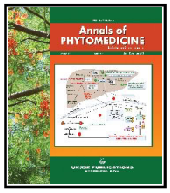
Annals of Phytomedicine 12(2): 720-729, 2023

DOI: http://dx.doi.org/10.54085/ap.2023.12.2.85

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885



Original Article : Open Access

# Formulation development and *in vivo* evaluation of nanoemulgel containing *Adhatoda vasaka* (L.) Nees extract for wound healing activity

**Nidhi Kumari, Juber Akhtar◆, Badruddeen, Mohammad Irfan Khan and Mohammad Ahmad**
*Faculty of Pharmacy, Integral University, Dasauli, Lucknow-226026, Uttar Pradesh, India*

**Article Info**

**Article history**
Received 20 July 2023
Revised 4 September 2023
Accepted 5 September 2023
Published Online 30 December 2023

**Keywords**
*Athatoda vasaka* (L.) Nees extract
Coconut oil
High Performance
Liquid Chromatography
Transmission Electron Microscopy
Interleukin-6
C-reactive protein

**Abstract**

Development, characterization and biological evaluation of nanoemulgel loaded with *Adhatoda vasaka* (L.) Nees (also called *Adhatoda vasica*) extract (VE) were done. Nanoemulgels have the potential for the delivery of numerous herbal medications. Traditional remedies may be favored over contemporary treatments due to their cost-effectiveness, limited adverse effects, and efficacy.

*A. vasaka* extract (VE) was prepared by the cold percolation technique, while the nanoemulgel containing VE was developed through homogenization. Chemical test; namely, Dragendroff's, Mayer's and Hager's tests were conducted to confirm the presence of alkaloids in the formulated nanoemulgel enriched with VE. High Performance Liquid Chromatography (HPLC) technique was used for the qualitative analysis of both *A. vasaka* extract. Among the six formulations, one formulation (VENEG4) exhibited stability, with no instances of phase separation or color alteration observed. The pH of VENEG4 nanoemulgel formulation was found to be 6.5 ± 0.98. Transmission Electron Microscopy (TEM) analysis revealed that the globules in VENEG4 were within the nano range (<100 nm). *In vivo* wound healing study, histological and biomarker analysis showed more effective wound healing in VENEG4 treated group than other groups. The result of our study indicates that nanoemulgel containing *A. vasaka* extract exhibits wound-healing potential.

## 1. Introduction

The process of wound healing is a highly intricate and crucially regulated series of carefully organized biochemical and cellular events aimed at restoring the skin's integrity. In the course of wound healing, the wound advances through three distinct but inter-related events: inflammation, proliferation (neo-angiogenesis, granulation, and re-epithelialization), and maturation (extracellular matrix remodeling). The choice of wound dressing materials can significantly impact wound management and the effectiveness of tissue occlusion during healing (Singh *et al.,* 2010). Numerous studies explore the role of traditional wound healing therapies in addressing the root causes of non-healing wounds, combining both experimental and clinical investigations.

Topical drug delivery for wound healing is reliable due to the accessibility of the skin, which serves as the primary route for such drug administration. Clinical evidence supports the safety and effectiveness of topical gels in managing skin-related diseases. Topical preparations are administered to the skin with the purpose of achieving a range of effects, including surface-level actions and localized therapeutic benefits. In certain instances, the base may be employed on its own due to its therapeutic attributes, such as its emollient, soothing, or protective properties. However, numerous topical preparations include active therapeutic ingredients that are either dispersed or dissolved within the base (Bhowmik, 2012).

Topical drug delivery has advantages including easy administration, patient cooperation, improved compliance, and avoiding first-pass metabolism (Algahtani *et al.,* 2020). Disadvantages of topical drug delivery include limited or slower absorption rates and cosmetic considerations (Qais *et al.,* 2019). Incorporating new drug delivery tools and penetration enhancers, can address some of these concerns effectively (Tadwee *et al.,* 2012).

Medicinal plants hold great therapeutic potential for wound healing due to the variety of active components present in it. These include flavonoids, essential oils, alkaloids, phenolic compounds, terpenoids, and fatty acids. Traditional medicines may be favored over modern therapy due to their affordability, minimal adverse effects, bioavailability, and effectiveness. Over the past decade, numerous nanoformulations comprised of novel drug carriers have emerged for effectively delivery of various herbal drugs (Kakkar *et al.,* 2016).

Wound healing activity and fungal infection (Chaughule *et al.,* 2023) has been reported for certain plants, including turmeric, aloe, walnut, coconut, and vasaka (Poorniammal *et al.,* 2022). *A. vasaka* commonly known as Malabar nut belonging to the Acanthaceae family, holds a prominent place in Ayurveda and Unani systems of medicine (Gangwar *et al.,* 2014). *A. vasaka* contains bioactive phytochemicals, such as vasicine (Jahangir *et al.,* 2023), a heterocyclic alkaloid with a

**Corresponding author: Dr. Juber Akhtar**
***Professor,*** *Faculty of Pharmacy, Integral University, Dasauli, Lucknow-226026, Uttar Pradesh, India*
**E-mail: juberakhtar@gmail.com**
**Tel.: +91-8050347455**

quinazoline nucleus (Nepali *et al.,* 2013) that was first isolated in 1924 (Basu *et al.,* 2013), along with vasicinone, vasicine acetate, vasicinolone, and 2-acetyl benzyl amine (Sayeed *et al.,* 2009). These compounds are reported to exhibit anti-inflammatory, antimicrobial, and wound healing properties. Vasicine has been explored for its potential in treating dengue-associated thrombocytopenia (Thangaraju *et al.,* 2022), inhibiting the activity of phosphodiesterase 7B (Balasundaram *et al.,* 2023), and addressing non-ulcer dyspepsia (Chaturvedi *et al.,* 1983). A semisynthetic derivative of vasicine has demonstrated a beneficial effect in the treatment of Alzheimer's disease (Bhanukiran *et al.,* 2023).

Numerous reports have highlighted the versatile properties of *A. vasaka* (Roy *et al.,*2013) including its potent anti-inflammatory (Kashyap *et al.,*2016; Singh *et al.,* 2013), protective effect against myocardial infarction (Jiang *et al.,* 2019), antimicrobial (Khambhatya *et al.,* 2023), antidiabetic (Sharma *et al.*, 2022), expectorant (Claeson *et al.,* 2000), bronchodilator/antitussive (Gupta *et al.*, 2010; Dhuley, 1999; Amin *et al.,* 1959; Cepae, 1999; Johri, 2000; Gholami *et al.,* 2022; Wenger *et al.,* 2023), antiasthmatic (Raghunath *et al., 2023)*, anticancer (Monika *et al.*, 2020; Singh *et al.,* 2023; Ahmad *et al.,* 2017; Manogaran *et al*., 2023), abortifacient (Nath *et al.,* 1992; Nirmala *et al.,* 2023), wound healing (Khan *et al.,*2020), and antiulcer (Shrivastava *et al.,*2006) activities. Additionally, it has been utilized to treat cold in pediatric patients (Sultana *et al.,* 2013). Furthermore, there is emerging evidence of its effectiveness in managing Covid-19 (Quazi *et al.*, 2023; Singh *et al.,* 2023; Kumar *et al.,* 2023; Ghoneum *et al.,* 2023; Fan *et al.,* 2023). Enhancing the effectiveness of medicinal plants in wound management involves employing nanosizing techniques or incorporating them into nanostructures, complementing their inherent benefits (Kumari *et al.,* 2021). Nanomaterials exhibit exceptional properties owing to their minute size and remarkable surface area-to-volume ratio. These unique characteristics make them a promising potential to increase the bioavailability and efficacy of these plant-derived compounds.

