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Design, Development and Characterization of Silver Nanoparticles Embedded Polyherbal Gel for Antimicrobial and Wound Healing Activity

Ku. Chhaya S. Gajbhiye, Prof Dr. Madhuri A. Channawar, Dr. Anil V. Chandewar
Dr. Nitin I. Kochar, Dr. Pramod Ingle
P. Wadhwani College of Pharmacy, Yavatmal (M.S), India
Corresponding Author: Ku Chhaya S. Gajbhiye[1]
chhayagajbhiye1979@gmail.com

Abstract: *Wound healing is a complex biological process often delayed by microbial infections and oxidative stress. Currently, a variety of treatment options exist for chronic wounds; however, many are ineffective and associated with high costs. The use of expensive antibiotics to manage wound infections frequently results in adverse effects for patients. This stresses the need to develop alternative antimicrobial agents for wound healing. The present study aimed to formulate, develope and characterized a novel polyherbal gel formulation containing Ehretia laevis mediated silver nanoparticles (AgNPs) in combination with polyherbal extract (Ehretia laevis, Aloe vera and Curcuma longa extract) for wound healing applications. AgNPs were synthesized using aqueous leaf extract of Ehretia laevis and characterized by UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), Scanning electron microscopy (SEM) and dynamic light scattering (DLS). The synthesized AgNPs exhibited spherical morphology with an average size of 45-60 nm and stable zeta potential. The nanoparticles were incorporated into a Carbopol- based polyherbal gel with Ehretia laevis, Aloe vera and Curcuma longa. The gel was evaluated for physicochemical parameters, antimicrobial activity, and in vivo wound healing efficacy in Wistar rats using excision wound models. Results demonstrated significant antimicrobial activity against Staphylococcus aureus, bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa. In vivo studies showed accelerated wound contraction, in animals treated with nanoparticle embedded polyherbal gel compared to control and standard marketed formulation. The study concludes that Ehretia laevis mediated AgNPs in polyherbal gel possess synergistic antimicrobial and wound healing potential, offering a promising natural therapeutic alternative.*

Keywords: Ehretia laevis, Silver nanoparticles, Polyherbal gel, Wound healing, Antimicrobial activity.

I. INTRODUCTION

Research on wound healing agents is a developing area in biomedical sciences . Wound healing is the complex cascade of cellular and biochemical actions that leads to the restoration of structural and functional integrity and the regain of strength in injured tissues. Chronic wounds are a major healthcare burden worldwide because they increase morbidity, treatment costs, and risk of severe infections. Therefore, the development of effective wound healing systems with enhanced antimicrobial and regenerative properties is of great importance. [1]

Today, there are a variety of treatment options for chronic wounds, and many of them are ineffective. Many high-cost antibiotics are used to treat wound infections. Patients have to face the adverse effects of higher antibiotic doses. Therefore, there is a need to develop alternative antimicrobial drugs for wound healing.

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Historical Perspective of Herbal Therapeutics

Herbal medicines have played a crucial role in wound care since ancient times, particularly due to the presence of phytoconstituents such as flavonoids, alkaloids, tannins, terpenoids and phenolic compounds, which possess antioxidant, anti-inflammatory, antimicrobial and collagen-stimulating activities. These phytochemicals help reduce oxidative damage, control microbial growth, and accelerate fibroblast proliferation and tissue regeneration. Plants serve as an alternative, effective, cheap, and safe antibacterial for the treatment of microbial infections. Plants are more potent healers because they promote natural repair mechanisms.[3,4]

As far as the Indian scenario is concerned, there is a need for standardization of medicinal plants for the preparation of medicines thus, traditionally used plant-derived natural products form the firm platform for drug discovery and development in modern medicine [22,23]. Many phytoconstituents, such as flavonoids, terpenes, and polyphenols, have poor aqueous solubility and low membrane permeability[30]. These issues are being overcome through the development and application of nanotechnology-based medicine [5,6]

Nanoparticles

"Nano" is derived from the Greek word "nanos", meaning "dwarf, tiny, or very small."[57] Nanoparticles (NPs) are nano-meter-sized (1- 100 nm, atomic or molecular scale solid particles having some excellent physical properties compared to the bulk molecules, depending on their size and morphology[7,8]

Silver Nanoparticles

Metal-based nanoparticles are widely utilised for their antibacterial, antimicrobial and anti-inflammatory effects. Among metal NPs, silver nanoparticles (AgNPs) are gaining enormous interest in the research community due to their wide scope of application in microbiology, chemistry, food technology, cell biology, pharmacology and parasitology.[9,10,11]

Their performance depends strongly on the synthesis route, size/shape distribution, surface chemistry, and colloidal stability, all of which, in turn, modulate silver-ion (Ag^+) releaseprotein corona formation, cellular uptake, and biodistribution. The solubility and bioactivity of the silver particles in the wound area depend on the size of the silver particles; smaller the size, the stronger the contact with the skin. [12]

