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GREEN SYNTHESIS OF ANTI-ACNE SILVER NANOPARTICLES GEL USING HYDROALCOHOLIC SEEDS EXTRACT FROM *EMBELIA RIBES*

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ABSTRACT

Acne vulgaris is one of the most common skin disorders in the world, affecting about 80 percent of teenagers during their lives. Antibiotic resistance is created by the creation of a particular relationship between antibiotics and bacteria through prolonged therapy. Medicinal and aromatic plants were commonly used in ancient times as medicine. Acne therapy has been considered a significant field of science in the field of medicinal and personal cosmetic care. The aim of the present work was to evaluate the phytochemical composition of *Embelia Ribes*, green synthesis of silver nanoparticle and to develop herbal topical gel formulation to treat acne. Phytochemical analysis revealed phytoconstituents such as flavonoids, phenol, proteins, carbohydrates, tannins and saponins are present in the hydroalcoholic extract. Silver nanaoparticle was synthesized using 1 mM aqueous silver nitrate solution. The resultant AgNPs were characterized using UV–visible spectroscopy, microscope and dynamic light scattering analysis. Synthesized silver nanoparticles was incorporated into gel base and evaluated for its physical properties such as pH, viscosity, spreadability and antiacne activity against *Propionibacterium acne*. The antiacne study of the developed formulation showed inhibitory activity against *Propionibacterium acne*. Synthesized silver nanoparticle of *Embelia Ribes* showed higher activity than the extract. Hence, silver nanoparticle of *Embelia Ribes* in aqueous gel-base can be used as an appropriate formulation for treatment of acne vulgaris.

KEYWORDS: Nanobiotechnology, *Embelia Ribes* extract, silver NPs, Green synthesis, Antiacne activity.

INTRODUCTION

Acne, also known as Acne Vulgaris, is a long-term skin disease that occurs when hair follicles are clogged with dead skin cells and oil from the skin. It is characterized by blackheads or whiteheads, pimples, oily skin, and possible scarring.[1] Acne Vulgaris is mostly triggered by *Propionibacterium acnes* in adolescence, under the influence of normal circulating Dehydro-Epiandrosterone (DHEA). It is the most common skin disease, and although it usually manifests during puberty and worsens throughout adolescence, epidemiological studies suggest that it can arise at any age. Apart from the classic belief that acne results from sebaceous gland hyperplasia, abnormal follicular differentiation with increased keratinization, microbial hyper-colonization of the follicular canal, and increased inflammation primarily through activation of the adaptive immune system may also be contributors.[2-3]

Nanotechnology and nanotools have gained much attention due to their wide range of applications in physics, chemistry, biology, material science, and medicine.[4] Metal nanoparticles like silver, gold, and copper have been used for diagnosis and treatment of disease because of their catalytic, optical, electronic, antimicrobial, and magnetic properties.[5] Green nanoparticle synthesis has been achieved using environmentally acceptable plant extract and ecofriendly reducing and capping agents. Plants and microbes are currently used for nanoparticle synthesis. The use of plants for synthesis of nanoparticles is rapid, low cost, eco-friendly, and a single-step method for biosynthesis process.[6] Among the various known synthesis methods, plant-mediated nanoparticles synthesis is preferred as it is cost-effective, environmentally friendly, and safe for human therapeutic use.[7] The most commonly reported biomoleculesresponsible for the reduction of precursor and stabilization of nanoparticles aremetabolites such as alkaloids, phenolic compounds, terpenoids, and water-solubleco-enzymes.[8]

Embelia ribes Burm. f. belongs to the family Myrsinaceae found in hilly parts of India up to 1500 m. elevation from outer Himalayas to Western Ghats. It is an endangered medicinal plant valued for its digestive, carminative, anthelmintic and laxative property since time immemorial.[9] It is also used in diabetes, heart related problems, nerval disorders, cancerous tumors and liver disorders. The seeds are also used for wound healing antioxidant, anti-inflammatory, analgesic and contraceptive activity.[10]

MATERIALS AND METHODS

Chemical reagents

Silver nitrate ($AgNO_3$) is purchased from Sigma-Aldrich Chemicals for this study. The pH buffer tablets were purchased from Himedia. Nutrient agar, nutrient broth, and agar agar media were purchased from Himedia Laboratories, Mumbai, India. Chemicals were obtained from Rankem Laboratory Chemicals Pvt. Ltd., Haryana, India, Himedia Laboratories Pvt. Ltd, Mumbai, India and Loba Chemie, Mumbai, India. All the chemicals used in this study were of analytical grade.

