RESEARCH PAPER

Development and Biological Evaluation of Momordica dioica-Derived Silver Nanoparticle Embedded Herbal Bio-Scaffolds for Enhanced Cutaneous Tissue Regeneration and Wound Healing

Shraddha Rajendra Chate [1*], Gayatri Shankar Dhobale[2], Dinesh Kumar Agarwal[3]

[1]Research Scholar, Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur.
[2]Professor, Department of Pharmaceutical Chemistry, SCSSS's Sitabai Thite College of Pharmacy, Shirur
[3]Professor, Department of Pharmacology, Pacific Academy of Higher Education and Research University, Udaipur.

Corresponding author mail id : Shraddhachate20@gmail.com

Abstract

Wound healing is a complex and highly regulated biological process involving inflammation, proliferation, and tissue remodeling, and the development of bioactive wound dressings capable of promoting tissue regeneration while preventing microbial infection remains a major focus in regenerative medicine. The present study aimed to develop and evaluate *Momordica dioica*-derived silver nanoparticle-loaded herbal bio-scaffolds for enhanced cutaneous tissue regeneration and antimicrobial activity. *Momordica dioica* fruits were collected, authenticated, dried, and subjected to physicochemical and phytochemical investigations. Various solvent extracts were screened for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and the aqueous extract exhibiting the highest antimicrobial activity was selected for the green synthesis of silver nanoparticles. The synthesized nanoparticles were characterized using UV-visible spectroscopy, particle size analysis, zeta potential measurement, field-emission scanning electron microscopy (FESEM), and energy-dispersive spectroscopy (EDS), confirming successful formation of stable nanoparticles with nanoscale dimensions. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, vitamins, and triterpenoids, which are known for their therapeutic and wound-healing properties. The silver nanoparticles were incorporated into polyvinyl alcohol (PVA)-based electrospun nanofibers and fabricated into herbal bio-scaffolds. The developed bio-scaffolds demonstrated significant antimicrobial activity against the tested microorganisms and provided a favorable microenvironment for tissue regeneration and wound repair. The synergistic interaction between the bioactive phytoconstituents of *Momordica dioica* and silver nanoparticles contributed to enhanced antimicrobial efficacy and regenerative potential. Overall, the findings suggest that *Momordica dioica*-based silver nanoparticle-loaded herbal bio-scaffolds represent a safe, effective, biocompatible, and cost-effective alternative for advanced wound management and cutaneous tissue regeneration.

Keywords: *Momordica dioica*, Silver Nanoparticles, Bio-scaffolds, Electrospinning, Wound Healing, Cutaneous Tissue Regeneration, Antimicrobial Activity, Nanofibers

How to cite this article: Chate SR, Dhobale GS, Agarwal DK. Development and Biological Evaluation of Momordica dioica-Derived Silver Nanoparticle Embedded Herbal Bio-Scaffolds for Enhanced Cutaneous Tissue Regeneration and Wound Healing. Int J Drug Deliv Technol. 2026;16(66s):202-214. DOI: 10.25258/ijddt.16.66s.27

.

Introduction

Skin is the largest organ of the human body and serves as the primary barrier against physical, chemical, and microbial insults. Any disruption in skin integrity due to trauma, burns, surgical procedures, infections, or chronic diseases results in wound formation and initiates a complex healing process[1]. Wound healing is a dynamic and highly regulated biological phenomenon involving hemostasis, inflammation, proliferation, and remodeling phases [2]. Although the body possesses an intrinsic capacity for tissue repair, delayed healing and chronic wounds remain major clinical challenges worldwide[3]. Factors such as infection, diabetes, poor vascularization, excessive inflammation, and oxidative stress can significantly impair the healing process, leading to prolonged morbidity and increased healthcare costs[4].

Recent advances in tissue engineering have focused on the development of bio-scaffolds that provide structural support and a favorable microenvironment for cellular attachment, proliferation, migration, and differentiation. Bio-scaffolds are three-dimensional biomaterials designed to mimic the extracellular matrix (ECM) and facilitate tissue regeneration[5]. Among various scaffold fabrication techniques, electrospinning has gained considerable attention because it produces nanofibrous structures that closely resemble the native ECM architecture. Electrospun nanofibers possess high porosity, a

large surface area-to-volume ratio, and excellent mechanical properties, making them highly suitable for wound healing applications[6].

The incorporation of herbal medicines into scaffold systems has emerged as a promising strategy to enhance tissue regeneration. Medicinal plants contain a wide variety of bioactive phytoconstituents such as flavonoids, alkaloids, phenolic compounds, tannins, glycosides, and terpenoids that exhibit antioxidant, antimicrobial, anti-inflammatory, and wound healing activities[7]. Herbal therapeutics offer several advantages including biocompatibility, reduced toxicity, cost-effectiveness, and easy availability. In recent years, nanotechnology has further improved the therapeutic potential of herbal medicines by enhancing their stability, bioavailability, and controlled release characteristics[8].