Synthesis Approaches

Different types of methods have been reported for AgNPs synthesis, which could be broadly divided into three different types, including physical, chemical and biological[13,14]

Several plant extracts, fungi, and bacteria have been utilized for the green synthesis of silver nanoparticles, showing numerous benefits for applications in different fields.[119-125] Plants serve as a valuable source of various components and biochemicals that can function as stabilizing and reducing agents for the synthesis of green nanoparticles. The methods employed for green synthesis are environmentally friendly, non-toxic, and cost-effective, and offer greater stability than biological, physical, and chemical methods. The advantages of using plants for the synthesis of nanoparticles include their availability, safety in handling, and the presence of a variety of metabolites[127]

Characterization of Silver Nanoparticles

Characterization of green-synthesized silver nanoparticles is essential to confirm their formation, determine their physicochemical properties, and to evaluate their stability and biological activity.[15] The following analytical techniques are used for this purpose.

- UV-Visible Spectroscopy
- Fourier Transform Infrared Spectroscopy (FTIR)
- X-ray Diffraction (XRD)
- Scanning Electron Microscopy (SEM)

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Polyherbal Formulations: Concept and Significance

Polyherbal formulations represent a unique therapeutic approach deeply rooted in Ayurveda, in which combining multiple herbs is preferred over single-plant preparations. The significance of polyherbalism largely arises from the **synergistic therapeutic effects** of multiple herbs. When combined, herbal constituents can act on distinct molecular pathways, producing additive or synergistic effects that exceed those of individual components.[16]

Pharmacological Importance of Selected Herbs

Among the vast diversity, ***Ehretia laevis*, *Aloe vera*, and *Curcuma longa*** (turmeric, with its principal bioactive compound curcumin) stand out for their wide ethnomedicinal applications. ***Ehretia laevis* (family: Boraginaceae)** is a deciduous tree distributed across tropical Asia. Traditionally, its leaves, bark, and roots have been used in Ayurvedic and folk medicine for treating wounds, ulcers, inflammation, and skin disorders. Phytochemical investigations of *E. laevis* reveal the presence of flavonoids, phenolic acids, alkaloids, saponins, tannins, and glycosides. Terpenoids and steroids have also been reported, contributing to its diverse pharmacological profile. These secondary metabolites are linked to its antioxidant and antimicrobial activities, which are critical for wound healing and tissue regeneration[17]

***Aloe vera* (family: Asphodelaceae)** is a succulent plant widely used in ethnomedicine and commercial skincare products. Its gel, derived from leaf parenchyma, contains a high proportion of bioactive polysaccharides, particularly acemannan, as well as anthraquinones, vitamins, minerals, and amino acids. The aloe vera plant is a well-known green healer and is thus widely used in several ethnomedicinal formulations as an active ingredient [18,19] *Aloe vera* gel promotes fibroblast proliferation, collagen deposition, and angiogenesis, all of which are essential for wound repair. Its polysaccharides form a protective barrier, maintaining moisture and preventing microbial invasion.

Curcumin (from Turmeric)

Curcuma longa, commonly known as a golden spice (family: Zingiberaceae), whose rhizomes contain curcuminoids, of which curcumin is the most extensively studied. It is a yellow polyphenolic compound responsible for the characteristic color and bioactivity of turmeric[20]

Synergistic Effects of *Ehretia laevis*, Aloe vera, and Curcumin

While each of these herbs independently exhibits wound-healing and anti-inflammatory effects, their combination offers significant synergistic potential. *E. laevis* offers flavonoid-mediated wound-healing and anti-inflammatory effects, *Aloe. vera* provides polysaccharide-driven immunomodulation and hydration, while curcumin contributes strong antioxidant, anti-inflammatory, and anticancer potential despite its bioavailability limitations. When combined, these three botanicals demonstrate synergistic activity in wound healing, skincare, and inflammation management, offering a natural and holistic alternative to synthetic drugs.[21]

Gels are important topical drug delivery systems that offer several advantages over conventional preparations such as ointments, creams, pastes, and lotions. Their hydrophilic nature, high water content, ease of application, and ability to incorporate and release therapeutic agents make them particularly suitable for wound-healing applications. Gels, particularly hydrogels, contain a high proportion of water and can maintain a moist environment at the wound surface. A moist wound environment supports cellular migration, re-epithelialization, and tissue regeneration, thereby contributing to the wound-healing process.[23,24] Due to their high-water content, gels provide a cooling and soothing sensation when applied to the skin. This property may help improve patient comfort and reduce the sensation of irritation associated with wound application.[25] Gels can retain active pharmaceutical ingredients and bioactive compounds at the site of application, thereby facilitating localized drug delivery. This can be particularly beneficial for wound-healing agents that require direct action on the injured tissue .

Gelling Agent

A **gelling agent** is a natural, semi-synthetic, or synthetic polymer that forms a continuous three-dimensional network when dispersed or dissolved in a suitable liquid. This network immobilizes the liquid phase through physical or chemical cross-linking, producing a semisolid preparation with desirable rheological properties.[26]

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Gelling agents improve viscosity, stabilize formulations, prolong drug residence time at the site of application, and enable controlled drug release.

