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Review Article

Green Synthesized Silver Nanoparticles of Artocarpus Heterophyllus Peel Extract and Its Evaluation for Anti-Inflammatory Activity

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Abstract

The aim of the study was to formulate Artocarpus heterophyllus peel extract into silver nanoparticles using green synthesis method and evaluating its Anti-inflammatory activity. It has various pharmacological benefits like anti-inflammatory, carcinogenic, anti-tubercular, anti-diabetic, anti-infective, anti-fungal, anti-viral, anti-oxidant. Artocarpus heterophyllus peel was extracted using methanol by maceration method and synthesized into silver nanoparticles using 1Mm silver nitrate by green synthesis. Then the nanoparticle was evaluated using particle size, zeta potential, UV-visible, and visual examination. The in vitro anti-inflammatory protein denaturation method using egg albumin in green synthesized Artocarpus heterophyllus peel extract silver nanoparticle was eco-friendly, non- toxic when compared to synthetic anti- inflammatory drugs.

Keywords: artocarpus heterophyllus; silver nanoparticles;green synthesis; anti-inflammatory

Silver Nanoparticles

The investigation of the therapeutic properties of AgNPs has allowed studies to be carried out on the mechanism for modulating cytokines. This research could allow the development of new treatments, define the effective therapeutic concentrations and indications for use as well as evaluate the cytotoxic, genotoxic and carcinogenic potential and associated environmental impact. AgNPs show a reduction in pro-inflammatory proteins and in the selective inhibition of the COX-2 pathway. It was concluded that AgNPs presents an anti-inflammatory action in vivo through mechanisms involving the increase of anti -inflammatory molecules.

Green Synthesis

Green synthesis is an emerging area in the field of science and provides both economic and environmental benefits. In this study, non- toxic reagents that are eco- friendly and biosafe are used. The use of herbal extracts in the green synthesis of metal oxide nanoparticles is an increasing approach.

Inflammation

Inflammation that can be triggered by a variety of factors including pathogens, damaged cells and toxic compounds. These factors may induce acute and chronic inflammatory responses in the heart, pancreas, lung, brain, intestinal tract and reproductive system potentially leading to tissue damage or disease. Both infectious and non -infectious agents and cell damage activate inflammatory cells and trigger inflammatory signaling pathways, most commonly the NK-B, MAPK and JAK STAT pathways. These signals activate LEUKOCYTE CHEMOTAXIS from the general circulation to sites of damage. These activated leukocytes produce cytokines that induce inflammatory responses.

Anti Inflammatory Activity

Anti-inflammatory drugs that inhibit H1 Histamine, COX enzyme and reduce the synthesis of prostanoids. Corticosteroids prevent the formation of both PGs and LTs. The inhibition of phospholipase A2 reduces arachidonic acid release and suppresses the inflammation.

Materials and Equipment

Materials

Artocarpus heterophyllus peel powder

Chemicals Reagents

Silver nitrate (AgNO3), distilled water.

Equipment Required

Magnetic stirrer, UV-Visible spectrophotometer, Homogenizer, Malvern Zeta size analyzer

Procedure:

Sample Collection

Artocarpus heterophyllus peels were collected from Coimbatore district.

Authentication

The plant specimen was identified and authenticated from Tamil Nadu Agriculture university.

Drying and Pulverizing

The peels were collected and shade dried. It was chopped into small pieces and kept for extraction.

Preparation of extract of peel of *Artocarpus heterophyllus*

Artocarpus heterophyllus fruits in total were collected, and each fruit's surface was cleaned with water and allowed to air dry. After cutting each fruit, the peel and pulp were separated. Carefully, the rinds, skin, and spines were separated. All of the fruits' spine, and skin samples were taken, chopped into tiny bits, and then blended. Each smashed piece was weighed separately. For 72 hours, 300 g of each were soaked in a 1:5 ratio (100 gm in 500 ml) of seven distinct solvents (polar-ethanol, methanol, ethyl acetate, non-polar chloroform, n-hexane, dimethyl sulfoxide, and petroleum ether) at room temperature. Filtration was used to separate the supernatant fluid, and a hot water bath was used to dry the residue. It was taken for analysis since the ethanolic extraction yield from all three sections was determined to be higher.

