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Formulation development and Evaluation of Silvernanopaticle as effective antimicrobial agent

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ABSTRACT

Interest in green nanotechnology in nanoparticle biosynthesis is growing among researchers. Nanotechnologies due to their physicochemical and biological properties have applications in diverse fields, including drug delivery, sensors, optoelectronics and magnetic devices. Green synthesis of nanoparticles is an eco-friendly approach, which should be further explored for the potential of different plants to synthesize nanoparticles. The sizes of AgNPs are in the range of 1 to 100nm. Multi drug resistance has become an emerging and challenging issue for pharmaceutical industry as more and more bacteria are developing drug resistance towards many antibiotics, also over use of these synthetic drugs causes toxicity leading to further detrimental effects on human body. This study focused on the synthesis of silver nanoparticles, which is necessary for safe and effective exploitation of silver nanoparticles in collaboration with plant derived metabolites as a substitute for harmful synthetic drugs. Silver nanoparticles were synthesized using flowers of *Cassia angustifolia*, synthesized silver nanoparticles were then subjected to check antibacterial activity against *Propionibacterium acnes* by well diffusion method. Phytochemical analysis of hydroalcoholic flowers of *Cassia angustifolia* revealed the presence of phenol, flavonoids, proteins, carbohydrates, tannins and saponins. The total flavonoids and phenol content of hydroalcoholic extract *Cassia angustifolia* was 0.876mg/100mg and 0.471mg/100mg/100mg respectively. Spectral techniques like UV-Vis, FT-IR, zetasizer, %EE and SEM were performed for the characterization of silver nanoparticles and results shows that AgNPs can be used as tool in combating the issue of drug resistance in future, as in comparison to broad-spectrum antibiotic, silver nanoparticles showed better bacteriostatic effect against bacteria.

Keywords: Green nanotechnology, Silver nanoparticles, *Cassia angustifolia,* Phytochemical analysis, Well diffusion method.

INTRODUCTION

The use of nanotechnology for the production of nanoscale products is the in the R&D divisions[1]. A wide variety of products accessible to an increasingly broad range of scientific industries can be developed using nanotechnology. Nanotechnology-related concepts are production, production,

and synthesis, which typically include materials that weigh less than 1 mm. The Greek word nanos, meaning dwarf, little, or very thin, or very small[2]. In general, nanotechnology is classified as wet, dry and computational. Wet nanotechnology, such as enzymes, proteins, membranes and other cellular materials, is associated with living organisms. Physical chemistry and the processing of inorganic products, such as silicon and carbon, are correlated with dry nanotechnology. Simulations of nanometer-sized structures[3] are concerned with computational nanotechnology. For optimum functionality, these three dimensions (wet, dry and computational) are dependent on each other. Nanotechnology serves a wide variety of specific sectors such as electronics, chemicals, pharmacy and parasitology, while offering a shared platform [4]. One such example is nanobiotechnology, where multiple scientific sectors, including nanotechnology, biotechnology, materials science, physics and chemistry, combine study and development[2, 5]. Through the collaboration of various natural science sectors, biologically synthesised nanoparticles with antimicrobial, antioxidant and anticancer properties are feasible. These nanotechnologies are capable of providing novel resources for assessing and developing new, safer and more effective drug formulations [6]. The use of plant extract in the synthesis of nanoparticles has therefore been a very new practise in recent times[7]. To date, nanoparticles of various sizes and shapes have been widely used in the synthesis of metal and metal oxide (silver, gold, platinum, titanium, iron and nickel) in various parts of plants such as leaves, fruit, bark, peels, roots and callus [8-15]. At present, in the synthesis of nanoparticles, chemical, electrochemical, radiation, photochemical, Langmuir-Blodgett and biological methods have been used extensively[16]. Out of these methods, plant-based biomimetic synthesis of silver nanoparticles is considered to be the best method as it meets with the least toxicity the demands of human health. In addition to being cost-effective and environmentally-friendly, plant-mediated nanoparticles can also be synthesised in a single-step process[17]. Studies by different research groups show that Alfalfa roots can absorb silver nanoparticles from the agar medium and transport them to the plant shoot at the oxidised state[18] In addition, silver nanoparticles synthesised with *Jatropha curcas* latex, Aloe vera, *Acalypha indica* and *Garcinia mangostana* leaf extracts have shown enormous therapeutic applications such as antioxidants, antimicrobials etc [19-20].

