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# *IN VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF SILVER NANOPARTICLE SYNTHESIZED FROM *Erythrina indica* FLOWER EXTRACT

A. Amargeetha and S. Velavan*

[1]Research Scholar, PG and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu, S. India

[2] PG and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu, S. India,

*Corresponding author

**ABSTRACT**

The aim of the study to investigate the *in vitro* antioxidant and anti-inflammatory activity of silver nanoparticles synthesized from *Erythrina indica* flower extract. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM AgNO3 solution through the extract of *Erythrina indica* flower extract as reducing as well as capping agent. The synthesized silver nanoparticle further investigated the antioxidant and anti-inflammatory activity and compared with standard. The result of the study conclude that antioxidant and anti-inflammatory activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in *Erythrina indica* flower extract (EIFE). Silver nanoparticle has potential activity than standard and plant extract. Overall, the silver nanoparticle is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including arthritis.

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***Corresponding author***
Dr. S. Velavan, P.G. and Research Department of Biochemistry, Marudupandiyar College, Tamil Nadu, India.

## INTRODUCTION

Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size (Prabhu et al., 2010). Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging, sensing, targeted drug delivery and gene delivery systems and artificial implants. The new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer and fight human pathogens like bacteria (Melgardt, 2008). Nowadays, a large number of noble metals like copper, zinc, titanium, magnesium, gold, alginate and silver have been used for the synthesis of nanoparticle but silver has acquired large attention due to its diverse applications (Arunachalam et al., 2012).

In recent years, the stabilizing development of efficient green chemistry methods employing natural reducing, capping, and agents to prepare silver nanoparticles with desired morphology and

size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents (Amanullah et al., 2005).

Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis without using toxic chemicals. Biological methods for nanoparticle synthesis using plants or plant extracts have been suggested as possible ecofriendly alternatives to chemical and physical methods The phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. The medicinal value of the chosen plant *Erythrina indica* flowers has not been extensively worked out. Therefore, the present study was to investigate the synthesis and evaluation of antioxidant and anti-inflammatory potential of silver nanoparticles from *Erythrina indica* flowers extract (EIFE).

## MATERIALS AND METHODS

### Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio barbituric acid (TBA), potassium hexa cyano ferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

### Collection of plant materials

The mature Erythrina indica flower were collected in April 2015 from Kodaikanal, Dindugal district, Tamil Nadu, India. The flower were identified and authenticated by Botanist, Prof. Dr. S. John Britto, Director, The Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamil Nadu, India.

### Preparation of flower extract

The dried flowers were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the flowers extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4◦C for further use. Doses such as 20, 40, 60 and 80µg/ml were chosen for *in vitro* antioxidant activity.

### Synthesis of Ag nanoparticles using flowers extracts

For the Ag nanoparticles synthesis, 5 ml of Erythrina indica is flowers extract was added to 45 ml of 1 mM aqueous AgNO3 solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without flowers 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 freeze dried using SEM analysis (Arunachalam et al., 2012).

### Antioxidant activity

### DPPH ASSAY

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al*. (1992).

### Determination of total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. (1999)

### Superoxide anion scavenging activity assay

The scavenging activity of the *Annona muricata* leaves towards superoxide anion radicals was measured by the method of Liu *et al*. (1997).

### Anti-inflammatory activity

Anti-inflammatory activity of the *Erythrina indica* extract and SNPs was evaluated by protein denaturation method as described by Padmanabhan and Jangle (2012).

**Statistical analysis:** Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, $IC_{50}$, was graphically estimated using a nonlinear regression algorithm.

## RESULTS AND DISCUSSION

### Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, 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 well known that silver nanoparticles exhibit brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles The appearances of

yellowish-brown colour (Figure 4) in the reaction vessels suggest the formation of silver nanoparticles (SNPs) (Thirumurgan et al., 2010). The synthesized nanoparticle further confirmed by SEM, XRD and EDX and the particle size determined in the range 10 – 20nm (Unpublished data).

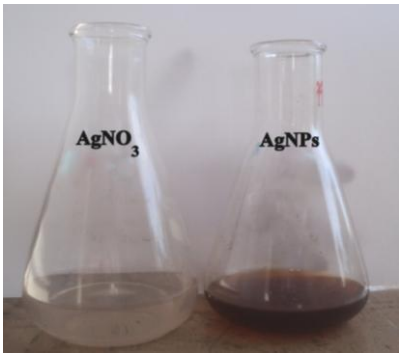



**Plate 1: Colour changes before (Plant extract) and after (AgNPs) the process of reduction of Ag+ to Ag nanoparticles (Brown colour) and control ($AgNO_3$)**

**Antioxidant activity**

The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for in vitro antioxidant study to understand the pharmacological role of medicinal plant and it's isolate. In vitro techniques have been used for detection of antioxidants, which are based on the ability of compounds to scavenge peroxy radicals (Acker *et al*., 2009).

**Total antioxidant activity**

The yield of the methanol extract of the plant extract and its total antioxidant capacity are given in Figure 8. Total antioxidant capacity of A. tetracantha is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.,* 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract, ascorbic acid and AgNPs. Among this AgNPs possess potential antioxidant activity as compared with plant extract and close to the standard (Figure 1).

