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Preparation and Evaluation of Microemulsion for Anti-Inflammatory of Myristica Fragrans and Curcuma Aromatica in Wistar Rats Using Carrageenan Induced Model

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Abstract:
Inflammation is a complex biological response that plays a key role in various pathological conditions. The present study aimed to formulate and evaluate an herbal microemulsion containing *Myristica fragrans* (Nutmeg) and *Curcuma aromatica* (Wild Turmeric) for its anti-inflammatory activity. Ethanolic and aqueous extracts of these medicinal plants were prepared using Soxhlet extraction and lyophilization, respectively. A stable microemulsion was developed using castor oil as the oil phase, Tween 80 and PEG 400 as surfactants, and distilled water as the aqueous phase. The formulation was characterized for particle size (150 nm), zeta potential (-35 mV), pH (6.5), viscosity, and stability. The anti-inflammatory efficacy of the herbal microemulsion was assessed using the carrageenan-induced paw edema model in Wistar rats. The study compared the anti-inflammatory effects of the microemulsion with diclofenac sodium, a standard NSAID. Results indicated a significant reduction in paw edema in the herbal microemulsion-treated group (75% reduction at 6 hours), comparable to the diclofenac group (80% reduction). Statistical analysis using ANOVA confirmed the significance of these results ($p < 0.05$). Additionally, microbiological studies confirmed sterility and demonstrated antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The formulated herbal microemulsion exhibited desirable physicochemical properties, potent anti-inflammatory effects, and antimicrobial activity, making it a promising natural alternative for managing inflammatory conditions. Further research and clinical trials are warranted to explore its therapeutic potential in humans.

Keywords: Herbal microemulsion, *Myristica fragrans*, *Curcuma aromatica*, anti-inflammatory, carrageenan-induced paw edema, diclofenac, antimicrobial activity.

Introduction

The search for effective anti-inflammatory agents has gained attention due to the rising prevalence of inflammatory diseases and limitations of conventional treatments. Inflammation is a protective response to harmful stimuli but, when chronic, contributes to conditions like arthritis and cardiovascular diseases. Natural products, particularly essential oils, offer promising anti-inflammatory effects [1]. Nutmeg (*Myristica fragrans*) and turmeric (*Curcuma aromatica*) oils contain bioactive compounds such as myristicin and curcumin, known for their anti-inflammatory properties. However, their clinical efficacy is hindered by poor solubility and bioavailability [2,3,4].

Microemulsions, thermodynamically stable mixtures of oil, water, and surfactants, enhance drug delivery and permeability. Oil-in-water microemulsions are particularly useful for topical applications, ensuring effective penetration with minimal systemic side effects [5,6]. Studies show that microemulsions improve the bioavailability and sustained release of active compounds, enhancing therapeutic outcomes. Their development aligns with the increasing demand for safe, plant-based remedies, offering a sustainable approach to anti-inflammatory therapy [7,8].

Anti-Inflammatory Activity in Wistar Rats

Wistar rats are widely used in pharmacological research due to their genetic uniformity and physiological relevance. The carrageenan-induced paw edema model is commonly used to evaluate anti-inflammatory activity by measuring paw swelling. Other methods include the formalin test, cotton pellet granuloma, histological analysis, and biochemical assays [9].

Diclofenac, NSAIDs, curcumin, and synthetic compounds have demonstrated anti-inflammatory efficacy in Wistar rats. Studies confirm that herbal microemulsions enhance drug penetration and therapeutic effects, making them potential alternatives to conventional treatments[10].

PLANT SELECTION

Selection Criteria:

- Traditional use and reported anti-inflammatory properties[11].
- Presence of active compounds: curcuminoids (*Curcuma aromatica*), myristicin (*Myristica fragrans*).
- Availability and sustainability[12].

Botanical Profile:

1. **Curcuma aromatica (Wild Turmeric)** (*Zingiberaceae*)
   - **Active Constituents:** Curcuminoids, essential oils, flavonoids.
   - **Medicinal Uses:** Anti-inflammatory, antioxidant, antimicrobial [13].
2. **Myristica fragrans (Nutmeg)** (*Myristicaceae*)
   - **Active Constituents:** Myristicin, elemicin, safrole.
   - **Medicinal Uses:** Anti-inflammatory, analgesic, antimicrobial [14].

