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Research Article

BIOSYNTHESIS, CHARACTERIZATION, AND ANTI-INFLAMMATORY STUDY OF *HEMIGRAPHIS COLORATA* LOADED SILVER NANOPARTICLES

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ABSTRACT

Objective: This study targeted at the synthesis, characterization, and evaluation of the anti-inflammatory effects of *Hemigraphis colorata* silver nanopartilces (HAgNPs).

Methods: The HAgNPs were photosynthesized and characterized by UV-visible spectroscopy. The *in vitro* anti-inflammatory properties of HAgNPs were assessed by evaluating inhibition of protein denaturation and membrane stabilization ability assay.

Results: HAgNPs exhibited an absorbance peak at 450 nm, characteristic for AgNPs, and their sizes ranged from 20 to 90 nm. The anti-inflammatory potential of HAgNPs when compared with *H. colorata* extract suggests that the AgNPs along with polyphenols and flavonoids from *H. colorata* can act as reducing or inhibiting agent on the release of acute inflammatory mediators.

Conclusion: The biologically synthesized HAgNPs could be of enormous use in the medical field for their proficient anti-inflammatory activity, which can be utilized as novel therapeutic agent for prevention and cure of inflammation due to biocompatible nature. This work clearly demonstrates HAgNPs as a budding source for anti-inflammatory drugs.

Keywords: Nanoscience, *Hemigraphis colorata*, Photosynthesis, Silver nanoparticles, Anti-inflammatory.

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INTRODUCTION

Indian traditional medicine, the oldest system, encompasses Ayurveda with numerous medicines based on metal mineral formulation, the herbo-metallic preparations with the particle diameter of 10–15 nm. Offers drug designing retaining contemporary perception of nano-medicine [1]. Silver used from prehistoric times for management of wounds, inflammation, and nanoparticles of silver industrialized with potent anti-inflammatory [2] and antioxidant activities [3]. The major phytochemicals responsible for spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, aldehydes, ketones, and amides [4,5]. Consequently, the application of plants as potential biological nano-factories has a heap of interest due to ecological, energy-efficient, economic, and biocompatible nature suitable for pharmaceutical and biomedical applications [6,7]. Therefore, study on plant systems is considered to be a potential bioreactor for synthesis of metal nanoparticles without using toxic chemicals.

Hemigraphis alternate (syn.) *Hemigraphis colorata* (Blume) H.G. Hallier (Acanthaceae)

Oxidative stress and inflammation are critical factors attributed with delay in wound repairing process [8]. In this scenario, various phytochemicals from our "oldest savior" plants and its commodities appear to play as a therapeutic representative or prophylactic antidote [9]. The phytoconstituents of *H. colorata* are phenols, saponins, flavonoids, terpenoids [10], coumarins, carbohydrates, carboxylic acid, xanthoproteins, tannins, proteins, alkaloids, steroids, and sterol [11]. Since time immemorial, man has used various parts of this plant for treatment and prevention of many ailments [12]. Leaf paste of *H. colorata* was shown anti-inflammatory effect on carrageenan-induced paw edema model [13]. One of the acute responses; in wound healing is inflammation that results in coordinated influx of neutrophils at the wound site. Although commenced as a defensive, loss of regulation of this multifaceted progression can lead to several inflammatory disorders such as cancer, arthritis, and neurological diseases [14-16].

Liver damage, gastric lesions, initiation of cardiovascular problems, renal failure, fluid retention, bronchospasm, and prolongation of bleeding time are experienced in using anti-inflammatory drugs (nonsteroidal anti-inflammatory drugs [NSAIDs]) [17,18]. Hence, there is a growing interest in search for alternative medicinal plants, because of their chemical composition and better ability to mitigate mediators of multiple mechanisms to treat symptoms related to painful inflammatory process more effectively [19]. Prolonged inflammation delay natural healing process; a good healing agent should possess anti-inflammatory activity for the proper wound [13,20,21]. The current study focused exploration of novel application of *H. colorata* for cost-effective green synthesis of *H. colorata* silver nanoparticles (HAgNPs) in a simplistic way, to characterize and determine the efficacy of HAgNPs for treatment of inflammatory disease using *in vitro* studies.