Cassia angustifolia (senna) and it belongs to Leguminosae family. Senna is used for the treatment of constipation mostly in Eastern and Western countries. The laxative activity of senna is due to the presence of two anthraquinone glycosides, i.e., sennoside A and sennoside B. *C. angustifolia* is also composed of rhein-8-diglucoside, sennosides C and D, rhein, rhein-8-glucoside, aloe-emodin and anthrone diglucoside, and napthalene glycosides such as tinnevellin glycoside and 6-hydroxy musizin glycoside, flavonoid (kaempferol), phytosterols, resin, and calcium oxalate[21-22]. It was reported that the first variety of senna was found along the Nile River in Egypt and Sudan.

Commercially, it is cultivated in Kutch (Gujarat) and Jodhpur (Rajasthan), India. However, the Flowers of the plant has not been reported for the synthesis of nanoparticles so far. Thus, the main goal of this study was to investigate antibacterial activity of AgNPs of flower extract of *Cassia angustifolia.*

MATERIALS AND METHODS

Plant material

The *Cassia angustifolia* were collected from local area of Bhopal (M.P.) in the month of January, 2020. The air dried sample was stored in close container before use.

Chemical reagents

Silver nitrate ($AgNO_3$) is purchased from Sigma-Aldrich Chemicals for this study. Dimethyl sulphoxide (DMSO) was purchased from Merck, India. The pH buffer tablets were purchased from Himedia. Nutrient Agar, Nutrient Broth, Agar Agar were purchased from Himedia Laboratories, Mumbai, India. The pathogenic bacteria used in the current study obtained from Microbial Culture collection, National Centre forcell science, Pune, Maharashtra, India. All the chemicals used in this study were of analytical grade.

Extraction of plant material

Dried powdered flowers of *Cassia angustifolia* has been extracted with hydroalcoholic solvent (ethanol: water: 75:25) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C and stored in an air tight container free from any contamination until it was used. Finally, the percentage yields were calculated of the dried extracts [23].

Qualitative phytochemical analysis of plant extract

The Hydroalcoholic extract of *Cassia angustifolia* obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate[24]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, protein and tannins.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution

was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm [25].

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method. 50 mg Gallic acid was dissolved in 50 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. 2 ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [25].

Green synthesis of silver nanoparticles

$AgNO_3$ powder was dissolved in distilled water to prepare 10 mM $AgNO_3$ stock solution from which a series of 1 mM, 2 mM and 3 Mm $AgNO_3$ solutions were prepared. The $AgNO_3$ solutions were mixed with the hydroalcoholic extract of flowers of *Cassia angustifolia* at a ratio of 1:1, and 1:2 (v/v) to a volume of 50 mL in a flask. The flask was wrapped with an aluminum foil and was then heated in a water bath at 60°C for 5 hours. Furthermore, the mixture was stored in the refrigerator for the further use[26].

Evaluation of silver nanoparticles [27- 29]

Microscopic observation of silver nanoparticles

An optical microscope (cippon, Japan) with a camera attachment (Minolta) was used to observe the shape of the prepared silver nanoparticle formulation.

Percentage yield

The prepared silver nanoparticle with a size range of 200-300nm were collected and weighed from different formulations. The measured weight was divided by the total amount of all non-volatile components which were used for the preparation of the nanoparticles.

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

Surface charge and vesicle size

The particle size and size distribution and surface charge were obtained by Dynamic Light Scattering method (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK). Zeta potential measurement of the nanoparticles was based on the zeta potential that was estimated according to Helmholtz–Smoluchowsky from electrophoretic mobility. For measurement of zeta

potential, a zetasizer was used with field strength of 20 V/cm on a large bore measures cell. Samples were diluted with 0.9% NaCl adjusted to a conductivity of 50 lS/cm.

Entrapment efficiency

The entrapment efficiency of the drug was defined as the ratio of the mass of formulations associated drug to the total mass of drug. Entrapment efficiency was determined by dialysis method. Silver nanoparticle entrapped extract were isolated from the free drug using dialysis method. The above said formulations were filled into dialysis bags and the free drug dialyzed for 24 hr. into 50 ml of buffer pH 1.2. The absorbance of the dialysate was measured against blank buffer pH 1.2 and the absorbance of the corresponding blank was measured under the same condition. The concentration of free Phenols could be obtained from the absorbance difference based on standard curve.

Formulation development of gel

Method of preparation

Measured amounts of methyl paraben, glycerin, polyethylene glycol and hydroalcoholic extract of flowers of *Cassia angustifolia* were dissolved in about 100 ml of water in a beaker and stirred at high speed using mechanical stirrer (or sonicator). Then Carbopol 940 was slowly added to the beaker which contained above liquid while stirring. Neutralized the solution by adding a slow, constantly stirring triethanolamine solution until the gel formed.