Synthesis of Silver Nanoparticles

In a typical reaction procedure, the 1 mM (mM) of AgNO3 aqueous solution was prepared in 100 ml Distilled water in 250 ml beaker and was used to produce silver Nanoparticles. The 40 ml of 1 mM AgNO3 solution was taken and treated with 10 ml of peel extract filtrate and heated the mixture for 1 hour at 40-50°C for reduction into Ag ions. For 1 mM silver nitrate solution the leaf extract acts as a reducing and stabilizing agent. The resulting solution becomes grey-black in colour after 48 hours, indicating the formation of nanoparticles. The concentrations of peel extract were also varied from 4 ml to 10 ml leaves extract as well as temperature and time duration are also varied.

Preformulation Studies

Solubility

Solubility test of *Artocarpus heterophyllus* peel were tested using different solvents such as ethanol, methanol, water and the results were given below.

SOLVENTS	SOLUBILITY
Water	Insoluble
Ethanol	Completely soluble
Methanol	Completely soluble

Table 1: Solubility of *Artocarpus heterophyllus* peel extract

Characterization of Synthesized *Artocarpus heterophyllus* Silver Nanoparticle

Characterization of *Artocarpus heterophyllus* silver nanoparticles is done to evaluate the functional properties. Various analytical techniques are utilized to confirm their formation and evaluate their characteristics. These techniques include UV-Visible spectroscopy, particle size analysis and zeta potential measurement.

Visual Examination

The primary confirmation of the synthesized Artocarpus heterophyllus silver nanoparticles is done by visual basis. The colour change of reaction mixture (silver nitrate solution and peel extract) with respect to time is observed.

UV-VIS spectroscopy

UV-VIS spectroscopy is a valuable technique to characterize the transmission, absorption and reflectivity of several compounds and for scientifically important material, such as coating and pigments etc. The ultraviolet and visible spectrum have wide features that are of use for sample identification but are extremely helpful for quantitative measurements.

Determination of particle size

The dried powders of Artocarpus heterophyllus Silver Nanoparticles dispersed in water to obtain proper scattering intensity of Artocarpus heterophyllus silver nanoparticles. The particle size was determined by the Malvern zeta size analyser.

Determination of zeta potential

The zeta potential was measured by using Zeta Sizer (Malvern Instruments) having zeta cells,

polycarbonate cell with gold-plated electrodes and using water as medium for sample preparation. Zeta potential determines the surface potential of silver nanoparticles and it is essential for the characterization of stability of nanoparticles.

In-Vitro Methods

Anti-arthritic studies by protein denaturation method

In Vitro anti-arthritic assay was conducted by protein denaturation method given by Mizushima and Kobayashi (1968). The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS with pH 6.4), and 2 ml of varying concentrations (10, 20,50, 10 ug/L) of plant extract. A similar volume of double distilled water is used as control. Then the mixture was incubated at 37 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 m by using the vehicle as blank.

The percentage of inhibition of protein denaturation was calculated by using the following formula;

Inhibition = AC660-AT660 x 100 AC600

AC = Absorbance of control solution, AT = Absorbance of the test sample.

Egg Albumin Assay

The anti-inflammatory activity of unknown crude extracts can be determined in vitro for inhibition of the denaturation of egg albumin (protein).

* 0.2 mL of 1-2% egg albumin solution (from fresh hen's egg or commercially available egg albumin powder), 2 mL of sample extract or standard (methotrexate) at varying concentrations, and 2.8 mL of phosphate buffer saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5 ml.

* A total volume of 5 mL of the control was created by combining 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline.

* The reaction mixtures were then incubated at 37±2°C for 30 min and will be heated in a water bath at 70±2°C for 15 min.

* After cooling, the absorbance was measured at 280 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank.

* The following equation was used to determine the percentage inhibition of protein denaturation:

Percentage Inhibition = (Absorbance of control - Absorbance of test sample) / Absorbance of control * 100

* Then plant extract/positive control concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition concerning control against concentration. All determinations were carried out in triplicate.

Result And Discussion

characterization of green synthesized *artocarpus heterophyllus* silver nanoparticles

Visual Examination

The colour of *Artocarpus heterophyllus* extract in the initial stage was yellow, after the addition of silver nitrate solution it turned into brown colour. There is no colour change after 90minutes, indicating that the reaction is finished. After 48 hrs the colour change from yellow to brown indicates the formation of silver nanoparticles. The image of *Artocarpus heterophyllus* extract is depicted in figure 6: The image of *Artocarpus heterophyllus* extract is depicted in figure 7: which denotes the formation of silver nanoparticles after 48hrs.

UV VISIBLE Spectral Analysis OF *Artocarpus heterophyllus* Silver Nanoparticles

Silver nanoparticles production and completion are characterized by UV-Visible spectral analysis, which makes use of a UV-Visible spectrophotometer.

