CHEMICALLLY AND BIOLOGICALLY SYNTHESIZED SILVER NANOPARTILCES

-SYNTHESIS, CHARACTERIZATION AND APPLICATION OF IN A NOVEL SKIN FORMULATION

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Key words: silver nanoparticles, green synthesis, antibacterial, ascorbic acid, skin lotion.

ABSTRACT:

The rapidly advancing field of nanomedicine is being explored by researchers as a tool to combat the alarming increase in resistant bacterial infections. Our skin is prone to many challenges like climate change, environmental stress due to which are skin becomes dry and rough, formation of dry callus, dullness etc. Therefore, it is very important to protect our skin from all of the above mentioned problem. The current study employs a novel approach in formulating a skin preparation possessing both antibacterial and anti-oxidant activity.

In the present study silver nanoparticles were chemically as well as biologically synthesized (green synthesis) and characterized by UV- visible spectroscopy and the maxima absorption was obtained at 430nm and 470nm respectively. Antimicrobial activity of the synthesized nanoparticles was tested by the agar cup diffusion assay against *E.coli* and *S.aureus* as the test organisms. MIC of the chemically synthesized silver nanoparticles was found to be 45.85 ppm whereas, for biologically synthesized silver nanoparticles (Papaya leaf) it was found to be 12.6 ppm. Atomic absorption spectroscopy studies revealed the concentration of silver nanoparticles 50.4ppm, 91.7ppm respectively. Scanning electron microscopy revealed that both chemically as well as biologically synthesized silver nanoparticles were spherical in morphology and approximately 67 nm and 87 nm in diameter respectively. An antibacterial lotion was prepared with the above-mentioned nanoparticles incorporated in it. Further, in order to impart antioxidant activity of the lotion, ascorbic acid was incorporated. Assays performed revealed the lotion to possess good antimicrobial and antioxidant activity.

Key words: silver nanoparticles, green synthesis, antibacterial, ascorbic acid, skin lotion.

INTRODUCTION:

Globally, a significant number of people suffer from skin infections at some point in their lives. Several antiseptic and antibiotic preparations are available however, growing resistance of microbes to these preparations is of constant concern. [4]. The word “Nano” has its origin in the Greek language, meaning “dwarf”. Nanotechnology is the study of nanoparticles. Nanoparticles are particles between 1 and 100 nanometres in size. The applications of nanotechnology and nanomaterials can be found in many cosmetic products including moisturisers, hair care products, make up and sunscreen. Titanium dioxide and zinc oxide are the main compounds used in these applications.

Silver is used since a long time as an active ingredient of a broad range of cosmetic formulations and products for its antimicrobial properties. Its use in the conventional form is allowed in Europe by the EU Cosmetic Directive. Nanoparticles have a large surface area to volume ratio than their conventional form. The large surface area affects the interaction of the elements and nanoparticles. Silver nanoparticles have been reported to possess a broad range of antimicrobial activity by previous researchers [5].

Since nanoparticles are extremely small in size, they can penetrate through the skin very easily and sometimes even enter through various organs, damaging cellular structures. This could be considered as one of the disadvantages in employing nanoparticles for human use. However, it has recently been shown that even if silver nanoparticles enter the body, they get flushed away from the blood stream, thus considerably reducing the associated toxicity [3].

The current study aimed to develop and characterize silver nanoparticles by chemical and biological synthesis and carry out investigation of their antimicrobial activity. Subsequent to this, an attempt was made to formulate a preparation for application on the skin, which would possess both antimicrobial as well as antioxidant activity and could therefore be considered 'borderline' between a medicinal and a cosmetic product. In the current study, this preparation employed both silver nanoparticles made chemically as well as biologically incorporated in combination with an antioxidant compound. Currently there is a large market for "anti-ageing" skin creams, which employ antioxidants to combat free oxygen radicals which, together with other factors, contribute to wrinkle formation [6]. In the current study, vitamin C or ascorbic acid, was incorporated in the lotion. Ascorbic acid is a potent antioxidant that can be used topically in dermatology to treat and prevent changes associated with photo ageing. It can also be used for the treatment of hyperpigmentation. [1]. Replenishing its levels directly in the skin can help combat collagen degradation and oxidative stress.

