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Design, Development and Evaluation of Ehretia Laevis Mediated Silver Nanoparticles Embedded in Polyherbal Gel for Its Antimicrobial and Wound Healing Acitivity

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Abstract: *Wound healing is a complex biological process often delayed by microbial infections and oxidative stress. The present study aimed to formulate, develope and evaluate 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, enhanced epithelialization and improved histopathological outcome 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*

INTRODUCTION:

Wound healing is a vital biological process involving hemostasis, inflammation, proliferation, and tissue remodelling, impaired wound healing remains a global health challenge, often complicated by microbial infections, oxidative stress, and inflammatory responses . Conventional therapies frequently show limited efficacy, resistance and adverse effects, necessitating the development of alternative strategies.

Herbal medicines have long been recognized for their wound healing properties. Ehretia laevis (Boraginaceae) traditionally used in Indian medicine, possesses, anti-inflammatory antimicrobial, and antioxidant activities. Aloe vera is widely known for its moisturizing, anti-inflammatory, and tissue regeneration effects, while Curcuma longa (Turmeric) is rich in curcumin, a polyphenolic compound, it is known for its potent antioxidant and antimicrobial activities.

Silver nanoparticles (AgNPs) possess broad-spectrum antimicrobial activity and promote tissue regeneration. Green synthesis using plant extracts provides a sustainable and eco-friendly method of nanoparticle preparation. Combining AgNPs with polyherbal extracts may provide synergistic effects for enhanced wound healing.

The present research aims to design, develop and evaluate a Carbopol-based polyherbal gel embedded with Ehretia laevis mediated AgNPs, targeting accelerated wound healing

MATERIAL & METHODS:

- **Selection, Collection and Identification of Plant Material**
- Ehretia laevis commonly known as khandu chakka is a medicinal herb. The plant has been reported to be used as antibacterial, keeping this in view, this plant's leaves are selected to formulate novel polyherbal preparations.

- The leaves collected from tree grown at home. Identification and authentication is to be done. Phytochemical investigation is to be done.
- Aloe vera and Curcuma longa are the other herbs having antibacterial activity, which are to be collected from authentic source.
- **Chemicals** -Polymer- Carbopol, HPMC, triethanolamine, glycerin, ethanol, methyl paraben, propyl paraben, silver nitrate, DI water.

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°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°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.

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	G1 gm	G2 gm	G3 gm	G4 gm	G5 gm
1	Ehretia laevis AgNPs	2.0			2.0	--
2	Ehretia laevis Extract	--	2.0	2.0	2.0	--
3	Aloe Vera Extract			2.0	2.0	--

4	**Curcuma longa extract**			2.0	2.0	--
5	**Carbopol 934 p**	1.0	1.0	1.0	1.0	1.0
6	**Glycerin**	5	5	5	5	5
7	**Triethanolamine**	qs	qs	qs	qs	qs
8	**Methyl paraben**	0.02	0.02	0.02	0.02	0.02
9	**Propyl paraben**	0.01	0.01	0.01	0.01	0.01
10	**Distilled water qs to**	100	100	100	100	100

1. The gel base was prepared using Carbopol 934 (1 % w/v) in 60 gm water, then glycerin was added under moderate shear and hydrate for 60 min, then preservative were added and mixed.
2. AgNPs , Ehretia laevis extract and/or polyherbal extract containing Ehretia laevis, aloe vera and curcuma longa extract were incorporated into it as per the formula. Neutralize with triethanolamine to convert into gel.
3. Finally make up volume with distilled water and filled into suitable container.
4. Formulation were evaluated for pH, viscosity, spreadability and drug content

EVALUATION OF GEL-All Formulations were evaluated for
1. Appearance-visually check for smooth, homogeneous preparation and its color
2. pH-measured the pH of all preparation using pH meter (compatible with skin pH)
3. Viscosity- Viscosity was measured using Brookfield viscometer
4. Spreadability-gel was spread between the two slides and fit into length of about 60 mm and fastened to a stand without any distraction. 20 gm weight were tied in the upper slide and the time taken for displacement of upper slide to the distance of 60 mm was noted under the impact of weight. By repeating the experiment 3 times, mean time was determined and the spreadability was calculated by using given formula

Spreadability=(weight x length)/Time

5. Stability study-Stability study was performed as per ICH guidelines. Stability study was performed on optimized formulation with 25±2 0C and 60±5% RH and 40±2 0C and 75±5% RH for 6 months. The sample were analysed at 0, 3, 6 months interval for color, physical appearance, pH and viscosity.
6. Antimicrobial study was performed by agar well diffusion method on 2 gram positive (staphylococcus aureus and Bacillus subtilis) and 2 gram negative (E. coli and Pseudomonas aeruginosa) bacteria. The zone of inhibition were measured compared G4 with other formulations.
7. In vivo wound healing study-wound healing study was done on optimized formulation (G4) Wistar rats were divided into groups