Carbopol as a Gelling Agent

Carbopol (Carbomer) is one of the most widely used synthetic gelling agents in pharmaceuticals. It is a large, cross-linked polymer made from acrylic acid that forms clear, thick gels when mixed with triethanolamine, sodium hydroxide, or other alkaline substances.

Carbopol swells well because its carboxylic acid groups become charged, causing the polymer chains to separate and absorb water, forming a gel. Even at low concentrations (0.5–2.0%), Carbopol makes thick gels that spread easily and stick well to surfaces. These features make it especially useful for wound-healing gels with herbal extracts and silver nanoparticles.

Role of Gels in Sustained Release, Enhanced Penetration and Patient Compliance

Gels have several benefits over traditional forms because they can release drugs slowly and steadily. Their structure can be designed to control how fast they swell and how the drug moves out, helping keep the right drug levels for longer. This means patients need fewer doses and may have fewer side effects, which is important for long-term treatments. Gels also help drugs get through the skin better by keeping it moist and making close contact with the skin. For patients, gels are non-invasive, simple to use, and look good, so people are more likely to use them than injections or pills that might cause discomfort or side effects [218,219,220.]

Polyherbal Wound-Healing Gel

For a formulation containing ***Ehretia laevis*** **extract, Aloe vera, Curcuma longa, and** ***Ehretia laevis*****-mediated silver nanoparticles.** Carbopol gel offers a good base for evenly spreading active ingredients, applying them to the skin, and controlling their release. Because of this, gel systems are especially useful for making polyherbal and nanoparticle wound-healing products

Integration of Plant-Mediated Silver Nanoparticles into Polyherbal Gel

Adding plant-made **silver nanoparticles (AgNPs) to a polyherbal gel** is a promising way to improve topical treatments, especially for wound healing. In this method, silver nanoparticles are made using a medicinal plant extract, then mixed into a gel base like Carbopol along with other herbal extracts. This mix brings together the healing effects of plant compounds and the antimicrobial power of tiny silver particles.. Making AgNPs with plant extracts is seen as an eco-friendly and sustainable method. Plant extracts have natural chemicals like phenolics, flavonoids, terpenoids, proteins, and sugars that help turn silver ions (Ag^+) into silver nanoparticles (Ag^0). These natural molecules can also stick to the nanoparticles and act as stabilizers, making the particles more stable and reducing the need for strong chemical agents [27,28]

In this formula, ***Ehretia laevis*** **leaf extract** acts as both the reducing and stabilizing agent to make AgNPs. The natural compounds in E. laevis help create and stabilize the nanoparticles and also offer antioxidant and healing benefits. These AgNPs are then added to a Carbopol gel with Ehretia laevis, Aloe vera, and Curcuma longa. This way, the nanoparticles and herbal extracts work together, possibly giving stronger antimicrobial, antioxidant, anti-inflammatory, and wound-healing effects.

Because AgNPs are very small and have a large surface area, they can interact closely with microbes and may work better as antimicrobials . AgNPs fight microbes in several ways: they attach to cell membranes, damage the membrane, create reactive oxygen species, interact with cell proteins, and disrupt important cell processes. These actions are especially useful in wound care, since infections can slow healing and keep wounds inflamed for longer

Putting plant-made AgNPs into a gel makes them even better for use on the skin. The gel spreads the nanoparticles evenly over the wound and keeps them in contact with the tissue for longer. It also keeps the wound moist, which helps

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healing, and lets silver and herbal compounds be released in a controlled way · Because of these benefits, combining green-made AgNPs with a gel is being studied as a promising method for wound care and local infection control

Adding AgNPs to a **polyherbal gel** can offer extra healing benefits. Aloe vera helps keep the skin moist and supports tissue repair, while Curcuma longa adds antioxidants and reduces inflammation [230]. Ehretia laevis also brings more antioxidants and wound-healing effects. This formula is designed to be multifunctional: AgNPs mainly fight microbes, and the herbal extracts help protect against oxidation, lower inflammation, boost cell activity, support collagen and tissue growth, and speed up healing.

II. MATERIAL & METHODS

Collection and Identification of Medicinal plants

E. laevis is a medicinal herb. The plant has been reported to be used as antibacterial, keeping this in view, leaves of this plant were selected to formulate novel polyherbal formulations. The leaves were collected from tree at my home. Identification authentication was done by Dr. Gawande, HOD, Botany department, Sant Gadge Baba Amravati University, Amravati.

Aloe vera and *C. longa* are the other herbs having antibacterial activity, which are to be collected from authentic source.

Chemicals – All chemicals of AR grade were procured from local vender.

