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Novel Approach towards Wound Healing using Curcumin Longa and Silver Nanoparticles

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Abstract—*Wound healing is a biological process related to the general phenomenon of tissue regeneration. Wound healing process, is a series of interdependent and overlapping stages, in which a variety of cellular matrix components act together to re-establish the integrity of damaged tissue and replacement of lost tissue. Plants have been a source of medicinal compounds and play an important role in maintaining human health since ancient times. Healing power of many plants has been used since ancient times.*

Nanotechnology is a field of science which involves the synthesis and applications of nanoparticles. Silver nanoparticles (AgNp) have applications in various fields due to their unique optical, physical, electrical and medicinal properties. In this study, silver nanoparticles were synthesized using *Ocimum sanctum leaf (Tulsi) and 100 mg/ml concentration was found optimal against both gram positive and gram negative bacteria. The bandage coated with AgNPs were analyzed under Scanning Electronic Microscope (SEM) and it was observed that AgNPs of size 53.35nm were formed hence confirming the formation of AgNPs.*

Turmeric is widely used as a home remedy to heal wounds and cut. It has been shown in laboratory that curcumin exhibits antibacterial activity and suppresses the growth of several bacteria like Streptococcus, Staphylococcus, Lactobacillus, etc.

Further Curcumin, nanoparticles were developed. Curcumin, the main component in Turmeric is a non-toxic, highly promising natural antioxidant compound. Ultrasonic bath or sonicator was used for making Curcumin nanoparticles. Nanocurcumin which was formed was freely dispersible in water whereas turmeric and Curcumin cannot be dissolved in water.

The natural lanoline is derived from the subcutaneous tissue of sheep and is allergenic in nature. It also has emollient and anti-microbial property. Thus a non-allergenic lanoline was produced by using column. The primary coating consisted of Hexane, Non-allergenic Lanoline and Glycerin.The final novel bandage formed showed positive results for both gram positive and gram negative.

1. INTRODUCTION

Wound Management is gaining importance in present times, with demand of advance technology in development of Medical devices and Medical textile fields. Ayurvedic 'Bhasms'(Alternative traditional medicine) application as the Ehono- nanomedicine for exploitation of our traditional knowledge, to develop efficient wound therapies, is also fast gaining popularity.In Ayurveda and Unani system, *Ocimum sanctum L***.** (Tulsi) has been used for thousands of years for its diverse healing properties. In these traditional medicines, herb extracts with unique metallic preparation are important tools for curing the diseases. Similarly, recent research findings showed that Tulsi herb extracts is a good source for the preparation of nanoparticles of silver metal and can be used in ethano-nanomedicine. It shows synergistic interactions of many different active phytochemicals along with wound healing; anti-inflammatory etc. reported the antimicrobial activity of silver nanoparticles (10-25nm size) that was synthesized by chemical method. Nano based silver particles are being used effectively for their antibiotic action in post-surgery effectively. Preparation of the nanoparticles synthesis by green chemistry is more active, biologically accepted.

Another herb, that plays a pivotal role in Ayurvedic, Unani Siddha and Tibetian system of medicines is Turmeric. It is also considered very auspicious and effective as a medicine.The most well known medicinal action of turmeric is its use as a powerful anti-inflammatory, the effectiveness of which is comparable to pharmaceutical medicines. However, it also acts as an alternative analgesic, antimicrobial, anti-inflammatory, anti-carcinogenic, anti-allergic, antioxidant, antiseptic, antispasmodic, astringent, carminative, cholagogue, digestive, diuretic, stimulant, and vulnerary. Modern science is beginning to recognize and understand the amazing healing qualities of turmeric and for the last many years turmeric has raised the inquisitive level of research fraternity and hence number of research works is being carried on to determine the, biological activities and pharmacological actions of turmeric and its extracts.[68]

The active ingredient in turmeric is curcumin. Curcumin is a non-toxic, highly promising natural antioxidant compound, which are important disease fighting substances that help to clean up unstable oxygen molecules (free radicals) that can damage cells and cause diseases such as cancer, having a wide spectrum of biological functions and exhibits anti-inflammatory properties.The active properties of curcumin are best called "protective properties". The only disadvantage that it suffers is of low aqueous solubility and poor bioavailability, as its retention time in the body is limited due to its rapid systemic elimination. To overcome this shortcoming, nanoparticles of curcumin (nanocurcumin) were developed and found to have a much greater dispersion in water in the

absence of any surfactants against *Staphylococcus aureus, Bacillussubtilis, E.coli, Pseudomonas aeruginosa, etc.*

2. MATERIALS AND METHODS

*Materials***:** Ultra pure silver nitrate (assay 99.8% ; Micro Photo Film Ambala Cantt) , Lanoline anhydrous IP (Rolex Lanoline Ltd Products Mumbai), Active Charcoal, Muller Hilton Agar and other reagents and solvents were of reagent grade.