The production of silver ions to silver nanoparticles was indicated by colour change from yellow to brown colour and it was reflected in spectral data obtained by using UV-Visible spectrophotometer. The silver nanoparticles Surface Plasmon Resonance (SPR) is characterized by the coloration. It shows an absorption peak at 425nm, confirming the formations of *Artocarpus heterophyllus* silver nanoparticles.

Particle size measurement:

The particle size is one of the most important parameters for Characterization nanoparticles. The mean particle size(z -average), polydispersity index (PI) of *Artocarpus heterophyllus* silver nanoparticles were determined by using a zeta size analyser (Malvern Instrument). The average particle size of *Artocarpus heterophyllus* was found to be 60.45nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.356 with intercept 0.845.

Zeta Potential

Zeta potential was used to determine the stability of prepared nanoparticles. Zeta potential was determined using a zeta-sizer instrument. The surface charge of the particles and stability of the solution was characterized by zeta potential. For *Artocarpus heterophyllus* silver nanoparticles, zeta potential was found to be 0.304mV with peak area 46.9 intensity. These values indicate that *Artocarpus heterophyllus* silver nanoparticles are stable.

Invitro Anti- Inflammatory Activity

Anti- inflammatory activity of standard drug aspirin and the *Artocarpus heterophyllus* silver nanoparticles were determined using protein denaturation assay. The anti- inflammatory activity of unknown crude extracts can be determined by using *in vitro* for inhibition of the denaturation of egg albumin. Different concentrations of drug sample and egg albumin solution are taken and mixed with phosphate buffer on PH 7.4 and incubated for 30min. Reaction mixture was heated at the water bath for 70°C for 15minutes. After cooling, the absorbance was measured at 280 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank. Aspirin is used as standard and the drug sample was compared with standard.

From the above standard drug is compared with our plant extract and the inhibition concentration value for inflammatory prepared nanoparticles of *Artocarpus heterophyllus* was slightly higher when compared to the standard drug aspirin.

It is believed that protein denaturation is the factor that promotes inflammation. Inhibition activity of *Artocarpus heterophyllus* silver nanoparticles against Rheumatoid were observed, hence it can be used as a potential alternative to synthetic drugs as a non-toxic safe way of green synthesis.

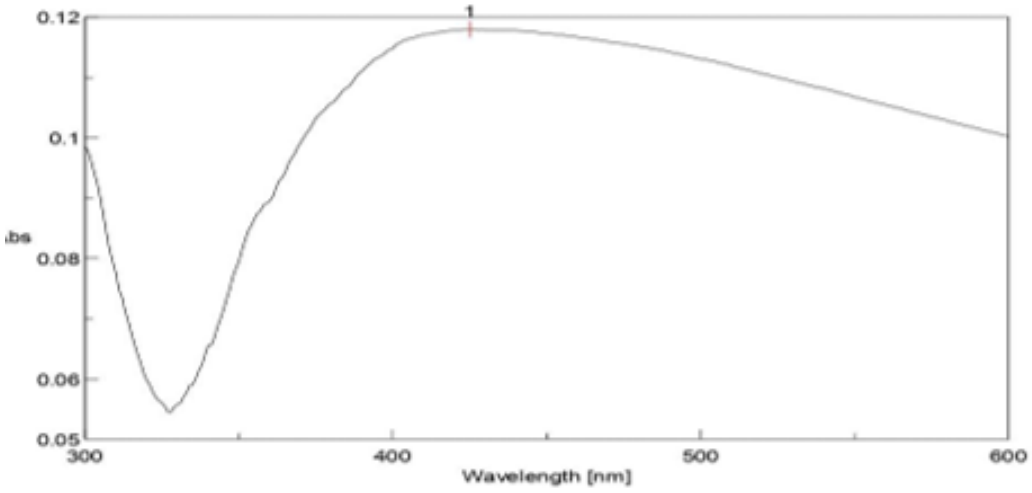

Figure 1: UV Visible spectrum of Formulation of *Artocarpus heterophyllus* Nanoparticles