Extraction of leaves of Ehretia laevis Roxb

The leaves of plant ***E. laevis* Roxb** were collected from plant at home and identified by expert taxonomist botany department in Amravati university. The leaf extract of plant was prepared as per standard procedure. Fresh leaves were rinsed and cleaned with tap water followed by distilled water (DW). Surface was sterilized using 4% NaOCl solution and finally cleaned with sterile distilled water. Leaves were shade dried at 60^{o}C for 6-7 days, then ground into a fine powder in a mixer grinder and sieved through the muslin cloth. The powder was extracted by soxhlet extraction using ethanol (70%) as solvent.

Preparation of Aloe vera gel:

AV leaves were collected, washed with tap water and DW thrice. Surface was sterelized using 4% NaOCl solution and finally cleaned with sterile DW. The leaves were then cut-open with a sterile blade or knife and the gel part was scrapped with the sterile scalpel and collected in an air tight bottle, homogenized with whisker or a hand blender and stored in a refrigerator at 4^{o}C for further use.

Extraction of Curcuma Longa-Curcuma longa rhizomes were dried, powdered and extracted with ethanol.

Green Synthesis Of Silver Nanoparticles (AgNPs)

The ***Ehretia. laevis* Roxb** extract was mixed with 1 mM silver nitrate solution, the solution turns dark brown in colour. This change in colour from colourless to dark brown confirms the synthesis of silver nanoparticles (AgNPs) . The formation of uniform colloid solution indicated stabilized synthesis of AgNPs using ***E. laevis* Roxb** extract. The stable synthesis of AgNPs was also supported by zeta potential determination.

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Figure : Color change from colorless to dark brown confirms the synthesis of silver nanoparticles

CHARACTERIZATION OF AgNPs

Characterization was performed using UV visible spectroscopy, FTIR, XRD, NTA and SEM

FORMULATION AND PREPARATION OF NANOPARTICLE EMBEDDED POLYHERBAL GEL

S. No	Name of Ingredients	NF1 gm	NF2 gm	NF3 gm	NF4 Gm
1	*E. laevis* AgNPs	0.5			0.5
2	*E. laevis* Extract	---	2.0	2.0	2.0
3	*C. longa* extract			2.0	2.0
4	Aloe Vera Extract			15	15
5	Carbopol 934 p	1.0	1.0	1.0	1.0
6	Glycerin	5	5	5	5
7	Polyethylene Glycol	2	2	2	2
8	Methyl paraben	0.2	0.2	0.2	0.2
9	Tri-ethanolamine	qs	qs	qs	qs
10	Distilled water qs	100	100	100	100

Procedure for Preparation of novel nanoparticles embedded herbal gels

The gel base was prepared using Carbopol 934 (1 % w/v) in 60 gm water, then glycerine was added under moderate shear and hydrate for 60 min, then preservative were added and mixed. ELAgNPs, *E. laevis* extract and/or polyherbal extract containing *E. laevis*, aloe vera and *C. longa* extract were incorporated into it as per the formula. Finally make up volume with distilled water till 100 gm and filled into suitable container. Neutralize with Tri-ethanolamine to convert into gel

Evaluation of Novel nanoparticles embedded herbal gels.

All prepared silver nanoparticles embedded gels (NF1-NF4) were subjected to physical evaluation to get optimized formula for silver nanoparticles embedded gel.

Appearance All prepared silver nanoparticles embedded gels (NF1- NF4) were inspected visually for color, clarity, homogeneity and texture

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7.10.2.2. pH: pH measurements of the formulated silver nanoparticles embedded gels (NF1-NF4) were carried out using a digital pH meter by dipping the glass electrode completely in the gel system to cover the electrode. pH was determined in triplicate and average value was calculated.

Viscosity: The viscosity of the prepared silver nanoparticles embedded gels (NF1-NF4) was determined by using Brookfield viscometer using spindle no 6 at 10 rpm speed. Sufficient quantity of gel was taken into a beaker and the spindle was dipped in it for about 5 minutes and then the reading was noted. Viscosity values of the silver nanoparticles embedded gels (NF1-NF4) are given in table

Spreadability: Two sets of glass slides of standard dimensions were taken. The silver nanoparticles embedded gels (NF1-NF4) formulation was placed over one of the slides. The other slide was placed on the top of the gel such that the gel was sandwich between the two slides in an area occupied by a slides. 100 gm weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to form thin layer. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip of freely by the force of weight tied to it. A 20gm weight was tied to the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. By repeating the experiment three times, the mean time was determined. Spreadability was calculated by using the following formula

$$S = m \times l/t$$

Where,

S = Spreadability,

M = weight tied to the upper slide (20g),

L = length of glass, t-time taken in second

Extrudability: 10 gms of the prepared silver nanoparticles embedded gels (NF1-NF4) formulations were filled in the standard collapsible tubes and locked by crimping to the end of tubes. Before placing in between the slides, weight was taken. The tubes were placed between two glass slides and fixed. 500gm weight was placed on the slides and the cap was removed. The amount of the extruded formulation was collected, weighted and percentage was calculated**.**

Drug Content: A specific quantity (1gm) of developed silver nanoparticles embedded gels (NF1-NF4) were taken and dissolved in 100 ml of phosphate buffer of pH 6.8. The volumetric flask containing gel solution was shaken for 2 hour on mechanical shaker in order to get complete solubility of the drug. This solution was filtered and estimated spectrophotometrically at 453 nm using phosphate buffer (pH 6.8) as blank

