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Formulation of Silver Nanoparticle of *Cassia angustifolia* by Using Green Synthesis Method and Screening for In-Vitro Anti-Inflammatory Activity

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ABSTRACT: The main objectives in developing nanoparticles as a delivery system are to manage particle size, surface characteristics, and the release of pharmacologically active substances to achieve the drug's site-specific action at the appropriate rate and dose. They can help boost medication stability and have helpful controlled release features, for example. Nowadays researchers are moving towards the green chemistry approach which is an alternative route that is eco-friendly, cheap and fast; in that plant extracts and microorganisms are used in the reduction of the metal salt which is fast gaining demand in the field of nanobiotechnology. In this study, silver nitrate was reduced to its "nanosilver form" through a one-step synthesis protocol using an extract of *Cassia Angustifolia.* Three different batches namely batch A, B, C of varying temperature and another three batches namely D, E, F of varying pH were synthesized. The prepared nanoparticles were optimized and characterized by practical yield determination, drug entrapment efficiency, particle size determination and measurement of zeta potential. The synthesized nanoparticles were screened for in vitro anti-inflammatory activity. Result found that the percentage practical yield of synthesized nanoparticles was within the range of 6.41-52.61%. The drug entrapment efficiency was found to be 99.875%. AgNPs inhibited protein denaturation and showed 75.52% inhibition at 500µg ml-1 whereas standard drug Aspirin exhibited 65.03% protein denaturation.

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INTRODUCTION

Nanotechnology is a new and realised field of research that has the potential to make a huge impact on society by assisting in the resolution of critical health and energy concerns. This is owing to metal nanoparticles' practical applicability in fields such as medicine [1], chemical sensing, catalysis, and electronics [2]. Nanotechnology is the control of shape and size at the nanoscale scale in the design and production of structures, devices, and systems [3]. Nanoparticles are the tiniest particles, ranging in size from 1 to 1000 nm, with exceptional properties due to their high surface area to volume ratio and small size[4]. Silver nanoparticles have drawn a lot of interest due to their appealing physical and chemical properties. More than a hundred years before the first metallic silver colloids were created. Chemical [5], electrochemical, γ-radiation[6], photochemical[7], laser ablation[8] and other processes can be used to make Ag nanoparticles. The Ag colloids were produced via chemical reduction of silver salts using sodium borohydride or sodium citrate. Even though this method of preparation is simple, extreme caution must be exercised to achieve a stable and repeatable colloid. The cleanliness of the glassware, as well as the purity of the water and reagents, are essential in the synthesis of nanoparticles. Particle size is affected by solution temperature, metal salt and reducing agent concentrations, and reaction time. Metal nanoparticles are difficult to manage in terms of size and shape[9]. Nanoparticles with size-induced properties are perfectly applied for the development of new applications or the modernization of existing methods in fields such as catalysis, optics, microelectronics, and many others. Silver nanoparticles possess unique properties not found in molecules or bulk metals. The absorption band in the invisible light area is one example. This band is caused by surface Plasmon-oscillation

modes of conduction electrons that are connected to external electromagnetic fields through the surface [9, 10].

The silver Nanoparticles were made using a green synthesis approach in this study. Green chemistry is the use of a set of concepts that will help to limit the use and creation of hazardous compounds during chemical product manufacturing. The goal of green chemistry is to conserve the environment by developing new methods that are less detrimental to society. In the chemical sciences, it is a rapidly growing and crucial field. Many researchers are interested in silver nanoparticles because of their remarkable physical and chemical characteristics. AgNPs reduce HIV-1 replication, according to a scientific journal. AgNPs have also been shown to exhibit antibacterial action in studies. Silver nanoparticles have been successfully synthesised using a variety of plant extracts. The possibility of employing *Cassia Angustifolia* extract to manufacture AgNPs using the green synthesis method is demonstrated in this paper [11, 12, 13, 14].