Antibacterial activity of silver nanoparticles

The well diffusion method was used to determine the antibacterial activity of the extract and silver nanoparticles gel prepared from the *Cassia angustifolia* using standard procedure of Bauer[30].
The drug used in standard preparation was ciprofloxacin of IP grade. The antibacterial activity was performed by using 24hr culture of *Propionibacterium acnes.* There were 3 concentration used which are 25, 50 and 100 mg/ml for each extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculums. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug. The diameter of zone of inhibition of each wall was recorded.

RESULTS AND DISCUSSIONS

The crude extract obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yields of extracts obtained from the hydroalcoholic extract are depicted in the Table 1. Phytochemical analysis of hydroalcoholic extracts of flowers of *Cassia angustifolia* showed the presence of phenol, flavonoids, proteins, carbohydrates, tannins and saponins while, alkaloid, Glycosides, and diterpines were not detected. The determination of the total flavonoid content, expressed as mg quercetin equivalents and per 100 mg dry weight of sample and total phenol content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. The total flavonoids and phenol content of hydroalcoholic extract of *Cassia angustifolia* was 0.876mg/100mg and 0.471mg/100mg respectively. Results are provided in Table 3. An optical microscope with a camera attachment was used to observe the shape of the prepared silver nanoparticle formulation. The % yield of prepared formulation F3 was found to be 69.98±0.74%. Average vesicle size, % entrapment efficiency and zeta Potential of optimized formulation of silver nanoparticle (F3) was found to be 220.5, 0.825±0.023 and - 38.5 mV respectively Table 4 and Fig. 1-2. Frurter optimized formulation of silvernanoparticle (F3) was incorporated into carbopol gel base (F1, F2, F3) and evaluated for Spreadability, Viscosity, Flavonoid Content, pH, and % Cumulative Drug Release. The Optimized gel formulation F2 release approx 7.85 percent drug within 15 minutes and approx 20.14 percent of drug release in 4 hours. When the regression coefficient values were compared, it was observed that 'r^2' values of first order were maximum i.e. 0.953 hence indicating drug releases from formulation follow first order release kinetics. Zone of inhibition against bacterial growth produced by AgNPs was compared to extract against *Propionibacterium acnes*. From the table 5, it is concluded that synthesized AgNPs gel exhibit zone of inhibition more as compared to extract.

Table 1: % Yield of flowers of *Cassia angustifolia*

S. No.	Extracts	% Yield
1.	Pet ether	2.68
2.	Hydroalcoholic	4.23

Table 2: Phytochemical screening of flowers of *Cassia angustifolia* extract

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	-ve
	Hager's Test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	+ve
	Fehling's Test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	-ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 3: Results total flavonoids and phenol content of flowers extract of *Cassia angustifolia*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.967	0.855