Nanogels are defined as minute particles resulting from the physical or chemical cross linking of polymer networks, which exhibit swelling characteristics in a compatible solvent. The term "nanogel" (Nanogel™) was initially coined to describe cross-linked networks formed from a positively charged polymer (polyethyleneimine or PEI) and a neutral polymer (polyethylene glycol or PEG), with the purpose of delivering polynucleotides (Aishwharyah *et al.*, 2012). The rapid advancements in nanotechnology have underline the necessity of developing nanogel systems, as they have demonstrated their capability to deliver drugs in a controlled, sustained, and targeted manner. The rapid growth of polymer sciences has made it imperative to create elegant nanosystems that can effectively contribute to both treatment and the advancement of clinical trials (Dorwal, 2012; Kumar *et al.,* 2022). In recent times, a novel approach called nanoemulgel, combining nanoemulsion and gel, has garnered attention for its numerous advantages, such as enhanced physical stability, non-toxicity, and non-irritating properties (Jung *et al.,* 2008; Mirza *et al.,* 2015). The utilization of nanoemulgel in topical drug delivery has the potential to improve the effectiveness, tolerability, and cosmetic appeal of topical formulations (Choudhury *et al.,* 2017; Donthi *et al.,* 2023; Tayah *et al.*, 2023; Atmakuri *et al.,*2023; Adem *et al.,* 2023; Mandal *et al.,* 2023; Dhawanand, 2023; Priyadarshini *et al.,* 2023; Date *et al.,* 2006; Katz *et al.,*1999). Nanoemulgel for topical drug delivery presents greater advantages compared to lipophilic formulations (Ting *et al.,* 2004; Bashir *et al.,* 2021; Sengupta *et al.*, 2017; Ojha *et al.,* 2022; Jeengar *et al.,* 2016). Nanoformulations containing alkaloids have demonstrated effective results in numerous diseases (Chellapa *et al.,* 2025; Contreras-Angulo *et al.,* 2023).Taking into account the factors mentioned earlier, our study concentrated on the utilization of *A. vasaka* extract to create a nanoemulgel formulation. This research aimed to investigate the potential benefits and applications of *A. vasaka* extract within the context of nanoemulsion based gels.

## 2. Materials and Methods

*A. vasaka* leaves were acquired from the local market in Aminabad, Lucknow, India. Parachute coconut oil was sourced from Marico India Limited located in Himachal Pradesh, India. Polyoxyethylene sorbitan monooleate (Tween 80), Span 80, and water were obtained from Merck in Germany. The Milli-Q water purification system (Millipore) was sourced from the USA. Polyethylene glycol 400, Carbopol 934, Ethanol, and other utilized reagents were of analytical reagent (AR) grade and were procured from Merck in Mumbai, India.

### 2.1 Plant material and extraction process

The extract from *A. vasaka* leaves was prepared using the cold percolation technique. This involved placing coarsely powdered *A. vasaka* leaves in an ethanol (Palshikar *et al.,* 2023) menstruum within a percolator for 48 h. Subsequently, the miscella (extract) filter obtained from the percolator was distilled on a water bath, resulting in the formation of a dark green residue. The extraction process was assisted by a vacuum pump (Duraipandiyan *et al.,* 2015; Karthikeyan *et al.,* 2009; Pankaj *et al.,* 2022).

### 2.2 Identification

#### 2.2.1 Phytochemical test for alkaloids

##### 2.2.1.1 Dragendorff's test

Upon mixing 2 ml of the extract with 1 ml of Dragendorff's reagent, an orange-red precipitate emerged.

##### 2.2.1.2 Mayer's test

The addition of a few drops of Mayer's reagent to 1 ml of the extract resulted in the formation of a yellowish-white precipitate.

##### 2.2.1.3 Hager's test

A yellow precipitate, indicative of the presence of alkaloids, was formed when a few drops of Hager's reagent were mixed with 1 ml of the extract indicating that *A. vasaka* extract contains alkaloids.

### 2.3 HPLC analysis

High Performance Liquid Chromatography (HPLC) technique was employed for analysis of plant extract. The analytical procedure involved the constant flow of a mobile phase through the analytical column. The mobile phase comprised 0.1% v/v trifluoroacetic acid (A) along with a mixture of methanol and acetonitrile (45:45) in 80:20 ratios (B). The flow rate was maintained at 0.5 mlf/min. To introduce the sample, a direct injection of approximately 20 µl was performed at the designated injection point. Detection of the components took place using a UV detector set at a wavelength of 282 nm (Nandhini *et al*., 2021). The optimized chromatographic conditions are detailed in Table 1.

**Table 1: Chromatographic conditions**

| Parameters | Setting applied |
|---|---|
| Mobile phase | 0.1% v/v trifluoroacetic acid (A) and methanol:acetonitrile (45:45) (B) with a ratio of (80:20) |
| Column | $C_{18}$ ODS analytical column with dimension 250 × 4.6 mm, 5 µm particle size |
| Flow rate | 0.5 ml/min |
| Wavelength | 282 nm |
| Injection volume | 20 µl |
| Software used | Shimadzu's LC solution |

### 2.4 Preparation of nanoemulsion

Nanomulsion was prepared by adding drug (1 g) in coconut oil (2.78 g), polyethylene glycol 400 (0.2 g), ethanol (0.08 g) and water followed by vortexing for 15 min and ulrasonication for 90 min using the bath sonicator.

### 2.5 Development of *A. vasaka* extract loaded nanoemulgel

The nanoemulgel was fabricated using a sequential process, starting with vortexing and followed by homogenization through sonication. The nanoemulsion containing the extract was gradually incorporated into a gel base consisting of 1 g of Carbopol 934, previously hydrated with an adequate volume of distilled water. This mixture was agitated using a mixer for 5 min until a uniform nanoemulgel was achieved (Ibrahim *et al*., 2012).

### 2.6 Stability study of prepared *A. vasaka* extract nanogel

The prepared nanoemulgels were placed in airtight glass vials and exposed to accelerated stability tests in accordance with WHO guidelines. A temperature of 40 ± 2°C and a relative humidity of 75 ± 5% was maintained at for duration of 6 months

### 2.7 Characterization of optimized nanoemulgel of *A. vasaka* extract

The pH, spreadability, viscosity and droplet size of *A. vasaka* extract nanoemulgel was determined.

#### 2.7.1 pH determination

The pH was measured by using a digital pH meter (HI-TECH WATER TECH. New Delhi, India). The pH meter was first calibrated using buffer tablet, the pH meter was dipped in a beaker containing *A. vasaka* extract nanoemulgel on post calibration. Maintaining the neutral pH is crucial to prevent skin irritation when applying a topical formulation (Asadinezhad *et al.,* 2019).

#### 2.7.2 Spreadability and extrudability

1 g of nanoemulgel was utilized for spreadability and 10 g for extrudability. The weighed quantity of nanoemulgel was applied to one glass slide, and another glass slide (25 cm × 25 cm) was placed on top of the nanoemulgel in such a way as to sandwich the sample between the two glass slides. The samples were then pressed between the upper and bottom glass slides using 100 g of weight, creating a consistent thin coating, and a portion of the excess sample was removed. Only the upper glass slide was removed effortlessly after being tied with a 20 g weight, as the other slide was securely fastened to the platform with minimal disruption. Spreadability was calculated from the length of time taken to slide the upper glass 7.50 cm over the thin sheet on the lower glass slide. Whereas to determine the extrudability of prepared nanoemulgel formulations, the amount of nanoemulgel extruding from collapsible tubes was evaluated. A weighed amount of each of the two types of gel formulation, *i.e.,* nanoemulgel and conventional gel, were placed in collapsible tubes. After that, the extrudability (g $cm^{-2}$) was calculated based on the weight (g) necessary to extrude a 1 cm ribbon of the formulations from the collapsible tubes.