- Group I- Control plain gel
- Group II- Standard (marketed silver sulfadiazine
- Group III- G4- AgNPs + polyherbal extract gel
- Group IV- Untreated

Excision wound were created, and gel was applied daily for 14 days
Wound contraction was measured over 14 days

RESULT AND DISCUSSION:

Characterization of AgNPs:

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

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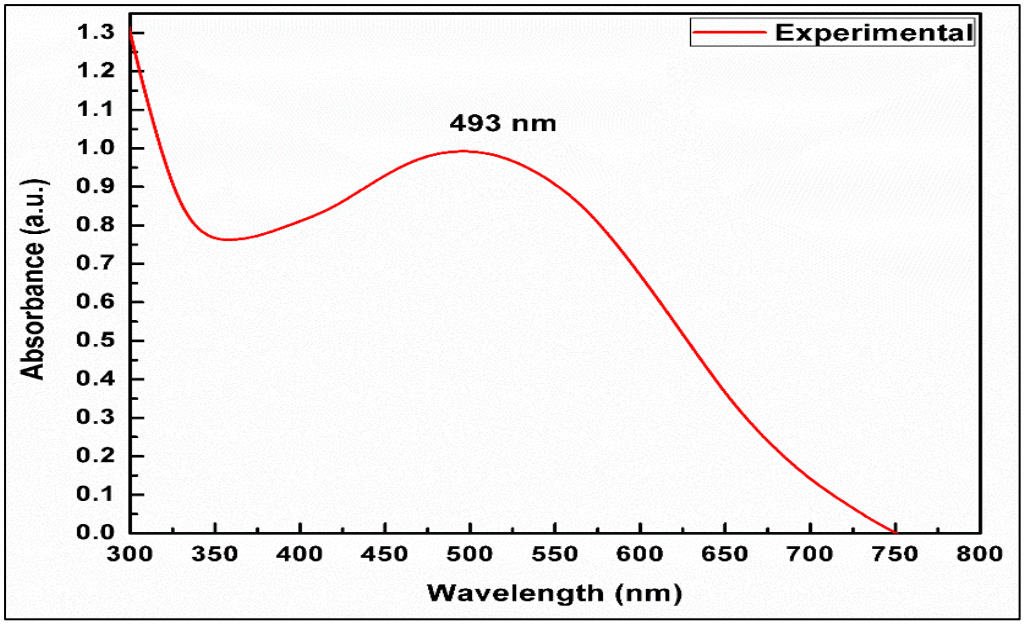

XRD confirmed crystalline nature of nanoparticles
SEM indicated spherical nanoparticles.
FTIR revealed functional groups responsible for reduction and stabilization
The FTIR analysis of AgNPs showed the major peaks at wavenumbers 3354 cm^{-1}, 1674 cm^{-1}, 1505 cm^{-1}, 1443 cm^{-1}, 1410 cm^{-1}, 1376 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

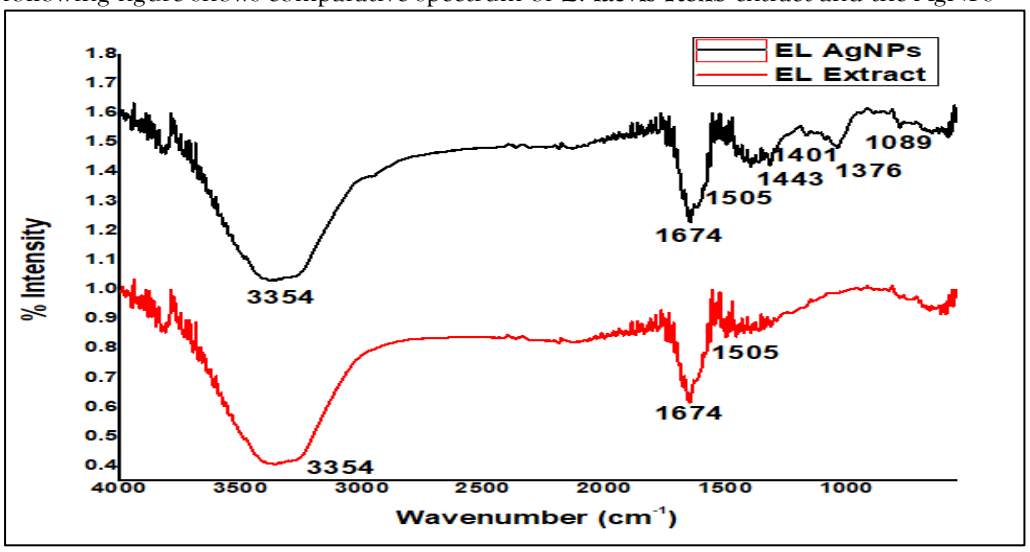

Fig. Comparative spectrum of E. laevis Roxb mediated AgNPs and plant extract as a control

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.

