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Article

Formulation and development of topical nano-emulgel TDDS for delivering *Vitex Negundo* as a painkiller: Enhancing effectiveness with natural essential oil permeation promoters in an innovative drug delivery system for rheumatoid arthritis treatment—A NDDS therapeutics approach

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Abstract: *Vitex negundo* is a powerful herbal anti-inflammatory with effects similar to those of non-steroidal anti-inflammatory drugs (NSAIDs), which are commonly used to treat rheumatoid arthritis. Despite its benefits, *Vitex negundo* shares some drawbacks with NSAIDs, including gastrointestinal side effects such as vomiting and poor transdermal penetration, which limits its effectiveness in transdermal and gastrointestinal drug delivery. This study aimed to enhance the bioavailability of *Vitex negundo* by developing nano-emulgel with reduced particle size and to assess their effectiveness in treating rheumatoid arthritis. The researchers utilised a modified emulsification-diffusion method to create nanosized dispersions of *Vitex negundo*, incorporating hydroxypropyl methylcellulose (HPMC) as a gelling agent. Essential oils were also included to improve skin penetration by interacting with the stratum corneum (SC), thus enhancing the absorption of both lipophilic and hydrophilic drugs. The prepared *Vitex negundo* nano-emulgel were evaluated for various parameters, including rheology, particle size, drug content, drug release percentage, and *in-vitro* diffusion. The results indicated favourable properties: the particle size was 120.10 nm, the zeta potential was ± 30 mV, drug content was 99.50%, drug release percentage was 98.92%, and drug diffusion was approximately 98%. The formulation also had a pH of 6.8 ± 0.1. Overall, the *Vitex negundo* nano-emulgel, formulated with HPMC and eucalyptus oil as a permeation enhancer, demonstrated potential as an effective topical treatment for oedema and rheumatoid arthritis. "The study illustrates the formulation process of a drug blended with a polymer matrix. The drug is uniformly dispersed within the polymer matrix through high-shear mixing, ensuring optimal integration and consistency in the final product. The process involves high-shear blending to achieve a homogenous mixture, enhancing the drug's stability and release characteristics within the polymer matrix."

Keywords: drug delivery; essential oils; eucalyptus essential oil; homogeneity; hydroxypropyl methylcellulose (HPMC); nano-emulgel formulation; painkiller; particle size; permeation enhancers; pH; spreadability; stability; synthetic membrane; transdermal drug delivery system (TDDS); transparency; *Vitex negundo*; zeta potential

1. Introduction

Traditional pain management and complete plant material extraction are not immediate and often require specialised skills. Therefore, developing an effective therapeutic formulation is crucial (**Figure 1**).

Gels are semi-solid materials that vary in consistency from soft and pliable to firm and durable. They consist of a cross-linked network that thickens a liquid phase, preventing constant flow while permitting partial liquid migration. Syneresis refers to the phenomenon where a gel contracts and expels liquid [1].

Traditional medicines are effective in managing complex diseases, including respiratory conditions like chronic obstructive pulmonary disease (COPD). *Vitex negundo L.*, a medicinal plant with notable ethnobotanical importance, is used for various health issues and impacts cellular processes. This shrub, rich in specialised metabolites, serves as a complementary therapy in many countries [2]. **Figure 1**.

Figure 1. Traditional management of pain, including the treatment of rheumatoid arthritis, often utilises various natural remedies. One such remedy is the *Neolamarckia kadamba* (Burflower- Tree) plant, which has been employed in traditional practices to help manage pain.

Its antihistamine properties contribute to its anti-itching effects, a traditional claim in Ayurvedic medicine [3]. Research shows that vitexin, derived from this plant, offers anti-senescence and anti-ageing benefits by inhibiting the SASP and suppressing the JAK2/STAT3 signalling pathway [4].

In the Vedic tradition, Nirgundi (*Vitex negundo*) is esteemed for its anti-inflammatory, analgesic, and wound-healing properties. Traditionally used to alleviate pain and treat skin disorders, the leaves, roots, and seeds of this plant have been vital in Ayurvedic medicine. These benefits have been handed down through generations, maintaining Nirgundi's role in traditional remedies in India [5].

The fresh leaves of *Vitex negundo* are noted for their anti-inflammatory and pain-relieving effects, attributed to their inhibition of prostaglandin synthesis, alongside their antihistamine, membrane-stabilising, and antioxidant properties [3].

Advancements in nanotechnology are enhancing treatments, with innovations like nano-emulgel and solid lipid nanoparticles (SLNs) improving drug delivery. Lycopene is also being effectively integrated into stable cosmetic formulations. Nonsteroidal anti-inflammatory drugs (NSAIDs), originating from willow bark, continue to be essential for pain and inflammation management [6]. Oxicams, a group

of long-acting NSAIDs, offer significant anti-inflammatory and analgesic effects, especially for conditions like rheumatoid arthritis and osteoarthritis. *Vitex negundo* is recognised for its comparable efficacy to conventional NSAIDs like aspirin, indomethacin, and naproxen in managing these conditions when used as directed [7,8].

Polymer matrices and carriers face challenges such as poor drug encapsulation, leakage during storage, and high-water content. Nanoemulgels, which use a solid lipid matrix to encapsulate liquid lipid compartments stabilised by surfactants, have emerged as a solution. Their irregular crystal structure enhances drug loading and prevents expulsion of encapsulated drugs [9].

Vitex negundo, a staple in Ayurvedic medicine for managing pain and conditions like rheumatoid arthritis and osteoarthritis, is now widely cultivated across Europe, Asia, North America, and the West Indies. Recent studies highlight its seeds' antineoplastic, antioxidant, and insecticidal properties. In southern China, *Vitex negundo* seeds have been traditionally used to treat various pains, including stomachaches and joint pain [10,11]. Among its extracts, the acetoacetate fraction showed the highest antinociceptive activity, with two majour lignans—6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde and vitedoamine A—demonstrating significant analgesic and anti-inflammatory effects. The flavonoid-rich fraction also exhibited anti-androgenic properties [10].

Alternative practices suggest that consuming small amounts of *Vitex negundo* juice may help reduce intestinal worms, though higher doses can cause vomiting. Some folklore also claims it has been used to combat leprosy in Indian villages or tribal communities. However, these claims lack scientific validation, and there is limited research on such traditional uses in Ayurvedic texts.

Topical application of *Vitex negundo* is considered safe and does not interfere with natural health, as per Ayurvedic guidelines, provided it is used in recommended doses.

Reactive oxygen species are known to contribute to various inflammatory conditions. *Vitex negundo*, recognised in Ayurvedic medicine for arthritis treatment, has been studied for its antioxidant and anti-inflammatory properties [8,11].