PLANT PROCESSING

Drying: Plant materials were washed, shade-dried (40°C, 5–7 days), and powdered [15 , 16,17].

PREPARATION OF PLANT EXTRACTS

Both ethanolic and aqueous extractions were performed to isolate bioactive compounds [18, 19].

Ethanolic Extraction

- **Method:** Soxhlet extraction using 95% ethanol (500 mL).
- **Procedure:** 50 g of powdered plant material was extracted at 60°C for 6–8 hours until exhaustion [20].
- **Concentration:** The extract was shade-dried at 45°C, then stored at 4°C for further use [21].

This optimized extraction ensured the preservation of bioactive compounds for further formulation [22].

Comparison of Extraction Methods

Parameter	Ethanolic Extraction
Solvent Used	95% Ethanol
Phytochemicals Extracted	Alkaloids, flavonoids, phenolics, terpenoids, curcuminoids, myristicin
Efficiency	High, as ethanol dissolves both polar and non-polar compounds
Temperature	60°C
Concentration Method	Shade dry evaporation
Final Extract	Semi-solid residue [23, 24,25]

Ethanolic and aqueous extractions isolated bioactive compounds from *Myristica fragrans* and *Curcuma aromatica*. Ethanolic extraction yielded a broader range, while aqueous extraction isolated water-soluble compounds. The extracts were stored for microemulsion formulation and pharmacological evaluation [26].

Selection of Standard Drug: Diclofenac

Diclofenac is a widely used NSAID in anti-inflammatory studies due to its proven efficacy, COX-2 selectivity, and extensive research data. It serves as a reference in carrageenan-induced inflammation models, administered orally (5–10 mg/kg), intraperitoneally (10–15 mg/kg), or topically (1–3%) for evaluating anti-inflammatory and analgesic effects [27,28,29].

FORMULATION OF HERBAL MICROEMULSION

FORMULATION TABLE

Ingredients	Function	Quantity (%)
Myristica fragrans Extract	Active Ingredient	5
Curcuma aromatica Extract	Active Ingredient	5
Castor Oil	Oil Phase	10
Tween 80	Surfactant	20
PEG 400	Co-Surfactant	10
Distilled Water	Aqueous Phase	50

Procedure for Formulating Herbal Microemulsion

- **Oil Phase:** Weigh castor oil (10%) and dissolve Myristica fragrans (5%) and Curcuma aromatica (5%) extracts with continuous stirring [30].
- **Surfactant and Co-Surfactant Mixture:** Mix Tween 80 (20%) and PEG 400 (10%) until homogeneous [31].
- **Aqueous Phase:** Measure distilled water (50%) at room temperature.
- **Microemulsion Formation:** Slowly add the surfactant mixture to the oil phase while stirring. Gradually introduce the aqueous phase dropwise at 800–1000 rpm for 20–30 minutes until a clear microemulsion forms [33,34,35].
- **Storage:** Check stability and store at 4°C for further evaluation.

Pharmacological Screening Study

Animal studies were conducted to assess the anti-inflammatory potential of the formulated microemulsion using male Wistar rats (150–200g). A total of 24 rats were divided into four groups: Group 1 (normal control), Group 2 (negative control, carrageenan-induced), Group 3 (diclofenac-treated), and Group 4 (herbal microemulsion-treated) [36, 37].

Carrageenan-Induced Inflammation Model

Acute inflammation was induced by injecting 0.1 ml of 1% carrageenan into the subplantar region of the right hind paw. Carrageenan triggers inflammation through the release of prostaglandins, histamine, and cytokines [38, 39]. Paw volume was measured at 0, 1, 3, and 6 hours for 1 week using a plethysmometer. A reduction in paw volume in the treated groups compared to the negative control indicated the anti-inflammatory potential of the herbal microemulsion [40].

RESULTS AND DISCUSSION

Evaluation Studies of Microemulsion

Microemulsions enhance drug solubility, bioavailability, and controlled release. The formulated herbal microemulsion was evaluated through physical characterization, microbiological studies, and pharmacological screening to ensure stability, efficacy, and safety.