Inflammatory bowel disease (IBD) is a term used to describe conditions in which the digestive tract is chronically inflamed. The following are examples of IBD types: 1. Ulcerative colitis is a condition in which the intestines become inflamed Crohn's disease is an illness that causes long-term inflammation and sores (ulcers) in the innermost lining of the large intestine (colon) and rectum. The lining of the digestive tract becomes inflamed, and the inflammation penetrates deep into the afflicted tissues. IBD symptoms vary based on where the inflammation is occurring and how severe it is. The signs and symptoms might range from minor to severe. Periods of active sickness will most likely be followed by periods of remission [15].

Senna glycoside or senna is mainly used in the treatment of constipation. This is used either through the mouth or rectum. This shows its result within twelve hours after being given by mouth and in minutes after being administered through the rectum. It shows weak laxative properties as compared to bisacodyl or castor oil [16].
Sennoside A, one of the sennosides found in laxatives, has recently been shown to block the human immunodeficiency virus (HIV) reverse transcriptase ribonuclease H (RNAse H) function [17].

Gut bacteria convert sennosides A and B into sennidins A and B. Gut bacteria use beta-glucosidase to convert sennidins A and B to rheinanthrone Rheinanthrone. Rheinanthrone is ingested into the systemic circulation, where it is converted into rhein Rhein and sennidins A and B by oxidation [18,19,20]. Rheinanthrone is the primary metabolite of sennosides A and B, rather it is the metabolite that imparts the drug its laxative effect. Rhein is an active metabolite with numerous defensive properties[21].

MATERIALS AND METHODS

Extract Preparation

1 g of the powdered drug of *Cassia Angustifolia* was weighed in a 250 ml conical flask. 100 ml of distilled water was added to the conical flask containing the powdered drug and macerated for 4 hours along with continuous stirring on a magnetic stirrer at 600 rpm. The temperature of 50^0c was maintained throughout the process. The mixture was then cooled and filtered with Whatman filter paper to obtain the extracts.

Preparation of Silver Nitrate Solution

The silver nitrate was issued of analytical grade (99.5 per cent purity). Using an analytical weighing balance, 3.3974 grammes of AgNO3 were weighed and then transferred to a 250 ml volumetric flask having 200 ml of distilled water. The mixture was agitated constantly till all the solid AgNO3 had dissolved. Silver nitrate concentration was determined to be 0.1 M.

Synthesis of Silver Nanoparticles (AgNPs) Using the Cassia Angustifolia extract

General Procedure

By combining silver nitrate solution (0.1M) with Cassia Angustifolia extract, silver nanoparticles were formed. 200 ml stock solution of 0.1 M silver nitrate was prepared. From the stock solution of 0.1 Molar silver nitrate, 40 ml of it was transferred in a 100 ml volumetric flask, 10 ml of *Cassia Angustifolia* extract then added with continuous stirring at 600 rpm for 1 hour. When a colour change was observed then it was proved that the silver nanoparticles were prepared (**Figure 1**). The pH was modified by applying 0.1 N HCl and 0.1 N KOH to obtain the deposit of silver nanoparticles which were then filtered and washed with distilled water. The washed silver nanoparticle was allowed to air dry. The above procedure was repeated to obtain 6 different batches of varying temperature and pH as follows

Preparation of 3 batches of varying temperature

Batch A: temperature 40^0c
Batch B: temperature 60^0c
Batch C: temperature 80^0c

200 ml stock solution of 0.1 M silver nitrate was prepared. From the stock solution of 0.1 M silver nitrate, 40 ml of it was placed in each 100 ml conical flask labelled A, B, C. 10 ml of *Cassia Angustifolia* extract was added to each respective flask with continuous stirring at 600 rpm for 1 hour. Temperatures of 400c, 600c, 800c were maintained constant for A, B, C flask respectively throughout the process with help of a temperature control magnetic stirrer. The appearance of silver nanoparticles was confirmed by a change in colour. All three batches A, B, C were adjusted at pH 4 by applying 0.1 N HCl and 0.1 N KOH to obtain the deposit of silver nanoparticles which was then filtered and rinsed by using distilled water. The washed silver nanoparticles were allowed to air dry.