METHODS

Plant collection and authentication

Healthy, disease-free, and plant leaves of *H. colorata* (Blume) collected from Ernakulam District, India and authenticated at Kerala Forest Research Institute, Peechi, Kerala.

HAgNPs

Green synthesis of HAgNPs

The synthesis of AgNP (HAgNP) was carried out by standard green synthesis procedures with slight modifications [22]. About 10 ml of *H. colorata* extract was taken in a conical flask, and to the 40 ml of 1 mM $AgNO_3$ (1:4 ratio of *H. colorata* extract to 1 mM $AgNO_3$) was added with constant stirring. The reaction was observed for change in color of the reaction mixture till 12 h. The solution turned from yellowish to bright yellow and to dark brown indicating the AgNP formation. The changing in the color was observed gradually as it turned deep brown at the end of 24 h [23].

Centrifugation

AgNPs solution was centrifuged at 15,000 rpm for 30 min. The pellets were washed 3 times with 20 ml of distilled water, to dispose of the free proteins/catalysts, remove any traces of unbound phytoconstituents that are not topping the AgNPs, and finally dried at 60°C in a dry oven, stored at 4°C [24].

Anti-inflammatory properties

Inhibition of protein denaturation

Anti-inflammatory activity of the *H. colorata* aqueous extract and HAgNPs to inhibit protein denaturation was studied through *in vitro* assay carried out by method of [25], with minor modifications. To 50 µl of different concentrations of tests, 5 ml of 0.2% w/v bovine serum albumin (BSA) added, heated at 72°C for 5 min and then cooled for 10 min.

5 ml of 0.2% w/v BSA solution with 50 µl water used as control and aspirin (100 µg/ml) in water with 5 ml 0.2% w/v BSA solution used as standard. The absorbance measured at wavelength 276 nm.

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Membrane stabilization ability assay

Anti-inflammatory activity can be assayed by 2 methods [26-28]: (1) Inhibition of protein denaturation. (2) Membrane stabilization ability assay. Anti-inflammatory activity assay by membrane stabilization ability test is also appropriate title.

i. Preparation of red blood cells (RBCs) suspension
   Blood collected in centrifuge tubes (from a healthy human volunteer who has not taken any NSAIDs for proceeding 2 weeks to experiment), centrifuged at 3000 rpm 10 min, washed thrice with normal saline and the remaining volume of blood measured and re-constituted as 10% v/v suspension with normal saline.

ii. Heat-induced hemolysis
   The reaction mixture (2 ml) consisted of 1 ml test sample, *H. colorata,* and HAgNP different concentrations (250 and 500 µg/ml) separately and 1 ml of 10% RBCs suspension. Standard aspirin, saline is taken as control. Centrifuge tubes containing reaction mixture, incubated in water bath at 56°C, 30 min, cooled under running tap water, followed by centrifugation at 2500 rpm for 5 min. The absorbance of the supernatants was measured at 560 nm.

$$\text{Haemolysis percentage inhibition} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

Statistical analysis

All data expressed as means ± standard deviations calculated from three independent experiments.

RESULTS

Green synthesis of nanoparticles using biological agents has been an important sustainable approach for the biosynthesis of various forms of biocompatible nanoparticles. The method adopted for the synthesis of nanoparticles from *H. colorata* in this study is a bioreduction process in which $AgNO_3$ was reduced to AgNPs. Formation of HAgNP was confirmed by the reduction of silver ions which was visibly evident from the color changes light yellow to dark brown of the solution associated with it.