#### 2.7.3 Viscosity

Viscosity measurements were performed utilizing a Brookfield viscometer equipped with spindle number R5, revolving at a rate of 5 rpm at 25 ± 0.5°C. Wait time for the operation was 30 min.

#### 2.7.4 Droplet size

The size and morphology of the nanoemulgel was analyzed using a transmission electron microscope operating at 200 kV. A drop of nanoemulgel was applied to a 300 mesh copper grid and was leave there for 1 min.

### 2.8 *In vivo* animal study of nanoemulgel for wound healing activity

#### 2.8.1 Animals

Sprague Dawley (SD) rats, with body weights ranging from 150 to 200 g, were sourced from the Animal House Facility at CSIR CDRI, Lucknow, India. These rats were accommodated in polypropylene cages, with five rats per cage, and were kept under standard laboratory conditions, which involved a 12 h light and 12 h dark cycle. They were provided with appropriate diets and purified reverse osmosis (RO) water. The temperature within the animal facility was maintained at 23 ± 2°C, while the relative humidity was held at 50 ± 15%. The rats were divided into three groups, each consisting of five animals. A treatment schedule is given in Table 2.

#### 2.8.2 Excision wound creation

The dorsal skin of animals was shaved with a pet trimmer. Under anaesthesia (2% Lignocaine injections), a predetermined region of the skin area (2 cm × 2 cm) was excised under aseptic conditions (Vinothapooshan *et al.,* 2010). Animals were treated with standard and test formulations for 21 days after the wound was developed as per treatment schedule given in Table 2.

**Table 2: Treatment schedules**

| Groups | Treatment |
|---|---|
| I (Normal control) | Placebo nanoemulgel was applied topically on wound (2 × 2 $cm^2$) for 21 days |
| II (Standard) | Silver nitrate (0.2% w/v) was applied topically on wound (2 × 2 $cm^2$) for 21 days |
| III (Test) | 1 g *A. vasaka* extract loaded nanoemulgel applied topically on wound (2 × 2 $cm^2$) for 21 days |

#### 2.8.3 Assessement of inflammatory biomarkers

On day 21 of the wound healing study, the animals were anesthetized, and blood was obtained using the retro-orbital method. The collected blood was then placed in Eppendorf tubes for testing inflammatory markers such as C-reactive protein and interleukin-6. It should be noted that interleukin-6 levels were observed to be higher than the normal range in non-healing animals, as per previous research (Patel *et al.,* 2016).

#### 2.8.4 Histological study

On day 21, after biomarker asserssement, animals were euthanized subsequently through cervical dislocation in a designated dissection area. The wounded skin samples from all experimental groups were then excised and preserved in a formalin solution for histological examination (Subhashini *et al.,* 2011).

### 2.9 Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons test using Graph Pad Instat software (Graphpad Software Inc., CA, USA), used to determine pharmacokinetic data of selected nanoemulgels. $p<0.05$ was considered as statistically significant.

## 3. Results

### 3.1 Identification test

Phytochemical tests showed presence of alkaloids in *A. vasaka* extract (Table 3).

**Table 3: Phytochemical tests for alkaloids**

| S. No. | Name of test | Procedure | Result |
|---|---|---|---|
| 1 | Dragendroff's test | 1 ml Dragendroff's reagent + 2 ml extract | Orange red precipitate was formed |
| 2 | Mayer's Test | Few drops of Mayer's reagent + 1 ml of extract | Yellowish white precipitate was formed |
| 3 | Hager's Test | Few drops of Hager's reagent + 1 ml of extract | Yellow precipitate was formed |

### 3.2 HPLC analysis

The HPLC results showed vasicine in *A. vasaka* extract as retention time of standard vasicine and *A. vasaka* extract were 5.276 and 5.225 min, respectively (Figure 1and Figure 2).

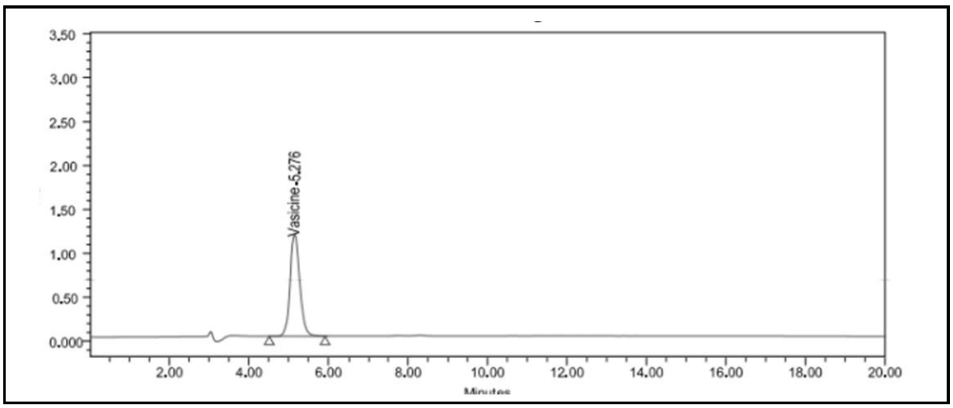



**Figure 1: Chromatogram of vasicine.**

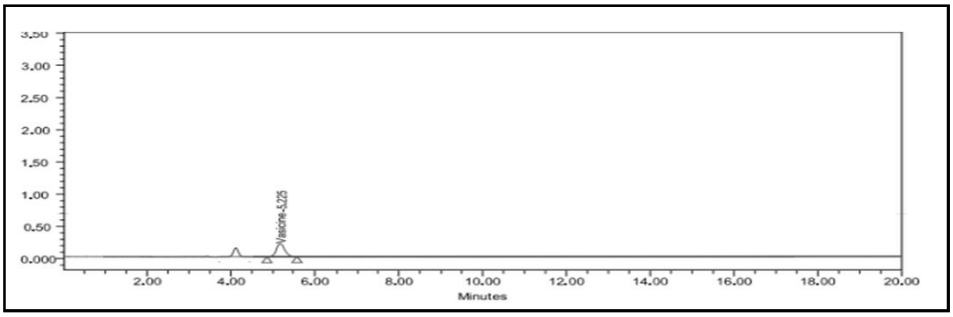



**Figure 2: Chromatogram of *A. vasaka* extract.**

**Table 4: Preparations of *A. vasaka* extract nanoemulgels**

| Ingredient (%w/w) | NEG1 | VENEG2 | VENEG3 | VENEG4 | VENEG5 | VENEG6 |
|---|---|---|---|---|---|---|
| VE extract | - | 1 | 1 | 1 | 1 | 1 |
| Carbopol 934 | 0.25 | 0.50 | 0.50 | 0.50 | 0.75 | 0.75 |
| Coconut oil | 3.75 | 2.50 | 3.75 | 2.50 | 2.78 | 2.75 |
| Tween 80 | 0.30 | 0.50 | 0.30 | 0.50 | 0.30 | 0.50 |
| Span 80 | 0.45 | 0.75 | 0.45 | 0.75 | 0.75 | 0.45 |
| Polyethylene glycol | 0.25 | 3.00 | 0.50 | 1.50 | 0.20 | 2.50 |
| Ethanol | 0.08 | 0.06 | 0.04 | 0.05 | 0.08 | 0.07 |
| Distilled water (q.s.) | 15 | 15 | 15 | 15 | 15 | 15 |

### 3.3 Development and optimization of *A. vasaka* extract loaded nanoemulgels

The six formulations were prepared and evaluated. One (NEG1) of them was placebo nanoemulgel (without VE extract) and rests were of VENEG2, VENEG3, VENEG4, VENEG5 and VENEG6, respectively, with other ingredients (Table 4).