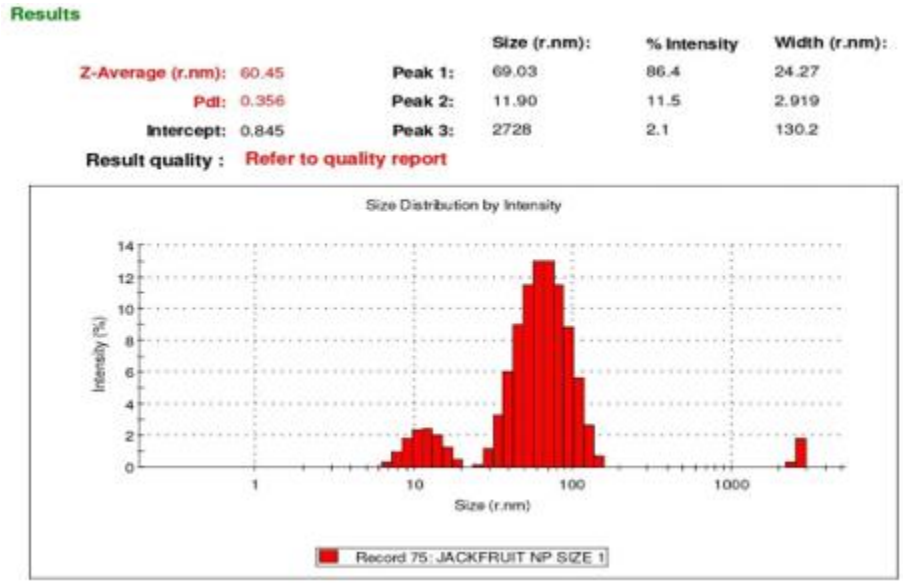

Figure 2: Determination of Particle Size of *Artocarpus heterophyllus* Silver Nanoparticle

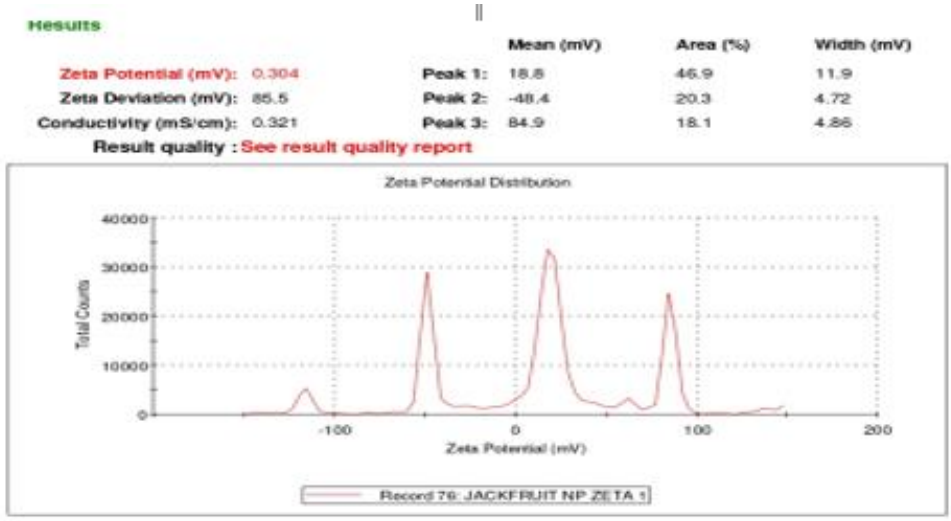

Figure 3: Determination of Zeta Potential of *Artocarpus heterophyllus* silver nanoparticles

Concentration (µg/ml)	Absorbance			% Inhibition			Average
	T1	T2	T3	T1	T2	T3	
10	0.0665	0.0652	0.0646	67.08	63.81	62.31	64.40
20	0.0618	0.0630	0.0627	58.27	58.29	57.03	57.03
30	0.0491	0.0496	0.0506	23.37	24.62	27.14	25.04
40	0.0659	0.0672	0.0661	65.57	68.84	66.08	66.82
50	0.0558	0.0573	0.0580	40.20	43.97	45.73	43.30

Table 2: Protein Inhibitory Data of Control Aspirin

Concentration (µg/ml)	Absorbance			% Inhibition			Average
	T1	T2	T3	T1	T2	T3	
10	0.0445	0.0450	0.0456	11.30	13.06	14.57	13.14
20	0.0623	0.0633	0.0634	56.53	59.05	59.55	58.37
30	0.0712	0.0725	0.0732	78.89	82.16	83.91	81.65
40	0.0841	0.0832	0.0830	111.30	109.04	108.54	109.62
50	0.1092	0.1097	0.1101	174.37	175.62	176.63	175.54

Table 3: Protein Inhibitory Data of *Artocarpus heterophyllus* Silver Nanoparticles

Conclusion

The study shows green synthesized silver nanoparticles of ***Artocarpus heterophyllyus*** has anti-inflammatory activity. These nanoparticles were characterized and evaluated by various test such as visual examination, UV-VIS Spectral analysis, zeta potential. Invitro anti-inflammatory activity was studied. Perform invivo methods for further confirmation of above mentioned property.

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