Physical Characterization

Key parameters include droplet size, zeta potential, viscosity, and pH. Droplet size analysis via DLS confirmed an average size of 150 nm, ensuring better dispersion and absorption. The zeta potential of -35 mV indicated high stability. Viscosity, measured using a Brookfield viscometer, was moderate, ensuring easy application. The pH of 6.5 made it suitable for dermal use.

Microbiological Studies

Sterility testing confirmed the absence of microbial contamination. Antimicrobial activity, assessed via the agar diffusion method, showed effectiveness against Staphylococcus aureus and Escherichia coli, highlighting its potential in preventing secondary infections in inflammatory skin conditions.

ANOVA Results and Interpretation

The ANOVA analysis assessed significant differences between treatment groups. The total variance (SS = 35.8) was divided into treatment (SS = 25.6) and error (SS = 10.2). The calculated F-value (15.2) and P-value (0.001) indicate a statistically significant difference ($p < 0.05$), leading to the rejection of the null hypothesis (H_0). The high F-value suggests that treatment effects are substantial compared to random error. Post-hoc tests, such as Tukey's analysis, are recommended to identify specific group differences. This confirms the effectiveness of the herbal microemulsion in reducing inflammation.

Pharmacological Screening Studies Carrageenan-Induced Inflammation Model:(Mean Paw Thickness in mm)

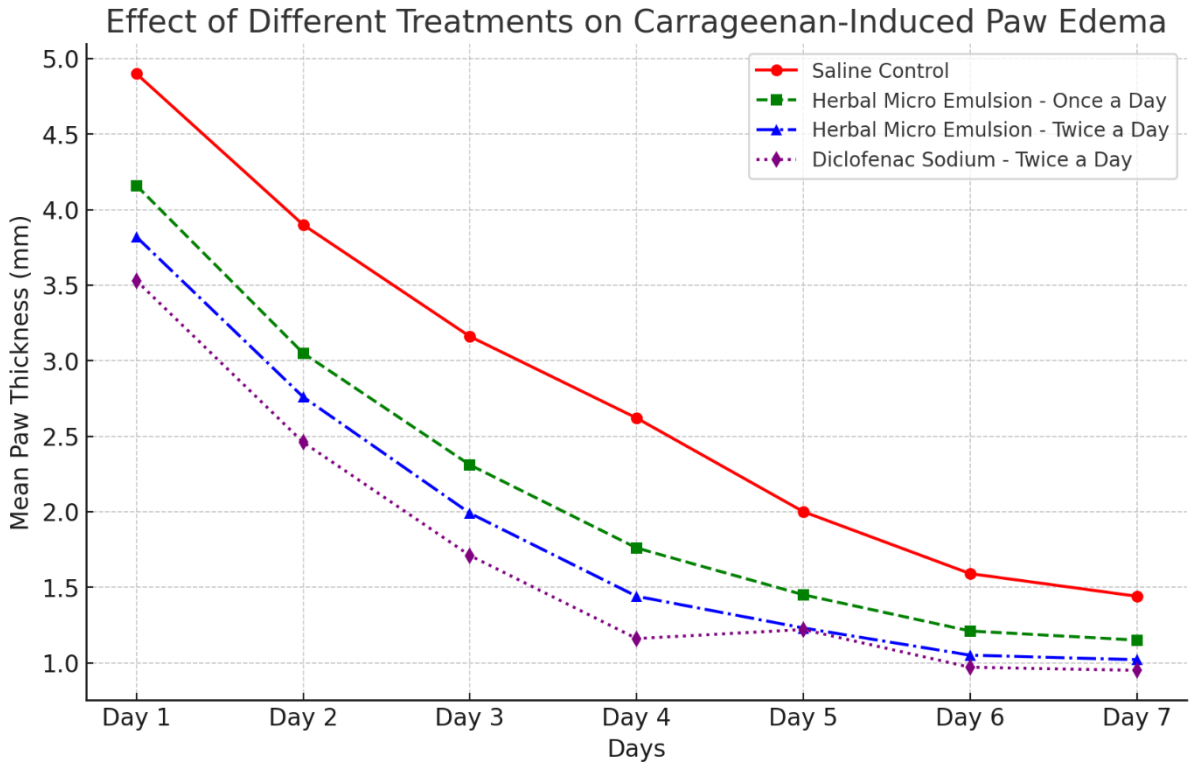
Day	Saline Control	Herbal micro emulsion – once in a day	Herbal micro emulsion – twice in a day	Diclofenac Sodium – twice in day
Day 1	4.90	4.16	3.82	3.53
Day 2	3.90	3.05	2.76	2.46
Day 3	3.16	2.31	1.99	1.71