Table 4: Characterization of optimized formulation of silver nanoparticle

Formulation	Average vesicle size (nm)	Zeta Potential (mV)
F1	220.5	- 38.5 mV

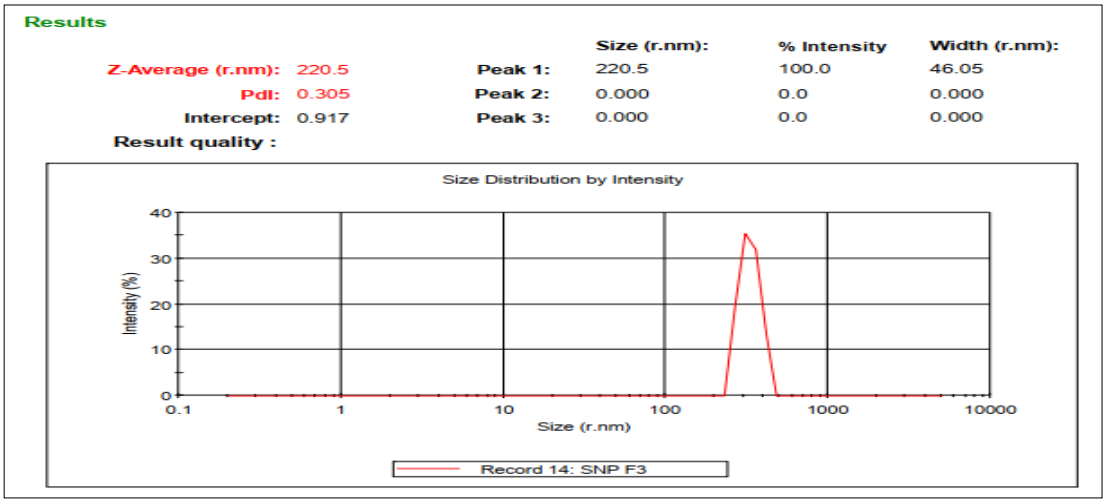

Fig. 1: Graph of average vesicle size

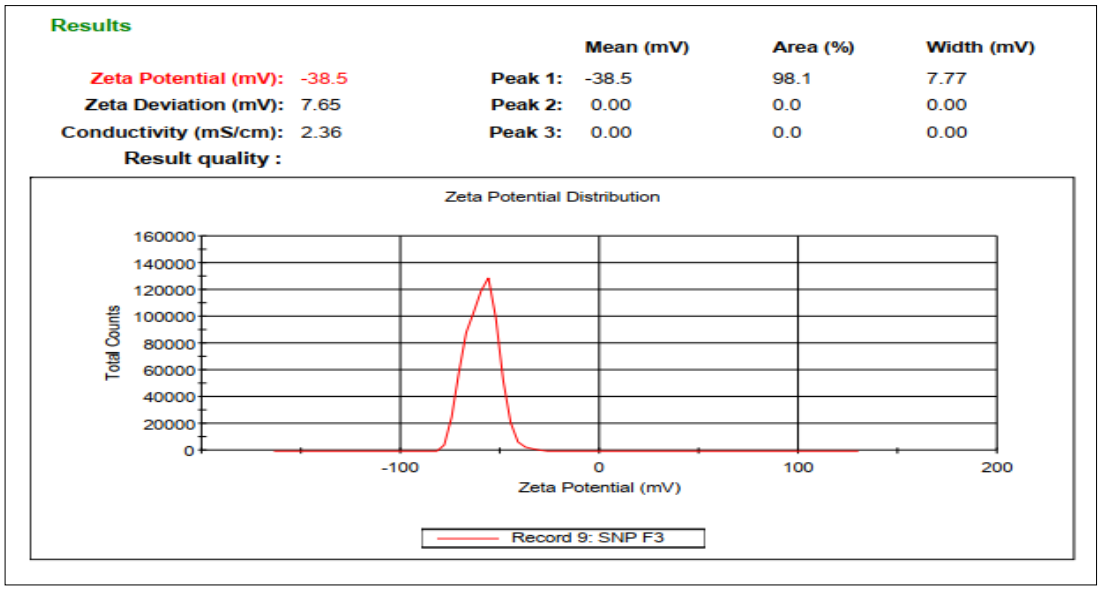

Fig. 2: Graph of zeta Potential

Table 5: Results of evaluation of nanoparticles incorporated gel

Formulation	Spreadability* (gcm/sec)	Viscosity* (cp)	Flavonoid Content (mg/100mg)	pH
F1	8.15±0.11	3515	0.589±0.054	6.95±0.02
F2	7.42±0.26	3045	0.854±0.032	7.00±0.01
F3	6.74±0.45	2878	0.789±0.042	6.87±0.02

*Average of three determinations (n=3 ±SD)

Table 6: Release Kinetics Regression values of formulation F1-F3

Formulation code	Zero order	First order
F2	0.953	0.852

Table 7: Antimicrobial activity against selected microbes

S. No.	Name of drug	Microbes	Zone of inhibition		
			25 mg/ml	50 mg/ml	100 mg/ml
1.	Extract	*Propionibacterium*	8±0.47	12±0.74	14±0.86
2.	Silver nanoparticles gel	*acnes*	9±0.94	13±0.5	17±0.57

CONCLUSION

The present investigation involved the screening and evaluation of flowers of *Cassia angustifolia* extract for the phytochemicals. Green synthesis of AgNPs using hydroalcoholic extract, characterization and antibacterial activity of synthesized nanoparticles were also carried out in the study. Outcome of all the experiments carried out suggests the existence of most of the phytochemicals in the flowers and are having some important biological activities. Further work is needed to isolate, purify and identify the exact active principle which is the cause for the biological activities. The Green synthesis is a simple, low cost and ecofriendly approach without any huge inputs in terms of energy. This is the first report of green synthesis of silver nanoparticles for this plant. Being exhibiting greater antibacterial activity, phytochemical based nanoparticles may stand as a potential remedy in developing drugs against antibiotic resistant bacteria.

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