Preliminary phytochemical screening

The phytochemical screening of the extracts was conducted using standard procedures described by Trease and Evans.[11]

Total phenol determination

The total phenolic content was determined by Folin Ciocalteau method.[12] A volume of 2ml of extract or each standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined by $AlCl_3$ method,[13] 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or each standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Biosynthesis 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.[14] The $AgNO_3$ solutions were mixed with the hydroalcoholic extract of seeds of *Embelia ribes* at a ratio of 1:1, 1:2 and 1:3 (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.

Optimization of formulation of Silver nanoparticles

Table 1: Different formulation of Silver nanoparticles.

Formulation Code	Extract (mg)	AgNO3 (mM)	Ratio
F1	250	1	1:1
F2	250	2	1:1
F3	250	3	1:1
F4	250	1	1:2
F5	250	2	1:2
F6	250	3	1:2
F7	250	1	1:3
F8	250	2	1:3
F9	250	3	1:3

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 microspheres.[15]

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

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 flavonoids could be obtained from the absorbance difference based on standard curve.[16]

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 silver 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.[17]

Formulation development of silver nanoparticles gel of optimized formulations (F3)

Measured amounts of methyl paraben, glycerin, polyethylene glycol and silver nanoparticles of *Embelia ribes* were dissolved in about 100 ml of water in a beaker and stirred at high speed using mechanical stirrer (or sonicator)[18] 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.

Table 2: Formulation of silver nanoparticles gel of optimized formulations (F3).

Ingredients (mg)	SNG1	SNG2	SNG3
Silver nanoparticle	100	100	100
Carbopol 940	250	500	750
Polyethylene Glycol 600	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0
Distilled Water	100 ml	100ml	100ml

Evaluation of silver nanoparticles gel of optimized formulations (SNG2)

Determination of Spreadability

A special apparatus was designed to study the formulations spreadability. Spreadability is expressed in terms of the time taken by two slides in seconds to slip off the surface, put between them, under the application of a certain load. The less time required for two slides to separate, the greater the spreadability.[19] Two normal dimensional glass slides (6x2) were chosen. The gel formulation the spreadability of which had to be determined was placed over one of the diapers. The second slide was mounted over the slide in such a way as to sandwich the formulation over the slide over a length of 6 cm between them. The upper slide had 20 grams of weight, so that the gel formulation between the two was placed uniformly to form a thin layer.

The weight was removed and the excess of the gel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cm and separate

away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each formulation.

$$Spreadability = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 gram)

l= length of glass slide (6cm).

t = time taken is seconds.

Determination of pH

Digital pH meter had calculated the pH of the anti-acne gels.[20] One gram of gel was dissolved in 25 ml of purified water and the electrode was then dipped into gel solution until steady reading was achieved. Measurements of pH were repeated twice for each formulation.

Flavonoid Content

The composition of the medication was measured by taking 1gm of gel mixed with methanol in 10 ml volumetric flask. 3 ml of stock solution has been mixed with 1 ml $AlCl_3$ solution of 2 per cent. The mixture was vortexed for 15s and allowed for the color production to stand at 40°C for 30min, using a spectrophotometer the absorbance was measured at 420 nm.

Viscosity

The viscosity of the prepared gel was determined by a Brookfield digital viscometer.[21] The viscosity was assessed using spindle no. 6 at 10 rpm at ambient room temperature of 25-30^{o}C. Reasonable large bottle for the mouth loaded the correct volume of gel. Usage of large mouth container to allow viscometer spindle within the jar. Viscosity value was noted down after stable of reading. Gel samples were allowed to settle more than 30minutes before the measurements at the constant room temperature.

Determination of Anti-acne activity

The prepared gel was evaluated for their anti-acne activity against *Propionibacterium acne* strains by agar well diffusion method.[22] The diameters of the inhibition zones were measured in mm.

In vitro diffusion profile (*In vitro* permeation in rat skin)

In vitro diffusion experiments were performed using franz diffusion cell for all formulations. Locally assembled as an open-ended cylindrical tube with an area of 3.7cm^2 and a height of 100 mm with a diffusion area of 3.8 cm^2. Phosphate buffer (pH 7.4) was used as substrate for receptors. Rat abdominal skin used as membrane for dialysis.[23-24] The skin was tied to the diffusion cell (donor cell) such that the stratum corneum side of the skin was in intimate contact with the release surface of the formulation in the donor cell. Isotonic phosphate buffer solution, pH 7.4 (100 ml) was added to a donor compartment prior to be mounted on the diffusion cell. A weighed quantity of formulation equivalent to 1g of gel was taken on to the rat skin and was immersed slightly in 100 ml of receptor medium, which was continuously stirred. The whole network had been held at 37±1^{o}C. At different time intervals of up to 4 hours, an aliquot of 5 ml was extracted, and spectrophotometrically measured at 265 nm. The diffusion media was replaced with an equal volume of fresh diffusion medium after each withdrawal. For each time period the total percent release was measured.