UV-visible spectrophotometric analysis

HAgNP was subjected to initial characterization by UV-visible spectroscopy and *H. colorata* extract taken as a control. It is generally recognized that UV-visible spectroscopy could be used to examine size and shape of controlled nanoparticles in aqueous suspensions. This analysis exhibited a sharp absorbance at 450 nm (Fig. 1), particular for AgNPs. The control could not form the characteristic peak indicating that the reaction conditions were optimum with no abiotic reduction of $AgNO_3$.

UV-visible spectra of *H. colorata* aqueous extract and HAgNP represent the formation of nanoparticles by *H. colorata* aqueous extract and showed the maximum absorption at a wavelength of 450 nm.

***In vitro* anti-inflammatory analysis of *H. colorata* aqueous extract and HAgNP**

Protein denaturation inhibition percentage

The anti-inflammatory activity or the ability of *H. colorata* aqueous extract and HAgNP to inhibit protein denaturation was studied

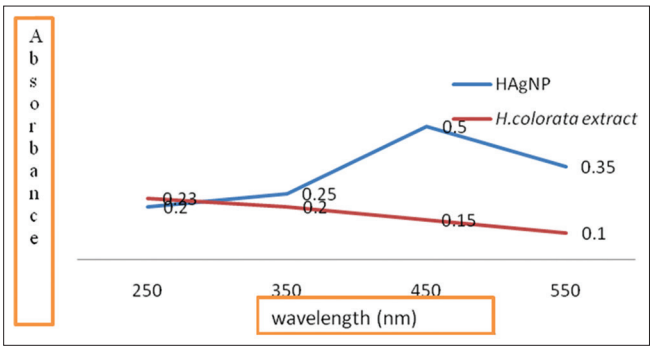

Fig. 1: UV-visible spectra of *Hemigraphis colorata* aqueous extract and *H. colorata* silver nanoparticles

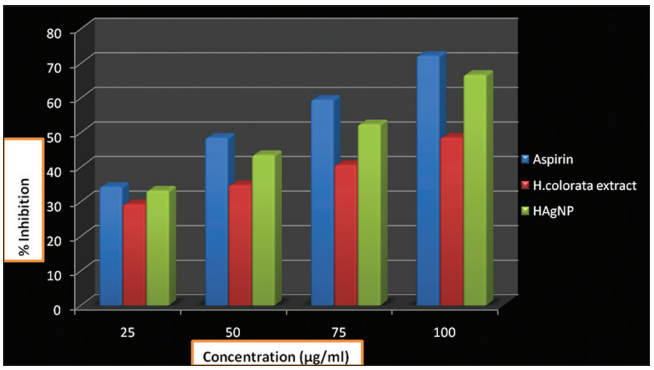

Fig. 2: *In vitro* anti-inflammatory activity of *Hemigraphis colorata* aqueous extract and *H. colorata* silver nanoparticles (HAgNP) by protein denaturation analysis. HAgNP shows better activity than *H. colorata* aqueous extract alone

Table 1: Inhibition of hemolysis analysis of *H. colorata* aqueous extract and HAgNP

S. No.	Sample	Concentration (µg/ml)	% Inhibition hemolysis
1.	Standard - Aspirin	100	68.7±1.0
2.	*H. colorata* aqueous extract	25	23.77±1.0
		50	27.6±1.3
		75	32.5±0.9
		100	39.67±1.7
3.	HAgNP	25	24.7±1.0
		50	29.8±1.2
		75	36.8±1.2
		100	49.5±0.9