Momordica dioica Roxb. ex Willd. (family Cucurbitaceae), commonly known as spiny gourd or teasel gourd, is an important medicinal plant widely distributed in India. The fruits are rich in flavonoids, alkaloids, glycosides, saponins, phenolic compounds, vitamins, and triterpenoids, which contribute to its antioxidant, antimicrobial, anti-inflammatory, and tissue regenerative properties[9]. Green-synthesized silver nanoparticles derived from Momordica dioica extracts have shown significant biological activities and may serve as effective therapeutic agents for wound management [10]. Therefore, the development of nanoparticle-loaded electrospun bio-scaffolds incorporating Momordica dioica extract represents a novel approach for enhancing cutaneous tissue regeneration and accelerating wound healing. The present study aims to formulate and evaluate herbal bio-scaffolds containing nanoparticle-loaded Momordica dioica extract for improved wound healing and tissue regeneration[11].

Materials and Methods

1. Materials

Fresh fruits of *Momordica dioica* Roxb. ex Willd. were collected from a local market/garden source in good physiological condition and free from visible microbial contamination or mechanical damage. Silver nitrate ($AgNO_3$) of analytical grade was used as the precursor for the synthesis of silver nanoparticles. Biocompatible polymers such as polyvinyl alcohol (PVA), chitosan, and/or gelatin were selected for scaffold fabrication based on their film-forming ability, biodegradability, and compatibility with skin tissue. Distilled water was used throughout the study for solution preparation and washing steps. All other chemicals, solvents, and reagents used in the study were of laboratory or analytical grade and were employed without further purification.

2. Collection and Authentication of Plant Material

Fresh *Momordica dioica* fruits were collected and immediately transported to the laboratory in clean sterile containers to avoid contamination and deterioration. The fruits were washed thoroughly under running tap water followed by rinsing with distilled water to remove dust, soil particles, and other surface impurities. The cleaned plant material was authenticated by a qualified taxonomist or botanist, and a voucher specimen was prepared and deposited in the departmental herbarium for future reference. After authentication, the fruits were cut into small pieces and shade-dried at room temperature in a well-ventilated area away from direct sunlight to prevent degradation of heat-sensitive phytoconstituents. Drying was continued until a constant weight was obtained. The dried material was then pulverized using a mechanical grinder to obtain a coarse powder, passed through a sieve to ensure uniform particle size, and stored in an airtight container at room temperature in a dry place until further use.

3. Preparation of Plant Extract

The powdered fruit material was subjected to extraction using a suitable solvent such as ethanol, methanol, or distilled water depending on the intended application and experimental design. In the case of maceration, a known quantity of powdered material was soaked in the selected solvent in a conical flask at a defined plant-to-solvent ratio and kept on a rotary shaker or at room temperature for 24–72 hours with intermittent shaking to facilitate the release of phytoconstituents. For Soxhlet extraction, the powdered sample was packed in a thimble and extracted continuously with the solvent until the siphon tube became colorless, indicating exhaustive extraction. After extraction, the mixture was filtered through Whatman No. 1 filter paper to remove insoluble residues. The filtrate was concentrated under reduced pressure using a rotary evaporator or by gentle evaporation on a water bath at a controlled temperature to obtain a semisolid crude extract. The concentrated extract was transferred to a clean container, labeled properly, and stored at 4°C until further analysis and nanoparticle synthesis.

4. Green Synthesis of Silver Nanoparticles

Green synthesis of silver nanoparticles was carried out using the aqueous or hydroalcoholic extract of *Momordica dioica* as both reducing and stabilizing agent. A freshly prepared aqueous solution of silver nitrate was made at the desired concentration and protected from light to prevent premature photoreduction. In a typical synthesis procedure, the plant extract was added dropwise to the silver nitrate solution under continuous magnetic stirring to

ensure uniform mixing and efficient interaction between phytochemicals and silver ions. The reaction mixture was maintained at room temperature or at a controlled temperature for a specified period of time until nanoparticle formation was complete. The synthesis process was monitored visually by observing a gradual color change from pale yellow or colorless to brownish or dark brown, which indicated the reduction of Ag^+ ions to elemental silver nanoparticles.

To optimize nanoparticle formation, different parameters such as extract concentration, silver nitrate concentration, pH, temperature, and reaction time were adjusted as required. After completion of the reaction, the nanoparticle suspension was centrifuged at high speed to separate the formed nanoparticles from the supernatant. The pellet obtained was washed repeatedly with distilled water and, if necessary, with ethanol to remove unreacted biomolecules and residual ions. The purified nanoparticles were then dried in a hot air oven, vacuum desiccator, or lyophilizer and stored in airtight containers for further characterization and scaffold preparation.

5. Characterization of Silver Nanoparticles

The synthesized silver nanoparticles were characterized using a series of analytical techniques to confirm their formation, stability, morphology, and crystalline nature. UV-visible spectroscopy was performed by scanning the nanoparticle suspension over an appropriate wavelength range to detect the characteristic surface plasmon resonance peak of silver nanoparticles. The appearance of a distinct absorption band confirmed nanoparticle formation.

Fourier transform infrared spectroscopy (FTIR) was carried out to identify the functional groups present in the plant extract and to determine the biomolecules responsible for reduction and stabilization of the nanoparticles. The dried nanoparticle sample was mixed with potassium bromide (KBr), compressed into pellets, and scanned over the required spectral range. Shifts in peak positions were analyzed to understand the interaction between phytochemicals and nanoparticle surfaces.