MATERIALS AND METHODS

Chemical synthesizes of silver nanoparticles

0.01 grams of silver nitrate was dissolved in 100 ml of distilled water. 5ml of 1% trisodium citrate was added drop wise and the solution was heated with constant stirring. The clear solution changed to dark yellow colour solution indicating the formation of silver nanoparticles.

Biological synthesis of silver nanoparticles

20-30 papaya leaves (*Carcia papaya*) was grinded with motor and pestle after washing them with D/W. The leaves were boiled for 10-15 minutes and the filter was extracted using a muslin cloth. To 5ml of the above extract 100ml of silver nitrate solution was added (Dissolve 0.01 gram of silver nitrate in 100 ml of D/W). The green colour solution changed to brownish-yellow colour indicating the formation of silver nanoparticles.

Characterization of Nanoparticles

Characterization of the synthesized nanoparticles was done using UV-visible spectrophotometer (Horiba). Quantification of the prepared silver nanoparticles was carried out by atomic absorption spectroscopy (Italab). Further, the morphology of the synthesized nanoparticles was studied using scanning electron microscope equipped with an Energy Dispersive X ray Analyser (Icon Analytical).

Assay of antimicrobial activity of silver nanoparticles

In vitro antimicrobial activity of the synthesized nanoparticles was first investigated by the agar cup diffusion method. Nutrient Agar plates were swabbed with the test culture and then the synthesized nanoparticles were added to wells. Appropriate controls were maintained. Size of zones of inhibition was measured after incubation at 37^0C for 24 hrs.

Determination of minimum inhibitory concentration of chemically as well as biologically synthesized silver nanoparticles

Using stock solution of the prepared silver nanoparticles various dilutions were prepared. 0.1 ml of the 24 hr old test culture (*S.aureus and E.coli*) was added to all dilutions used. Positive and negative controls were maintained. Tubes were incubated at 37^{o}C for 24 hrs and the lowest concentration that did not show growth corresponded to the minimum inhibitory concentration (MIC).

Preparation of lotion

250 ml of almond oil was combined with 150 ml of coconut oil.150 gram of bees wax was added in a double boiler and all ingredients added and heated. The preparation was stirred occasionally, cooled, put in a jar and refrigerated till further use.

Checking the antioxidant activity of the lotion

The antioxidant activity of the lotion was determined by phosphomolybdeum method. Here 6ml of reagent solution (2ml of 0.6M of sulphuric acid, 2ml of 28mM sodium dihydrogen phosphate, 2ml 4mM ammonium molybdate) was combined with 0.6ml of different concentration of the standard solution (Vitamin C) and sample each (Lotion). The tubes were capped and then incubated at 95 degree Celsius for 90 minutes. After cooling the tubes at RT absorbance of the standard and the test solution was measured at 670 nm using colorimeter.

Checking the efficiency of the lotion using disc diffusion assay

The antimicrobial activity of the lotion was tested by placing Whatman number 1 filter paper discs incorporated with the following formulations, on nutrient agar plates swabbed with the test organisms:

- Silver nanoparticles (chemically as well as biologically synthesized) alone in lotion
- Ascorbic acid alone in lotion
- Mixture of both the synthesized silver nanoparticles along with ascorbic acid in lotion.
- Only lotion (control)

Zones of inhibition were measured after 24hrs incubation at 37^{o}C.

All experiments (microbiological and chemical analysis) were performed in triplicate and repeated three times. Mean values have been reported.

RESULTS AND DISCUSSION

Nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state and refractive index near the nanoparticle surface, which makes UV/Vis/IR spectroscopy a valuable tool for identifying, characterizing, and studying these materials.

Silver Nanoparticles exhibited yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations. The conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band occurring due to the collective oscillation of electrons of silver nanoparticles in resonance with the light wave. When the frequency of the electromagnetic field becomes resonant with the coherent electron motion, a strong absorption takes place, which is the origin of the observed colour. The absorption peak (SPR) of the chemically synthesized silver nanoparticles was obtained in the visible range at 430 nm whereas, for biologically synthesized silver nanoparticles it was obtained in the visible range of 470nm.

