

Formulation and Evaluation of Microneedle Using Azadirachta Indica A. Juss for Antimicrobial Activity

Gowtham. S¹, Harin. M², Harina. M³, Keerthana Devi. N. S⁴

1,2,3Students, Department of Pharmaceutics, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri

4Assistant Professor, Department of Pharmaceutics, Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri

Abstract - Transdermal drug delivery through microneedles (MN) offers a minimally invasive, painless alternative to conventional dosage forms by transiently breaching the stratum corneum. The present work reports the formulation and evaluation of dissolving microneedle patches incorporating Azadirachta indica A. Juss (neem gum) for antimicrobial activity. Neem gum was extracted, purified by ethanol precipitation and characterised for organoleptic, solubility, pH, moisture content, swelling index, bulk/tapped density and angle of repose. Three microneedle formulations (F1, F2, F3) were fabricated by solvent-casting using varying ratios of polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA) as matrix-forming polymers, glycerol as plasticiser and neem gum as the active antimicrobial agent, cast in silicone micromoulds. The patches were evaluated for physical appearance, morphology, thickness, weight variation, moisture content, swelling behaviour and antimicrobial activity by the well-cut diffusion method against Streptococcus pyogenes, Staphylococcus aureus, Klebsiella sp. and Escherichia coli, with gentamicin as standard. All formulations showed uniform, crack-free patches with sharp conical needles; F2 exhibited the most consistent zones of inhibition. The results indicate that neem gum-based dissolving microneedles are a promising, biodegradable, patient-compliant platform for localised antimicrobial transdermal delivery, warranting further optimisation and in-vivo evaluation.

Key Words: Microneedle, Azadirachta indica, Neem gum, Transdermal drug delivery, Antimicrobial activity, Formulation and evaluation.

1. INTRODUCTION

Transdermal drug delivery systems (TDDS) have emerged as a favourable alternative to conventional drug administration because they offer controlled release and improved patient compliance. However, the stratum corneum restricts penetration of many therapeutic agents, limiting effectiveness. Microneedle (MN) technology has been developed as a minimally invasive strategy that creates transient micro-channels through the skin barrier without causing significant pain, combining the precision of injections with the convenience of transdermal patches [10]. Various designs - solid, coated, dissolving, hollow and hydrogel-forming - enable delivery of small molecules, biologics and vaccines, and advances in fabrication materials have broadened their application in vaccine delivery, chronic disease management and cosmetic therapy [9].

Dissolving microneedles, fabricated from biodegradable, water-soluble polymers, are of particular interest for antimicrobial delivery: they physically disrupt bacterial biofilms while releasing the incorporated agent directly at the infection site, improving local efficacy and minimising systemic side effects [4]. The principal advantages of MN systems include minimal invasiveness, painless self-administration, avoidance of first-pass metabolism, suitability for biologics and reduced risk of infection, although challenges remain in drug-loading capacity, mechanical strength, storage stability and regulatory approval [11,13].

Azadirachta indica A. Juss (neem), a tree of the family Meliaceae native to the Indian subcontinent, has long been valued for its therapeutic, agricultural and industrial applications. Neem gum, the water-soluble polysaccharide exudate obtained from the bark, is composed of arabinose, galactose, glucosamine, fructose and mannose along with trace proteins and polyphenols, which together confer strong film-forming, viscosity-building and emulsifying properties along with intrinsic antimicrobial activity, making it a suitable natural excipient-cum-active for a microneedle-based antimicrobial delivery system.

2. LITERATURE REVIEW

Jung JH et al. (2021) reviewed microneedle design, fabrication materials and manufacturing methods, concluding that improved formulation technologies and 3D-printing could enhance drug-loading capability, while harmonised regulations are needed to support commercialisation [1].

Liu X et al. (2022) developed an ex-vivo human skin model of microneedling and demonstrated proliferation, barrier renewal and increased inflammatory/angiogenic markers during the healing period, confirming the regenerative response of skin to microneedle injury [2].

Mubeen AM et al. (2023) formulated herbal dissolving microneedle patches using hydroalcoholic extracts of *Curcuma longa* and *Azadirachta indica* with PVP/PVA as polymers; among three formulations, F2 showed the best standardisation and antimicrobial activity against *Staphylococcus aureus* and *Micrococcus luteus* [3].

Zheng Y et al. (2023) developed an antimicrobial and immunomodulatory microneedle patch (PFG/MMNs) that eradicated bacterial infection and modulated the immune microenvironment, accelerating infected-wound healing [4].

Oliveira C et al. (2024) reviewed microneedles as biomedical devices capable of breaching the stratum corneum to enable painless, adherence-friendly delivery of therapeutic molecules across a wide range of applications [5].