The research focused on the plant's total methanol extract, standardised for polyphenol content. The standardised extract, given at 100 mg/kg, reduced oedema about as much as diclofenac sodium (25 mg/kg) did in a rat model using the carrageenan-induced paw oedema method to test its anti-inflammatory effects. The extract showed robust free radical scavenging activity according to the 1,1-diphenyl-2-picrylhydrazyl method and significantly inhibited lipid peroxidation, as indicated by reduced formation of thiobarbituric acid-reacting substances. These findings strongly suggest that the extract's anti-inflammatory properties may be attributed, at least in part, to its ability to quench free radicals [8].

The extract of *Vitex negundo* showed a lipid peroxidation inhibitory effect, reducing thiobarbituric acid-reacting substances (TBARs) by 14.87% at a concentration of 1.87 mg/mL. In comparison, curcumin extract achieved a 94.22% reduction in TBARs at 0.74 mg/mL [8].

In anti-inflammatory tests, diclofenac sodium (25 mg/kg) reduced oedema by about 10.82% after four hours, while *Vitex negundo* extract (50% methanol) at 100

mg/kg reduced oedema by approximately 21.52%. These findings highlight *Vitex negundo's* significant anti-inflammatory and antioxidant properties, which support its traditional use in treating inflammatory and arthritic conditions. The plant's ability to protect tissues from oxidative damage likely enhances its anti-inflammatory effects, validating its traditional medicinal applications [8].

Nanoemulsions (NE) are stable formulations consisting of ultra-small oil droplets dispersed in water, stabilised by surfactants. They enhance drug delivery by improving solubility, bioavailability, and controlled release, and protect drugs from degradation. NE are versatile for oral, topical, and injectable applications, benefiting both pharmaceuticals and cosmetics [12].

Oral nanoemulsions face absorption challenges, with Zeta potential being a crucial factor. Negative Zeta potential enhances mucus permeation, while positive Zeta potential improves cellular uptake. Using phosphorylated surfactants like N, N′-bis(polyoxyethylene) oleylamine bisphosphate (POAP) can shift Zeta potential from negative to positive, thereby enhancing drug delivery. POAP's design allows for significant changes in Zeta potential, optimising NE performance and advancing oral drug delivery strategies [12].

The extracts of *Vitex negundo* leaves contain phenolic acids and flavonoids as the primary antioxidant components. The phenolic acids mainly consist of derivatives of caffeic acid, including monocaffeoylquinic acids like neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid; di-caffeoylquinic acids such as isochlorogenic acid A, B, and C, along with the isomer P26; and tricaffeoylquinic acid (P20). Various isomers of lithospermic acid, which are polymers of caffeic acid like P14, P17, P26, and P29, are present. The key flavonoids identified include luteolin derivatives, such as orientin and isoorientin (C-glucosides) and cynaroside and scutellarin (O-glucosides). Other notable flavonoids are isoquercetin, casticin, and apigenin-7-glucoside. Moreover, derivatives of p-hydroxybenzoic acid, including P1, P2, P4, P32, and P37, are significant antioxidant components. Agnuside, a key compound and derivative of p-hydroxybenzoic acid, is the principle active ingredient in *Vitex negundo* leaves [13].

A study validated the oral anti-inflammatory, analgesic, and antihistamine effects of mature fresh leaves (MFL) of *Vitex negundo L.* (*Verbenaceae*) as per Ayurvedic claims. Rats were administered a water extract of the leaves, which significantly reduced carrageenan-induced paw oedema in a dose-dependent manner with an EC (50) of g/kg. In the formaldehyde-induced paw oedema test, inflammation was notably suppressed on days 4–6 with doses of 2.5 g/kg. The hot plate test revealed significant, dose-dependent analgesic effects at 1 h post-treatment with 2.5 and 5 g/kg, although no effects were observed in the tail flick test, with an EC (50) for analgesic activity of 4.1 g/kg. The formalin test showed significant pain reduction at doses of 1.25, 2.5, and 5 g/kg, comparable to aspirin. The leaves also demonstrated dose-dependent antihistamine properties, in addition to *in vitro* inhibition of prostaglandin synthesis, membrane stabilisation, and antioxidant activities [3].

From an ethanol extract of *Vitex negundo* L.'s aerial parts, researchers isolated polyoxygenated ursane-type triterpenoids (vitnegundins A-G), three novel triterpenoid saponins (vitnegundins H-J), and 17 known compounds. Vitnegundins A, B, and E were confirmed by single-crystal X-ray diffraction. Vitnegundins B-D are unique

pentacyclic triterpenoids with cis-fused C/D rings, while Vitnegundins C-H are new ursane-type triterpenoids featuring a 12, 19-epoxy group. In biological activity tests, vitnegundins A and E, along with swinhoeic acid, inhibited LPS-induced NO release in BV-2 microglial cells, with IC_{50} values of 11.8, 44.2, and 19.6 μM, respectively [14].

Vitexin, a natural flavonoid glycoside sourced from the leaves and seeds of *Vitex negundo*, is noted for its substantial anti-tumour, anti-inflammatory, and anti-hypertensive properties. Key antioxidant compounds in *Vitex negundo* leaves include isoorientin, chlorogenic acid, agnuside, cynaroside, and scutellarin [4,13].

2. Materials and methods

Fresh *Vitex negundo* plants were obtained in Nashik, India, and maintained as herbarium specimens by pressing them between absorbent papers (type 4) and weighing them with books for inspection. Mature leaves were then picked from the specimens and preserved in plastic or polyethylene containers. Impurities were removed from the crude leaf medicine by thoroughly washing it with distilled water. Additional materials were purchased.

2.1. *Vitex negundo* synonym: Nirguḍi, Nirgundi, Lagundi, Chaste Tree

1) Other variants:

Vitex negundo var. *glabrescens* (a variety name used in specific botanical references) [15].

2) Botanical references:

Vitex negundo L. (*Linnaean* nomenclature) [15].

3) Scientific synonyms:

i. *Vitex trifolia* (*Linnaeus* name).
ii. *Vitex negundo var. heterophylla* (used in some classifications).
iii. *Vitex altissima* (a name used in some regions) [15].

4) Nano-emulgel preparation:

Vitex negundo nano-emulgel were created using a modified emulsification-diffusion process. Initially, 30 mg of *Vitex negundo* was dissolved in 20 mL of PROPYLENE GLYCOL + WATER + GLYCERINE+ETHANOL, **Table 1** along with the polymer hydroxypropyl methylcellulose (HPMC), using steady stirring at speeds ranging from 5,000 to 10,000 rpm with a T-10 basic Ultra Turrex high-speed homogeniser or disperser, which is commonly used in laboratories for mixing. The aqueous phase containing the drug-polymer mixture was slowly introduced into a 10 mL organic phase containing Tween 80, polyethylene glycol, and eucalyptus oil with other oils in appropriate ratios at a controlled rate of 0.5 mL/min using a syringe with a needle inserted directly into the aqueous stabiliser solution. After agitation at 10,000–25,000 rpm for 6 min, the dispersion was sonicated for 5–10 min.

