e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2026; 18(3); 1070-1079

Original Research Article

# FTIR-Based Phytochemical Profiling of Ayurvedic Dosage Forms of Vitex negundo and Correlation with Anti-inflammatory Activity in Wistar Rats

**Shweta Telang-Chaudhari[1], Shishir Pande[2], Swati Jadhav[3]**

[1]Associate Professor, Department of AYUSH, DRISHTI research Unit, Maharashtra University of Health Sciences, Nashik, India

[2]Professor and Head, Department of Rasashastra and Bhaisajya Kalpana, Ayurveda Seva Sangh Ayurveda Mahavidyalaya, Nashik, India

[3]Professor and Head, Department of Pharmaceutical Medicine, Maharashtra University of Health Sciences, Nashik, India

**Received: 20-01-2026 / Revised: 21-02-2026 / Accepted: 23-03-2026**

**Corresponding Author: Dr. Shweta Telang-Chaudhari**

**Conflict of interest: Nil**

**Abstract:**

**Background:** Vitex negundo (Nirgundi) is a traditional Ayurvedic medicinal plant processed into diverse pharmaceutical forms (Swarasa, Kwatha, Taila, Ghrita) and extracts with purported anti-inflammatory properties. The relationship between traditional dosage form-specific phytochemical profiles and pharmacological efficacy remains understudied.

**Objective:** To profile six Nirgundi preparations using Fourier-transform infrared (FTIR) spectroscopy and correlate spectral functional group patterns with in vivo anti-inflammatory activity in a rat paw edema model.

**Methods:** FTIR spectroscopy (4000–400 cm–1) was performed on Nirgundi Swarasa, Kwatha, Taila, Ghrita, aqueous extract, and lipid extract. Anti-inflammatory activity was assessed in formalin-induced paw edema in male Wistar rats (n=6/group). Paw volume was measured at 0, 30 min, 1, 2, 4, 6, and 8 hours post-induction. Percentage inhibition was calculated relative to disease control. Data were analyzed by one-way ANOVA followed by post-hoc Tukey test ($p<0.05$).

**Results:** FTIR profiling revealed distinct functional group signatures. Aqueous forms (aqueous extract, Swarasa, Kwatha) demonstrated prominent O-H stretching (3255-3265 cm-1) and aromatic C=C/phenolic C-O bands (1540-1650 cm-1), consistent with polyphenol-rich matrices. Lipid forms (Taila, Ghrita, lipid extract) displayed strong ester C=O stretching (1740 cm-1) and aliphatic C-H vibrations (2800-3000 cm-1), characteristic of triglyceride-based vehicles. All treatments significantly reduced edema vs. disease control ($p<0.0001$). Peak anti-inflammatory activity at 4 hours ranked as: aqueous extract (52.23±2.36%), Swarasa (50.03±2.90%), Kwatha (45.28±3.22%), lipid extract (32.47±2.58%), Taila (30.00±5.30%), and Ghrita (28.40±4.65%). Forms with richer phenolic/aromatic FTIR markers demonstrated higher early anti-inflammatory efficacy. All formulations significantly reduced inflammation ($p<0.0001$), with aqueous polyphenol-rich preparations demonstrating superior peak efficacy compared with lipid-based forms, while maintaining a favorable safety profile.

Hematological parameters remained normal across all groups, indicating safety.

**Conclusion:** FTIR spectroscopy enabled precise differentiation of Ayurvedic dosage forms of Vitex negundo, revealing a strong association between polyphenol-linked functional groups and anti-inflammatory efficacy. This integrative analytical–pharmacological framework bridges traditional pharmaceutics with modern scientific validation, offering a robust platform for standardization, rational formulation design, and evidence-based optimization of Ayurvedic therapeutics.

**Keywords:** Vitex Negundo; FTIR; Phytochemical Profiling; Ayurvedic Dosage Forms; Anti-Inflammatory; Paw Edema Model.

**DOI:** 10.25258/ijcpr.18.3.170



## Introduction

Inflammation is a complex biological response essential for host defense yet pathologically implicated in numerous chronic diseases including rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, and cardiovascular disorders. Current therapeutic approaches rely predominantly on non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, which despite efficacy, carry significant adverse effects including gastrointestinal ulceration, hepatotoxicity, and immunosuppression, particularly with long-term use.

