

In-Vitro Anti-Arthritic Activity of Green Synthesized Silver Nanoparticles – *Justicia gendarussa* Burm. F.

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Abstract

The present study was aimed to evaluate the *in-vitro* anti-arthritic activity of *Justicia gendarussa* leaves extract and silver nanoparticle synthesis *Justicia gendarussa* leaves extract (AgNPs) of using inhibition of protein denaturation model and human red blood cell Membrane stabilization model. Diclofenac sodium was used as a standard drug. Results revealed that the *Justicia gendarussa* leaves extract and AgNPs at different concentrations possessed significant anti-arthritic activity as compared to standard drug. The results obtained in the present investigation Indicate that AgNPs showed anti-arthritic activity.

Key words: *Justicia gendarussa*, Nanoparticles, AgNPs, Anti-arthritic activity, Diclofenac sodium

Nano-biotechnology is an increasing area of investigate that inculcates the purpose of nano-materials in the field of biomedical research as their enhanced property are foremost to the rapid improvement and discovery in the medical, optics and electronic industries. The recent advancement of nano-materials is based on explicit individuality such as size, distribution and morphology [1-2]. Green synthesis is a process of nanomaterial fabrication subsequent eco-friendly and sustainable approaches without usage of any toxic chemicals. Various biological entities like plant extracts, bacteria, algae, fungi, lichens and enzymes are used for green synthesis of nanoparticles [3]. Green synthesis of nanoparticles is specific due to their ability in shape confinement due to the diversified chemical, optical and electrical properties [4]. Silver nanoparticle synthesis is notably interesting due to their application in the field of drug delivery, energy systems and optics. Because of their surface properties. These silver nanoparticles are clinically used in cancer treatment and diagnosis. Generally, nanoparticles preparation involves chemical and physical methods that are expensive and emits hazardous chemicals to the environment causing pollution and various biological risks. The recent development of biotechnological processes for nanoparticle synthesis is evolving as the plant phytoconstituents can act as both reducing and capping agents [5].

Among the different types of nanoparticles, Ag NPs have a wide range of applications. In recent years, silver nitrate nanoparticles have received considerable interest due to their unique antibacterial, antifungal, UV filtering properties, high catalytic and photochemical activity. When compared with other nanoparticles, Ag nanoparticles are less toxic and safe so they find increased applications in food industries, cosmetic industry, solar cells and semiconductors.

Rheumatoid arthritis is a form of arthritis that causes pain swelling, stiffness, and loss of function in joints. It is a chronic condition with multiple conditions with multiple causes and defects the people in their most active period of life. The deformities that may develop due to the chronic forms stand as the greatest cripper of mankind [6].

Justicia gendarussa (Acanthaceae) is an important medicinal crop of India. *Justicia gendarussa* is a shade loving, quick growing; ever green plant which is mostly found in moisture areas. It is considered to be a native of China and is distributed widely in India, Sri Lanka and Malaysia. In the Indian and Chinese traditional medicines, the leaves of the plant are recommended to treat ailments such as fever, hemiplegia, rheumatism, arthritis, headache, ear ache, muscle pain, respiratory disorders and digestive troubles. Leaf extracts actively participate in the bio-reduction process to convert the silver ions to silver and silver nitrate nanoparticles have utilized the leaf extract of *Justicia gendarussa* for the preparation of Ag Nanoparticles. In this regard, we have effectively synthesized the Ag NPs from *Justicia gendarussa* extract. The anti-arthritic activities of the Ag NPs were determined.

Hence, the present study was aimed leaf of *Justicia gendarussa* and to determine anti-arthritic effect by using *in-vitro* pharmacological models by RBC membrane stabilization and Egg albumin in controlled experiment conditions and subjected determination of absorbance to assess the anti-arthritic property Diclofenac sodium was used reference drug. The present findings exhibited a concentration dependent of protein denaturation by the *Justicia gendarussa* leaves extract and AgNPs.

MATERIALS AND METHODS

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All chemicals, reagents, medium components are procured from Merck, India. *Justicia gendarussa* leaves were collected from Kaadukkaval, Thanjavur District, Tamil Nadu. Leaves were removed from flesh, shade dried then rinsed with water three times and then cleansed with Milli-Q water to remove the fine dust particles. Finally, the leaves were air dried for one week to remove all moisture completely, coarsely powdered, packed in air tight container and stored under normal temperature for further use [7].