Double-distilled water was then gently added to the dispersion, followed by 1 h of stirring to allow organic solvent diffusion into the continuous phase, culminating in the creation of nano-emulgel. The pH was changed. Triethanolamine is required for further analysis in order to obtain the necessary results.

Table 1. Nano-emulgel composition for 30 gm of *Vitex negundo* nano-emulgel.

Sr. No	Drug/Excipients	Quantity	Enhancements and benefits of the formulation	Role of ingredient
1	*Vitex negundo* (mg) and castor oil, drug extracted + chloroform q.s.	1 mg	Combines the anti-inflammatory properties of *Vitex negundo* with the moisturising and healing benefits of castor oil.	*Vitex negundo* (mg) nano particles. Analgesic. Utilises the anti-inflammatory effects of *Vitex negundo* and the skin-nourishing properties of castor oil.
2	Concentrated onion juice, concentrated aloe vera, curcumin, garlic, and ginger extract, eugenol	5%	Synergistic anti-inflammatory and antioxidant effects enhance therapeutic potential.	Analgesic, rubefacient. Provides enhanced anti-inflammatory and antioxidant benefits for improved formulation efficacy.
3	Hydroxypropyl methylcellulose (HPMC)	q.s.	Ensures optimal gel consistency and stability.	Polymer. Maintains desired gel consistency and stability; alternatives include carbomers, xanthan gum, PVA, sodium alginate, Pluronic F127, PEG, ethylcellulose, and gelatine.
4	Propyl paraben	0.5 mg	Maintains formulation's shelf life and protects against microbial contamination.	Pharmaceutical excipient. Preserves shelf life and prevents microbial growth.
5	Methyl paraben	0.7 mg	Maintains formulation's shelf life and protects against microbial contamination.	Pharmaceutical excipient. Preserves shelf life and prevents microbial growth.
6	Propylene glycol	2 mL	Functions as a humectant to retain moisture in the gel.	Release modifier. Helps retain moisture and improve gel texture.
7	Tween 80	1.5 mL	Serves as a surfactant to evenly distribute essential oils in the gel.	Surfactant. Ensures uniform mixing of essential oils in the gel.
8	Essential oils blend (Camphor, Cinnamon oil, Eucalyptus oil, Lavender oil, Peppermint oil, Rosemary oil, Thyme oil)	1 mL each, or q.s.	Provides combined anti-inflammatory, analgesic, and aromatic properties.	Analgesic, rubefacient. Enhances therapeutic effect and user experience with anti-inflammatory, analgesic, and aromatic benefits.
9	Water + Glycerine + Ethanol	20 mL	Serves as the base and solvent, ensuring smooth application and absorption.	Vehicle. Provides a medium for other ingredients, ensuring effective application and absorption.
10	Triethanolamine	q.s. or as specified in the U.S.P. or pharmacopoeia.	Adjusts pH for optimal skin compatibility and gel stability.	pH modifier. Ensures the gel is skin-compatible and maintains stability.
11	Perfume (rose oil, lemon grass oil)	q.s.	Adds aromatic qualities for enhanced sensory pleasure.	Scientifically to improve sensual pleasure or for sensual pride. Also, aromatic. Ordinarily, aromatic or for fragrance. Enhances sensory appeal and user experience of the formulation.
Total Weight		30 gm		

2.2. Characterisation of *Vitex negundo* nano-emulgel

Vitex negundo nano-emulgel were characterised using several physicochemical criteria for topical use.

2.2.1. Homogeneity

Visual inspection of the filled flint-coloured vial containers revealed no aggregates in the topical *Vitex negundo* nano-emulgel formulations.

2.2.2. Spreading ability

The Spreading ability or spreading capacity of topical *Vitex negundo* nano-emulgel was assessed by inserting 0.5 g between two slides (5 cm^2) for 1 min and measuring the diameter of the spread circle to compare formulations [1].

An increasing force of F = 0.0049 N, F = 0.0098 N, F = 0.0147 N, F = 0.0196 N, F = 0.0245 N, and F = 0.0294 N was vertically directed such that each time the hydraulic press is functional to act over the surface and the required displacement action under controlled and designed fashion that can be graphically measurable. These units were graphically recorded, and the gain of value was recorded and mathematically calculated to count the force of action and its resultant. During each time of test measures, the direction of action was unidirectional and supported the flow of action vertically downwards that had a resultant action of unelastic or inelastic displacement and deformation.

2.2.3. Grittiness

A microscopic analysis was performed to confirm no particles in the formulation [1].

2.2.4. pH Determination

The pH of prepared *Vitex negundo* nano-emulgel was tested with a calibrated pH meter (METTLER). Each batch (1.0 g) was dissolved in 20 mL of distilled water, swirled for 10 min on a magnetic stirrer at room temperature, and pH readings were taken three times following calibration with standard pH buffer solutions (pH 4, 7, and 9.2) [1,16,17].

2.2.5. Drug content

To test drug content and entrapment efficiency, 1 g of *Vitex negundo* nano-emulgel was dissolved in 10 mL of PROPYLENE GLYCOL+WATER+GLYCERINE+ETHANOL. The formulation was centrifuged at 5,000 rpm for 15 min (Remi-900), and 1 mL of the supernatant was extracted and diluted with PROPYLENE GLYCOL+WATER+GLYCERINE+ETHANOL to a final concentration of 10 mL. UV spectrophotometry (Shimadzu UV-1900, Japan) compared the diluted supernatant solution to a blank/control of PROPYLENE GLYCOL+WATER+GLYCERINE+ETHANOL at 320 nm wavelength for calculation [1].

$$Drug\ content\ (\%) = \frac{\text{Total amount of Nano} - \text{emulgels} \times \text{Amount of drug in 0.1gms}}{\text{Amount of nano gel in gms}} \times 100$$

2.2.6. Viscosity study

The viscosity of *Vitex negundo* nano-emulgel (50 g) was measured at room temperature (25 ℃ ± 1 ℃) with torque ranging from 10 to 100% using a Brookfield DV-MHA viscometer (spindle number C75). Viscosity readings were measured in Pascal seconds (Pa.s.) for each formulation tested in triplicate [1,12,17].

2.2.7. Zeta potential

The prepared *Vitex negundo* nano-emulgel had a zeta potential of 298 K at room temperature. Before measuring, each LSLNs suspension (1 mL) was diluted tenfold (10 mL) with distilled water. A serial dilution was measured for evaluation purposes [12].