### 3.4 Stability study of *A. vasaka* extract nanoemulgel

One specific nanoemulgel (VENEG4) amongst others nanoemulgels demonstrated stability, as there was absence of any signs of phase separation or alteration in color of the nanoemulgels.

### 3.5 Characterization of optimized nanoemulgel of *A. vasaka* extract

#### 3.5.1 pH determination

The pH of the optimized nanoemulgel of *A. vasaka* extract (VENEG4) was 6.5 ± 0.98. The almost neutral pH is suitable for human acceptance and less irritating to human skin.

#### 3.5.2 Spreadability and extrudability

The spreadability and extrudability of *A. vasaka* extract nanoemulgel (VENEG4) was found to be 52 ± 3.75 $gcmS^{-1}$ and 78 ± 6.42 $gcm^{-2}$. The large diameter signifies better spreadability and extrudability.

#### 3.5.3 Viscosity

The viscosity of *A. vasaka* extract nanoemulgel (VENEG4) was 60813 ± 251 cP which showed better consistency of VENEG4.

#### 3.5.4 Droplet characterization

Transmission electron microscopy (TEM) was employed to examine the size and morphology of the nanoemulgel. The TEM of VENEG4 exhibited low brightness, giving the nanoemulgel a subdued appearance. The results fell within the nanosize range (70 nm to 100 nm), as depicted in Figure 3.

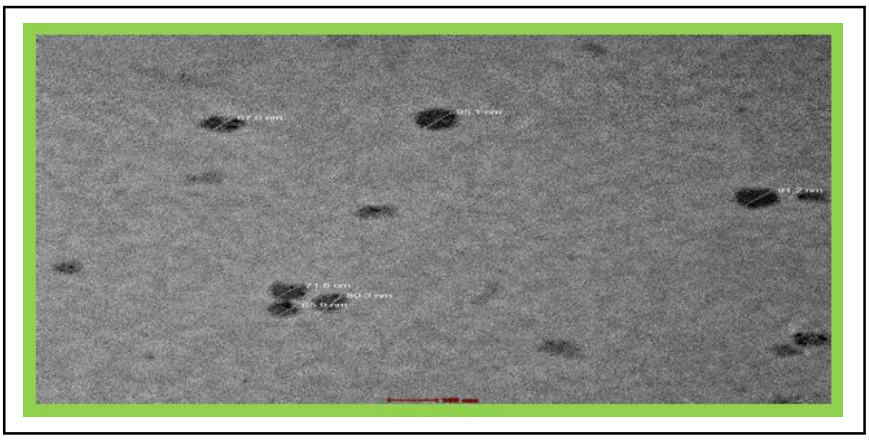


**Figure 3: Transmission electron microscopy of nanoemulgel (VENEG4).**

Only VENEG4 was selected for further *in vivo* evaluation as it showed stability for six months ($p<0.05$). The pH (6.5 ± 0.98), spreadability (52 ± 3.75 $gcmS^{-1}$) and extrudability (78 ± 6.42 $gcm^{-2}$), viscosity (60813 ± 251 cP) and droplet size (70-100 nm) of VENEG4 were in nanorange.

### 3.6 *In vivo* wound healing activity

#### 3.6.1 Effects of optimized nanoemulgel (VENEG4) on wound closure and healing

The wound healing effect of the developed nanoemulgel formulation was assessed by the excision wound model in rats. Use of nanoemulgel for initial 7 days demonstrate an acceleration in wound healing process as revealed by wound contraction in figure 4. Additionally, application of nanoemulgel for a duration of 14 days of post wounding showed almost closure of wound as compared to marketed conventional gel and untreated control group. An improved healing pattern and decrease in the period of epithelization were observed in nanoemulgel treated wounds. Figure 4 depicts the wound

contraction recorded on day 0, day 7, day 14 and on day 21. Nanoemulgel treated wound showed epithelization in 14 days and the conventional gel treated group showed epithelialization in 21 days. A considerably fast healing was observed in animals treated nanoemulgel in comparison to the conventional gel.

The application of both nanoemulgel (VENEG4) and standard gel, *i.e.* Silver nitrate (0.2% w/v) on rats resulted in a substantial increase in percentage wound contraction over the 21 days period compared to control groups ($p<0.01$). Specifically, the use of nanoemulgel for 7 days markedly accelerated wound healing, leading to a 70.73% increase in wound contraction (Table 5). By day 14 post-wounding, the test gel demonstrated nearly 93.14% wound closure, reaching close to 100% by day 21, unlike the control group ($p<0.01$). Nanoemulgel treatment exhibited a superior healing pattern and significantly reduced the epithelization period. Rats treated with nanoemulgel showed a notable acceleration in wound healing compared to those treated with the standard gel (Table 5, Figure 4).

**Table 5: Effects of optimized nanoemulgel (VENEG4) on wound healing**

| Groups | No. of animals (n = 5) | Dosage, route of administration and duration | Percentage of wound contraction | | | |
|---|---|---|---|---|---|---|
| | | | Day 0 | Day 7 | Day14 | Day 21 |
| I (Normal control) | 5 | Placebo nanoemulgel was applied topically on wound for 21 days | 0 | 32.12 | 47.41 | 67.22 |
| II (Standard) | 5 | Standard dose of silver nitrate (0.2% w/v) was applied topically ($2 \times 2$ cm$^2$) on wound for 21 days | 0 | 68.41 | 90.70 | 100 |
| III (Test ) | 5 | 1g *A. vasaka* extract loaded nanoemulgel applied topically ($2 \times 2$ cm$^2$) on wound for 21 days | 0 | 70.73 | 93.14 | 100 |