Preparation of leaves extract from *Justicia gendarussa*

The dried leaves of *Justicia gendarussa* were pulverized well with pestle and mortar to make a fine powder. Twenty grams of powder sample was mixed into one hundred milliliter of deionized water and the mixture was boiled for 10 min. After cooling the leaf extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4°C for further use.

Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticle synthesized by the method [8]. In this method, 5 ml of *Justicia gendarussa* leaves 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 five hours (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without *Justicia gendarussa* leaves extract. The AgNPs solution thus attained 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 silver nanoparticles were collected for further use.

In-vitro anti-arthritis activity

Inhibition of egg albumin denaturation

Reagent

1. Egg albumin
2. Phosphate Buffer (pH 6.4)
3. Diclofenac sodium as standard

Procedure

The reaction mixture (5ml) consisted of 0.2ml of egg albumin (from fresh hen's egg), 2.8ml of phosphate buffered saline (PBS, pH6.4) and 2ml of varying concentrations of AgNPs and *Justicia gendarussa* leaves extract (100, 200, 300, 400 and 500µg/ml respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in an incubator for 15min and then heated at 70 °C for 5 minutes. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100- 500µg/ml) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (\text{Absorbance of test sample} / \text{Absorbance of control} - 1) [9].$$

Assay of RBC membrane stabilizing activity

Anti-arthritis activity evaluated by Membrane stabilizing activity as described [10].

Reagents

1. Phosphate buffer (pH 7.4)
2. Hypotonic saline
3. Diclofenac sodium

Procedure

1 ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of AgNPs and *Justicia gendarussa* leaves extract extracts of various concentrations (100, 200, 300, 400 and 500 µg/ml) and 0.5 ml of 10% w/v human red blood cells. All the assay mixtures were incubated at 37°C for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was separated and the hemoglobin content was estimated by a spectrophotometer at 560nm. Diclofenac sodium (100 to 500 µg/ml) was used as reference drug.

RESULTS AND DISCUSSION

Anti – arthritis activity

Anti – arthritis activity of egg albumin

To screen *in vitro* anti-arthritis activity of *Justicia gendarussa* leaves extract and AgNPs the protein denaturation method was carried out. The extract at different concentrations was incubated with egg albumin and RBC membrane in controlled experiment conditions and subjected to determination of absorbance to assess the anti-arthritis property. Diclofenac sodium was used as the reference drug.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the *Justicia gendarussa* leaves extract and AgNPs.

The lowest activity of *Justicia gendarussa* leaves extract, AgNPs and Diclofenac sodium were 14.92±0.73, 17.18±1.45 and 20.37±1.38% in the concentration of 100µg/ml recorded. While the highest activity of *Justicia gendarussa* leaves extract AgNPs and Diclofenac sodium were 91.75±1.92, 94.12±1.41 and 96.45±2.81% in the concentration of 500µg/ml recorded respectively. The significant result of AgNPs (500µg/ml) was found to be nearby standard diclofenac sodium. The half inhibition concentration (IC₅₀) of *Justicia gendarussa* leaves extract AgNPs and diclofenac sodium were 288.38, 270.03 and 255.66 µg/ml recorded respectively. The higher the concentration, the higher the observed activity. Additionally, the comparison with Diclofenac sodium, a standard drug, indicates that AgNPs, particularly at 500 µg/ml, exhibit a significant activity comparable to the standard drug. The values you've provided suggest that Diclofenac sodium has the lowest IC₅₀, followed by AgNPs and then *Justicia gendarussa* leaves extract. The present study it can be concluded that AgNPs showed marked *in vitro*-anti arthritis effect against the denaturation of protein (Table 1, Fig 1).

Table 1 Anti-arthritis activity of egg albumin

Concentrations (µg/ml)	% of inhibitions		
	<i>Justicia gendarussa</i> leaves	AgNPs	Std. (Diclofenac)
100	14.92 ± 0.73	17.18 ± 1.45	20.37 ± 1.38
200	28.99 ± 0.69	35.77 ± 1.33	37.89 ± 1.75
300	52.39 ± 2.07	56.79 ± 2.26	60.41 ± 2.07
400	73.39 ± 1.22	75.09 ± 2.13	77.29 ± 2.56
500	91.75 ± 1.92	94.12 ± 1.46	96.45 ± 2.81
IC ₅₀ Value (µg/ml)	288.38	270.03	255.66