Antimicrobial study:

Antimicrobial study was carried out on silver nanoparticles embedded gels (NF1-NF4) by agar well diffusion method on two gram positive (*S. aureus* and *B. subtilis*) and two gram negative (*E. coli* and *P. aeruginosa*) bacteria. The purpose of study was to find out effective concentration of *E. laevis*. Nutrient agar and nutrient broth media were used for bacterial culture. All the glassware were sterilized in an autoclave before initiating the experiment. The required amount of Mueller-Hinton agar was measured and was prepared according to manufacturer's instructions and poured into conical flask and covered by wrapping with aluminium foil then sterilized by autoclaving at 121 ^{0}C for 15 minutes. A sterile swab was used to pick the bacteria into appropriate solidified media. After sterilization, the nutrient agar was poured into petri plates in a laminar air flow cabinet and allowed to solidify. Two gram positive and two-gram negative bacteria were inoculated using sterile swab sticks on different culture plates and spread. Wells of 6 mm in in diameter were bored into the agar plates using a sterile cork borer. The well was filled with tested formulation of each concentration. Povidone Iodine was positive control. The plates were then incubated at 37 ^{0}C for 48 hours. The zone of inhibition was measured and compared with standard marketed preparation (Povidone iodine). The antimicrobial assessment was performed as per the latest CLSI M100 (Clinical Laboratory Standard Institute) guidelines

Wound healing study - Wound healing study was done on optimized formulation (NF4) on Wistar rats, which were divided into 3 groups of 3 in each group. Excision wound model were used. The rats were anesthetized by diethylether[483] Hairs from the dorsal thoracic region were removed by application of a hair removing cream which was taken from our medical store. Approximate 500 mm^2 area was marked by an indelible ink and rubber seal. Then it was

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cleaned by normal saline water and skin was excised and circular wound was created by cutting throughout the marked area through the skin. The wound were created, and gel under test was applied daily for 15 days. Wound contraction was measured over 15 days. The study of wound was done by marking the wound area on a transparent paper on 0^{th}, 4^{th}, 8^{th}, 15^{th} day using millimeter scale

Graph paper.

The percent of wound contraction was calculated by given formula [487]

% wound contraction = {(wound area on 0^{th} day - wound area on n^{th} days)/(wound area on 0^{th} day)}x100

Where n= no of days (4, 8, 15)

Group I- under test gel-(NHG4) AgNPs + polyherbal extract gel

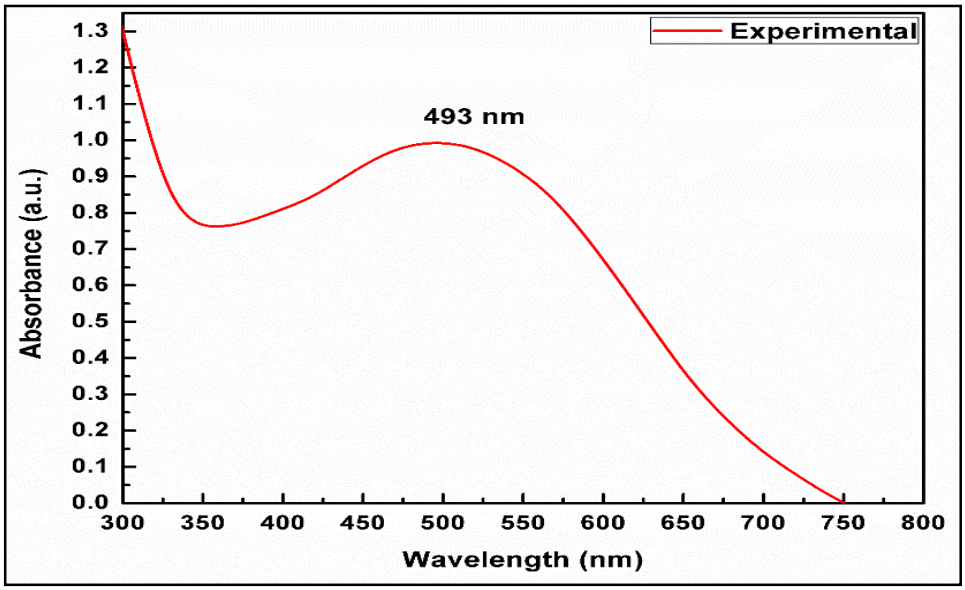
Group II- Standard marketed preparation

Group III- Untreated

III. RESULT AND DISCUSSION

Characterization of AgNPs

UV-Visible spectrum showed a peak at near 493 nm (surface plasmon resonance of AgNPs) confirming AgNPs formation.



XRD confirmed crystalline nature of nanoparticles

SEM indicated spherical nanoparticles.