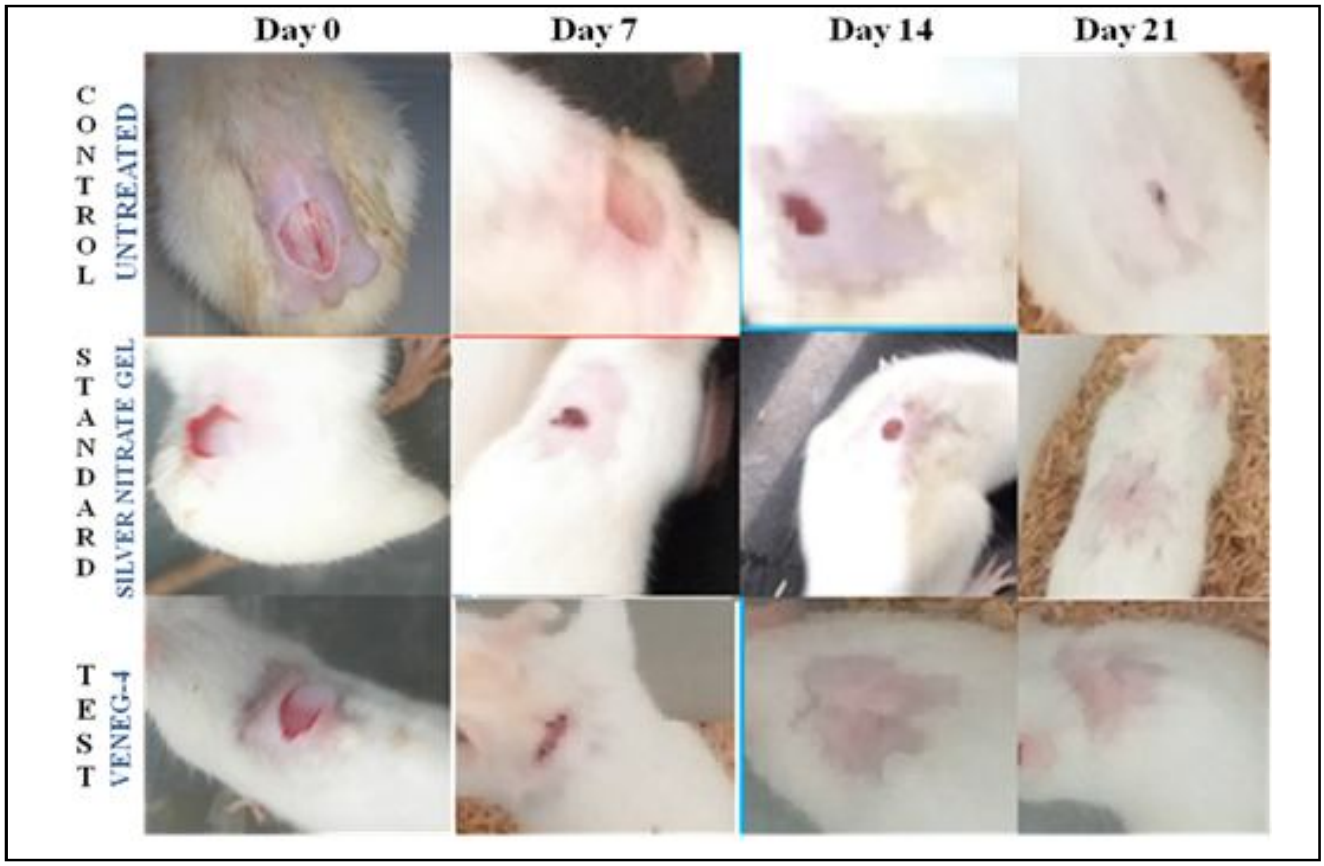



**Figure 4: Wounds photographs of a representative rat from each group.**

### 3.6.2 Effects of optimized nanoemulgel on inflammatory biomarkers

On 21 day of wound healing study, the amount of both interleukin-6 (IL-6) and C-reactive protein was less (18.10 ± 2.66 pg/ml) in VENEG4 as compared to normal control, *i.e.,* placebo nanoemulgel (44.20 ± 4.24 pg/ml) and standard, *i.e.,* silver nitrate (0.2% w/v) gel (24.30 ± 3.26 pg/ml). This indicates that the test (VENEG4) treated animals were less susceptible to infection than placebo nanoemulgel and silver nitrate (0.2% w/v) gel (Table 6).

**Table 6: Serum interleukin 6 (IL-6) and C- reactive protein assays**

| S. No. | Sample name | Serum interleukin-6 | C-reactive protein assay |
|---|---|---|---|
| 1 | Normal control | 44.20 ± 4.24 pg/ml | 1000 ± 24 microg/l |
| 2 | Standard | 24.30 ± 3.26 pg/ml | 600 ± 18 microg/l |
| 3 | Test | 18.10 ± 2.66 pg/ml | 360 ± 12 microg/l |

All the values were expressed as mean ± SD (n=3).

### 3.6.3 Histopathology of rat skin

Histological study reports on day 14 post wounding demonstrate that the skin of control group rat had delay in skin healing. However, both standard (marketed silver nitrate gel) and test (nanoemulgel) formulation treated groups had marked skin healing with remarkable epithelization and remodeling of tissues as revealed in Figures 5A, 5B, and 5C.

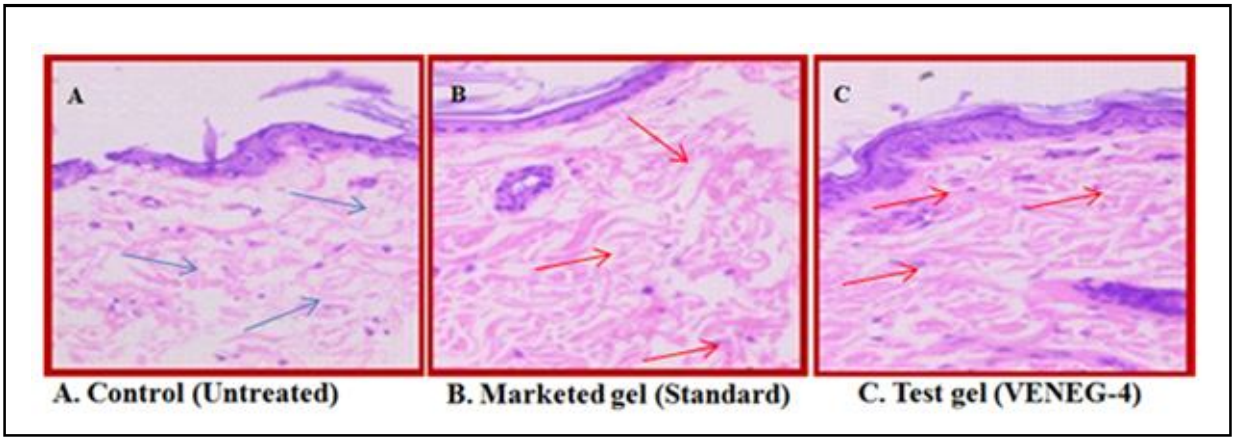



**Figure 5: Histological examination at day 14 post-wounding in rats.**

## 4. Discussion

The wound healing process involves three basic phases: inflammation, proliferation, and maturation, each of which plays a critical role in the body's natural healing process. In addition, the choice of dressing materials is of the utmost importance, as it directly affects the efficiency and speed of wound healing, while minimizing the risk of infection and complications. Topical drug delivery stands out as a highly effective approach to promoting wound healing, primarily due to its remarkable ease of administration, the improved cooperation it encourages from patients, and its ability to circumvent the complexities associated with hepatic first-pass metabolism. This method not only simplifies the application of therapeutic agents to the affected area, but also minimizes potential systemic side effects and ensures targeted and effective treatment to improve wound healing outcomes. Medicinal plants such as *A. vasaka* have shown promise in wound healing due to their bioactive principles that are responsible for the therapeutic activities. The use of nanoformulations of herbal drugs can significantly increase their efficacy in ways that have gained considerable attention and interest in the fields of medicine and pharmacy. These innovative nanostructures enable improved properties of herbal medicines, including improved solubility, increased bioavailability, sustained release, and improved targeting to specific cells or tissues. Consequently, the use of nanoformulations holds great promise for unleashing the full therapeutic potential of herbal medicines, which will ultimately lead to more effective and efficient treatments for various medical conditions. *A. vasaka* has versatile properties, including anti-inflammatory, antimicrobial and wound healing properties (Talukdar *et al.,* 2023). Nanogels and nanoemulgels are advanced drug delivery systems that offer controlled and targeted drug delivery. In this context, our study focused on the use of vasaka extract to create a nanoemulgel formulation for potential wound healing applications.