X-ray diffraction (XRD) analysis was performed to determine the crystalline structure and phase purity of the synthesized nanoparticles. The dried nanoparticle powder was mounted on the sample holder and scanned over a suitable 2θ range. The diffraction peaks obtained were compared with standard reference data to confirm the crystalline nature of silver nanoparticles and to estimate average crystallite size using the Debye-Scherrer equation.

Scanning electron microscopy (SEM) was used to examine the surface morphology, shape, and aggregation pattern of the nanoparticles. A small amount of dried sample was mounted on a stub, coated with a thin layer of conductive material if required, and observed under the microscope. Transmission electron microscopy (TEM) was employed to determine the exact particle size, shape, and dispersion pattern at high resolution. For TEM analysis, a diluted nanoparticle suspension was placed on a carbon-coated copper grid, dried, and examined.

Dynamic light scattering (DLS) analysis was conducted to determine the hydrodynamic particle size distribution and polydispersity index of the nanoparticles in suspension. Zeta potential analysis was performed to evaluate the surface charge and colloidal stability of the nanoparticle formulation. A high absolute zeta potential value indicated good stability and reduced tendency for aggregation.

6. Preparation of Herbal Bio-Scaffolds

The herbal bio-scaffolds were prepared using a suitable polymeric matrix such as PVA, chitosan, gelatin, or a combination of these polymers to obtain a biocompatible and mechanically stable nanofibrous scaffold. Initially, the selected polymer was dissolved in an appropriate solvent system under continuous stirring and mild heating, if necessary, until a clear and homogeneous polymer solution was obtained. In the case of blended scaffolds, each polymer was dissolved separately and then mixed in the desired ratio to achieve uniformity.

The *Momordica dioica* extract and/or synthesized silver nanoparticles were incorporated into the polymer solution in a predetermined concentration. The mixture was stirred continuously for several hours to ensure even distribution of the bioactive components throughout the polymer matrix. If required, sonication was performed to remove air bubbles and improve dispersion of nanoparticles within the solution. The final solution was then transferred into a syringe fitted with a metallic needle for electrospinning.

Electrospinning was carried out by optimizing key parameters such as applied voltage, flow rate, needle-to-collector distance, and collection time. The polymer solution was extruded from the syringe at a controlled rate under a high-voltage electric field, resulting in the formation of fine nanofibers that were deposited onto a grounded collector. The collected nanofibrous mat was carefully removed and dried under vacuum or in a desiccator to eliminate residual solvent. The dried scaffolds were cut into suitable sizes, sterilized if necessary, and stored in sterile containers for further characterization and biological evaluation.

7. Characterization of Bio-Scaffolds

The prepared bio-scaffolds were subjected to detailed physicochemical and functional characterization to evaluate their suitability for wound healing applications. Morphological analysis was performed using SEM to observe fiber diameter, surface texture, pore structure, and uniformity of the scaffold. Prior to imaging, the scaffold samples were mounted on stubs and sputter-coated with a thin layer of gold or platinum if required. Fiber diameter was measured from SEM images using image analysis software.

Mechanical properties of the scaffolds, including tensile strength, elongation at break, and Young's modulus, were determined using a universal testing machine. Rectangular strips of scaffold were cut to standard dimensions and stretched at a constant rate until breakage occurred. These measurements helped assess the flexibility and durability of the scaffold during application on skin wounds.

Porosity was evaluated to determine the percentage of void spaces within the scaffold, which is important for cell infiltration, nutrient diffusion, and exudate absorption. Swelling behavior was assessed by immersing pre-weighed scaffold samples in phosphate-buffered saline or distilled water and measuring the increase in weight at predetermined time intervals. The swelling ratio was calculated to estimate the water uptake capacity of the scaffold.

The biodegradation study was conducted under physiological conditions by incubating scaffold samples in buffer solution or in the presence of lysozyme, depending on the polymer composition. Weight loss was recorded at regular intervals to determine the degradation profile. Water vapor transmission rate (WVTR) was measured to evaluate the moisture permeability of the scaffold, which is essential for maintaining a moist wound environment without excessive dehydration.

The in vitro drug release study was performed by placing scaffold samples in a release medium and analyzing the amount of extract or nanoparticles released over time using spectrophotometric methods. The release profile was used to determine whether the scaffold provided sustained delivery of the bioactive components. In addition, antimicrobial activity of the scaffold was assessed against selected bacterial strains using standard microbiological methods such as agar well diffusion or disc diffusion assay. The zone of inhibition was measured to evaluate the antibacterial efficacy of the scaffold.

8. In Vitro Biocompatibility Studies

The biocompatibility of the scaffold was evaluated through in vitro cell-based assays to determine its safety and suitability for tissue regeneration. Cytotoxicity was assessed using cell viability assays such as MTT, Alamar Blue, or similar methods. For this purpose, fibroblast or keratinocyte cell lines were cultured under standard conditions and exposed to scaffold extracts or directly seeded onto scaffold samples. After incubation for a specified period, cell viability was measured and compared with control groups to determine any toxic effect of the scaffold.