HAgNP showed almost similar results of standard aspirin and extract alone resulted in scarcer values signifying the encouraging anti-inflammatory activity of HAgNP. HAgNP: *Hemigraphis colorata* silver nanoparticles, *H. colorata: Hemigraphis colorata*

through inhibiting heat-induced albumin denaturation. *In vitro* anti-inflammatory activity of *H. colorata* aqueous extract and HAgNP at a concentration of 25–100 µg/ml was carried out by analyzing their ability to inhibit protein denaturation (Fig. 2). Denaturation of proteins is a well-established cause of inflammation. Albumin denaturation inhibition was highest in HAgNP than *H. colorata* aqueous extract at a concentration of 100 µg/ ml. The percentage inhibition rate exhibited by HAgNP is 66.7±1.2%, and *H. colorata* extracts 55±1.9%, both had given more than 50% inhibition. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 76.2±0.9% at the concentration of 100 µg/ml. *H. colorata* aqueous extract and HAgNP showed a dose-dependent increase in percentage inhibition same as the control drug. The results of this investigation clearly show that HAgNP has better abilities than extract alone, can be explored in the search for natural anti-inflammatory drug.

Membrane stabilization test

Stabilization of the RBCs membrane was considered to further confirm the anti-inflammatory ability of *H. colorata* aqueous extract and HAgNP. The results of inhibition of hemolysis showed that HAgNP and extract at a concentration range of 25–100 µg/ml effective in inhibiting the heat-induced hemolysis and protect the erythrocyte membrane against lysis are shown in Table 1. *H. colorata* aqueous extract exhibited the maximum inhibition 39.67±1.7% and HAgNP 49.5±0.9% at 100 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum of 68% at the concentration of 100 µg/ml (Table 1) and the biosynthesized HAgNP, was much effective in stabilization of the RBCs membrane than extract alone even at lower concentration of 25 µg.

DISCUSSION

In the current study, biosynthesis of HAgNPs was carried out using a bottom-up approach that mostly involves reduction/oxidation reactions that takes place in one step; therefore, compounds like plants which hold dual characteristics, i.e. reducing and capping agents are preferred [29] for the synthesis of shape and size-controlled nanoparticles. The improvement of resourceful green chemistry methods is employing for natural reducing, capping, and stabilizing agents like plant parts such as leaf [30] to organic AgNPs in ideal morphology and size.

Analysis of HAgNP-color change

The addition of extract to the silver nitrate solution changed the color of the solution from light yellow to dark brown, confirmed the reduction of silver nitrates into the silver nanoparticles HAgNP (Fig. 1). This color change has been observed earlier by several researchers [31]. Ibrahim [32] suggested that the color change appeared due to surface plasmon resonance of deposited AgNPs. This change in color of reaction mixture was considered as a primary indication for formation of AgNPs. In the current study, HAgNP may be synthesized by higher total phenolics content in *H. colorata* which are strong antioxidants with high reducing capacity [33]. The aqueous silver ions treated with herbal extracts reduced in solution, resulting in formation of silver hydrosols [34]. The higher content of total phenolic content in *H. colorata* leaves extract facilitates reduction of silver ions to nanoscale-sized silver particles due to the electron-donating ability of phenolic compounds. The oxidation of the phenol group in leaf extract creates quinoid compound that can adsorb on surface of nanoparticles, ensuing their stabilization [35].

HAgNP UV-visible spectroscopic analysis

The biosynthesized HAgNPs primarily characterized by UV-visible spectroscopy. The absorbance at 450 nm recorded due to localized surface plasmon resonance and confirmed the formation of AgNPs (Fig. 2). When a specific wavelength is matched to the size of NPs dipole oscillation is generated, compensated form of inducing polarization and electrons in the NPs resonate, presenting a sturdy absorption [36]. Plant-based materials seem best candidates for eco-friendly large-scale nanoparticles production, alternatives to chemical methods [29]. Characteristic absorbance peak of AgNPs is between 400 and 450 nm. If the peak raises above 450 nm, indicates aggregation or precipitation resulting particle with large size but if peak shifts below 400 nm, the nano solution contains other participants such as impurities, organic species, and solvent [37]. The shift in absorption peak may be extremely indicative to size, morphology, quantity, and NPs growth, signifying the current study HAgNP is formed in adequate size and shape.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Most biological proteins lose their efficacy when denatured. The ability of a substance to inhibit the denaturation of protein implies the obvious potential for anti-inflammatory activity. The capacity of *H. colorata* and HAgNP to inhibit protein denaturation of albumin was ranging from 48.55±1.9 for *H. colorata*, 66.7±1.2 for HAgNP against standard aspirin 76.2±0.9% inhibition in this assay had therefore provided another evidence for its promising anti-inflammatory properties at concentration of 100 µg/ml. The results are almost similar [38,39].

