32.5%	0.045	33.8%
5.	50µl	0.040	33.3%	0.056	34.9%

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