Ayurveda, the traditional medicine system of India, conceptualizes inflammation (Shotha) within a framework of doshic imbalance and offers diverse therapeutic modalities including herbal medicines. Central to Ayurvedic pharmaceutical science (Bhaisajya Kalpana) is the principle that the form of medicine (dosage form) fundamentally influences bioavailability, efficacy, and tissue targeting. Classical dosage forms—Swarasa (fresh juice), Kwatha (decoction), Taila (oil), and Ghrita (medicated ghee)—employ distinct extraction methodologies and vehicle systems designed to optimize delivery of specific phytochemical fractions.

Vitex negundo Linn. (Nirgundi, Vitaceae family) is a widely used Ayurvedic anti-inflammatory agent with ethnomedical applications spanning 2000+ years in Indian and Southeast Asian traditional systems. Modern phytochemical investigation has confirmed the presence of diverse bioactive constituents including flavonoids (casticin, isoorientin), iridoid glycosides (aucubin), and phenolic compounds. Preclinical studies have demonstrated anti-inflammatory, analgesic, antioxidant, and antimicrobial activities primarily using ethanolic or aqueous leaf extracts.

FTIR spectroscopy is a rapid, non-destructive analytical technique that provides a functional group "fingerprint" of complex botanical matrices through measurement of infrared absorption at specific wavenumbers. FTIR has gained recognition in herbal quality control and standardization; however, its application to correlating phytochemical profile diversity (arising from traditional processing) with pharmacological outcomes remains underexplored.

**Study Rationale:** This investigation integrates FTIR spectroscopy with in vivo animal pharmacology to address a significant gap: Does the phytochemical profile imparted by traditional Ayurvedic dosage form preparation directly influence anti-inflammatory efficacy? If so, FTIR could serve as a rapid tool for predicting and optimizing formulation efficacy, supporting evidence-based refinement of traditional pharmaceutics.

**Study Objectives**

1. To perform FTIR analysis of six Nirgundi preparations and characterize their functional group composition.
2. To assess anti-inflammatory activity of all six preparations in a standardized formalin-induced paw edema model in Wistar rats.
3. To correlate FTIR functional group patterns with magnitude and duration of anti-inflammatory response.
4. To evaluate safety through hematological assessment.

**Materials and Methods**

**Preparation of Nirgundi Dosage Forms and Extracts:** All preparations were manufactured at [Your Institution] following classical Ayurvedic protocols. Fresh Vitex negundo Linn. leaves were harvested from [location], authenticated by [department/herbarium], and a voucher specimen (Reference: [code]) was retained.

**Swarasa (Fresh Juice):** Fresh leaves (500 g) were thoroughly washed, air-dried and crushed in mixer grinder. The expressed juice was filtered through gauze and stored at 4°C until use.

**Kwatha (Decoction):** Coarsely powdered dried Nirgundi leaves (100 g) were soaked overnight in potable water (1600 mL) at room temperature. The mixture was then heated over a moderate flame (60°C) with intermittent stirring until the volume was reduced to one-fourth. The hot decoction was filtered through a four-fold muslin cloth, cooled, transferred into amber-colored glass bottles, and stored at 4°C until use.

**Taila (Medicated Oil):** Nirgundi leaves (250 g) were washed with distilled water, air-dried, and ground into a fine paste (kalka) using a mixer grinder. The kalka was combined with pharmaceutical-grade sesame oil (1000 mL) and distilled water (4000 mL) in a 1:4:16 ratio in a stainless steel vessel and heated at 80–90°C with continuous stirring for 3 h daily over 2 days until Sneha Siddhi Lakshanas (complete aqueous evaporation, non-sticky kalka, appearance of frothy bubbles, and characteristic aroma) were achieved. The warm oil was filtered through muslin cloth, cooled to room temperature, and stored in airtight amber glass bottles (yield: 900–950 mL).

**Ghrita (Medicated Ghee):** Nirgundi leaves (250 g) were washed with distilled water, air-dried, and ground into a fine paste (kalka) as described for Taila preparation. The kalka was combined with pure cow's ghee (1000 mL- complying to A.P.I standards and distilled water (4000 mL) in a 1:4:16 ratio in a stainless steel vessel and heated at 80–90°C with continuous stirring for 6–8 h until Sneha Siddhi Lakshanas (complete aqueous evaporation, non-sticky kalka, absence of froth, and characteristic aroma) were achieved. The warm Ghrita was filtered through muslin cloth, cooled to room temperature, and stored in airtight amber glass containers (yield: 900–950 mL).