Preparation of 3 batches of varying pH

Batch D: pH 5
Batch E: pH 7
Batch F: pH 9

200 ml stock solution of 0.1 M silver nitrate was prepared. From the stock solution of 0.1 M silver nitrate, 40 ml of it was placed in each 100 ml conical flask labelled D, E, F. 10 ml of *Cassia Angustifolia* extract were added to each respective flask with continuous stirring at 600 rpm for 1 hour. The temperature of 40^0c was maintained constant for all three batches D, E, F throughout the process with help of a temperature control magnetic stirrer. A colour change was observed indicating the formation of silver nanoparticles. Batches D, E, F were adjusted at pH 5, 7, 9 respectively by using 0.1 N HCl and 0.1 N KOH to obtain the deposit of silver nanoparticles which was then filtered and rinsed by distilled water. The washed silver nanoparticles were allowed to air dry.

Optimization of Nanoparticles

Practical yield

The Practical yield in per cent of all prepared Silver nanoparticles of Cassia Angustifolia (Batch A, B, C, D, E, F) were calculated. For determination of % practical yield following formula was used-

(%) Yield = (Practical yield)/ (Theoretical yield) × 100

Drug entrapment efficiency

The concentration of free drugs in the dispersion media was used to evaluate the entrapment efficiency of nanoparticles. The unentrapped drug was assessed by dissolving 0.1 gm of nanoparticles in 9.9 ml ethanol (95 per cent) and centrifuging the resulting suspension for 45 minutes at 6,000 rpm. After separating the supernatant, it was filtered via filter paper. Using ethanol as a diluent, the filtrate was diluted and spectrophotometrically analysed at 430nm. The following formula was used to compute the entrapment efficiency.

% EE= [(total drug content-unentrapped drug)/ total drug content] × 100

Thus, the best batch was optimized by performing the above test and further sent to carry out characterization. Characterization of the optimized batch was carried out by performing Particle size and zeta potential.

Characterization

Measurement of Zeta potential

The zeta potential is the most essential metric for nanoparticle physical stability. The stability and the electrostatic repulsion between the particles were directly proportional. The optimised nanoparticle suspension's zeta potential was measured using the (HORIBA scientific S-Z100). For the test, 0.1gm of the sample was diluted in water to make 10ml, then 5ml of the diluted sample was transferred to a cuvette and the zeta potential was determined.

Measurement of particle size

The Particle size is the most important parameter required to know the accurate size of the synthesized nanoparticle. Particle size determination was done by using (HORIBA scientific SZ-100). For the measurement sufficient sample was dissolved in water, The particle size of this sample was assessed after it was transferred to a cuvette.

***In Vitro* Anti-inflammatory potential of Silver nanoparticles synthesized from *Cassia Angustifolia* extract**

Required Chemicals: Bovin serum, 1N HCl, Distilled water.
Required Apparatus: Test tube, volumetric flask of 10ml , beaker .
Required Instrument: Incubator, Cystronic UV AU2701.

Procedure

1 Prepare 1% Bovine serum : 1 gm in 100 ml double distilled water.
2 Prepare Stock solution of nanoparticles of concentration 50 microgm/ ml, 100 microgm/ml, 150 microgm/ml, 200microgm/ml, 500 microgm/ml.
3 Take 5 volumetric flasks of 10 ml.
4 In volumetric flask take 0.5 ml Bovin serum and adjust pH (6.4) and add 3 ml of stock solution (50,100,150,200,500 microgram) each. Mix well.
5 Incubate at 37^0c for 20 min.
6 After removing from the incubator heat the mixture for 20 min on the water bath.
7 Allow the mixture to cool, check the absorbance at 660nm.
8 Repeat the above same procedure on the standard anti-inflammatory drugs that is by preparing (50, 100, 150, 200, 500 micrograms) of solutions.
9 Then Percentage inhibition of protein denaturation was calculated as follow

% inhibition = [Abs of control - Abs of Test/ Abs of control] $_x$ 100

Figure 1. Synthesis of Silver Nanoparticles (AgNPs) Using the *Cassia angustifolia* extract.