2.2.8. Particle size

The particle size distribution of *Vitex negundo* nanoemulgel formulations was assessed using photon correlation spectroscopy (PCS) with Zetasizer Ver. 6.50. For the analysis, 0.1 mL of the formulation was mixed with 50 mL of a solution containing propylene glycol, water, glycerine, and ethanol. The mixture was shaken vigorously, and light scattering was recorded at 25 ℃ ± 0.5 ℃ at a 90° angle to evaluate the droplet size distribution. This method differs from the conventional approach used in the research [16].

2.2.9. Transmission Electron Microscopy (TEM): JOEL JEM T-30

The morphology of *Vitex negundo* nano-emulgel formulations was examined using a Transmission Electron Microscope (JOEL JEM T-30). A diluted drop of the dispersion was placed on a carbon-coated 300-mesh copper grid for 1 min. Excess dispersion was removed by blotting with filter paper. The grid was then rinsed twice with deionised water for 3–5 s each time. Finally, a drop of 2% aqueous uranyl acetate solution was briefly applied before imaging. **Figures 2** and **3.**

2.2.10. In-vitro permeation study

For the *in-vitro* permeation study, a Franz diffusion cell with an artificial cellophane membrane (molecular weight cutoff 1000 Da) was utilised. This cell consists of two compartments: the donor and the receptor compartments. The donor compartment, which holds the *Vitex negundo* nanoemulgels supported by a backing membrane, is open to the atmosphere. The system was maintained at a constant temperature of 37 ± 0.5 ℃, and the receptor compartment included a sampling port.

Phosphate buffer at pH 7.5 was used as the diffusion medium. Before the experiment, the cellophane membrane was soaked in phosphate buffer (pH 7.5) for 24 h. The donor and receptor chambers were secured together with a clamp to prevent leakage (see **Figure 4**). To avoid drug accumulation behind the cellophane membrane, the receptor compartment, containing 30 mL of phosphate buffer (pH 7.5), was stirred at 37 ± 0.5 ℃ using a magnetic stirrer.

Samples of 1 mL were collected at specified intervals and replaced with fresh buffer. The drug concentration in these samples was measured at 365 nm using a UV spectrophotometer [1,16].

2.2.11. Stability study

The stability of the *Vitex negundo* nano-emulgel was tested under two conditions: 25 ℃ with 60% relative humidity (RH) and 40 ℃ with 75% RH, over a 90-day period. Samples were taken at 0, 30, 60, and 90 days to evaluate any changes in clarity, homogeneity, pH, viscosity, and spreadability of the nano-emulgel formulation [1,16].

3. Results

3.1. Assessment of topical Vitex negundo nano-emulgel

Table 2 presents the evaluation findings for the topical *Vitex negundo* nano-emulgel formulation. The nano-emulgel resembled a transparent chartreuse gel with a smooth texture, no little particles, and excellent spreading ability. The drug concentration and entrapment efficiency (EE) of the nano-emulgel were calculated using a standard calibration curve for *Vitex negundo* in a solution of propylene glycol, water, glycerine, and ethanol. The standard curve-derived equation was used to compute drug content from measured absorbance.

3.2. Transmission electron microscopy (TEM): JOEL JEM

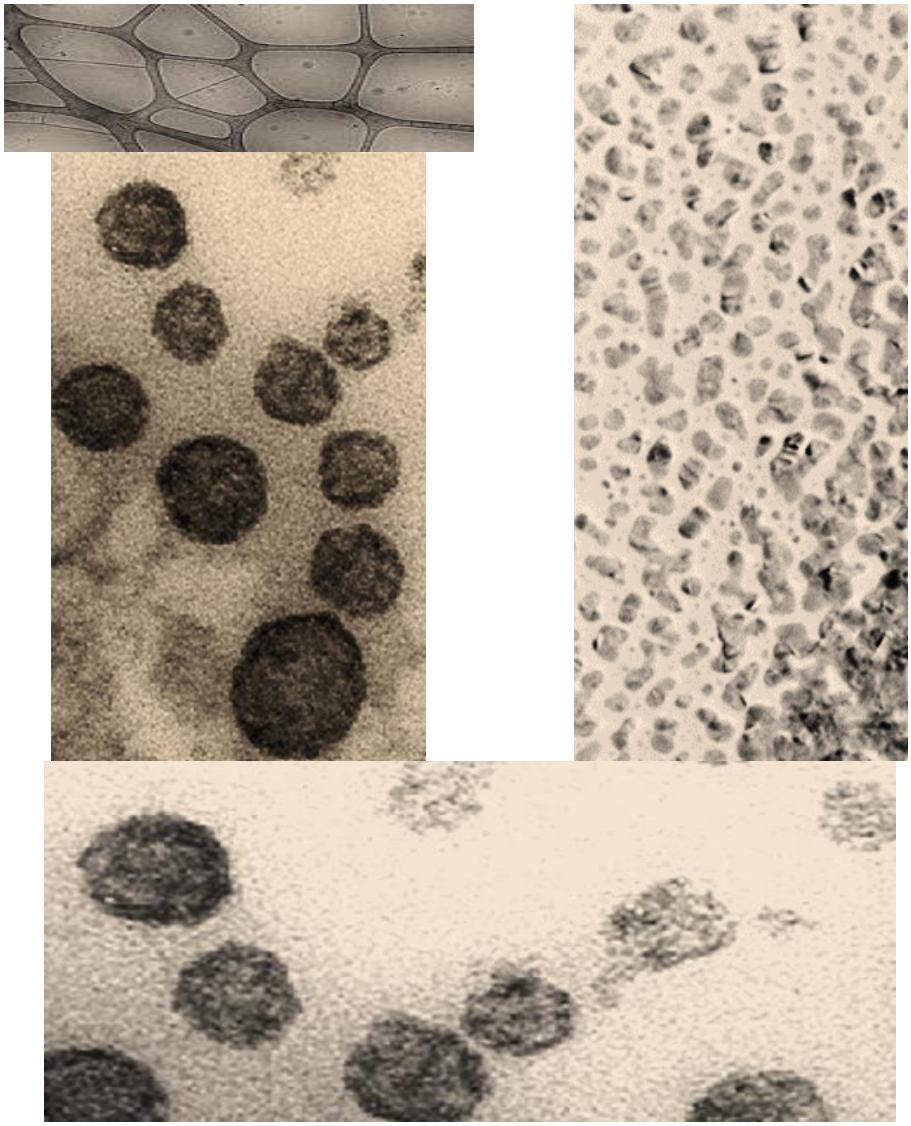


Figure 2. Photomicrograph of the nano-emulgel with Transmission Electron Microscopy (TEM): at 0.5 μm.