*A. vasaka* extract (VE) (in ethanol) (Thomas *et al.,* 2023) was prepared using cold percolation technique, while nanoemulgel containing VE was developed by homogenization. To verify the presence of alkaloids in the carefully formulated nanoemulgel enriched with *A. vasaka* extract (VE), a series of chemical tests; namely, Dragendroff's, Mayer's and Hager's tests were rigorously performed. Sophisticated high-performance liquid chromatography (HPLC) was used for rigorous quantitative analysis of *A. vasaka* extract (VE) and nanoemulgel containing VE. This advanced analytical method highlights the promise of obtaining accurate and reliable measurements, ensuring the precise determination of components in the VE extract and their incorporation into the nanoemulgel formulation. Among the six formulations tested, only one formulation (VENEG4) stood out for its stability, showing no signs of phase separation or color changes. The pH range measured within the VENEG4 nanoemulgel formulation was consistently near the neutral value, confirming its compatibility for safe and effective skin application. In particular, comprehensive transmission electron microscopy (TEM) analysis offered conclusive confirmation of the globule size within VENEG4, with all globules thoughtfully kept well below the desired size threshold of <100 nm. In addition, effects of VENEG4 on wound healing, inflammatory biomarkers and skin histology were performed, which demonstrated a remarkable improvement in wound healing compared to other groups. This indicates the potential of nanoemulgel

loaded with *A. vasaka* extract as an effective wound healing agent. This study provides conclusive evidence that nanoemulgel enriched with *A. vasaka* extract has significant wound healing activity. This finding underscores its potential to serve as a valuable therapeutics in wound care and treatment.

## 4. Conclusion

This study concluded that the developed and optimized nanoemulgel containing *A. vasaka* extract was stable and efficacious, which was characterized by pH, viscosity, droplet size, and surface morphology using TEM analysis. The efficacy (wound healing activity) of nanoemulgel containing *A. vasaka* extract was assessed by using a well-established experimental model involving excision wounds in rats and results indicated that the nanoemulgel formulation exhibited substantial potential in promoting wound healing in rats. Our study suggests the potential application of *A. vasaka* extract loaded nanoemulgel as an effective therapeutic agent in wound care.

## Acknowledgements

The authors offer their sincere thanks to Integral University for providing technical support and assigning Communication Number (IUL/R&D/2023-MCN0001890).

## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

## References

**Adem, A.A.; Belete, A.; Lai, K.K.; Hage, C.; Neubert, R.H. and Gebre-Mariam, T. (2023).** Nanoemulgel formulation for topical delivery of plant glucosylceramide: Characterization and optimization. Journal of Drug Delivery Science and Technology, **79**:104056.

**Ahmad, M.; Aga, M.A.; Bhat, J.A.; Kumar, B.; Rouf, A.; Capalash, N.; Mintoo, M.J.; Kumar, A.; Mahajan, P.; Mondhe, D.M. and Nargotra, A. (2017).** Exploring derivatives of quinazoline alkaloid L-vasicine as cap groups in the design and biological mechanistic evaluation of novel antitumor histone deacetylase inhibitors. Journal of Medicinal Chemistry, **60**(8):3484-3497.

**Aishwharyah, M.V. and Balagurusamy, K. (2012).** A review on safe and effective selected herbs for common pediatric ailments. I.J.F.A. N.S., pp:1793-1797.

**Algahtani, M.S.; Ahmad, M.Z. and Ahmad, J. (2020).** Nanoemulgel for improved topical delivery of retinylpalmitate: formulation design and stability evaluation. Nanomaterials, **10**(5):848.

**Amin, A.H. and Mehta, D.R. (1959).** A bronchodilator alkaloid (vasicinone) from *Adhatoda vasica* Nees. Nature, **184**(4695):1317.

**Asadinezhad, S.; Khodaiyan, F.; Salami, M.; Hosseini, H. and Ghanbarzadeh, B. (2019).** Effect of diferent parameters on orange oil nanoemulsion particle size: Combination of low energy and high energy methods. Journal of Food Measurement and Characterization, **13**(4):2501-2509.

**Atmakuri, S.; Nene, S.; Jain, H.; Joga, R.; Devabattula, G.; Godugu, C. and Srivastava, S. (2023).** Topical delivery of tofacitinib citrate loaded novel nanoemulgel for the management of 2, 4-Dichlorodinitrobenzene induced atopic dermatitis in mice model. Journal of Drug Delivery Science and Technology, **80**:104145.

**Balasundaram, A. and David, D.C. (2023).** Evaluating phosphodiesterase 7B inhibition by vasicine using nuclear magnetic resonance spectroscopy. Research Journal of Pharmacy and Technology, **16**(1):103-106.

**Bashir, M.; Ahmad, J.; Asif, M.; Irfan, M.; Ibrahim, A.Y.; Asghar, S.; Khan, I.U.; Iqbal, M.S.; Haseeb, A.; Khalid, S.H. and Abourehab, M.A. (2021).** Nanoemulgel, an innovative carrier for diflunisal topical delivery with profound anti-inflammatory effect: *In vitro* and *in vivo* evaluation. International Journal of Nanomedicine, **16**:1457.

**Basu, S.; Pant, M.; Priyanka, K.M. and Saluja, S. (2011).** Review and future perspectives of using vasicine, and related compounds. Indo Global Journal of Pharmaceutical Sciences, **1**(1):85-98.

**Bhanukiran, K.; Gajendra, T.A.; Krishnamurthy, S.; Singh, S.K. and Hemalatha, S. (2023).** Discovery of multi-target directed 3-OH pyrrolidine derivatives through a semisynthetic approach from alkaloid vasicine for the treatment of Alzheimer's disease. European Journal of Medicinal Chemistry, **249**:115145.

**Bhowmik, D. (2012).** Recent advances in novel topical drug delivery system. The Pharma Innovation, **1**(9):12.

**Cepae, B.A. (1999).** WHO monographs on selected medicinal plants. Geneva: World Health Organization.

**Chaturvedi, G.N.; Rai, N.P.; Dhani, R. and Tiwari, S.K. (1983).** Clinical trial of Adhatodavasica syrup (vasa) in the patients of non-ulcer dyspepsia (Amlapitta). Ancient Science of Life, **3**(1):19.

**Chaughule, R.S. and Barve, R.S. (2023).** Role of herbal medicines in the treatment of infectious diseases. Vegetos, pp:1-1.

**Chellapa, P.; Mohamed, A.T.; Keleb, E.I.; Elmahgoubi, A.; Eid, A.M.; Iss,a Y.S. and Elmarzugi, N.A. (2015).** Nanoemulsion and nanoemulgel as a topical formulation. IOSR. J. Pharm., **5**(10):43-47.

**Choudhury, H.; Gorain, B.; Pandey, M.; Chatterjee, L.A.; Sengupta, P.; Das, A.; Molugulu, N. and Kesharwani, P. (2017).** Recent update on nanoemulgel as topical drug delivery system. J. Pharm. Sci., **106**:1736-1751.

**Claeson, U.P.; Malmfors, T.; Wikman, G. and Bruhn, J.G. (2000).** Adhatodavasica: a critical review of ethnopharmacological and toxicological data. Journal of Ethnopharmacology, **72**(1-2):1-20.

**Contreras-Angulo, L.A.; Gutiérrez-Grijalva, E.P.; Cabanillas-Bojórquez. L.A.; Jiménez-Ortega, L.A. and Heredia, J.B. (2023).** Nanoformulations applied to the delivery of alkaloids. In Phytochemical Nanodelivery Systems as Potential Biopharmaceuticals Elsevier, pp:257-281.

**Date, A.A.; Naik, B. and Nagarsenker, M.S. (2006).** Novel drug delivery systems: potential in improving topical delivery of antiacne agents. Skin Pharmacology and Physiology, **19**(1):2-16.

**Dhawan, S. and Nanda, S. (2023).** Implementation of quality by design (QbD) concept for the development of emulsion based nanotailored gel for improved antiphotoageing potential of silymarin. Journal of Drug Delivery Science and Technology, 104201.

**Dhuley, J.N. (1999).** Antitussive effect of Adhatodavasica extract on mechanical or chemical stimulation-induced coughing in animals. Journal of Ethnopharmacology, **67**(3):361-365.