Values are expressed as Mean± SD for triplicates

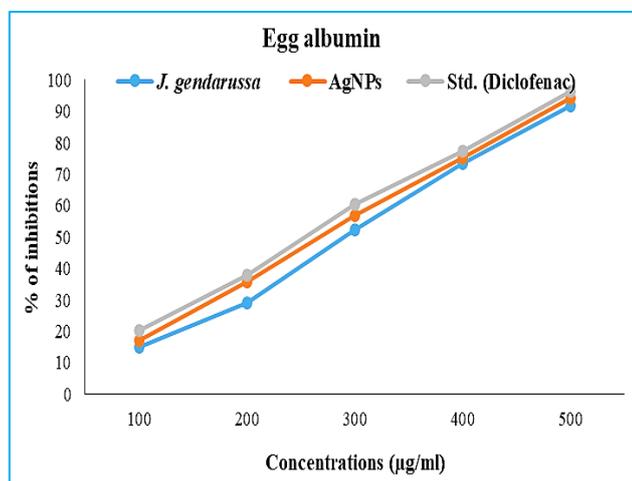


Fig 1 Anti-arthritis activity of egg albumin

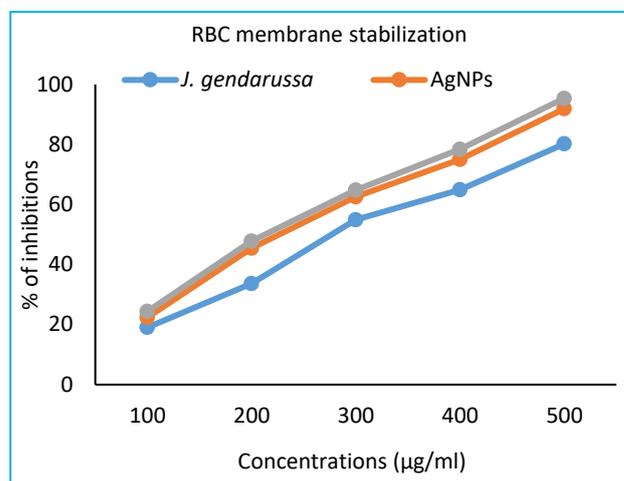


Fig 2 Anti-arthritis activity of RBC membrane stabilization

Anti arthritic activity of RBC membrane stabilization

The present findings exhibited a concentration inhibition of protein denaturation by the *Justicia gendarussa* leaves extract and AgNPs. The lowest activity of *Justicia gendarussa* leaves extract, AgNPs and Diclofenac sodium were 19.04±1.36, 22.40±0.77 and 24.38±0.98% in the concentration of 100µg/ml respectively. While the highest activity of *Justicia gendarussa* leaves extract AgNPs and Diclofenac sodium were 80.20±2.86,

91.90±1.73 and 95.39±2.65% in the concentration of 500µg/ml respectively. The half inhibition concentration (IC₅₀) of *Justicia gendarussa* leaves extract, AgNPs and ascorbic acid were 296.36, 243.76 and 229.43 µg/ml⁻¹ recorded respectively. The greatest effect of AgNPs (500µg/ml) was found to be nearby standard diclofenac sodium from the present study it can be concluded that AgNPs showed marked in anti-arthritic activity effect against the denaturation of protein (Table 2, Fig 2).

Table 2 Anti-arthritic activity of RBC membrane stabilization

Concentrations (µg/ml)	% of inhibitions		
	<i>Justicia gendarussa</i> leaves	AgNPs	Std. (Diclofenac)
100	19.04 ± 1.36	22.40 ± 0.77	24.38 ± 0.98
200	33.65 ± 1.74	45.46 ± 1.97	47.81 ± 1.37
300	54.96 ± 2.10	62.57 ± 1.75	64.85 ± 1.98
400	64.90 ± 1.99	75.03 ± 1.50	78.46 ± 2.47
500	80.20 ± 2.86	91.90 ± 1.73	95.39 ± 2.65
IC ₅₀ Value (µg/ml)	296.36	243.76	229.43

Values are expressed as Mean± SD for triplicates

To screen present work *in vitro* anti-arthritis activity of *Justicia gendarussa* leaves extract and AgNPs the protein denaturation method was carried out. The extract at different concentrations was incubated with egg albumin and RBC membrane in controlled experiment conditions and subjected to determination of absorbance to assess the anti-arthritis property Diclofenac sodium was used as the reference drug. The half inhibition concentration (IC₅₀) of *Justicia gendarussa* leaves extract AgNPs and diclofenac sodium were 288.38, 270.03 and 255.66 µg/ml recorded respectively. The present study AgNPs showed marked in vitro-anti arthritic effect against the denaturation of protein.