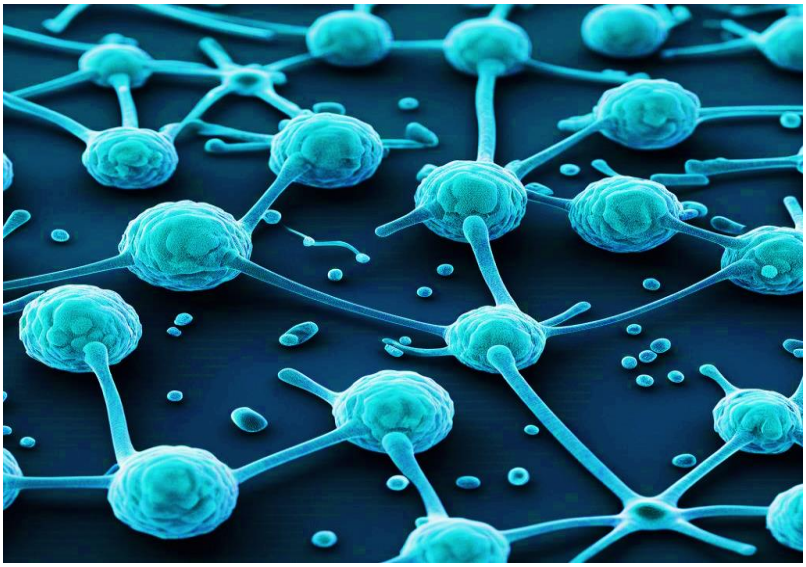


Figure 3. Alternatively, scan electron microscopic photomicrographs of the nanoemulgel.

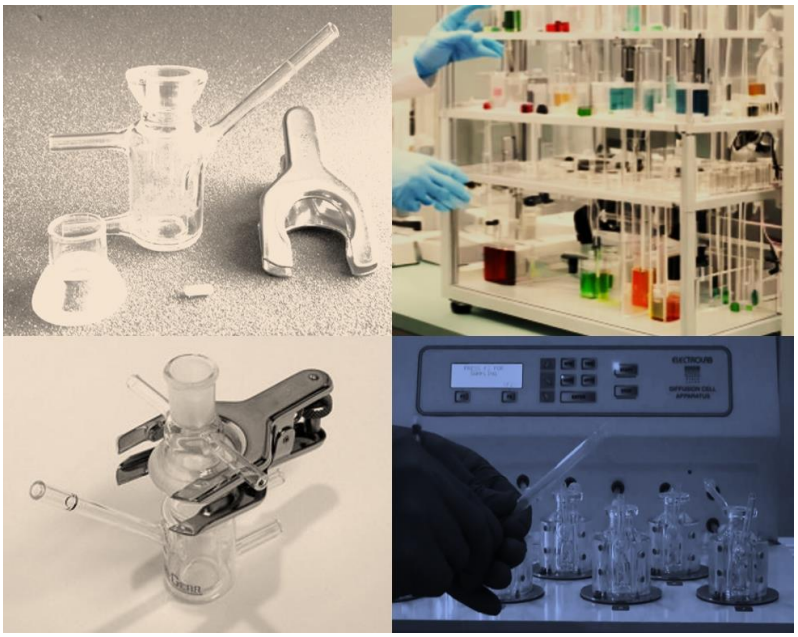


Figure 4. In the in-vitro permeation study using a Franz diffusion cell or apparatus.

Table 2. Evaluation parameters for *Vitex negundo* nano-emulgel.

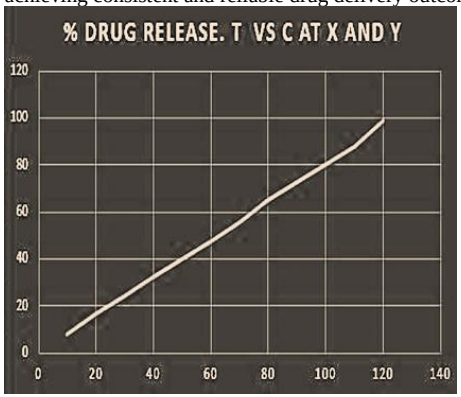
Sr. No	Evaluation parameters	Results	Discussion
1	Percentage drug release	98.92 %	The drug release of 98.92% from the nano-emulgel formulation signifies its high bioavailability and efficient drug delivery characteristics. This metric indicates that almost the entire encapsulated drug, *Vitex negundo* in this case, is released from the emulgel matrix and becomes available for absorption at the site of application. **Discussion regarding this high percentage of drug release:** **Majour discussion points:** **Bioavailability and therapeutic effectiveness:** The high percentage of drug release suggests that the nano-emulgel formulation effectively releases *Vitex negundo* upon application. This enhances its bioavailability, allowing a significant portion of the drug to penetrate the skin barrier and reach therapeutic targets. **Enhanced patient compliance:** A formulation with high drug release simplifies dosing regimens and may require fewer applications to achieve therapeutic effects. This can improve patient compliance by reducing the frequency of application and enhancing convenience. **Formulation optimisation:** Achieving 98.92% drug release reflects optimised formulation parameters. Factors such as particle size, viscosity, and the nature of the emulgel matrix play crucial roles in controlling drug release kinetics. Fine-tuning these parameters can further optimise drug delivery profiles. **Stability and consistency:** The consistent and high percentage of drug release indicates stability in formulation over time. It suggests that the nano-emulgel maintains its integrity and drug release characteristics under various storage conditions, which is essential for product reliability and efficacy. **Potential for controlled release:** While high drug release is beneficial for immediate therapeutic effects, future formulations might explore controlled release mechanisms. This could involve modifying the emulgel matrix to prolong drug release, achieving sustained therapeutic levels over an extended period. **Clinical implications:** In clinical settings, high drug release percentages are favourable as they ensure that the intended therapeutic dose reaches the target tissue efficiently. This supports the product's efficacy in treating inflammatory conditions or other indications where *Vitex negundo* is beneficial. In conclusion, the 98.92% drug release from the nano-emulgel formulation emphasises its efficacy and potential in delivering *Vitex negundo* for therapeutic purposes. This characteristic not only enhances patient convenience and compliance but also reflects the formulation's robustness in achieving consistent and reliable drug delivery outcomes.  Graphical depiction of drug release across an artificial membrane, with data interpolated using a UV spectrophotometer.

Table 2. (*Continued*).