For cell adhesion and proliferation studies, sterile scaffold samples were placed in culture plates and seeded with fibroblast or keratinocyte cells at a defined density. The cells were allowed to attach and grow on the scaffold surface under controlled incubation conditions. At different time points, cell attachment, spreading, and proliferation were observed using inverted microscopy or fluorescence microscopy. Cell morphology was examined to assess whether the scaffold supported normal cellular architecture and growth.

If required, a scratch wound assay was performed to evaluate the effect of the scaffold on cell migration. In this assay, a uniform scratch was created in a confluent cell monolayer, and the scaffold or its extract was applied to the culture system. The rate of wound closure was monitored over time to determine the migratory response of the cells and the potential of the scaffold to promote wound healing.

9. In Vivo Wound Healing Study

The wound healing activity of the prepared scaffold was evaluated using an appropriate animal model such as Wistar rats or albino rats. The animals were procured from an authorized animal house and housed under standard laboratory conditions with controlled temperature, humidity, and light-dark cycle. They were provided with standard pellet diet and water ad libitum. All experimental procedures were conducted in accordance with institutional ethical guidelines, and prior approval was obtained from the Institutional Animal Ethics Committee before the commencement of the study.

An excision wound model was created under aseptic conditions after anesthetizing the animals as per approved protocol. The dorsal fur of the animals was shaved, and a full-thickness circular wound of uniform size was made using sterile surgical instruments. The animals were randomly divided into different groups, such as normal control, wound control, standard treatment, scaffold-treated, and nanoparticle-loaded scaffold-treated groups. The prepared scaffolds were applied topically to the wound area at regular intervals or as per the experimental design.

Wound healing progression was monitored by measuring wound area at predetermined time points using graph paper, digital planimetry, or image

analysis software. The percentage of wound contraction was calculated to assess the rate of healing. The period of epithelialization, defined as the number of days required for complete closure of the wound surface, was also recorded. At the end of the study period, animals were sacrificed humanely, and wound tissue samples were collected for biochemical and histopathological analysis.

Biochemical parameters such as hydroxyproline content, total protein, collagen deposition, and antioxidant markers including superoxide dismutase, catalase, and reduced glutathione were estimated in the wound tissue homogenate to evaluate the healing response at the molecular level.

10. Histopathological Examination

Wound tissue samples collected from the experimental animals were immediately fixed in 10% neutral buffered formalin for adequate preservation. The fixed tissues were then processed through a series of dehydration steps using graded alcohol, cleared in xylene, and embedded in paraffin wax. Thin tissue sections were cut using a microtome and mounted on glass slides for staining.

The sections were stained with hematoxylin and eosin (H&E) to observe general tissue architecture and cellular organization. Additional special stains such as Masson's trichrome may also be used to evaluate collagen deposition and connective tissue formation. Histological examination was carried out under a light microscope to assess epithelialization, fibroblast proliferation, collagen fiber arrangement, angiogenesis, inflammatory cell infiltration, and reorganization of the dermal layer. The histopathological findings were compared among the different treatment groups to determine the extent of wound healing and tissue regeneration.

11. Statistical Analysis

All experiments were performed in triplicate or in the required number of replicates to ensure reproducibility and reliability of the results. Data obtained from the study were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using appropriate statistical software. One-way analysis of variance (ANOVA) followed by suitable post hoc tests was used to compare the differences among the experimental groups. A p-value of less than 0.05 was considered statistically significant. The statistical evaluation helped determine the effectiveness of the herbal bio-scaffold in comparison with control and standard treatment groups.

Results and discussion

1.Individual plant study.

1.Physicochemical constant Parameter –

Different physicochemical parameters such as Loss on drying, total ash value, acid insoluble ash value, water soluble ash value, extractive value, were determined and recorded. The above studies enable the identification of plant materials for further investigation and forms an important aspects of drug studies. The determination of ash value is helpful in detecting the quality and purity of crude drug especially in powder form. It showed the presence of inorganic matters. Extractive values are useful for

Table No.1.1. Physicochemical constant Parameter

Sr.no	Parameters	Observation
1	Foreign matter	5.0%
2	Total ash	11%
3	Water soluble ash	7%
4	Acid insoluble ash	3%
5	Loss on drying	8.4%
6	Swelling index	More than 1 cm
7	Foaming index	Less than 1 cm
8	Water soluble extractive value	18%w/w
9	Alcohol soluble extractive value	11%w/w

2.Preliminary screening by qualitative chemical tests

The extract obtained from successive solvent extraction were characterized by preliminary phytochemical tests for various secondary metabolites. As per (Table1) *Momordica dioica* fruit Aqueous extract shows the presence of Alkaloid, Flavonoid, Tannin & Phenolic, Saponin. And Alcoholic extract shows flavonoids and glycoside.

Table no.1 Shows the phytochemical screening analysis of crude Aqueous: Alcoholic extract obtained from Soxhlet extraction.