The size of the nanoparticles plays an important role in changing the entire property of a material. SEM analysis of chemically synthesized silver nanoparticles revealed the particle size to be ranging from 62nm to 88nm with spherical morphology (Fig1). Analysis using EDX showed the presence of high silver content (Fig 2). Whereas, the Biologically synthesized Silver Nanoparticle from *Carcia papaya* leaf revealed the particle size to be form **87nm to 100nm** with spherical morphology (Fig 3). The quantitative analysis using EDX showed the presence of high silver conten 43.44%. (Fig 4). The spectrum also showed the presence of Carbon, Chlorine, Oxygen of 16.48%. 34.89%, 5.18% respectively.

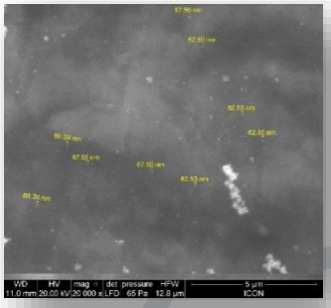


Figure 1: Scanning electron microscopy micrograph of chemically synthesized silver nanoparticles (AgNPs)

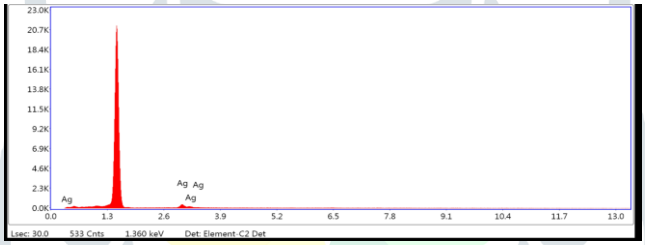


Figure 2: EDX characterization spectrum obtained for chemically synthesized silver nanoparticles. Visible peak shows the presence of silver substance in the tested sample.

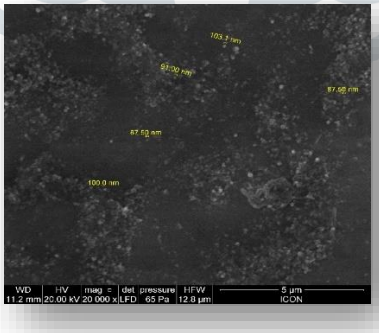
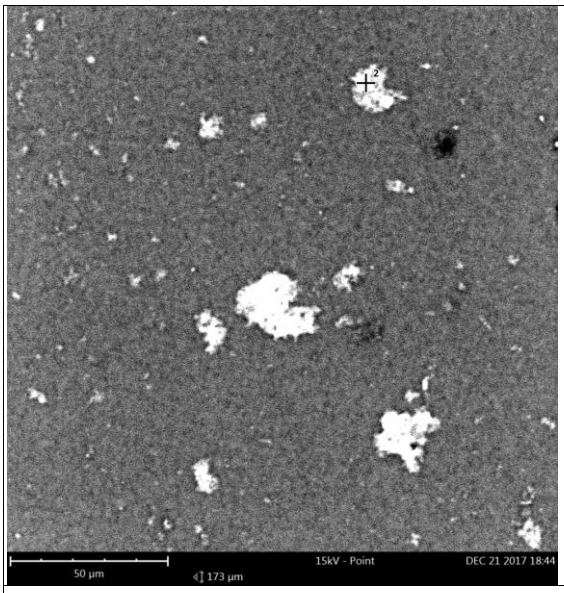


Figure 3: Scanning electron microscopy (SEM) micrograph of biologically synthesized silver nanoparticles from *C. papaya* leaf.



Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
6	C	Carbon	3.01	16.48
17	Cl	Chlorine	19.38	34.89
8	O	Oxygen	6.38	5.18
47	Ag	Silver	71.23	43.44