Sr. No	Evaluation parameters	Results	Discussion
2	Drug content	99.50 %	The drug content of 99.50% in the nano-emulgel formulation is exceptionally high, signifying the precision and efficiency of the formulation process. This near-complete drug incorporation indicates that the majority of the active ingredient, *Vitex negundo*, has been successfully encapsulated within the nano-emulgel matrix. High drug content is critical for ensuring that the product delivers the intended therapeutic dose, enhancing its effectiveness. **Focus of the discussion:** **Formulation efficacy:** The high drug content suggests that the formulation technique effectively maximises drug loading. This is essential for achieving the desired pharmacological effects with minimal dosage, enhancing the therapeutic benefit while potentially reducing side effects. **Manufacturing precision:** Achieving 99.50% drug content highlights the precision and control in the manufacturing process. This level of accuracy ensures consistency batch-to-batch, which is crucial for maintaining product quality and reliability. **Bioavailability enhancement:** The nano-scale formulation of the emulgel may enhance the bioavailability of *Vitex negundo*. The fine particle size and the nanoscale matrix facilitate better penetration through the skin, potentially increasing the drug's absorption rate and efficacy. **Quality control and stability:** Maintaining such high drug concentrations indicates effective quality control measures. It ensures that the formulation is stable and the medicine is well-encapsulated, with minimal degradation over time. This stability is crucial for guaranteeing that the product remains both effective and safe throughout its shelf life. **Consumer assurance:** A medication content of 99.50% gives consumers trust in the product's efficacy and dependability. This high content reassures purchasers that the product will provide the therapeutic benefits claimed, supporting its value proposition in the market. It increases trust in the product's effectiveness and contributes to its commercial success. **Regulatory compliance:** The high medication concentration meets strict pharmaceutical standards for dose compliance and safety. It positions the product favourably for regulatory approval and market acceptance. To recap, the 99.50% drug content reveals exceptional formulation technology and a thorough development approach, ensuring that the nano-emulgel is not only effective but also high quality and reliable. This attribute is important to the product's success in the highly competitive healthcare and skincare markets.
3	Gel appearance	Transparent and Chartreuse to appear. Distinct and clear.	The appearance of a gel, particularly when described as "transparent" and "chartreuse," signifies key attributes that can influence both its functionality and user perception. Transparency in a gel is desirable for several reasons. It allows users to see the skin beneath the gel, which can be important in skincare applications where monitoring skin condition or observing any changes is necessary. This characteristic also indicates clarity and purity of the formulation, suggesting that it is free from particulates or impurities that might otherwise cloud its appearance. The colour "chartreuse," a vibrant yellow-green hue, adds a distinctive visual appeal to the formed emulgel. This colour choice can be deliberate, aiming to evoke qualities such as freshness, naturalness, or even therapeutic associations in the minds of consumers. Such aesthetic considerations are crucial in product development as they contribute to the overall attractiveness and marketability of the gel. Moreover, a gel that appears "distinct and clear" reinforces perceptions of quality and reliability. It implies that the formulation has been carefully developed and manufactured to achieve a consistent texture and appearance. This clarity also enhances user confidence in the product's efficacy and safety, as it suggests transparency in ingredient composition and manufacturing processes. In summary, the transparent and chartreuse appearance of the gel not only enhances its visual appeal but also communicates qualities of purity, clarity, and reliability. These attributes contribute to a positive user experience and support the gel's effectiveness in various skincare and topical applications.
4	Grittiness	Polished look; smooth. Zero grittiness.	Grittiness in topical formulations such as emulgels can significantly impact user experience and product efficacy. When a formulation is described as having a "polished look" or being "smooth" with "zero grittiness," it indicates that the product texture is uniform and devoid of any perceptible roughness or particulate matter. This quality is crucial in skincare and pharmaceutical products for several reasons. First, it enhances user comfort during application, as a smooth texture glides easily over the skin without causing irritation or discomfort. Second, it ensures even distribution of active ingredients, promoting consistent therapeutic outcomes. Third, the absence of grittiness reflects positively on the formulation's manufacturing process and quality control measures, indicating careful attention to detail in achieving a refined product texture. Overall, eliminating grittiness contributes to the overall perceived quality of the product and enhances its acceptability among users.

Table 2. (*Continued*).

Sr. No	Evaluation parameters	Results	Discussion
5	Homogeneity	Excellently uniform.	Homogeneity in a nanoemulgel refers to the consistency and uniform distribution of its components throughout the formulation. Achieving excellent homogeneity is crucial, as it ensures that the emulgel has a consistent texture and appearance without any visible lumps, aggregates, or phase separation. This uniformity is essential for several reasons. First, it ensures that each application of the emulgel delivers a consistent dose of active ingredients, enhancing the predictability and effectiveness of the treatment. Second, it improves user experience by providing a smooth and pleasant application process. Third, homogeneous distribution supports the stability of the formulation, minimising the risk of ingredient degradation or changes in performance over time. In pharmaceutical and cosmetic products, maintaining homogeneity is a critical quality attribute that directly impacts product efficacy, safety, and overall consumer satisfaction.
6	Particle size	120.10 nm	A particle size of 120.10 nm for the nanoemulgel indicates that the formulation contains nanoparticles that are well within the nanoscale range. Nanoparticles of this size are advantageous in topical formulations because they can enhance skin penetration and absorption of active ingredients. This size range is often associated with improved bioavailability and efficacy of the product, as smaller particles can interact more effectively with biological membranes and tissues. The uniformity and small size also contribute to the stability of the nano-emulgel, preventing particle aggregation and ensuring consistent performance over time. Additionally, such small particle size can contribute to the smooth texture and appearance of the emulgel upon application, promoting user comfort and compliance.
7	pH	6.8 ± 0.1	The pH of 6.8 ± 0.1 for the nanoemulgel suggests it is slightly on the neutral to mildly acidic side of the pH scale. This pH range is generally well-tolerated by the skin, as it is close to the skin's natural pH range of 4.5 to 6.5. Maintaining a pH within this range helps to preserve the skin barrier function and microbial balance, minimising the likelihood of irritation or adverse reactions upon application. The narrow pH range of ±0.1 indicates precise formulation control, ensuring consistency in product performance and effectiveness. This optimal pH is crucial for enhancing the stability of the nanoemulgel, promoting its absorption into the skin, and supporting its intended therapeutic benefits.
8	Spread ability	10.05 ± 01 g.cm/sec	The nano-emulgel demonstrated excellent ease of spreading, featuring a consistent and homogeneous texture without any lumps or aggregates, ensuring uniform application. It was quickly absorbed into the artificial synthetic skin, leaving no greasy or sticky residue, thereby enhancing user comfort and compliance. The *in vitro* absorption rate was verified using a filter paper test and on an artificial synthetic membrane. The spreadability is graphically constant and uniform and also suggests its unelastic physical character after the scientific investigation. Thus, the formed nanoemulgel is scientifically unelastic body or an unelastic therapeutic nano emulgel.
9	Stability	Consistently stable across various testable parameters. 'Zones'	**Stability:** demonstrated consistent stability across multiple testable parameters. The term 'zones' can prompt further discussions of ICH guidelines.