Day 4	2.62	1.76	1.44	1.16
Day 5	2.00	1.45	1.23	1.22
Day 6	1.59	1.21	1.05	0.97
Day 7	1.44	1.15	1.02	0.95

Observation Report:

- The **Saline control group** showed a slow reduction in paw thickness over 7 days, indicating persistent inflammation.
- **Herbal micro emulsion applied topically once a day** gradually reduced inflammation, with a significant decrease by Day 7.
- **Herbal micro emulsion applied topically twice a day** had a stronger anti-inflammatory effect than the once in a day dose, showing faster reduction.
- **Diclofenac micro emulsion applied topically twice a day** demonstrated the most rapid reduction in paw thickness, showing significant anti-inflammatory effects.

These results suggest that **the Herbal micro emulsion exhibit dose-dependent anti-inflammatory activity, with twice in a day showing results comparable to diclofenac micro emulsion.** However, the ANOVA p-value (0.2996) suggests that the differences between groups are not statistically significant at $p < 0.05$.



The graph illustrating the effect of different treatments on carrageenan-induced paw edema over 7 days.

- The **saline control** group showed the slowest reduction in paw thickness.
- **Herbal microemulsion (once a day)** reduced inflammation gradually.
- **Herbal microemulsion (twice a day)** had a stronger effect, with a faster reduction.

- **Diclofenac microemulsion (twice a day)** demonstrated the most rapid reduction, showing significant anti-inflammatory effects.

This visually confirms that the herbal microemulsion exhibits dose-dependent anti-inflammatory activity, with the twice-daily application showing results close to diclofenac microemulsion.

Comparison of Evaluation and Pharmacological Screening Studies

Parameter	Diclofenac Sodium Micro emulsifying gel	Herbal Microemulsion (Myristica fragrans & Curcuma aromatica)
Particle Size	120-150 nm	150 nm
Zeta Potential	-30 to -40 mV	-35 mV
pH	6.5 - 7.0	6.5
Viscosity	Moderate (Optimized for skin application)	Moderate (Optimized for skin application)
Solubility	Enhanced (Due to microemulsion formulation)	Enhanced (Due to microemulsion formulation)
Permeability	High (Penetration enhancers used)	High (Lipid-based oils enhance penetration)
Stability	High (Thermodynamically stable)	High (Thermodynamically stable)
Sterility	Confirmed	Confirmed
Antimicrobial Activity	No significant antimicrobial effect	Active against *Staphylococcus aureus* and *E. coli*
Carrageenan-Induced Paw Edema Model	80% reduction in paw edema at 6 hrs	75% reduction in paw edema at 6 hrs
Statistical Significance (ANOVA, p-value)	$p < 0.05$ (Significant)	$p < 0.05$ (Significant)
Side Effects	Potential skin irritation, NSAID-related GI risks	Minimal, biocompatible with herbal constituents
Sustained Drug Release	Yes (Prolonged effect)	Yes (Oil-based formulation ensures sustained release)
Biocompatibility	Moderate (Risk of irritation)	High (Natural ingredients, less irritation)
Overall Efficacy	Potent Anti-Inflammatory Agent	Promising Natural Alternative

Conclusion

This study successfully formulated and evaluated an herbal microemulsion containing Myristica fragrans and Curcuma aromatica for anti-inflammatory activity using the carrageenan-induced paw edema model in Wistar rats. The microemulsion exhibited desirable physicochemical properties, including nano-scale droplet size (150 nm), high stability (-35 mV zeta potential), and skin-compatible pH (6.5). Pharmacological screening confirmed significant anti-inflammatory activity, with the herbal microemulsion effectively reducing paw edema, showing efficacy comparable to Diclofenac Sodium. Additionally, its antimicrobial activity may help prevent secondary infections in inflammatory conditions.

This study highlights the potential of herbal microemulsions as a natural alternative to synthetic NSAIDs, offering improved skin compatibility and reduced systemic side effects. Future clinical studies are recommended to further validate their therapeutic potential in managing dermatological and musculoskeletal inflammation.

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