RESULT AND DISCUSSION

Percentage practical yield

Table 1 shows the findings of % practical yield investigations. The silver nanoparticles of Cassia Angustifolia were synthesized by the green synthesis method. At three different pH and three different temperatures. It was found that batch A of temperature 40^0c shows maximum percentage yield. It was also found that the green synthesis method gives the practical yield in the range of 6.41-52.61%. The maximum yield was found 52.61% in batch A. Yield of batch F was found least thus we can conclude that pH 9 for the synthesis of a nanoparticle is not favourable.

Table 1: Percentage practical yield.

BATCH	PERCENTAGE YIELD
A	52.61%
B	27.59 %
C	46.84 %
D	51.97 %
E	14.75 %
F	6.41 %

Entrapment efficiency of nanoparticles

The ultracentrifugation method was used to determine the entrapment efficiency of synthesized nanoparticles. Drug Cassia Angustifolia of batch A was found to be the maximum drug entrapped. Batch A shows maximum drug content capacity. The drug entrapment efficiency was found to be 99.875%. **Table 2** shows the findings of these results.

Table 2: Entrapment efficiency of nanoparticles

Sr. No	BATCH	% ENTRAPMENT
1	**A**	**99.875 %**
2	**B**	**99.52%**
3	**C**	**99.632 %**
4	**D**	**99.202 %**
5	**E**	**99.012 %**
6	**F**	**------**

Thus, by performing the above test for optimization of the best sample, Batch A was optimized. Further study and characterization were carried out using A batch as a sample. Thus, particle size and zeta potential tests were carried out and results were found to be as follows.

Particle size analysis

The result of particle size analysis (**Table 3 and Figure 2**) is as follows:

Performed on HORIBA Scientific SZ-100

Measurement Results

Date : 29 March 2019 2:44:25 PM
Measurement Type : Particle Size
Sample name : Sample A size
Scattering Angle : 90
Temperature Of the Holder : 24.9^0 C
Dispersion Medium Viscocity : 0.897mPa.s
Transmission Intensity before Meas. : 33064
Distribution Form : Standard
Distribution Form (Dispersity) : Monodisperse
Representation Of Results : Scattering Light Intensity
Count Rate : 35 kCPS

Table 3. Calculation Results

Peak No	S.P.Area Ratio	Mean	S.D	Mode
1	1.00	821.1 nm	194.4 nm	785.4 nm
2	------	-------	------	-------
3	------	-------	------	-------
Total	1.00	821.1 nm	194.4 nm	785.5 nm

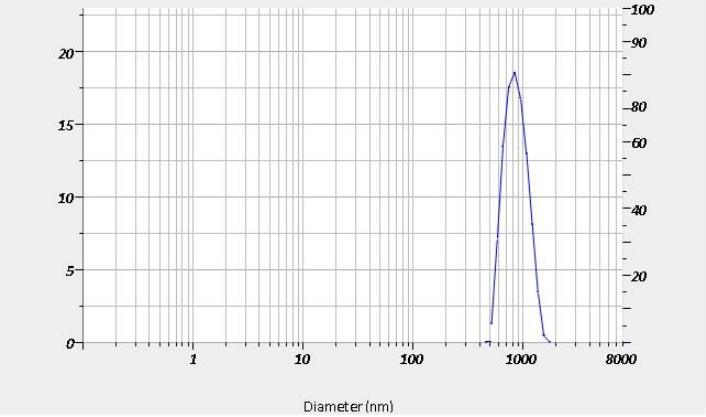

Figure 2. Particle size analysis

Zeta potential

The result (**Table 4** and **Figure 3**) of zeta potential was found to be as follows:

Performed on HORIBA Scientific SZ-100

Measurement Results:

Date : 29 March 2019 2:28:16 PM
Measurement Type : Zeta Potential
Sample name : Sample A
Temperature Of the Holder : 25.0^0 C

Dispersion Medium Viscocity : 0.896mPa.s
Conductivity : 0.141 mS/cm
Electrode Voltage : 304 V

Table 4. Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-17.6 mv	-0.000136 cm^2/Vs
2	-------	-------
3	-------	--------