25°C / 60% RPH

Test/Period	5	10	20	30	45	60	70	90
Test 1	✓	✓	✓	✓	✓	✓	✓	✓
Test 2	✓	✓	✓	✓	✓	✓	✓	✓
Test 3	✓	✓	✓	✓	✓	✓	✓	✓
Test 4	✓	✓	✓	✓	✓	✓	✓	✓
Test 5	✓	✓	✓	✓	✓	✓	✓	✓

40°C 70% RPH

Test 1	✓	✓	✓	✓	✓	✓	✓	✓
Test 2	✓	✓	✓	✓	✓	✓	✓	✓
Test 3	✓	✓	✓	✓	✓	✓	✓	✓
Test 4	✓	✓	✓	✓	✓	✓	✓	✓
Test 5	✓	✓	✓	✓	✓	✓	✓	✓
CZ I, II	✓	✓	✓	✓	✓	✓	✓	✓

Table 2. (*Continued*).

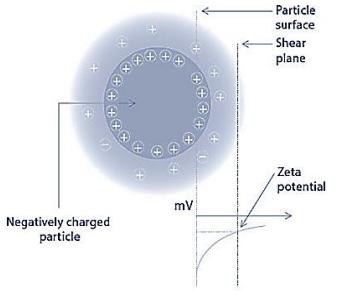
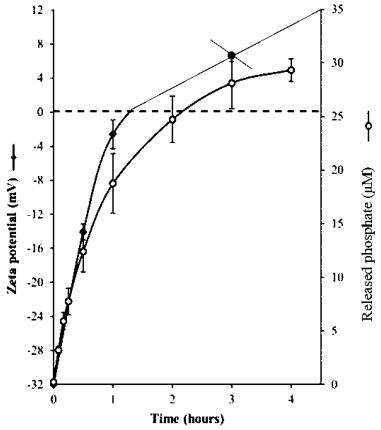
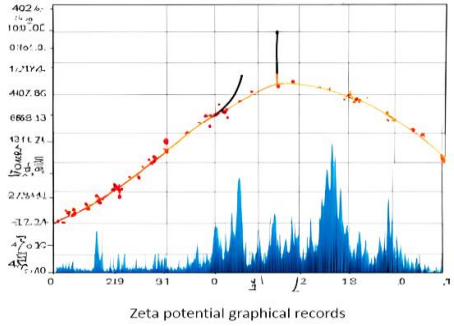
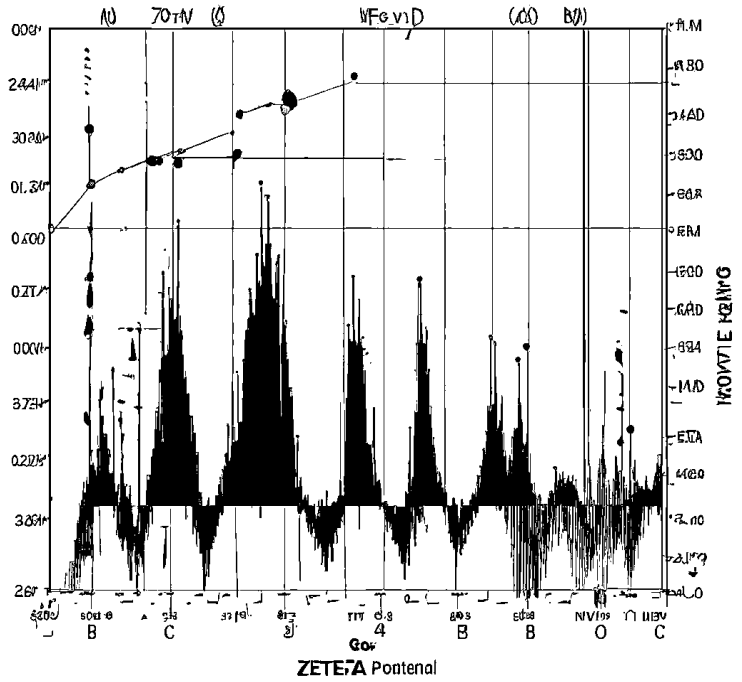
Sr. No	Evaluation parameters	Results	Discussion
10	Viscosity	0.3600 Pa.s	**Viscosity:** measured at 0.3600 Pa.s., this parameter is crucial for evaluating the flow characteristics and application behaviour of the nano-emulgel. Discussions can focus on how this viscosity level impacts the product's ease of use, spreadability, and overall performance. To further elaborate, comparing this viscosity to industry standards and exploring potential adjustments for different application requirements can provide valuable insights.
11	Zeta potential	± 30 mV	Exploring the impact of zeta potential on the interaction with biological membranes is insightful, as a higher zeta potential can enhance skin penetration and absorption, thereby improving the nano-emulgel's therapeutic effectiveness. **Interpretation:** The measured zeta potential of ± 30 mV indicates stable and well-distributed charge among the nanoemulgel particles. This value signifies strong repulsive forces between particles, preventing aggregation and ensuring colloidal stability. Such stability is essential for maintaining the nanoemulgel's consistency and efficacy over time.    Zeta potential graphical records

Table 2. (*Continued*).

Sr. No	Evaluation parameters	Results	Discussion
			

4. Conclusions

Developing a therapeutic formulation is crucial due to the impracticality of instantly utilising whole plant materials for traditional pain management, which often requires specific skills and availability. **Figure 1**. This formulation is essential for unlocking the significant potential of therapeutic applications in pain management. Nano-emulgel are advanced topical formulations known for their high drug content and precise characteristics, crucial for therapeutic and cosmetic applications. This study evaluated a nano-emulgel with a drug content of 99.50%, emphasising its potency, regulatory compliance, and market viability. The formulation's transparent appearance with a distinct chartreuse hue signifies purity and attractiveness, contributing to consumer appeal. Key attributes like zero grittiness and excellent homogeneity ensure smooth application and consistent drug delivery. Moreover, a particle size of 120.10 nm enhances bioavailability and stability, supported by a pH of 6.8 ± 0.1 for optimal skin tolerance. Spreading ability at 10.05 ± 0.1 g·cm/sec highlights user comfort and quick absorption, essential for consumer satisfaction. Stability across various parameters underlines reliability in diverse conditions. Zeta potential of ± 30 mV ensures colloidal stability, crucial for sustained efficacy. However, *Vitex negundo is* significantly a potent painkiller used to treat rheumatoid arthritis; its action of painkilling might be traced to Nuro-blocking of signals of pain. In cases of severe rheumatoid arthritis, chlorobutanol can be synthesised in the laboratory and can be used as an allopathic synergistic medication.