FOV: 173 µm, Mode: 15kV - Point, Detector: BSD Full, Time: DEC 21 2017 18:44

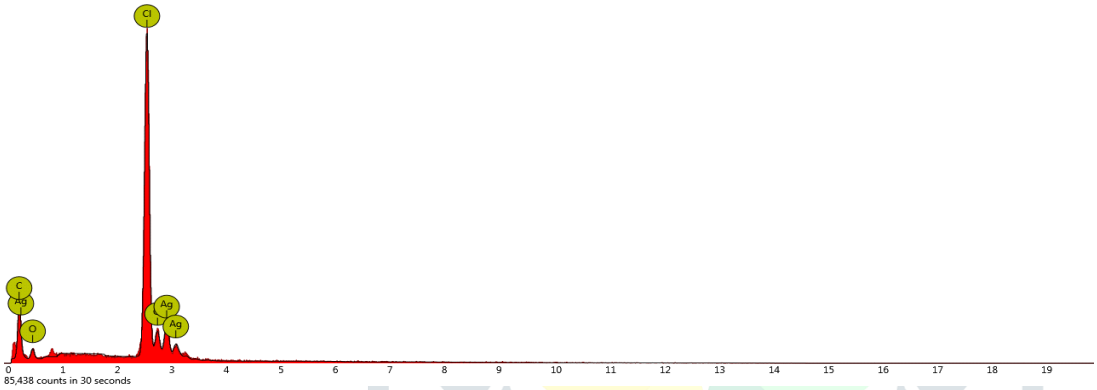


Figure 4: EDX charcterization spectrum obatined for biologically synthesized silver nanoparticles from *C. papaya* leaf. Visible peaks shows the presence of silver substance in the tested sample.

Atomic absorption spectroscopy studies were done and the concentration of the chemically synthesized silver nanoparticles was found to be 50.4ppm. Whereas, biologically synthesized silver nanoparticle solution had a concentration of 91.7ppm. In a previous study by Garima Singhal et al (2011) employing a similar method for synthesizing silver nanoparticles, the concentration of Nano silver obtained was 5.5ppm (Garima Singhal et.al, 2011).

The total anti-oxidant activity of the ascorbic acid incorporated in the lotion and tested after 20 and 60 minutes was found to be 59 μg/ml and 110 µg/ml respectively, by the phosphomolybdenum method. This assay is based on the reduction of Mo (VI) to Mo (V) in the presence of an antioxidant compound because of which a coloured complex is formed at an acidic pH. Formation of the coloured complex was estimated calorimetrically at 670nm.

Antimicrobial activity of the chemically synthesized silver nanoparticles(CAgNps) as well as biologically synthesized silver nanoparticles (BAgNPs) against *Escherichia coli* and *Staphylococcus aureus* was determined using agar cup diffusion assay (Table 1).

Table 1: Zones of inhibition observed for *Escherichia coli* and *Staphylococcus aureus* (standard deviation is mentioned).

Culture	Zone of Inhibition with CAgNPs	Zone of Inhibition with BAgNPs
Escherichia coli	21 ± 1mm	9± 1mm
Staphylococcus aureus	21± 2mm	18± 1mm

MIC of chemically synthesized silver nanoparticles and biologically synthesized silver nanoparticles against *S.aureus* was found to be 0.45mg/ml and 0.12mg/ml respectively. Whereas, against *E.coli* it was found to be 0.45mg/ml and 6.3mg/ml respectively, after 24hrs of incubation at 37^0 C.

An antimicrobial lotion was prepared by using by using bee wax, coconut oil and almond oil as the oil part and water was incorporated in to the blender to form the oil- water phase. After the formation of the lotion, Nano silver particles was added to it.
The formulated lotion was subjected to various standard evaluation procedures. pH of the prepared lotion was found to be 6 which is recommended as a suitable pH in lotion formulations. The formulated lotion showed slight fruity odour, and dark yellow colour. Also, the cream was not greasy and sticky after applying on the skin. There was no change in the colour and consistency of the lotion upon keeping it for a longer time until the end of the study period.

In order to determine the antimicrobial activity of the formulated lotion with and without the incorporation of synthesized nanoparticles disc diffusion assay was performed. Results are depicted in Table 2.

Table 2: Zones of inhibition (ZOI) obtained with various lotion formulations.

Culture	ZOI with lotion	ZOI with CAgNPs+ lotion	ZOI BAgNPs+ lotion	ZOI with ascorbic acid + lotion	ZOI with both NPs + ascorbic acid.
E.coli	-	20mm± 2mm	8mm± 2mm	1mm	19mm± 2mm
S.aureus	-	20mm± 1mm	18mm± 1mm	-	24mm± 1mm

From the above data it is evident that the lotion with both chemically synthesized silver nanoparticles as well as biologically synthesized silver nanoparticles along with ascorbic acid incorporated in it, showed enhanced antimicrobial effect and further studies must be carried out to investigate the broad-spectrum activity of the lotion and its potential application in medicinal and cosmetic use. Currently, there are many antibiotics used to treat various skin infections and lotions that possess antioxidant and anti-ageing property. The lotion formulated during the current study is a novel concept in cosmetics and dermatology and could serve both these purposes.

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