Table 1.2. Phytochemical analysis of crude Aqueous: Alcoholic extract

Sr.no	**Typeof phytoconstituents**	**Aqueous extract**	**Alcoholic extract**
1	Alkaloids	+	-
2	Tannins	+	-
3	Flavonoids	+	+
4	Saponin	+	-
5	Glycosides	-	+

+ positive, -Negative

1.2.Selection of fraction for Nanoparticle Formulation-:

Fig.No.01. fraction for Nanoparticle Formulation

1.3.Zone of inhibition (in mm)

Table No.2. Zone of inhibition

Sr.no	Microorganism	Control	Ethyl acetate	**Aqueous**	Pet ether	Diethyl ether	N - butanol
1	E.coil	10	15	**20**	8	9	8
2	S. aureus	7	17	**17**	4	6	3

3	P.aspergus	8	15	**15**	6	6	7

The anti-microbiology activity of *M. dioica*Roxb. (Cucurbitaceae) fruit was done. The different extracts of *M. dioica* were evaluated for antimicrobial activity by pour plate method. The diameter of the zone of inhibition against various Gram-positive and Gram-negative bacteria was measured. Turmeric was used as positive control. The results indicate all the extract of Momordica dioica fruits have antimicrobial property.

1.4.Characterization of Nanoparticles -

UV analysis –

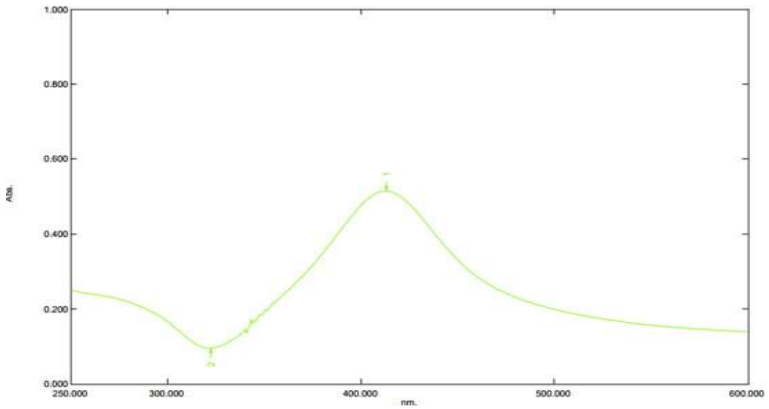


Fig.no.2. UV analysis

1.5.Particle Size Analysis-:

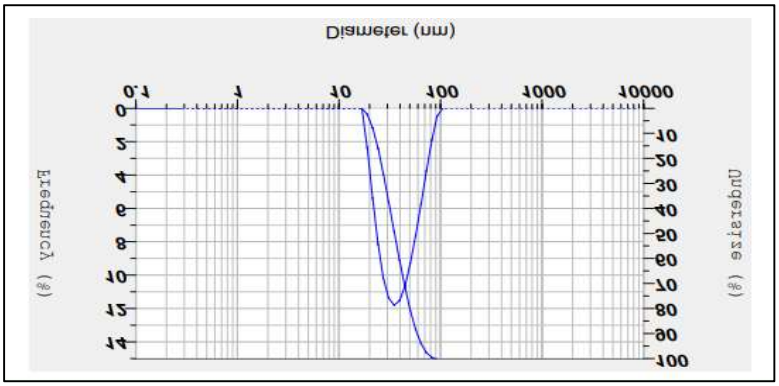


Fig.no.3. Particle Size Analysis

Two different populations were measured through DLS for variations. The population of sample was characterized by a larger mean size, amounting to wide range, having a very narrow scatter range.

1.6.Zeta potential -:

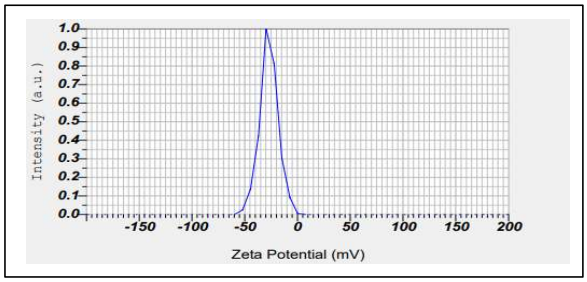


Fig.no.4. Zeta potential

1.7.FESEM-:(Filed emission scanning electron microscopy)

Field Emission Scanning Electron Microscopy (FE-SEM) was used to determine the morphological characteristics of the nanoparticles. Mainly the shape of the biosynthesized AgNPs were spherical, however, some irregularly shaped particles were marked. In addition, a few agglomerations were noticed.

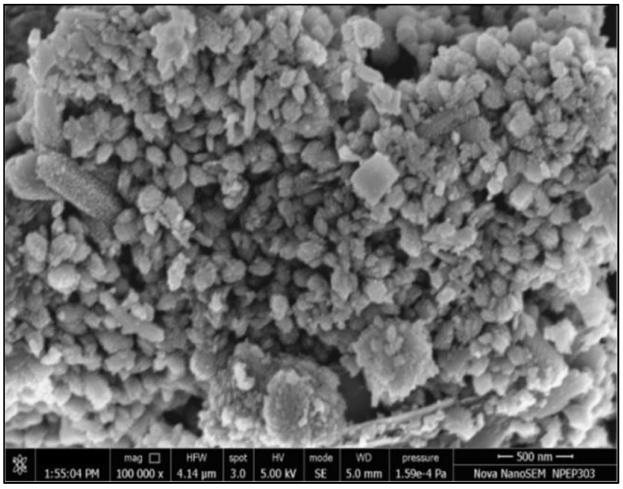


Fig.no.5. Filed emission scanning electron microscopy

1.8.EDS -:

Field Emission Scanning Electron Microscopy (FE-SEM) was used to determine the morphological characteristics of the nanoparticles. Mainly the shape of the biosynthesized AgNPs were spherical, however, some irregularly shaped particles were marked. In addition, a few agglomerations were noticed.