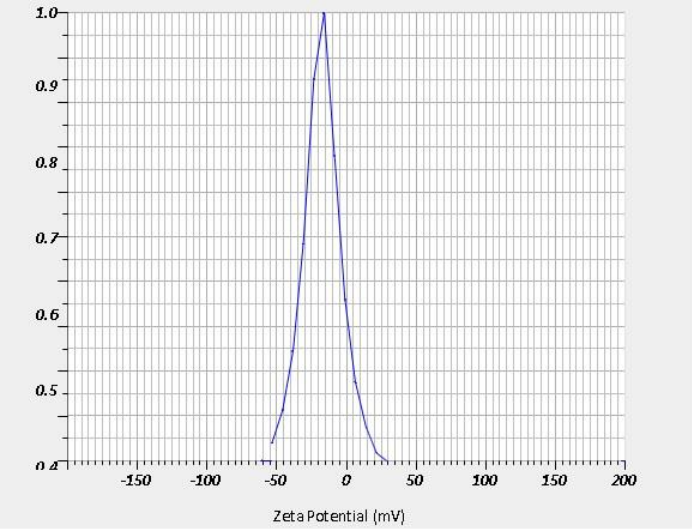

Figure 3. Zeta potential

Result of In-Vitro Anti-inflammatory activity study

In this study, it was found that in a dose-dependent manner AgNPs inhibited protein denaturation and showed 75.52% inhibition at 500µg ml-1 whereas standard drug Aspirin exhibited 65.03% protein denaturation. AgNPs displayed significant antiproteinase activity at different concentrations as shown in **Table 5** and **Figure 4**.

Table 5. Results of in vitro anti-inflammatory activity

Sr no.	Concentration Microgram/ml	% inhibition of standard	% inhibition of AgNPs
1	50	11.11%	41.25%
2	150	33.56%	49.64%
3	100	50.34%	48.24%
4	200	52.44%	72.02%
5	500	65.03%	75.52%

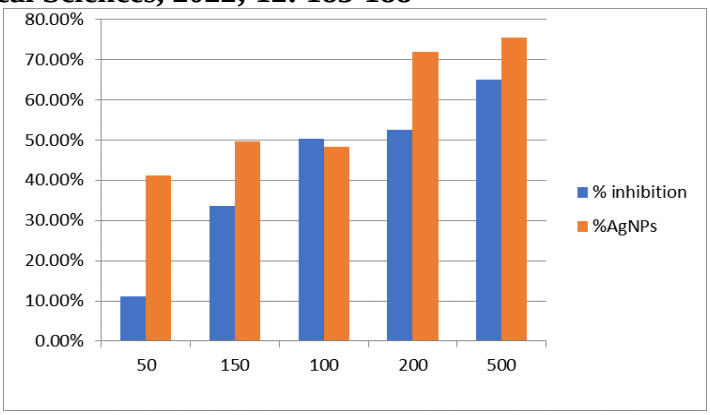

Figure 4. Results of in vitro anti-inflammatory activity

CONCLUSION

Silver nanoparticles were successfully Formulated by the green synthesis method with extract of *Cassia Angustifolia.* Successfully 6 different batches of varying pH and temperature were formulated, also optimization and characterization were successfully performed. Among which batch A was optimized having the highest percentage yield and entrapment efficiency.

Particle size analysis and zeta potential validated the development of silver nanoparticles, which seem to be spherical in shape. The silver nanoparticle size was estimated to be 785.4 nm. For the first time the electrochemical technique was used to properly characterize biosynthesized silver nanoparticles, the result shows reversible peaks corresponding to -17.6mV.

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AUTHOR CONTRIBUTION

Miss. Shubdha Dalvi and Mr Vishwajit Dhaygude done the laboratory work. Miss. Sanmati D. Shete: Monitor the lab work. Miss. Priyanka Annaso. Patil: Manuscript writing and communicated to the journal.

ETHICS STATEMENT

The authors have taken all the necessary permissions as per ethical guidelines wherever applicable. The authors will be responsible for all the technical content mentioned in the manuscript. Journal and Publisher will not be responsible for any copyright infringement and plagiarism issue.

DATA AVAILABILITY

Not declared.

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CONFLICT OF INTEREST

The authors have no known conflict of interest about the present article.

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