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Anti-inflammatory activity of silver nanoparticles synthesized from *Eichhornia crassipes*: An *in vitro* study

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Abstract
This paper presents an empirical analysis on the use of aqueous extract of *Eichhornia crassipes* for the production of silver nanoparticles (AgNPs) from silver nitrate. The *Eichhornia crassipes* leaf extract, AgNPs and Diclofenac sodium at different concentrations was incubated with egg and Bovine serum albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein denaturation by *Eichhornia crassipes* extract and AgNPs. The effect of AgNPs was found to be high when compared with the *Eichhornia crassipes* extract and near to the diclofenac sodium. From the present study it can be concluded that AgNPs possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein.

Keywords: Silver nanoparticles, *Eichhornia crassipes* extract, diclofenac sodium, anti-inflammatory effect

Introduction
Herbs and herbal extracts have been used to treat various ailments since ages. Their derivatives have attracted tremendous attention therapeutically and are promising as remedies to treat diseases of diversified origin. Herbs especially have fallen into limelight, anticipating their replacement with sophisticated drugs. More than 50% of modern drugs existing in clinical use today are derived from plants. Metal nanoparticles have proved to be of significance due to their lesser volume to surface area ratio along with their catalytic, optical, electrical and magnetic characteristics (Nelson *et al*., 2010) [2], that are extensively used owing to their anti-microbial properties. Silver nanoparticles are highly conductive, chemically stable and highly economical (Niraimathi *et al*., 2014) [3]. The plant extract was used for the preparation of silver nanoparticles owing to its least toxicity and lesser need for elaborate purification as compared to the chemical methods. The present work essentially deals with increasing therapeutic efficacy of the selected drug in its nanoparticle form. The present study aims to synthesis silver nanoparticles using the aqueous leaf extract of *Eichhornia crassipes* and evaluation of its anti- inflammatory activity.

Materials and Methods
Plant materials
The *Eichhornia crassipes* leaves were collected in January 2015 from Koraiyaru River, Mannargudi, Thiruvarur district, Tamil Nadu. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen (SJCBO12335) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of leaves extract
The dried leaves 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 leaves extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4 ^{0}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 leaves extracts
For the Ag nanoparticles synthesis, 5 ml of *Eichhornia crassipes* leaves extract was added to 45 ml of 1 mM aqueous $AgNO_3$ 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 leaves 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) [4].

In vitro anti-inflammatory activity

Anti-inflammatory activity of the *Dodonaea angustifolia* leaves extract and SNPs was evaluated by protein denaturation method as described by Padmanabhan and Jangle (2012) [5].

Results and Discussion

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the *Eichhornia crassipes* leaves extract and AgNPs. The lowest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 17.105%, 21.052% and 21.03% in the concentration of 100µg/ml respectively, while the highest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 81.578%, 88.157% and 89.65% in the concentration of 500µg/ml respectively. The greatest effect of AgNPs (500µg/ml) was found to be near to standard diclofenac sodium. The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract, AgNPs and diclofenac sodium were 308.24, 279.34 and 237.11µg/ml respectively. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 1 and Figure 1).

Table 1 Effect of *Eichhornia crassipes*, AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

Groups	Concentrations	% of inhibition		
		Eichhornia crassipes	**AgNPs**	**Diclofenac sodium (Standard)**
Group I	100µg/ml	17.105±1.197	21.052±1.473	21.03±1.47
Group II	200µg/ml	32.894±2.302	36.842±2.578	51.65±3.61
Group III	300µg/ml	47.368±3.315	52.631±3.684	62.35±4.36
Group IV	400µg/ml	64.473±4.513	68.421±4.789	76.25±5.33
Group V	500µg/ml	81.578±5.710	88.157±6.170	89.65±6.27
IC_{50} (µg/ml)		308.24	279.34	237.11

Values are expressed as Mean ± SD for triplicates

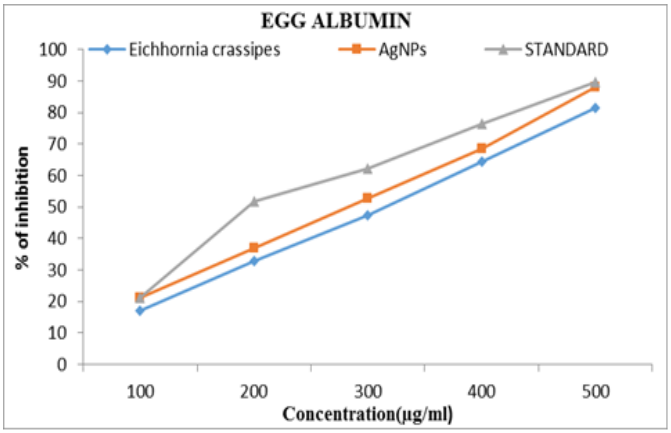

Fig 1: Effect of *Eichhornia crassipes*, AgNPs and Diclofenac sodium on protein denaturation (Fresh egg albumin)