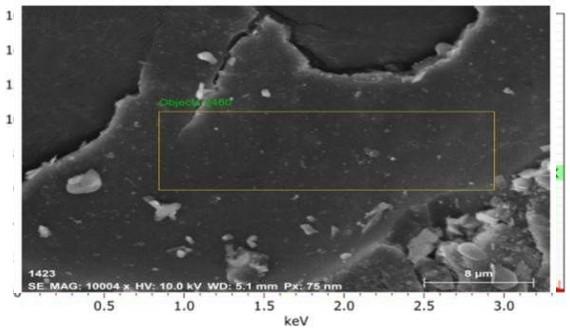


Fig.No.7.EDS

1.9.Nanofiber Preparation by Electrospinning Technique -:

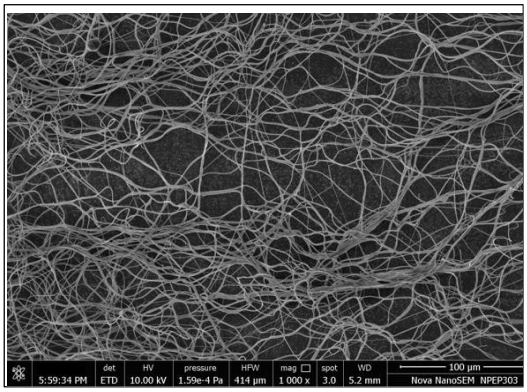


Fig.no.8. Nanofiber Preparation by Electrospinning Technique

FESEM (Field emission scanning electron microscopy) images reveals that there is the formation of smooth nanofibers with a uniform diameter as well as there is no bead formation.

2.Preparation of Bio- Scaffolds -:

The anti-microbiology activity of *M. dioica* Roxb. (Cucurbitaceae) fruit was done. The different extracts of *M. dioica* were evaluated for antimicrobial activity by agar disc diffusion test. The diameter of the zone of inhibition against various Gram-positive and Gram-negative bacteria was measured. Turmeric was used as positive control. The results indicate preparation of bio-scaffolds have antimicrobial property. The bio-scaffolds 48 (hrs) shows better anti-microbial activity as compare to bio-scaffolds 24 (hrs) of *M. dioica* Fruits.

2.1.Agar Disc Diffusion Test -:

Fig.no.9. Agar Disc Diffusion Test

Table no.3: Agar Disc Diffusion Test

SR. No	Micro-organism	control	Bio-scaffolds (24hrs)	Bioscaffolds (48hrs)

1	**E.coil**	10	14	16
2	**S.aureus**	7	15	16
3	**P.aspergus**	8	12	15

3.Pharmacological

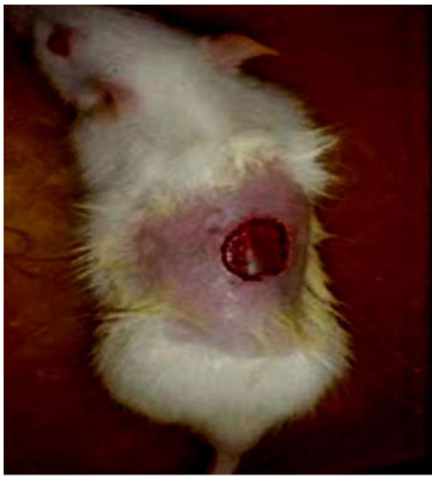
study -: Tissue Regeneration activity-:

The significant increase in the wound-healing activity was observed in the animals treated with the *aqueous* extracts and bio-scaffolds compared with those who received the placebo control treatments. In the excision wound model, aqueous extracts and bio scaffolds treated animals showed a significant reduction in the wound area and epithelialization period. The effect of bio scaffolds treated animals showed significant healing in 12 to 15 days, whereas the aqueous extract also showed healing in 12 to 15 days as compared control but less significant than bio scaffolds.

Table no:4: Tissue Regeneration activity

Sr.no	Groups	Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
1	Negative	10mm	12mm	12.5mm	12.5mm	14mm	14mm
2	Standard	10mm	10mm	8mm	7mm	5mm	2mm
3	Aq. extract	10mm	10mm	8.2mm	7.5mm	6.8mm	6.3mm
4	Bio - scaffolds	10mm	8.5mm	7.1mm	5.4mm	3mm	1.2mm

The significant increase in the wound-healing activity was observed in the animals treated with the *aqueous* extracts and bio-scaffolds compared with those who received the placebo control treatments. In the excision wound model, aqueous extracts and bio scaffolds treated animals showed a significant reduction in the wound area and epithelialization period. The effect of bio scaffolds treated animals showed significant healing in 12 to 15 days, whereas the aqueous extract also showed healing in 12 to 15 days as compared control but less significant than bio scaffolds.