FTIR revealed functional groups responsible for reduction and stabilization

The FTIR analysis of AgNPs showed the major peaks at wavenumbers 3354 cm^{-1}, 1674 cm^{-1}, 1505 cm^{-1}, 1443 cm^{-1}, 1410 cm^{-1}, 1376 cm^{-1}, 1089 cm^{-1} which are assigned to the keto compounds, aromatic compounds, phenolic, tertiary alcohol, phosphates and aliphatic chloro compounds. Thus, all probable compounds on the surface of the AgNPs had major contribution of negatively charged functional groups. These imparted net negative charge to the AgNPs and significantly supported the negative value of zeta potential for the AgNPs. There was a significant resemblance observed with the previous reports. The following figure shows comparative spectrum of ***E. laevis*** **Roxb** extract and the AgNPs

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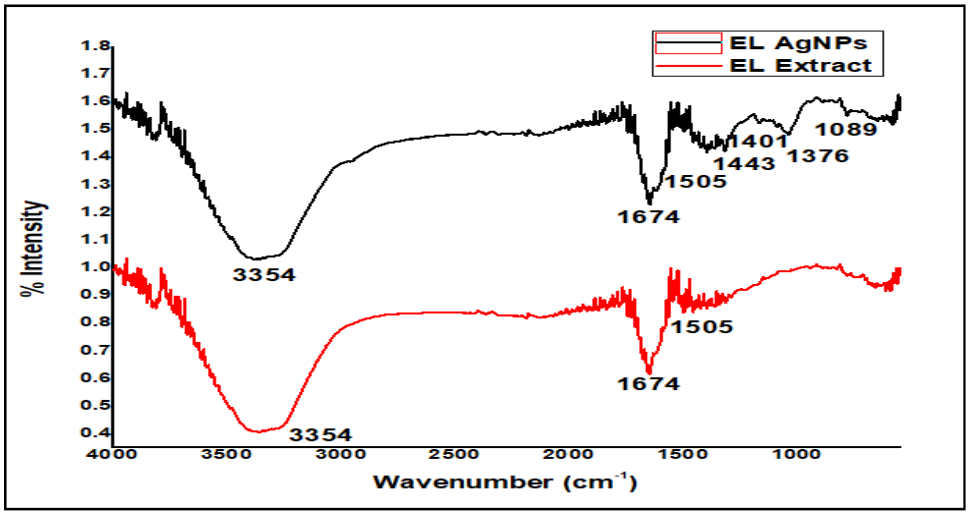

Fig. Comparative spectrum of *E. laevis* Roxb mediated AgNPs and plant extract as a control

Zeta Potential analysis

The zeta potential analysis showed a major peak with the average zeta potential of -7.72 mV with the standard deviation of 10.1 mV. This indicated the synthesis of stable AgNPs using ***E. laevis* Roxb** extract. The zeta potential value was in the range of stability i.e. -30 mV to +30 mV. The size analysis using zetasizer predicted the average size of ***E. laevis* Roxb** mediated AgNPs as 82.84 nm. The size determined by zetasizer and NTA were comparatively similar. The polydispersivity index value of 0.193 and the single moderately broad peak formation indicated that the synthesized AgNPs were less polydispersed.

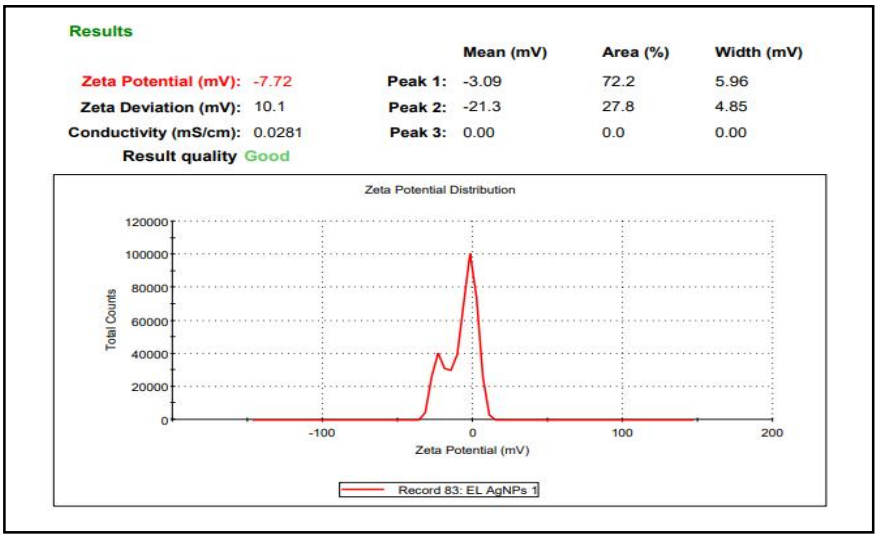

Fig. Size determination using zetasizer indicated synthesis of AgNPs with average size of 82.84 nm