Membrane stabilization

The human RBC membrane stabilization has been employed as a method to study *in vitro* anti-inflammatory activity of *H. colorata* extract and HAgNP, because the erythrocyte membrane is similar to the lysosomal membrane [40] and its stabilization entails that they may well stabilize lysosomal membranes. Maintenance of lysosomal membranes is substantial in averting the inflammatory reaction by checking the liberation of lysosomal components of activated neutrophil, such as bacterial enzymes and proteases, which forms further basis for tissue inflammation and damage on cellular discharge. The lysosomal enzymes released during inflammation are believed, related to acute or chronic inflammation. Results of *H. colorata* and HAgNP with maximum inhibition of 39.67±1.7 for *H. colorata*, 49.5±0.9 for HAgNP and 68.7±1.0 for standard aspirin at a concentration of 100 µg/ml are comparable with [41] and are entitling *H. colorata* and particularly HAgNP with better results, as potent anti-inflammatory drug.

Results indicate that extracts of *H. colorata* and HAgNP possess excellent anti-inflammatory properties. It was formerly described AgNPs could successfully decrease the infiltration of inflammatory cells, obstruct the creation of inflammatory cytokines, upregulate the expression of matrix metalloproteinase and moreover, AgNPs possessed anti-inflammatory activity in post-operative peritoneal adhesion model [2]. These activities may be due to strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. The extract elements function as free radical inhibitors or scavengers by executing as primary oxidants, inhibited the heat-induced albumin denaturation and stabilized the RBCs membrane. Yilmaz *et al*. [42] showed that silver-polyvinyl pyrrolidone nanoparticles exhibited anti-inflammatory activity by reducing tumor necrosis factor-α. The potential mechanism of AgNPs-mediated anti-inflammatory property was due to intracellular blocking of inflammatory pathways and downregulating pro-inflammatory cytokines [43]. It is well-known that in chronic and sub-acute inflammation, ROS play an important role in modulating extent of the inflammatory response, consequent tissue, and cell injury; antioxidants are considered as possible protecting agents reducing oxidative damage of the human body from ROS and retarding the progress of many diseases [44]. The results of previous *in vitro* antioxidant studies and quantitative determination of the total phenolic and flavonoids [45,46], strongly support the high anti-inflammatory activity of HAgNPs like many other plants. From all these findings, HAgNP can be used as lead compound for designing a potent anti-inflammatory natural drug to be used for treatment of various ailments without side effects.

CONCLUSION

Green nanobiotechnology encompasses the practice of energy-efficient ecological approaches for the production of nanoparticles using bio-sustainable approaches and biodegradable constituents.

Current work demonstrates sustainable green synthesis of HAgNPs in an eco-friendly manner using aqueous extract of *H. colorata* leaf. Synthesis of HAgNP is owed to the higher content of total phenolic content in *H. colorata* leaf extract that expedites reduction of silver ions to AgNPs through electron-donating ability of phenolic compounds. The study demonstrated *H. colorata,* and especially HAgNP has significant anti-inflammatory activities, proved in the *in vitro* model. Experimental findings collectively show that HAgNPs are effective in reducing inflammation and accordingly delivers further indication that contributes to the understanding of the anti-inflammatory properties and due to their biocompatible nature, propose HAgNPs as an innovative therapeutic agent for prevention and cure of inflammation.

AUTHORS' CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

There were no conflicts of interest.

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