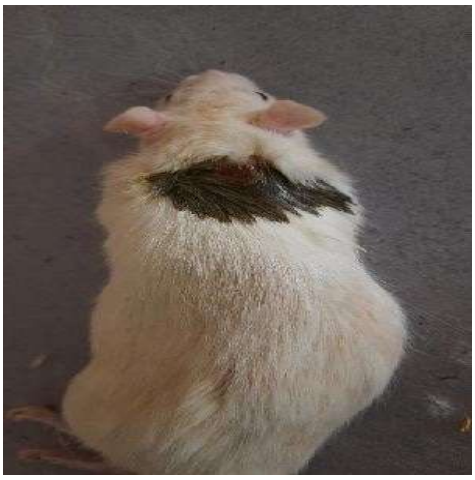


Fig.no.10. excision wound model

Discussion

The present study was carried out to develop and evaluate a herbal bio-scaffold containing green synthesized silver nanoparticles from *Momordica dioica* fruit extract for improved cutaneous tissue regeneration and wound healing. The study successfully demonstrated the potential of combining herbal bioactive compounds with nanotechnology and scaffold-based delivery systems for enhanced wound management.

Preliminary phytochemical screening revealed the presence of important secondary metabolites such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, and glycosides in the extracts of *Momordica dioica*. These phytoconstituents are known to possess antioxidant, antimicrobial, anti-inflammatory, and wound healing activities. The presence of these bioactive compounds supports the traditional medicinal use of *Momordica dioica* in the treatment of skin disorders and tissue injuries.

Among the different solvent fractions evaluated, the aqueous extract exhibited the highest antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas* species. The superior activity of the aqueous fraction may be attributed to the higher concentration of water-soluble phenolics, flavonoids, and glycosides that possess broad-spectrum antimicrobial effects. Therefore, the aqueous extract was selected for nanoparticle synthesis.

Green synthesis of silver nanoparticles using the aqueous extract was successfully achieved, as evidenced by the characteristic brown-yellow coloration and UV-visible spectroscopic analysis. Particle size analysis indicated the formation of nanoparticles with a narrow size distribution, suggesting uniformity and stability of the synthesized system. The zeta potential value of approximately –52 mV indicated excellent colloidal stability due to strong electrostatic repulsion between particles.

Morphological characterization by FESEM demonstrated predominantly spherical nanoparticles with minimal aggregation. EDS analysis confirmed the presence of elemental silver, indicating successful reduction of silver ions into metallic nanoparticles. These findings are consistent with previous reports on plant-mediated synthesis of silver nanoparticles and confirm the suitability of *Momordica dioica* extract as a reducing and stabilizing agent.

Nanofibers prepared by the electrospinning technique showed smooth morphology, uniform diameter, and absence of bead formation. Such structural characteristics are desirable for wound dressing applications because they mimic the extracellular matrix and provide an ideal microenvironment for cell attachment, proliferation, and tissue regeneration. Incorporation of silver nanoparticles within the nanofibers further enhanced their antimicrobial potential.

The prepared bio-scaffolds demonstrated significant antimicrobial activity in the agar disc diffusion assay. The zone of inhibition increased after 48 hours compared with 24 hours, indicating sustained release of active constituents from the scaffold matrix. The enhanced antimicrobial activity can be attributed to the synergistic effect of phytoconstituents and silver nanoparticles, which effectively inhibit microbial growth and reduce the risk of wound infection.

In the in vivo wound healing study, animals treated with nanoparticle-loaded bio-scaffolds exhibited accelerated wound contraction and reduced epithelialization time compared to the control group. Complete healing was observed

within 12–15 days, indicating enhanced tissue regeneration. The improved healing response may be due to the combined effects of antimicrobial action, antioxidant activity, stimulation of collagen synthesis, fibroblast proliferation, and enhanced angiogenesis. The scaffold structure also provided a moist wound environment that favors rapid tissue repair.

Overall, the results confirm that the developed herbal bio-scaffold is an effective wound healing system capable of promoting tissue regeneration while simultaneously preventing microbial infection. The integration of herbal medicine, green nanotechnology, and scaffold engineering offers a promising strategy for advanced wound care applications.

Conclusion

The present investigation successfully developed and evaluated a novel herbal bio-scaffold containing silver nanoparticles synthesized from *Momordica dioica* fruit extract for cutaneous tissue regeneration. Phytochemical studies confirmed the presence of biologically active constituents responsible for antimicrobial and wound healing activities. The aqueous extract exhibited superior antimicrobial activity and was selected for nanoparticle synthesis. The synthesized silver nanoparticles showed good stability, appropriate particle size distribution, spherical morphology, and high purity as confirmed by UV spectroscopy, particle size analysis, zeta potential measurements, FESEM, and EDS studies.

Electrospun nanofibers loaded with silver nanoparticles were successfully fabricated and incorporated into bio-scaffolds. The prepared bio-scaffolds demonstrated sustained antimicrobial activity against pathogenic microorganisms and significantly enhanced wound healing in the excision wound model.

The bio-scaffold treatment accelerated wound contraction, reduced epithelialization time, and promoted effective tissue regeneration compared with untreated controls. The combined action of phytoconstituents, silver nanoparticles, and nanofiber scaffolds contributed to improved healing outcomes.