Curcumin: Extracted from the rhizome part of the turmeric and fresh Tulsi leaves

Common human pathogenic bacteria *Escherichia coli (E. coli)* and *Staphylococcus aureus (S.aureus)* were used for assessment of antimicrobial activity of synthesized silver and curcumin nanoparticles. Growth and maintenance of bacterial strains was done using nutrient agar. The suspension culture was prepared using nutrient broth. Muller Hilton Agar was used for assessing antimicrobial activity.

Preparation of Ocimum sanctum leaf broth: 25 g of finely cut fresh Ocimum sanctum (Tulsi) leaves were mixed with 100 ml of distilled water. This mixture was boiled for 5 minutes and the decantation of the mixture was done. a The extract was filtered and stored at 4ºC.

Synthesis of silver nanoparticles (AgNPs) and Preparation of different concentrations of AGNPs: Tulsi leaf extract mixed with $AgNO_3$ aqueous solution (5ml each) was left for few hours. For studying the maximum effectiveness, AgNPs in different concentrations were made.

Developing Curcumin Nanoparticles

To make the nanoparticles of curcumin 5mg of curcumin was dissolved in 15ml distilled water in a phalcon. It was kept for 1- 1½ hours in an ultrasonic bath or sonicator for agitation of the particles.

Primary coating of Bandage fabric: 100ml Hexane solution was made with 5% lanoline, 1% Glycerin & 0.5% Active Charcoal. These chemicals were mixed and bandage fabric (10cm x 10cm) was soaked for 10minutes and dried in Hot air oven at 50^{o}C for 30 minutes.

Coating of primary coated fabric with AgNPs: The primary coated fabric of size 2cm x 1cm were taken, each were coated with different concentration of AgNPs solution.

Coating of primary coated with curcumin nanoparticles: The primary coated fabrics were coated with different concentration of curcumin nanoparticles

Assessment of anti-bacterial activity: In order to access the antimicrobial activity of AgNPs and curcumin nanoparticles E. coli bacteria and S. aureus were used. The zone of inhibitions was measured after a gap of 24 hours for a period of four days.

*Final Novel Bandage:*The final novel bandage was prepared using *Silver nanoparticles (AgNPs)* and curcumin nanoparticles were poured in an optimized concentration to prepare matrix using primary coating of hexane and active charcoal.

3. RESULTS

Through this extraction method, an attempt was made to combine the inherent antimicrobial activities of silver and Tulsi for enhanced antimicrobial activity. The green color extract of Tulsi, on adding the clear aqueous silver nitrate solution to it and keeping aside for 30minutes, turned brown. Turning brownish color of the solution (Fig 1)was physical indication of silver nitrate being converted into AgNPs.The bandage fabric coated with these AgNPs were analyzed under SEM and it was observed that AgNPs of size 53.35nm were formed and was good, biocompatible for medical application (Fig. 2), hence confirming the formation of AgNPs.

Fig. 1: Extract turning brown

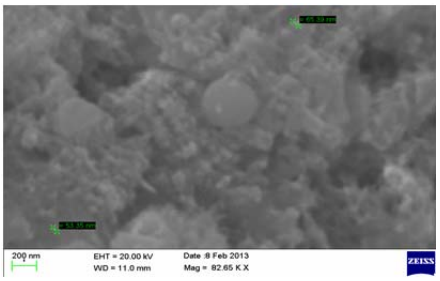
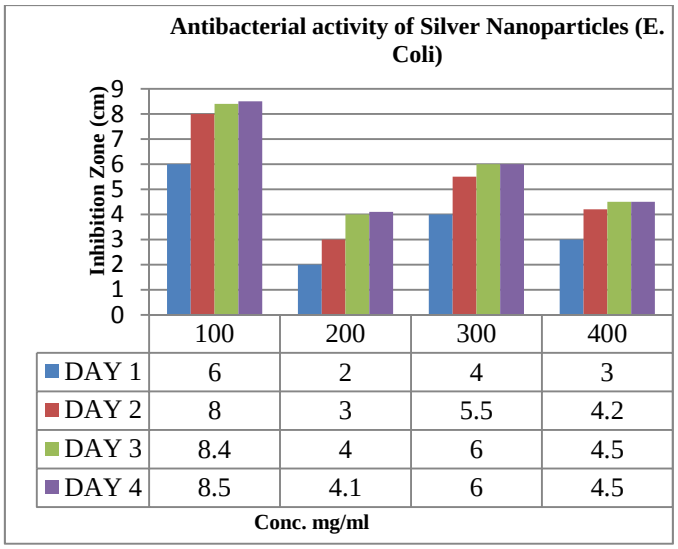


Fig. 2: Ag. NPs under SEM at 200 nm (82.65 KX)