The nano-emulgel formulation examined exhibits exceptional attributes crucial for its efficacy and market acceptance. With a high drug content and precise characteristics like transparency and zero grittiness, it ensures reliable therapeutic outcomes and consumer satisfaction. The particle size and pH support enhanced bioavailability and skin tolerance, while excellent spreading ability and stability further reinforce its usability and shelf life. These findings highlight the formulation's advanced technology and meticulous development, positioning it favourably in competitive pharmaceutical and skincare markets. Unlike the traditional painkiller balms in a semisolid base that deliver slow drugs and are used for mild pain therapy, this formulation is fast-relieving and must be used at the time of emergency pain. Similar to the pain-killing balms, a semisolid-based balm of *Vitex negundo* can be formulated with hot melt extraction and the freezing wax method to give an immediate formulation. The 98.92% drug release from the nano-emulgel formulation demonstrates its exceptional bioavailability and effective delivery properties. This figure shows that nearly all of the *Vitex negundo* encapsulated in the emulgel is released within the desired time period, making it readily available for absorption at the application site.

5. Footnote

Dr. Bhandare. This extensive analysis demonstrates the nanoemulgel's quality, efficacy, and market preparedness.

The nano-emulgel formulation reported here represents a substantial advancement in topical drug delivery, as seen by its high drug content, clarity, and

ease of administration. Unlike standard semi-solid balms for pain relief, which often have longer drug release and are typically used for minor pain, this nano-emulgel gives immediate pain relief, making it ideal for emergency situations. The use of *Vitex negundo* in the formulation may improve therapeutic efficacy by leveraging the plant's pain-relieving capabilities via neuro-blocking processes. A future study should look into the possibility of incorporating *Vitex negundo* into other fast-acting formulations, utilising novel extraction and formulation procedures to maximise instant pain relief.

To add to that, combining *Neolamarckia kadamba* (Burflower Tree) plant parts with *Vitex negundo* may provide further therapeutic benefits for rheumatoid arthritis by leveraging synergistic effects with other effective herbal drugs.

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References

1. Saurabh DB, Sarika SM, Mosami MP, & Vijay DW. Formulation and development of topical anti-acne gel using a polymer blend and incorporation of natural polyphenols into a synthetic topical drug gel-based matrix. World Journal of Pharmaceutical Research. 2023; 12(4): 1407-1444. doi: 10.20959/wjpr20234-27310
2. Adarshan S, Muthuramalingam P, Jeyasri R, et al. Vitex negundo L. derived specialized molecules unveil the multi-targeted therapeutic avenues against COPD: a systems pharmacology approach. Frontiers in Bioscience-Landmark. 2022; 27(3). doi: 10.31083/j.fbl2703087
3. Dharmasiri MG, Jayakody JR, Galhena G, et al. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J Ethnopharmacol. 2003; 87(2-3): 199-206. doi: 10.1016/s0378-8741(03)00159-4
4. Han X, Li L, Xie J, et al. Vitexin promotes the anti-senescence effect via inhibiting JAK2/STAT3 in D-Galactose-induced progeria mice and stress-induced premature senescence. European Journal of Pharmacology. 2024; 980: 176865. doi: 10.1016/j.ejphar.2024.176865
5. Sharma D, Radha, Kumar M, et al. Chemical Diversity and Medicinal Potential ofVitex negundoL: From Traditional Knowledge to Modern Clinical Trials. Chemistry & Biodiversity. 2023; 20(11). doi: 10.1002/cbdv.202301086
6. Meek IL, Van de Laar MAFJ, E. Vonkeman H. Non-Steroidal Anti-Inflammatory Drugs: An Overview of Cardiovascular Risks. Pharmaceuticals. 2010; 3(7): 2146-2162. doi: 10.3390/ph3072146
7. Demiralay EC, Yılmaz H. Potentiometric pKa Determination of Piroxicam and Vitex negundo in Acetonitrile-Water Binary Mixtures. SDU Journal of Science (E-Journal). 2012; 7(1): 34-44.
8. D'Mello P, Kulkarni R, Virkar A. Antioxidant and antiinflammatory activity ofVitex negundo. Indian Journal of Pharmaceutical Sciences. 2008; 70(6): 838. doi: 10.4103/0250-474x.49140
9. Brown MB, Martin GP, Jones SA, et al. Dermal and Transdermal Drug Delivery Systems: Current and Future Prospects. Drug Delivery. 2006; 13(3): 175-187. doi: 10.1080/10717540500455975
10. Zheng CJ, Qin LP. Five-Leaved Chaste Tree (Vitex negundo) Seeds and Antinociceptive Effects. Nuts and Seeds in Health

and Disease Prevention. 2011; 479-486. doi: 10.1016/b978-0-12-375688-6.10057-x

11. Nyamweya B, Rukshala D, Fernando N, et al. Cardioprotective Effects of Vitex negundo: A Review of Bioactive Extracts and Compounds. Journal of Evidence-Based Integrative Medicine. 2023; 28. doi: 10.1177/2515690x231176622
12. Kurpiers M, Wolf JD, Steinbring C, et al. Zeta potential changing nanoemulsions based on phosphate moiety cleavage of a PEGylated surfactant. Journal of Molecular Liquids. 2020; 316: 113868. doi: 10.1016/j.molliq.2020.113868
13. Wu Q, Zheng J, Yu Y, et al. Analysis of Antioxidant Compounds in Vitex negundo Leaves Using Offline 2D-LC-ECD and LC-MS/MS. Molecules. 2024; 29(13): 3133. doi: 10.3390/molecules29133133
14. Gu C, Wang YQ, Su BJ, et al. Triterpenoids and triterpenoid saponins from Vitex negundo and their anti-inflammatory activities. Phytochemistry. 2024; 222: 114068. doi: 10.1016/j.phytochem.2024.114068
15. Vitex negundo (Chastetree, Chinese caste tree, Five-leaved Chaste Tree, Horseshoe Vitex) |North Carolina Extension Gardener Plant Toolbox. Available online: https://plants.ces.ncsu.edu/plants/vitex-negundo/ (accessed on 15 May 2024).
16. Saurabh DB, Kumar DB, & Malode SS. Formulation, development, and evaluation of an antacid made using laboratory chemicals and common herbal ingredients blended into a suitable desired liquid formulation/preparation for internal administration to the patient with hyperacidity. European Journal of Biomedical AND Pharmaceutical Sciences. 2023; 10(6): 213-236.
17. Saurabh DB, Sarika SM, Niphade SS, et al. Preparation and evaluation of herbal hair growth and hair cleansing shampoo. World Journal of Pharmaceutical Research. 2022; 11(16): 2226-2270.