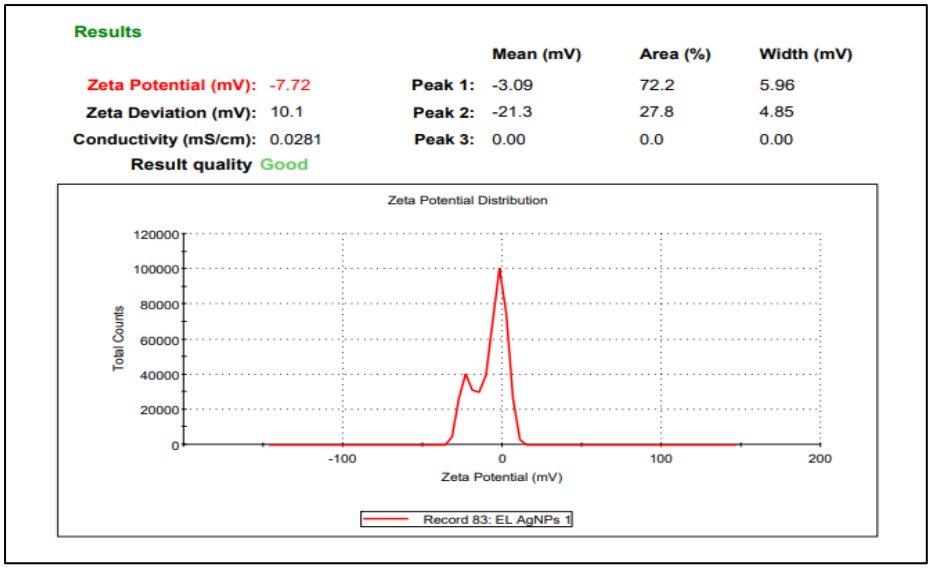

Fig. Size determination using zetasizer indicated synthesis of AgNPs with average size of 82.84 nm

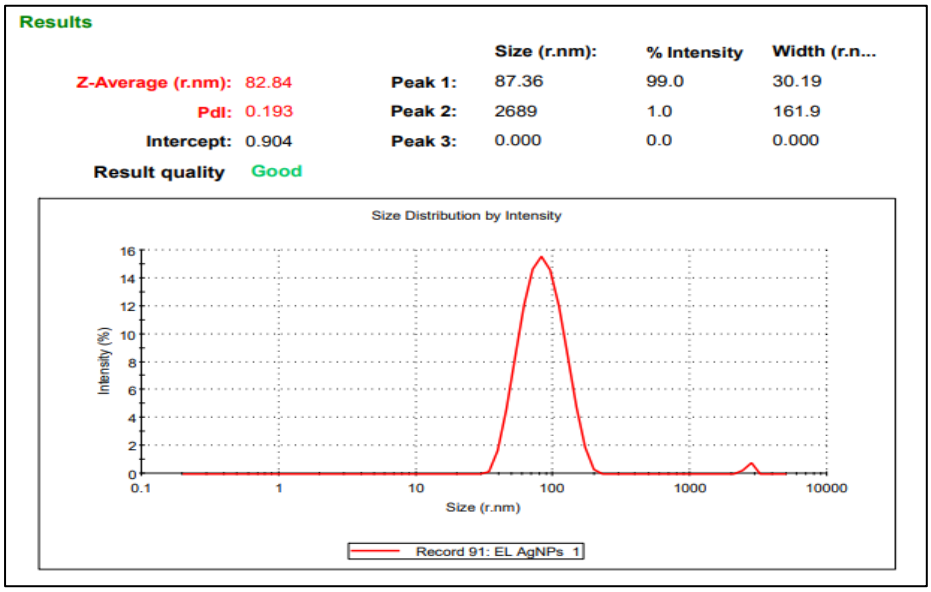

Fig. Size determination using zetasizer indicated synthesis of AgNPs with average size of 82.84 nm

Polyherbal gel evaluation:

Appearance – all formulations were smooth, homogeneous, white, yellow, yellowish green color

pH- pH of all formulations were found in between (6.8-7.2) which is skin compatible

Spreadability-found to be good

Viscosity- found to be suitable

Antimicrobial activity

The 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.

WOUND HEALING STUDY

The group IV (G4 formulation) showed 95 % wound contraction by day 14, compared to other groups. Histopathology confirmed faster re-epithelization and collagen deposition

CONCLUSION:

The developed G4 formulation containing Ehretia laevis mediated silver nanoparticles embedded in polyherbal gel, demonstrated excellent antimicrobial and wound healing properties compared to

conventional formulation. The combination of herbal bioactives and nanotechnology provides a promising therapeutic approach for effect wound management. The synergistic effect of AgNPs and polyherbal extract enhances wound contraction and tissue regeneration, making it a promising alternative to conventional wound dressing.

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