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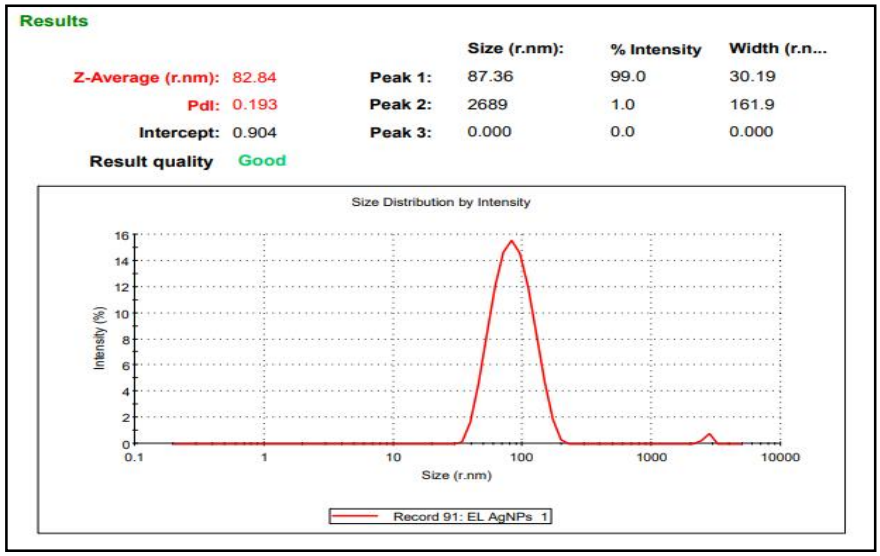

Fig. Size determination using zetasizer indicated synthesis of AgNPs with average size of 82.84 nm

Evaluation of nanoparticles embedded polyherbal gel (NF1-NF4)

Appearance- The preparations were found to be smooth, homogenous and various color depending upon presence of herbal extract like white, green, yellow, yellowish green.

pH – pH of liquid *in situ* gel was found to be acidic and after neutralization it was found to be in range of 5.8-6.5 as given in table

Viscosity of all formulations were studied using Brookfield viscometer before gelation and after gelation. Viscosity was measured using 10 rpm speed. Readings are given in table 8.15

Spreadability- The spreadability of the formulation was 17-20±0.05 g.cm/sec, which is within the prescribed range and sufficient for easy spreadability. Readings are given in table

Result for pH, Viscosity, Spreadability, After feel, drug content of nanoparticles embedded polyherbal gel

Formulation	Viscosity	Spreadability	PH	After feel	%drug content
NF1	34.230	18.01±0.04	5.5	Emollient	96±0.047
NF2	35.090	17.20±0.05	5.7	Emollient	97±0.072
NF3	37.330	15.04±0.22	5.8	Emollient	95±0.038
NF4	41.160	19.04±0.26	5.8	Emollient	97±0.028

Antimicrobial Study polyherbal gel-

Antimicrobial study was carried out on (NF1-NF4) formulations. Antimicrobial activity of the formulations was analyzed from the zones of inhibition. Zone of inhibition of formulations (NF1-NF4) was compared with standard marketed preparation. Nanoparticles embedded polyherbal gel exhibited significant inhibition of zone against tested microorganism with enhanced activity compared to pure extract or AgNPs alone indicating synergistic antimicrobial effect. From antimicrobial study G4 formulation was selected for wound healing study.

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Table Showing zone of inhibition by different conc of nanoparticles embedded polyherbal gel on 4 bacteria

S. No	Formulation	Zone of Inhibition			
		S. Aureus (I)	*B. subtilis (II)*	*E.coli (III)*	*P. aeruginosa (IV)*
A	**NF1**	21±0.20	19±0.15	18±0.40	22±0.28
B	**NF2**	13±0.20	11±0.20	14±0.11	15±0.05
C	**NF3**	22±0.28	18±0.20	19±0.15	22±0.35
D	**NF4**	24±0.47	23±0.53	20±0.53	24±0.61
E	**Standard**	25±0.05	25±0.20	26±0.15	24±0.67