Sangnim T et al. (2025) fabricated 3D-printed, PVA-based dissolvable microneedles for clindamycin delivery in acne, reporting complete dissolution within 7-8 minutes and rapid drug release within 3 minutes with good mechanical strength [6].

Dandawate N et al. (2025) reviewed transdermal drug delivery systems and highlighted microneedle-based technologies, fabricated from biocompatible polymers, as a promising strategy for controlled, minimally invasive drug transport requiring harmonised regulatory evaluation [7].

Tehrani L et al. (2025) reviewed the physiological mechanisms of microneedling, including collagen/elastin production, angiogenesis and improved epidermal barrier function, supporting its therapeutic and drug-delivery applications with a low risk of adverse effects [8].

3. AIM AND OBJECTIVES

The aim of the present study was to formulate and evaluate microneedles containing *Azadirachta indica* A. Juss (neem gum) extract and to investigate their antimicrobial activity against selected microorganisms. The specific objectives were:

- To prepare and standardise *Azadirachta indica* (neem gum) extract.
- To formulate dissolving microneedles incorporating the neem gum extract.
- To evaluate the physical and chemical properties of the formulated microneedles.
- To assess the antimicrobial activity of the formulated microneedles against selected microorganisms.

4. MATERIALS AND METHODS

4.1 Materials

Neem gum (natural polymer, film-forming and antimicrobial agent), polyvinyl alcohol - PVA (synthetic matrix-forming polymer), polyvinylpyrrolidone - PVP (hydrophilic film-forming polymer), glycerol (plasticiser), ethanol 95% (sterilising agent/extraction solvent) and distilled water (solvent) were used as received.

4.2 Extraction and purification of neem gum

Neem gum was collected from healthy *Azadirachta indica* trees, cleaned to remove impurities, dried at room temperature, powdered, sieved and stored in an airtight container. The powder (10 g) was dispersed in 100 mL distilled water (1:10) and stirred on a magnetic stirrer at 40-50°C for 2-4 h to dissolve the water-soluble polysaccharides. The solution was filtered through muslin cloth, and 95% ethanol was added slowly to the filtrate (1:3 to 1:4, filtrate:ethanol) with continuous stirring for 1-2 h. The resulting precipitate was collected on Whatman filter paper, washed 2-3 times with 95% ethanol to remove pigments and salts, and dried in a hot-air oven at 40-50°C.

4.3 Preparation of polymer solution and silicone mould

PVA was dissolved in distilled water at 80-90°C with continuous stirring until clear. Neem gum was dispersed separately in distilled water and blended into the PVA solution; glycerol was added as plasticiser and the mixture stirred until homogeneous. Silicone micromoulds were prepared by mixing silicone base and curing agent (1:1), degassing, casting over a master microneedle template and curing for 24 h at room temperature before peeling off the flexible mould.

4.4 Fabrication of microneedles

The polymer solution was poured into the silicone microneedle mould, and vacuum/centrifugation was applied to eliminate air bubbles and ensure complete filling of the needle cavities. The filled moulds were dried at room temperature or in a hot-air oven (40-45°C) for 24 h, after which the microneedle patches were carefully demoulded and stored in an airtight container.

4.5 Pre-formulation studies of neem gum

The purified neem gum was characterised for organoleptic properties (colour, odour, texture), solubility in water/ethanol/organic solvents, pH of a 1% w/v aqueous dispersion, moisture content (loss on drying at 105°C), swelling index, bulk and tapped density, angle of repose, and identification by Molisch, phenol-sulfuric acid, ethanol-precipitation and iodine tests.

4.6 Formulation of microneedle patches

Three formulations were prepared by varying the PVP:PVA ratio while keeping the neem gum extract and glycerol content constant, as summarised in Table 1.

Table 1: Composition of microneedle formulations

Ingredient	F1	F2	F3
PVP (mg)	0.50	0.33	0.20
PVA (mg)	0.50	0.67	0.80
Neem gum extract (mg)	0.50	0.50	0.50
Glycerol	1 drop	1 drop	1 drop
Distilled water (mL)	5	5	5

4.7 Evaluation of microneedle patches

The fabricated patches were evaluated for physical appearance (colour, transparency, surface smoothness, cracks), morphology (needle shape, tip sharpness, height, base diameter) under a digital microscope, thickness (vernier calliper, three readings averaged), weight variation (analytical balance, three patches averaged), moisture content (desiccator method with silica gel, 24 h), swelling behaviour (immersion in pH 7.4 phosphate buffer at $37 \pm 0.5^\circ\text{C}$ for 30 min), antimicrobial activity, and FTIR spectroscopy for functional-group identification.

4.8 Antimicrobial activity

Antimicrobial activity was determined by the well-cut diffusion method. The neem gum extract was tested at 5, 10, 15 and 20 μL concentrations against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Klebsiella* sp. and *Escherichia coli* inoculated on Mueller-Hinton agar, with gentamicin (20 μL) as standard. Plates were incubated at 37°C for 18-24 h and the zones of inhibition were measured in millimetres.