Therefore, the developed *Momordica dioica*-based herbal bio-scaffold can be considered a promising, cost-effective, biocompatible, and sustainable wound dressing system for cutaneous tissue regeneration. Further studies involving detailed histopathological evaluation, toxicity assessment, and clinical investigations are recommended to establish its therapeutic potential for human wound care applications.

Acknowledgement

The authors sincerely thank the Department of Pharmaceutical Chemistry, SCSSS's Sitabai Thite College of Pharmacy, Shirur, for providing research facilities. The authors are also grateful to Mr. Gayatri Shankar Dhobale and Prof. Dinesh Kumar Agarwal, Department of Pharmacology Pacific Academy of higher education and research university, Udaipur. for their valuable guidance and support.

References

1. Ahmed, S., Ahmad, M., Swami, B.L. and Ikram, S. (2016) 'Green synthesis of silver nanoparticles using plant extracts and their applications', *Journal of Advanced Research*, 7(1), pp. 17–28.
2. Atiyeh, B.S., Costagliola, M., Hayek, S.N. and Dibo, S.A. (2007) 'Effect of silver on burn wound infection control and healing', *Burns*, 33(2), pp. 139–148.
3. Augustine, R., Kalarikkal, N. and Thomas, S. (2016) 'Electrospun PCL membranes incorporated with biosynthesized silver nanoparticles as antibacterial wound dressings', *Applied Nanoscience*, 6(3), pp. 337–344.
4. Balaji, A., Vellayappan, M.V., John, A.A. and Subramanian, A.P. (2020) 'Biomaterials-based nanoscaffolds for skin tissue engineering applications', *Journal of Drug Delivery Science and Technology*, 55, p. 101452.
5. Bhardwaj, N. and Kundu, S.C. (2010) 'Electrospinning: A fascinating fiber fabrication technique', *Biotechnology Advances*, 28(3), pp. 325–347.
6. Bhattacharya, S. and Sen, S.K. (2018) 'Green synthesis of silver nanoparticles and their antimicrobial activity', *International Journal of Pharmaceutical Sciences and Research*, 9(4), pp. 1201–1210.
7. Boateng, J.S., Matthews, K.H., Stevens, H.N.E. and Eccleston, G.M. (2008) 'Wound healing dressings and drug delivery systems: A review', *Journal of Pharmaceutical Sciences*, 97(8), pp. 2892–2923.
8. Dash, G.K. and Subramanium, J. (2010) 'Phytochemical and pharmacological evaluation of Momordica dioica fruits', *Indian Journal of Natural Products and Resources*, 1(1), pp. 95–100.
9. Dhiman, N.K., Sharma, M. and Kumar, A. (2012) 'Medicinal properties of Momordica dioica: A review', *International Journal of Pharmaceutical Sciences Review and Research*, 12(1), pp. 35–39.
10. Gupta, A., Mumtaz, S., Li, C.H., Hussain, I. and Rotello, V.M. (2019) 'Combatting antibiotic-resistant bacteria using nanomaterials', *Chemical Society Reviews*, 48(2), pp. 415–427.
11. Iravani, S. (2011) 'Green synthesis of metal nanoparticles using plants', *Green Chemistry*, 13(10), pp. 2638–2650.

12. Jain, A. and Singhai, A.K. (2011) 'Pharmacological and phytochemical profile of Momordica dioica Roxb.', *Asian Journal of Traditional Medicines*, 6(2), pp. 56–72.
13. Lansdown, A.B.G. (2004) 'A review of the use of silver in wound care', *International Wound Journal*, 1(1), pp. 59–79.
14. Rai, M., Yadav, A. and Gade, A. (2009) 'Silver nanoparticles as a new generation of antimicrobials', *Biotechnology Advances*, 27(1), pp. 76–83.
15. Singer, A.J. and Clark, R.A.F. (1999) 'Cutaneous wound healing', *New England Journal of Medicine*, 341(10), pp. 738–746.
16. Kuppusamy, P., Yusoff, M.M., Maniam, G.P. and Govindan, N. (2016) 'Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – an updated report', *Saudi Pharmaceutical Journal*, 24(4), pp. 473–484.
17. Li, W.J., Laurencin, C.T., Caterson, E.J., Tuan, R.S. and Ko, F.K. (2002) 'Electrospun nanofibrous structure: a novel scaffold for tissue engineering', *Journal of Biomedical Materials Research*, 60(4), pp. 613–621.
18. Pham, Q.P., Sharma, U. and Mikos, A.G. (2006) 'Electrospinning of polymeric nanofibers for tissue engineering applications: a review', *Tissue Engineering*, 12(5), pp. 1197–1211.
19. Percival, S.L., Bowler, P.G. and Russell, D. (2005) 'Bacterial resistance to silver in wound care', *Journal of Hospital Infection*, 60(1), pp. 1–7.
20. Rujitanaroj, P.O., Pimpha, N. and Supaphol, P. (2008) 'Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles', *Polymer*, 49(21), pp. 4723–4732.
21. Sondi, I. and Salopek-Sondi, B. (2004) 'Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria', *Journal of Colloid and Interface Science*, 275(1), pp. 177–182.