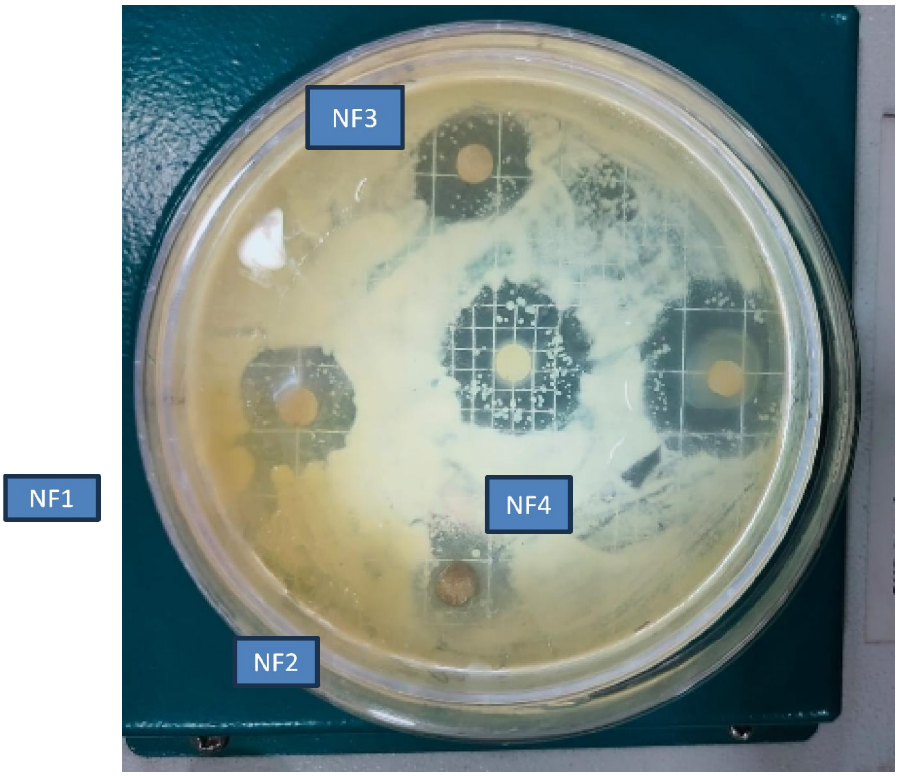

Figure : Antimicrobial activity of nanoparticle embedded polyherbal gel formulation against selected bacterial pathogens [NF1-silver nanoparticles only, NF2-E.laevis only, NF3-polyherbs extract, NF4-nanoparticles embedded]

WOUND HEALING STUDY

The percent wound contraction after applying optimized novel herbal gel (nanoparticle embedded polyherbal gel, standard marketed preparation (povidone-iodine), and control (without treatment) were shown in table and supported by animal photos.

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Group I – treated with nanoparticles embedded polyherbal gel showed 95 % wound contraction by day 15, compared to standard marketed preparation. This is due to synergistic antimicrobial activity of silver nanoparticles and antimicrobial activity of *Ehretia laevis* and other two extract. All three herbs also have antiinflamatory, antioxidant activity, which help for better wound contraction and faster healing.It is a promising formulation for further research.

Table: Result showing percent wound contraction

Days after wounding	Percent wound contraction		
	Group I -NF4	Group II-Standard	Group III-Untreated
Day 0	0.00	0.00	0.00
Day 8	48.36±0.03	42,61±0,666	30.42±0.822
Day 15	90.5±0.033	85.11±0.066	66.48±0.92

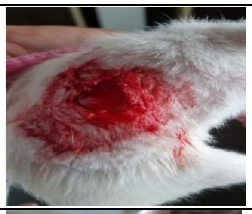
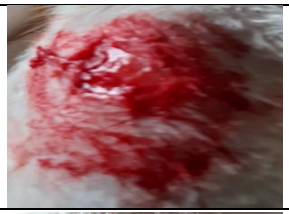
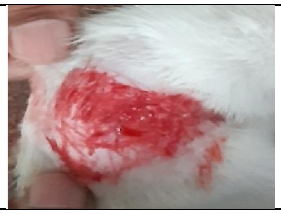
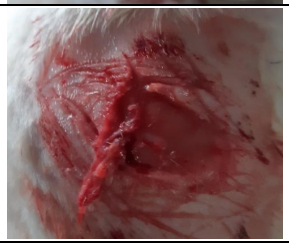
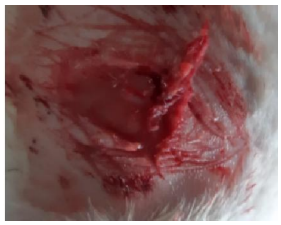
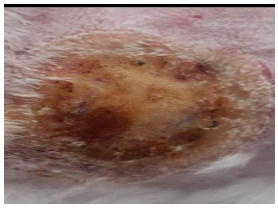
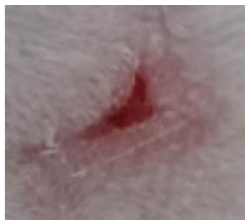
Days	**Group I (Novel Gel)**	**Group II (standard)**	**Group III (untreated)**
0			
4			
8			

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Figure :Comparative Healing of wound in Novel nano gel, standard and untreated mice.

IV. CONCLUSION

Ehretia laevis mediated silver nanoparticles (ELAgNPs) showed antimicrobial activity similar to polyherbal gel but less than standard preparations. Silver nanoparticles embedded polyherbal gel (NF4) showed enhanced antimicrobial activity similar to standard marketed products. This might be due to synergistic effect of silver nanoparticles, and polyherbal extract. Wound healing study support this.

Aloe vera is associated with hydration, epithelialization, and tissue repair, while *Curcuma longa* provides curcuminoids and other phytoconstituents with antioxidant and anti-inflammatory properties. Thus, the formulation can be designed as a multifunctional system in which AgNPs embedded polyherbal gel, enhance antimicrobial activity, while the combined herbal extracts support antioxidant protection, reduction of inflammation, fibroblast activity, collagen formation, epithelialization, and tissue regeneration.

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