**DPPH Radical scavenging activity**

DPPH free-radical scavenging activity Free radicals are harmful by-products generated during normal cellular metabolism, which could initiate oxidative damage to body (Halliwell , Gutteridge 1999). Antioxidants are believed to play a significant role in the body's defense system against free radicals. Recently, numerous reports have described antioxidants and compounds with radical-scavenging activity present in fruits, vegetables, herbs and cereals extracts (Hou *et al*., 2005). The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.*, 2003).

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The antioxidant activity of A. tetracantha and AgNPs were shown in Figure 2. The A. tetracantha and AgNPs exhibited a significant dose dependent inhibition of DPPH activity. AgNPs possess probable antioxidant activity as compared with plant extract and near to standard.

**Superoxide anion radical scavenging activity**

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka *et al*., 1978). The superoxide anion radical scavenging activity A. tetracantha assayed by the PMS-NADH system is shown in Figure 3. The superoxide scavenging activity of A. tetracantha and AgNPs were increased markedly with the increase of concentrations. The superoxide scavenging capacity is high in AgNPs. These results suggested that AgNPs had superior superoxide radical scavenging effect.

**Fig 1- % of Total antioxidant activity of *Erythrina indica* extract, AgNPs and ascorbic acid at different concentrations**

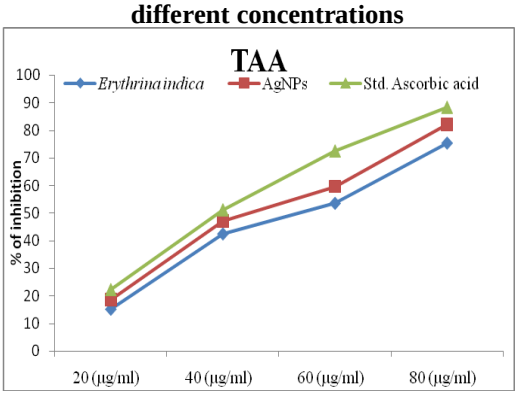


**Fig 2- % of DPPH Radical scavenging activity of *Erythrina indica* extract AgNPs and ascorbic acid at different concentrations**

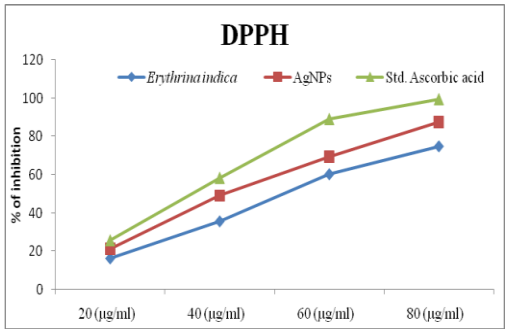
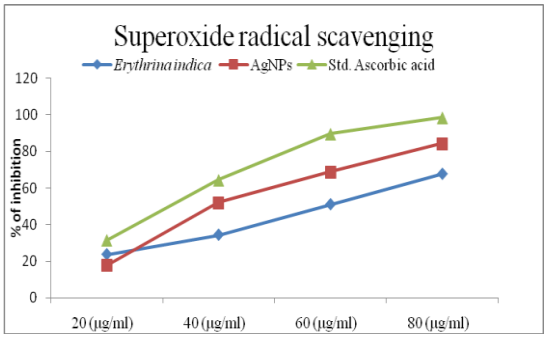



**Fig 3- % of Superoxide Radical scavenging activity of *Erythrina indica* extract AgNPs and ascorbic acid at different concentrations**




## Anti-inflammatory activity

Inflammation is a host defence mechanism of the body and it's an essential immune response that enables the body to survival during infection or injury and maintains tissue homeostasis in noxious conditions. According to the modern concept, inflammation is a healthy process resulting from some disturbance or disease. Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from localized response to a generalized one. In other words "Inflammation is the major and complex reaction of the body against infection upon tissue injury (Medzhitov, 2010)."

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of Erythrina indica flower extract, AgNPs and standard as diclofenac. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo (Opie, 1962; Umapathy et al., 2010). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development.

Some literature reported that denaturation of protein is one of the cause of rheumatoid arthritis (Vane et al., 1995). Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Rahman et al., 2012). In our present study, Erythrina indica flower extract, AgNPs and standard as diclofenac inhibited heat induced protein denaturation and may be one of the reason of possessing anti-inflammatory activity (Fig 4). The anti-inflammatory activity has potent in AgNPs as compared with plant and standard.

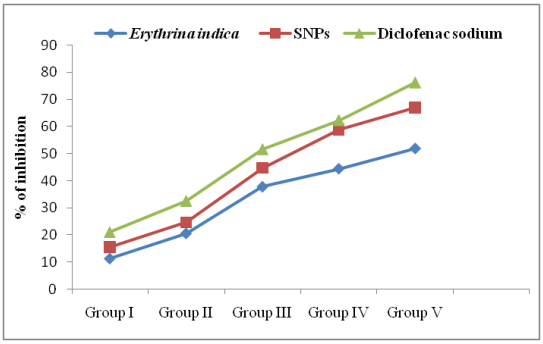



**Figure 4: Effect of *Erythrina indica* and AgNPs on protein denaturation (Fresh egg albumin)**

In the present study, we have demonstrated, for the first time, extracellular green synthesis of AgNPs using Erythrina indica flower extract. The synthesized nanoparticles exhibited potent antioxidant and anti-inflammatory activity by attenuating production of superoxide anion. This study further proves the importance of green technology for nanoparticle production and future applications in control of various human diseases.

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