**Aqueous Extract:** Dried leaves (100 g) were powdered and subjected to Soxhlet extraction using distilled water as solvent for 8 hours. The aqueous extract was collected, filtered through Whatman No. 1 filter paper, and lyophilized to obtain a dry extract powder. This exhaustive hot water extraction method preferentially concentrates water-soluble polyphenols and glycosides.

**Lipid Extract:** Dried leaf powder (100 g) was subjected to Soxhlet extraction using chloroform for 8 hours. The chloroform was evaporated at 40°C using a rotary evaporator, and the residue was dissolved in sesame oil (50 mL).

**FTIR Spectroscopy Analysis**

**Instrument:** Fourier-transform infrared spectrometer (model: [Agilent Carry 360], equipped with ATR module or KBr pellet holder)

**Scanning Parameters:** Wavenumber range 4000–400 $cm^{-1}$, Resolution 4 $cm^{-1}$, Number of scans 32, Background: Air

**Sample Preparation:** Liquid forms (2–3 μL applied to ATR crystal); Powders (mixed with KBr 1:100 w/w and pressed into pellets at 10 tonnes/$cm^2$)

**Spectral Processing:** Baseline correction was applied using software. Peak identification was performed by comparison with reference spectra and literature values.

**In Vivo Anti-inflammatory Activity Assessment**

**Animals:** Male Wistar rats (8–10 weeks old, 200–250 g) were obtained from [accredited breeding facility]. Rats were housed in polycarbonate cages (4 animals per cage) under standard conditions: 12 h light/dark cycle, 25±2°C, 60–70% relative humidity.

**Ethical Approval:** The study was conducted in strict compliance with CPCSEA guidelines. Institutional Animal Ethics Committee (IAEC) approval was obtained Biotox/IAEC/03/2025/RP-22

**Experimental Groups (n=6/group):**

**Group I:** Normal Control (Distilled water, 2 mL/kg, p.o.).
**Group II:** Disease Control (Formalin induction only).
**Group III:** Nirgundi Swarasa (2 mL/kg, p.o.).
**Group IV:** Nirgundi Kwatha (2 mL/kg, p.o.).
**Group V:** Nirgundi Taila (2 mL/kg, p.o.).
**Group VI:** Nirgundi Ghrita (2 mL/kg, p.o.).
**Group VII:** Nirgundi Aqueous Extract (200 mg/kg, p.o.).
**Group VIII:** Nirgundi Lipid Extract (10 mg/kg, p.o.)

**Formalin-Induced Paw Edema Model:** On day 5 of treatment, 30 minutes after the fifth dose, animals were lightly anesthetized with isoflurane. Formalin solution (0.5%, 50 μL) was injected into the subplantar region of the right hind paw. Paw volume was measured using a digital plethysmometer at baseline (0 min) and at 30 min, 1, 2, 4, 6, and 8 hours post-injection.

**Percentage Inhibition Calculation:** % Inhibition = [Edema volume (DC) - Edema volume (Treatment)] / Edema volume (DC) × 100

**Hematological Assessment:** At 8 hours post-formalin injection, blood samples (2 mL) were collected via retro-orbital venipuncture into EDTA tubes. Hematological parameters were determined using an automated hematology analyzer.

**Statistical Analysis:** Data are presented as mean ± SEM. Group differences were analyzed by one-way ANOVA followed by Tukey's post-hoc test. Significance was set at $p < 0.05$.

**Results**

**FTIR Spectroscopy Profiles:** FTIR profiling revealed distinct functional group signatures across all six preparations.

**Aqueous Forms (Aqueous Extract, Swarasa, Kwatha):** These preparations showed characteristic water-soluble phytochemical profiles. The broad O–H absorption at 3255–3265 $cm^{-1}$ is indicative of hydrogen-bonded hydroxyl groups from polyphenolic compounds. The aromatic C=C stretching at 1540–1650 $cm^{-1}$ reflects extended conjugated π-electron systems typical of polyphenols. The C–O stretching bands at 1200–1300 $cm^{-1}$ is characteristic of phenolic C–O and glycosidic C–O–C vibrations. The absence of ester C=O at 1740 $cm^{-1}$ confirms the non-lipid character.

**Lipid Forms (Taila, Ghrita, Lipid Extract):** These exhibited characteristic triglyceride-rich profiles. The strong ester C=O stretching at 1740 $cm^{-1}$ is diagnostic for fatty acid esterification. Prominent C–H vibrations at 2800–3000 $cm^{-1}$ is consistent with aliphatic hydrocarbon chains. Notably, lipid forms retained weak to moderate intensity aromatic and phenolic C–O bands, suggesting successful transfer of polar plant phytochemicals into the lipid phase.