The present findings exhibited a concentration dependent inhibition of protein (Bovine serum albumin) denaturation by the *Eichhornia crassipes* leaves extract and AgNPs. The lowest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 18.210%, 20.351% and 23.75% in the concentration of 100µg/ml respectively while the highest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 77.368%, 84.210% and 91.52% in the concentration of 500µg/ml respectively. The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract, AgNPs sand ascorbic acid were 345.09, 312.03 and 216.59µg/ml respectively. The greatest effect of AgNPs (500 µg/ml) was found to be near to standard diclofenac sodium. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein (Table 2 and Figure 2).

Table 2 Effect of *Eichhornia crassipes*, AgNPs and Diclofenac sodium on protein denaturation (Bovine serum albumin)

Groups	Concentrations	% of inhibition		
		Eichhornia crassipes	**AgNPs**	**Diclofenac sodium(Standard)**
Group I	100µg/ml	18.210±1.274	20.315±1.422	23.75±1.66
Group II	200µg/ml	30.105±2.107	31.473±2.203	58.65±4.10
Group III	300µg/ml	40.00±2.80	44.315±3.102	64.54±4.51
Group IV	400µg/ml	52.631±3.68	60.315±4.222	74.83±5.23
Group V	500µg/ml	77.368±5.415	84.210±5.894	91.52±6.40
IC_{50} (µg/ml)		345.09	312.03	216.59

Values are expressed as Mean ± SD for triplicates

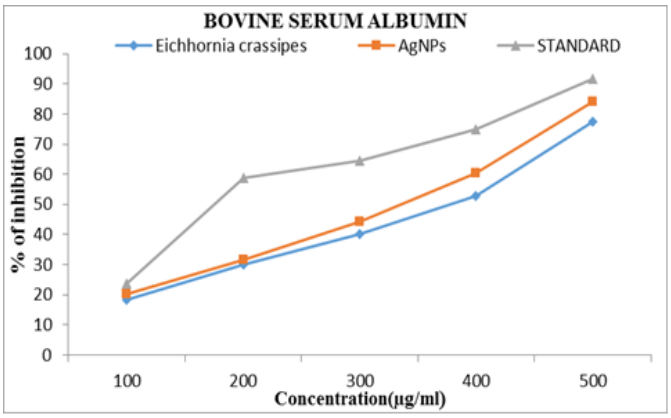

Fig 2: Effect of *Eichhornia crassipes*, AgNPs and Diclofenac sodium on protein denaturation (*Bovine serum* albumin)

The use of nano-herbal-technology to synthesize compounds with improved anti-inflammatory properties is an area of current research by many scientists. In our study, we report the non-toxic, practical and environmentally benevolent approach for the synthesis of silver nanoparticles using the aqueous leaf extract of *Eichhornia crassipes* with potent anti-inflammatory activity. The synthesize and characterization of AgNPs from *Eichhornia crassipes* leaf extract showed the particle size between 10-80nm as well the cubic structure of the nanoparticles reported in our earlier report (Prabakaran and Mani, 2017) [6].

The increments in absorbances of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein denaturation by *Eichhornia crassipes* leaf extract, AgNPs and reference drug diclofenac sodium. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the *Eichhornia crassipes* leaves extract and AgNPs. The lowest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 17.105%, 21.052% and 21.03% in the concentration of 100µg/ml respectively, while the highest activity of *Eichhornia crassipes* leaves extract, AgNPs and Diclofenac sodium were 81.578%, 87.157% and 89.65% in the concentration of 500µg/ml respectively. The greatest effect of AgNPs (500µg/ml) was found to be near to standard diclofenac sodium. The half inhibition concentration (IC_{50}) of *Eichhornia crassipes* leaves extract, AgNPs and diclofenac sodium were 308.24, 279.34 and 237.11µg/ml^{-1} respectively. From the present study it can be concluded that AgNPs showed marked *in vitro* anti-inflammatory effect against the denaturation of protein. Our result agrees with the earlier report (Aparna Mani *et al*., 2015; Giridharan *et al*., 2014) [7, 8].

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Conclusion

The synthesised silver nanoparticles are capped by the phytochemicals of *Eichhornia crassipes* leaf extract especially flavonoids and show significant anti-inflammatory effects. It was concluded that combining the benefits of phytomedicine with nano medicne can result in the formation of more efficient silver nanoparticles. This finding suggests that the synthesis of AgNPs using *Eichhornia crassipes* leaf extract could be a good source for developing green nano-medicine for the management of inflammation.

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