**Donthi, M.R.; Munnangi, S.R.; Krishna, K.V.; Saha, R.N.; Singhvi, G. and Dubey, S.K. (2023).** Nanoemulgel: A novel nano carrier as a tool for topical drug delivery. Pharmaceutics, **15**(1):164.

**Dorwal, D. (2012).** Nanogels as novel and versatile pharmaceuticals. Int. J. Pharm. Sci., **4**(3):67-74.

**Duraipandiyan, V.; Al-Dhabi, N.A.; Balachandran, C.; Ignacimuthu. S.; Sankar, C. and Balakrishna, K. (2015).** Antimicrobial, antioxidant, and cytotoxic properties of vasicine acetate synthesized from vasicine isolated from *Adhatod avasica* L. BioMed Research International, **2015**:727304.

**Fan, G.; Zhang, K.; Wang, L.; Peng, J.; Sangji, K.; Luo, Y.; Zeng, Y. and Zeweng, Y. (2023).** Traditional Tibetan medicine to fight against COVID-19: Basic theory and therapeutic drugs. Frontiers in Pharmacology, **14**:287.

**Gangwar, A.K. and Ghosh, A.K. (2014).** Medicinal uses and pharmacological activity of *Adhatoda vasica*. Int. J. Herb. Med., **2**(1):88-91.

**Gholami, A.; Minai-Tehrani, D. and Eftekhar, F. (2022).** Bromhexine and its inhibitory effect on lipase-kinetics and structural study. Archives of Physiology and Biochemistry, **128**(6):1687-1692.

**Ghoneum, M.; Abdulmalek, S. and Fadel, H.H. (2023).** Biobran/MGN-3, an arabinoxylan rice bran, protects against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): An *in vitro* and *in silico* study. Nutrients, **15**(2):453.

Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms. https://www.paho.org/hq/dmdocuments/2008/6 Annex 5 report 34 pdf (Accessed october, 2021).

**Gupta, A. and Prajapati, P.K. (2010).** A clinical review of different formulations of Vasa (*Adhatoda vasica*) on Tamaka Shwasa (Asthma), **31**(4):520.

**Ibrahim, M.M. and Shehata, T.M. (2012).** The enhancement of transdermal permeability of water soluble drug by niosome-emulgel combination. Journal of Drug Delivery Science and Technology, **22**(4):353-359.

**Jahangir, M.; Abdel-Farid, I.B.; Verpoorte, R.; Khan, I. and Peng, J. (2023).** NMR-Based metabolomics for geographical discrimination of *adhatoda vasica* leaves. Plants, **12**(3):453.

**Jeengar, M.K.; Rompichar, S.V.; Shrivastava, S.; Chella, N.; Shastri, N.R.; Naidu, V.G. and Sistla, R. (2016).** Emuoil based nano-emulgel for topical delivery of curcumin. International Journal of Pharmaceutics, **506**(1-2):222-236.

**Jiang, T.; Zhang, L.; Ding, M. and Li, M. (2019).** Protective effect of vasicine against myocardial infarction in rats via modulation of oxidative stress, inflammation, and the PI3K/Akt pathway. Drug Design Development and Therapy, 3773-3784.

**Johri, R.K. (2000).** Mechanism of action of 6, 7, 8, 9, 10, 12-hexahydro-azepino-[2, 1-b] quinazolin-12-0ne-(rlx) a novel bronchodilator. Rkjohri and Zutshi.Indian J Physiol Pharmacol., **44**(1):75-81.

**Jung-Kwon, O.; Ray, D. and Daniel, J. (2008).** The development of microgels/nanogels for drug delivery applications, Prog. Polym. Sci., **33**:448–477.

**Kakkar, V.; Modgill, N. and Kumar, M. (2016).** Novel drug delivery systems for herbal antidepressants. Herbal Medicine in Depression: Traditional Medicine to Innovative Drug Delivery, 529-556.

**Karthikeyan, A.; Shanthi, V. and Nagasathaya, A. (2009).** Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica*. L. International Journal of Green Pharmacy, **3**(1):78-80.

**Kashyap C.A.; Gogoi, D.; Chetia, P.; Sarma, D. and Kumar, B.A. (2016).** An *in silico* approach for identification of potential antimycobacterial targets of vasicine and related chemical compounds. Combinatorial Chemistry and High Throughput Screening, **19**(1):14-24.

**Katz, M.A.; Cheng, C.H. and Nacht, S. (1999).** Heron Therapeutics LLC, assignee. Methods and compositions for topical delivery of benzoyl peroxide. United States Patent US, 5:879:716.

**Khambhatya, Y. and Bondada, S. (2023).** Pharmacological evaluation and kinetics of in vitro drug release efficacy of biofabricated silver nanoparticles using medicinally important Justicianeesii Ramamoorthy. Indian Journal of Experimental Biology, **61**(03):185-195.

**Khan, R.; Shamsi, Y. and Nikhat, S. (2020).** Medicinal benefits of *Adhatoda vasica* Nees-in unani and contemporary medicine. Cellmed, **10**(2):13.

**Kumar, D.S.; Dash, D.P.; Panda, P.K.; Sadana, S.; Kumar, R.M.; VenkateshHari, K. and Divya K. (2023).** The efficacy of a plant based formulation in the symptomatic management of mild COVID-19 cases: A double blind, randomized controlled trial. Archives of Clinical and Medical Case Reports, **7**:5-12.

**Kumar, S.; Singh, N.; Devi, L.S.; Kumar, S.; Kamle, M.; Kumar, P. and Mukherjee, A. (2022).** Neem oil and its nanoemulsion in sustainable food preservation and packaging: Current status and future prospects. Journal of Agriculture and Food Research, **7**:100254.

**Kumari, N.; Akhtar, J.; Ahmad, M.; Badruddeen. and Khan, M.I. (2021).** An outline on vasicine, its ethnomedical and nanoformulation approach. Journal of Biologically Active Products from Nature, **11**(1):42-59.

**Mandal, S.; Vishvakarma, P. and Mandal, S. (2023).** Future aspects and applications of nanoemulgel formulation for topical lipophilic drug delivery. European Journal of Molecular and Clinical Medicine, **10**(01):3986-4003.

**Manogaran, P.; Beeraka, N.M.; Paulraj, R.S. and Sathiyachandran, P. (2023).** Impediment of cancer by dietary plant-derived alkaloids through oxidative stress: Implications of PI3K/AKT pathway in apoptosis, autophagy, and ferroptosis. Current Topics in Medicinal Chemistry, **23**(10):860-877.

**Mirza, M.A.; Khan, M.J. and Iqbal, Z. (2015).** Nanoemulsion technology in unani medicine. Int. J. Adv. Pharm. Med. Bioallied. Sci., **3**:70-74.

**Monika, P. and Chandraprabha, M.N. (2020).** Functional bionanomaterials: from biomolecules to nanoparticles. Springer, **1**:11-28.

**Nandhini, S. and Ilango, K. (2021).** Development and characterization of a nano-drug delivery system containing vasaka phospholipid complex to improve bioavailability using quality by design approach. Research in Pharmaceutical Sciences, **16**(1):103.

**Nath, D.; Sethi, N.; Singh, R.K. and Jain, A.K. (1992).** Commonly used Indian abortifacient plants with special reference to their teratologic effects in rats. Journal of Ethnopharmacology, **36**(2):147-154.

**Nepali, K.; Sharma, S.; Ojha, R. and Dhar, K.L. (2013).** Vasicine and structurally related quinazolines. Medicinal Chemistry Research, **22**:1-5.