RESULTS AND DISCUSSION

Preliminary phytochemical screening reveals the presence of flavonoids, phenol, proteins, carbohydrates, tannins and saponins while alkaloids, glycosides and diterpenes are negative (Table 3). The result of total flavonoid and total phenols contents of hydroalcoholic crude extract is given in Table 4. Gallic acid solution of concentration (10-50 µg/ml) conformed to Beer's Law at 765 nm with a regression co-efficient (R^2) = 0.998. Total phenolic content was calculated with the help of calibration curve of Gallic acid as standard and expressed as mg GAE/100mg dry extract weight. Quercetin solution of concentration (5-25 µg/ml) conformed to Beer's Law at 420 nm with a regression co-efficient (R^2) = 0.998 (Figure 2). Total flavonoids content was calculated with the help of calibration curve of Quercetin as standard and expressed as mg QE/100mg dry extract weight. The amount of phenolics compounds was present in seed (0.951mg of GAE/100mg of crude extract) and the flavonoids a compound was in *Embelia ribes* extract (1.048mg of QE/100mg) table 4.

Further Three Different formulation of Carbopol Gel was prepared and evaluated. The Optimized gel formulation SNG2 release approx 10.32 percent drug within 15 minutes and approx 99.85 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. 0.728 hence indicating drug releases from formulation follow first order release kinetics.

Table 3: Phytochemical screening of extract *of* seeds of *Embelia ribes.*

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

Table 4: Estimation of total flavonoids and phenol content of seeds of *Embelia ribes.*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	1.048	0.951

Characterization of silver nanoparticles

Table 5: % Yield and Percentage entrapment efficiency of all formulations.

Formulation	% Yield	Percentage entrapment efficiency (Flavonoid mg/100mg quercetin equivalent)
F1	62.23±0.12	0.658±0.025
F2	64.58±0.32	0.642±0.032
F3	68.89±0.25	0.715±0.014
F4	70.23±0.74	0.856±0.025
F5	76.65±0.65	1.002±0.036
F6	69.95±0.31	0.908±0.012
F7	62.46±0.35	0.678±0.025
F8	59.98±0.41	0.774±0.011

Table 6: Particle size and Particle size of optimized formulation (F5).

Formulation	Average Particle size (nm)	Particle size (mV)
F5	210.32	- 25.65 mV

Table 7: Results of silver nanoparticles gel optimized formulation SNG2.

Formulation	Spreadability*(gcm/sec)	Viscosity* (cp)	Flavonoid Content (mg/100mg)	pH
SNG1	11.23±0.45	3225±14	0.745±0.054	6.56±0.45
SNG2	10.36±0.32	3014±23	0.985±0.065	7.01±0.32
SNG3	9.85±0.14	2785±21	0.856±0.042	6.32±0.14

Table 8: *In vitro* drug release study of prepared gel formulation.

S. No.	Time (hr)	% Cumulative Drug Release		
		SNG1	SNG2	SNG3
1	0.25	14.45	10.32	6.65
2	0.5	20.32	15.65	12.23
3	1	42.56	36.65	22.14
4	1.5	65.58	45.56	36.65
5	2	92.23	58.89	42.23
6	2.5	96.65	69.98	52.56
7	3	98.85	86.65	65.58
8	4	99.45	99.85	76.65

Table 9: Release Kinetics Regression values of formulation SNG2.

Formulation code	Zero order	First order
SNG2	0.986	0.728

Anti-acne activity of extract, optimized silver nanoparticles gel (SNG2) and clintop marketed gel

Table 10: Anti-acne activity against *Propionibacterium acnes.*

Name of drug	Microbes	Zone of inhibition		
		25 mg/ml	50mg/ml	100 mg/ml
Extract	*Propionibacterium acnes*	9±0.57	10±0.5	12±0.047
Silver nanoparticles gel (SNG2)		12±0.94	17±0.57	18±0.94
Clintop marketed gel		13±0.86	14±0.74	16±0.5

CONCLUSION

AgNPs has an excellent antibacterial agent due to its non-toxic effect on the human cells. Medicinal plants have been used as a home remedy from ancient time due to its variety of metabolites and its phytoconstituents. These phytoconstituents and metabolites can reduce the silver ions and assist synthesize of AgNPs from plant extracts. These AgNPs are having strong binding affinity with many functional groups of the plant extracts. The present study

reveals a simple, rapid and economical method to synthesize AgNP silver nanoparticle from *Embelia ribes*. The antibacterial activity is well demonstrated by agar well diffusion method.

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