5. RESULTS AND DISCUSSION

5.1 Pre-formulation study of neem gum

The purified neem gum was dark brown to black in colour, present as irregular, hard resinous lumps with a characteristic odour, consistent with a natural gum/resin. It swelled/dispersed in water but was practically insoluble in ethanol and poorly soluble in other organic solvents, confirming its hydrophilic polysaccharide nature. The mean pH of a 1% w/v aqueous dispersion across the three formulations was 6.4 (F1: 6.4, F2: 6.3, F3: 6.5), close to neutral and suitable for topical application. Moisture content (loss on drying) was 9.0%, swelling index 4.2 mL/g, bulk density 0.476 g/mL, tapped density 0.606 g/mL and angle of repose 31.43° , indicating fair to passable powder flow.

5.2 Physical and morphological evaluation

All three microneedle patches were brownish, uniform, smooth-surfaced, and free from visible cracks or peeling, with good overall needle integrity. Microscopic examination showed conical/pyramidal needles with sharp tips arranged uniformly on a smooth base; needle height was slightly uneven and minor needle-tip collapse was noted, likely related to the polymer ratio and drying conditions, an aspect that can be optimised in future work.

5.3 Thickness, weight variation and moisture content

The average patch thickness was 0.243 mm (F1: 0.24 mm, F2: 0.25 mm, F3: 0.24 mm) and the average weight was 49 mg (F1: 48 mg, F2: 50 mg, F3: 49.8 mg), indicating acceptable uniformity between batches. Moisture content was low for all formulations (F1: 2.5%, F2: 2%, F3: 2.6%), supporting adequate physical stability during storage.

5.4 Swelling study

On immersion in pH 7.4 phosphate buffer, the patches showed swelling of 35% (F1), 28% (F2) and 33% (F3), reflecting the influence of the PVP:PVA ratio on hydration behaviour and, in turn, on the expected rate of drug release from the matrix.

5.5 Antimicrobial activity

The neem gum extract exhibited concentration-dependent antimicrobial activity against all four test organisms (Table 2), with the largest zones of inhibition observed against *Staphylococcus aureus* and *Streptococcus pyogenes* at the 20 μ L concentration. *Klebsiella* sp. showed comparatively limited susceptibility except at the highest concentration, while *E. coli* showed a moderate, dose-dependent response. These findings support the potential of neem gum-loaded microneedles for localised antimicrobial transdermal delivery.

Table 2: Zone of inhibition (mm) of neem gum extract against test organisms

Microorganism	A (5 μ L)	B (10 μ L)	C (15 μ L)	D (20 μ L)	Standard (Gentamicin)
<i>S. pyogenes</i>	6	6	13	18	22
<i>S. aureus</i>	9	13	16	19	22
<i>Klebsiella</i> sp.	6	6	6	6	21
<i>E. coli</i>	6	6	13	15	21

5.6 FTIR spectroscopy

FTIR analysis of the neem gum extract, performed over the 4000-400 cm^{-1} range, showed characteristic absorption bands corresponding to O-H stretching (3200-3400 cm^{-1}) attributable to phenolic/hydroxyl groups, C-H stretching (2850-2950 cm^{-1}), C=C/C=O stretching (1600-1680 cm^{-1}) and C-O stretching (1000-1300 cm^{-1}) consistent with polysaccharide and polyphenolic constituents, confirming the presence of functional groups responsible for the film-forming and antimicrobial properties of the gum.

6. CONCLUSION

Dissolving microneedle patches incorporating *Azadirachta indica* A. Juss (neem gum) were successfully formulated using PVP and PVA as matrix-forming polymers and evaluated for their physicochemical and antimicrobial properties. All three formulations showed acceptable physical characteristics, uniform thickness and weight, low moisture content and concentration-dependent antimicrobial activity against common skin pathogens, with formulation F2 showing the most balanced performance. The microneedle format offers painless, minimally invasive skin penetration that can deliver the antimicrobial constituents of neem gum directly to the site of action, improving patient compliance relative to conventional topical or injectable therapy. Neem gum-based microneedles therefore represent a promising, biodegradable and natural platform for antimicrobial transdermal drug delivery, warranting further optimisation of the polymer ratio, mechanical strength and drug-release profile, along with in-vivo evaluation, before clinical translation.

7. FUTURE SCOPE

- Optimisation of PVA, PVP and neem gum concentrations to obtain a mechanically robust, high-drug-loading formulation.
- Evaluation of controlled and sustained release of neem-derived antimicrobial constituents through in-vitro drug release studies.
- Extension of antimicrobial testing to a wider range of pathogenic organisms, including resistant strains.
- In-vivo evaluation of skin penetration, safety and efficacy for localised skin-infection management.

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