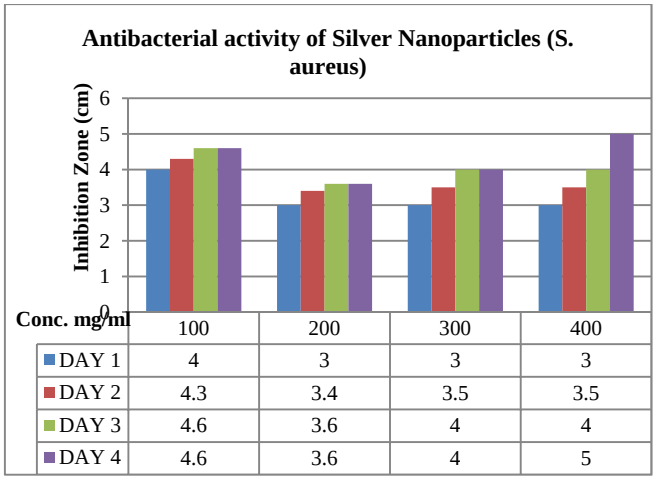


Fig. 3: The Zone of inhibition

Thus it was concluded that 100 gm/ml is the optimal concentration of AgNPs that is adequate to inhibit the growth of both gram positive and gram negative group of bacteria.

It was studied while reviewing the literature that the active ingredient in turmeric is curcumin. Curcumin is a highly potent, nontoxic, bioactive agent. It is a highly promising natural antioxidant compound and has important disease fighting substances and has a wide spectrum of biological functions and exhibits anti-inflammatory and antimicrobial properties.

The only disadvantage that it suffers is of low aqueous solubility and poor bioavailability. The insolubility of curcumin in water restricts its use to a great extent which can be overcome by the synthesis of curcuminin nanoparticles. Unlike turmeric or curcumin, that does not dissolve in water, nanocurcumin (Figur4:) formed was found to be freely dispersible in water in the absence of any surfactants

Fig. 4: Nanocurcumin

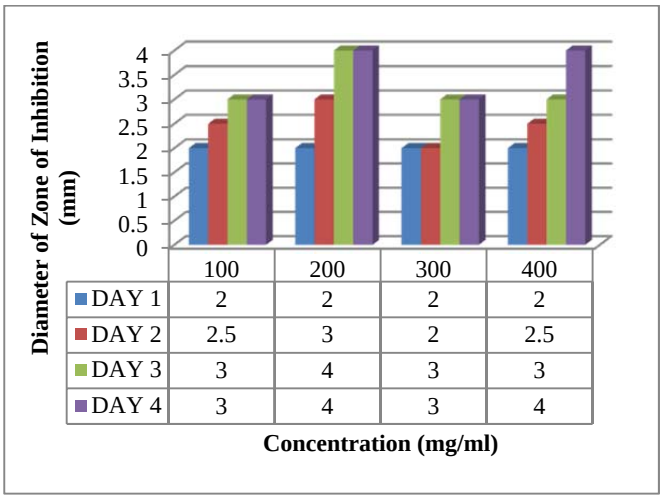


Fig. 5: Antimicrobial activity of nanocurcumin against

E. coli

Thus the optimum concentration of nanocurcumin that inhibits the growth of *Staphylococcus aureus and Escherichia coli* and was concluded as 200 mg/ml.

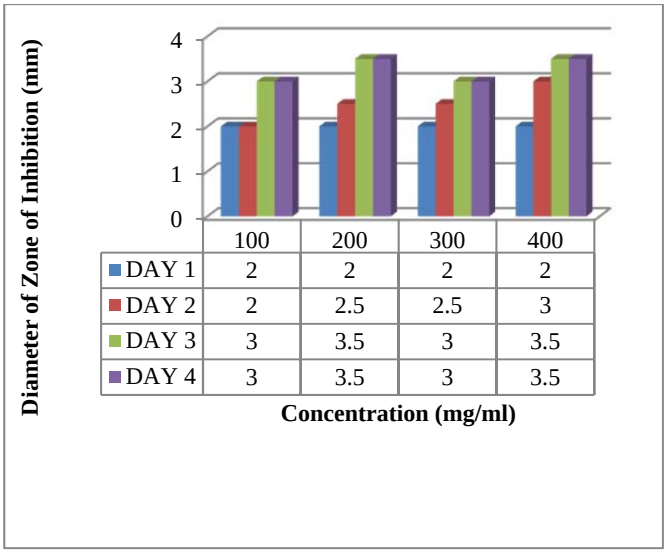


Fig. 6: Antimicrobial activity of nanocurcumin against *S. aureus* by Agar Diffusion Method.

The natural lanoline has an emollient and anti microbial property but is allergenic in nature. Thus a non-allergenic lanoline was produced by using column. The bandage was prepared by applying the primary coating on gauze fabric. The primary coating consisted of Hexane, Active Charcoal, Non-allergenic Lanoline and Glycerin. Hexane, which is a non-polar solvent, was used basically to dissolve non-allergenic Lanoline. Being a volatile substance, it evaporates without leaving any traces on to the gauze fabric when kept to dry.

The final coated fabric was also sent to an independent NABL accredited Laboratory for testing. There also, the coated fabric resulted in zero colony formation units (cfu) after 1 hour, in the case of bacteria including MRSA and fungi.

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