**Nirmala, S.; Sabapathi, P.N. and Sravanthi, Y. (2023).** Anticancer activity of *Adathoda vasica* in Hela, HepG2, MCF-7, MDAMB-231 cell lines - *in vitro*. Current Overview on Pharmaceutical Science, **2**:57-67.

**Ojha, B.; Jain, V.K.; Gupta, S.; Talegaonkar, S. and Jain, K. (2022).** Nanoemulgel: A promising novel formulation for treatment of skin ailments. Polymer Bulletin, **79**(7):4441-4465.

**Palshikar. and Gautam, D.P. (2023).** Variations in vasicine content of vasaka-an indian medicinal plant. Journal of Pharmaceutical Negative Results, **14**(1):97-104.

**Pankaj, H.; Naikwadi, J.; Narendra, D.; Phatangare. And Mane D.V.. (2022).** Ethanopharmacological anti-inflammatory study of phytol in pet ether extract of *Woodfordia floribunda* Salisb. Ann. Phytomed., **11**(2):426-437.

**Patel, S.; Maheshwari, A. and Chandra, A. (2016).** Biomarkers for wound healing and their evaluation. Journal of Wound Care, **25**(1):46-55.

**Poorniammal, R. and Prabhu, S. (2022).** Antimicrobial and wound healing potential of fungal pigments from Thermomyces sp. and Penicillium purpurogenum in wistar rats. Ann. Phytomed., **11**(1):376-382.

**Priyadarshini, P.; Karwa, P.; Syed, A. and Asha, A.N. (2023).** Formulation and evaluation of nanoemulgels for the topical drug delivery of posaconazole. Journal of Drug Delivery and Therapeutics, **13**(1):33-43.

**Qais, F.A.; Khan, M.S.; Ahmad, I. and Althubiani, A.S. (2019).** Potential of nanoparticles in combating Candida infections. Letters in Drug Design and Discovery, **16**(5):478-491.

**Quazi, S.H. and Pathak, S.S. (2023).** Ayurgenomics in antiviral therapy: A literature synthesis. Cureus Journal of Medical Science, 15(2):e34649

**Raghunath, I.; Koland, M.; Vadakkepushpakath, A.N.; Kumar, L. and Shenoy, S.C. (2023).** Herbal bioenhancers with nanocarriers: A promising approach for oral peptide delivery. Int. J. Pharm. Investigation., **13**(1):7-13.

**Roy, D.C.; Shark, M. and Faruquee, H.M.**A. **(2013).** Brief review on phytochemistry and pharmacological properties of *Adhatoda vasica*. Journal of Tropical Medicinal Plants, 14.

**Sayeed, A.; Madhukar, G.; Maksood, A.; Mhaveer, S.; Athar, M.T. and Ansari, S.H. (2009).** A phytopharmacological overview on *Adhatoda zeylanica* Medic. syn. *A. vasica* (Linn.) Nees. Natural Product Radiance, **8**(5):549-554.

**Sengupta, P. and Chatterjee, B. (2017).** Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. International Journal of Pharmaceutics, **526**(1-2):353-365.

**Sharma, D.K. and Jain, V.K. (2022).** Enumeration on therapeutic and ethnopharmacological properties of adosa (*Adhatoda Vasica* (L.) Nees). Central Asian Journal of Theoretical and Applied Science, **3**(8):93-102.

**Shrivastava, N.; Srivastava, A.; Banerjee, A. and Nivsarkar, M. (2006).** Antiulcer activity of *Adhatoda vasica* (L.) Nees. Journal of Herbal Pharmacotherapy, **6**(2):43-49.

**Singh, B. and Sharma, R.A. (2013).** Anti-inflammatory and antimicrobial properties of pyrroloquinazoline alkaloids from *Adhatoda vasica* Nees. Phytomedicine, **20**(5):441-445.

**Singh, L.; Singh, A.; Chhavi, A.; Srivastav,a R.P.; Pandey, S.; Dixi.t P.; Verma, P.C. and Saxena, G. (2023).** Overview of COVID-19 pandemic: Its management and prevention in light of the Indian traditional medicine system. Current Traditional Medicine, **9**(4):1-8.

**Singh, V.K.; Mishra, V.; Tiwari, S.; Khaliq, T.; Barthwal, M.K.; Pandey, H.P.; Palit, G. and Narender, T. (2013).** Antisecretory and cytoprotective effects of peganine hydrochloride isolated from the seeds of Peganumharmala on gastric ulcers. Phytomedicine, **20**(13):1180-1185.

**Subhashini, S. and Arunachalam, K.D. (2011).** Investigations on the phytochemical activities and wound healing properties of *Adhatoda vasica* leave in Swiss albino mice. Afr. J. Plant Sci., **5**(2):133-145.

**Sultana, F.; Manirujjaman, M.; Imran-Ul-Haque, M.A. and Sharmin, S. (2013).** An overview of nanogel drug delivery system. J. Appl. Pharm. Sci., **3**(8):95-105.

**Tadwee, I.K.; Gore, S. and Giradkar, P. (2012).** Advances in topical drug delivery system: A review. Int. J. of Pharm. Res. and All. Sci., **1**(1):14-23.

**Talukdar, A.; Sarkar, D. and Sarkar, D. (2023).** Bromhexine: Into the spotlight. Viral, Parasitic, Bacterial, and Fungal Infections. Academic Press, pp:719-731.

**Tayah, D.Y. and Eid, A.M. (2023).** Development of miconazole nitrate nanoparticles loaded in nanoemulgel to improve its antifungal activity. Saudi Pharmaceutical Journal, pp:20-23.

**Thangaraju, P.; Gurunthalingam, M.P.; Venkatesan, S. and Thangaraju, E. (2023).** Molecular docking analysis of *Adhatoda vasica* with thromboxane A2 receptor (TXA2R)(6IIU) and Antiviral molecules for possible dengue complications. Infectious Disorders Drug Targets, 23(1):e18072 2206836

**Thomas, D.; Latha, M.S. and Thomas, K.K. (2023).** Evaluation of the antibacterial activity of calcium alginate beads modified with ethanolic extract of *Adhatoda Vasica* leaf extract on S*taphylo-coccus Aureus* and *Escherichia Coli*. Asian J. Pharm. Clin. Res., **11**(3):68-70.

**Ting, W.W.; Vest, C.D. and Sontheimer, R.D. (2004).** Review of traditional and novel modalities that enhance the permeability of local therapeutics across the stratum corneum. International Journal of Dermatology, **43**(7):538-547.

**Singh, V.A.; Gade, W.; Vats, T.; Lenardi, C. and Milani, P. (2010).** Nanomaterials: New generation therapeutics in wound healing and tissue repair. Current Nanoscience, **6**(6):577-586.

**Vinothapooshan, G. and Sundar, K. (2010).** Wound healing effect of various extracts of *Adhatoda vasica*. International Journal of Pharma and Bio Sciences, **1**(4):530-536.

**Wenger, N.M.; Qiao, L.; Nicola, T.; Nizami, Z.; Martin, I.; Halloran, B.A.; Tanaka, K.; Evans, M.; Xu, X.; Dinan, T.G. and Kakilla, C. (2023).** Clinical trial of a probiotic and herbal supplement for lung and gut health. Frontiers in Nutrition, **10**:1-13.

**Citation** **Nidhi Kumari, Juber Akhtar, Badruddeen, Mohammad Irfan Khan and Mohammad Ahmad (2023). Formulation development and *in vivo* evaluation of nanoemulgel containing *Adhatoda vasaka* (L.) Nees extract for wound healing activity. Ann. Phytomed., 12(2):720-729. http://dx.doi.org/10.54085/ap.2023.12.2.85.**