Similar to the present study [11] the *in vitro* bioassay results of antiarthritic effect of *Selaginella myosurus* silver nanoparticles assessed against denaturation of egg albumin are summarized. All tested concentrations significantly ($P < 0.001$) inhibited the denaturation of egg albumin. The maximum inhibition percentage obtained was 99% at a concentration of 0.2mg/mL. The acetylsalicylic acid used as a standard drug exhibited an inhibition of 65% at a concentration of 5mg/mL recorded.

Similar to the present investigation to earlier work of [6] reported *Justicia gendarussa* methanolic extracts, toluene fraction and compound La of leaf of *Justicia gendarussa* were capable of controlling the production of auto antigen and

thereby it inhibits the denaturation of proteins. The result was compared with the standard drug indomethacin. Based on our finding, it was proved that all different fractions having one common compound, that may be compound La. Therefore, it can be said that effect may be because of this common compound. While methanolic extract and compound Ra of root of *Justicia gendarussa* were capable of controlling the production of auto antigen and thereby it inhibits the denaturation of proteins and its effect was compared with the standard drug indomethacin. Protein denaturation inhibitory assay showed that the IC₅₀ value of compound-La of leaf and compound-Ra of root of *Justicia gendarussa* 24.74 and 25.75 µg/ml as compared with standard drug Indomethacin 19.41 µg/ml.

Most of the investigators have reported [12] that denaturation of the protein is one of the causes of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study, it can be stated that Compound La from methanolic extract of leaf of *Justicia gendarussa* is capable of controlling the production of auto-antigens due to *in vivo* denaturation of proteins in rheumatic diseases.

Hence, our finding justifies the usefulness of *Justicia gendarussa* for the management and treatment of inflammation associated diseases like arthritis. Based on our results, obtained in the present studies, compound isolated from plant and AgNPs extract of leaf of *Justicia gendarussa* possess significant *in-vitro* anti-arthritic activity which is comparable to synthetic anti-inflammatory agents.

The present findings exhibited a concentration inhibition of protein denaturation by the *Justicia gendarussa* leaves extract and AgNPs. The half inhibition concentration (IC₅₀) of *Justicia gendarussa* leaves extract, AgNPs and ascorbic acid were 296.36, 243.76 and 229.43 µg/ml⁻¹ recorded respectively. The greatest effect of AgNPs (500µg/ml) was found to be nearly standard diclofenac sodium from the present study it can be concluded that AgNPs showed marked in anti-arthritic activity effect against the denaturation of protein results were recorded.

Earlier reported [13] the lysosomal enzyme released during inflammation produces a variety of disorders. The extracellular activity of these enzymes is said to be related to acute or chronic inflammation. The non-steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since, Human Red Blood Cell (HRBC) membrane is similar to lysosomal membrane, their study was undertaken to check the stability of HRBC membrane

by the extracts to predict the anti-inflammatory activity *in vitro* [14]. *Justicia gendarussa* showed significant anti-inflammatory activity in a concentration dependent manner. *Justicia gendarussa* methanolic extract was determined the concentration of 100, 500 and 250 mg mL⁻¹. The maximum concentration was observed the methanolic extract (1000mg mL⁻¹) 76.20, 72.46 and 73.62% protection of HRBC in hypotonic solution respectively. All the results were compared with standard Diclofenac at 50 mg mL⁻¹, which showed 74.14% protection of HRBC in hypotonic solution.

CONCLUSION

Our results strongly support the anti-arthritic potential of the plant *Justicia gendarussa* and its use in traditional medicine. *In vitro* analysis of silver nanoparticles over arthritis represents a better cure by inhibiting protein denaturation the major cause for the leading to arthritics. The findings of the present study revealed that green synthesized silver nanoparticles from *Justicia gendarussa* extract harbor potent anti-arthritic activity. The method used in this study is very simple, ecofriendly and economically viable. The results clearly demonstrated that AgNPs synthesized by green route can definitely complete commercial antibiotic used for treatment of arthritics and sometimes even better.

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