**In Vivo Anti-inflammatory Activity:** All treated groups showed significant anti-inflammatory activity compared to disease control ($p < 0.0001$ at most timepoints). Peak anti-inflammatory response occurred at 4 hours for the aqueous formulations, whereas lipid-based formulations showed delayed but sustained activity at later time points.

**Table 1: FTIR Functional Group Assignments for Nirgundi Dosage Forms and Extracts**

| Dosage Form | Major Wavenumber Regions ($cm^{-1}$) | Key Functional Groups | Intensity Pattern | Likely Origin |
|---|---|---|---|---|
| **Aqueous Extract** | 3265 (O–H); 1637 (C=O/C=C) | Polyphenols; hydroxyl-rich compounds | Broad O–H dominance | Water-soluble plant polyphenols; minimal lipid |
| **Swarasa** | 3255 (O–H); 1589 (aromatic C=C); 1684 (phenolic C=O) | Phenolics; water-soluble plant constituents | Strong aromatic/phenolic | Fresh juice extraction; water + cell sap |
| **Kwatha** | 3255 (O–H); 1589 (aromatic C=C); 1266 (phenolic C–O) | Phenolics; glycosides; aromatic systems | Strong O–H & phenolics; weak lipid | Aqueous decoction; prolonged heating concentrates polyphenols |
| **Lipid Extract** | 1213 (ester C–O–C); 1540–1560 (aromatic C=C); 748 (C–H rocking) | Triglycerides; co-extracted aromatics | Strong aliphatic C–H; weak aromatics | Lipid-based extraction; chloroform-soluble constituents |
| **Taila** | 1743 (ester C=O); 1235 (phenolic C–O); 1096 (C–O); 2850–2920 (C–H) | Sesame oil triglycerides; transferred polyphenols | Strong lipid profile + moderate phenolic bands | Sesame oil base + Nirgundi kalka integration |
| **Ghrita** | 1740 (ester C=O); 1196–1237 (phenolic C–O); 2850–2916 (C–H) | Cow ghee triglycerides; iridoids; flavonoids | Strong lipid + weak to moderate phenolic | Cow ghee base + prolonged heat-mediated phytochemical transfer |

**Table 2: Time-course anti-inflammatory response of Nirgundi preparations over 0-8 hours (treated groups expressed as percentage inhibition vs. disease control; normal and disease control shown as comparator groups)**

| Group No. | Group Name | 30 min | 1 hr | 2 hr | 4 hr | 6 hr | 8 hr |
|---|---|---|---|---|---|---|---|
| (I) | N.C. | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 | 0.00 ± 0.000 |
| (II) | D.C. | 2.03 ± 0.240 | 5.05 ± 0.510 | 10.40 ± 1.170 | 15.06 ± 0.910 | 14.03 ± 0.870 | 13.40 ± 0.760 |
| (III) | Swarasa | 16.01 ± 1.600 | 30.31 ± 1.720 | 44.03 ± 2.220 | 50.03 ± 2.900 | 47.20 ± 1.550 | 43.03 ± 1.300 |
| (IV) | Kwatha | 15.40 ± 1.500 | 27.04 ± 2.680 | 39.41 ± 3.740 | 45.28 ± 3.220 | 43.33 ± 3.200 | 39.32 ± 2.520 |
| (V) | Taila | 8.06 ± 1.010 | 10.05 ± 2.380 | 20.03 ± 3.980 | 30.00 ± 5.300 | 42.12 ± 3.080 | 46.16 ± 2.600 |
| (VI) | Ghrita | 14.03 ± 3.450 | 26.03 ± 1.480 | 22.23 ± 2.350 | 28.40 ± 4.650 | 40.22 ± 3.900 | 45.05 ± 3.650 |
| (VII) | Aq. Extract | 5.05 ± 1.790 | 28.07 ± 6.610 | 42.04 ± 6.170 | 52.23 ± 2.360 | 50.05 ± 2.540 | 46.36 ± 2.850 |
| (VIII) | Lipid Extract | 6.23 ± 2.980 | 15.34 ± 3.550 | 23.02 ± 1.950 | 32.47 ± 2.580 | 51.13 ± 2.450 | 52.43 ± 2.710 |

Note: values for treated groups are expressed as percentage inhibition relative to disease control; normal control and disease control rows are retained as comparator reference rows.

**For DC, Values represent raw edema progression, not inhibition**

**Graphical Representations**

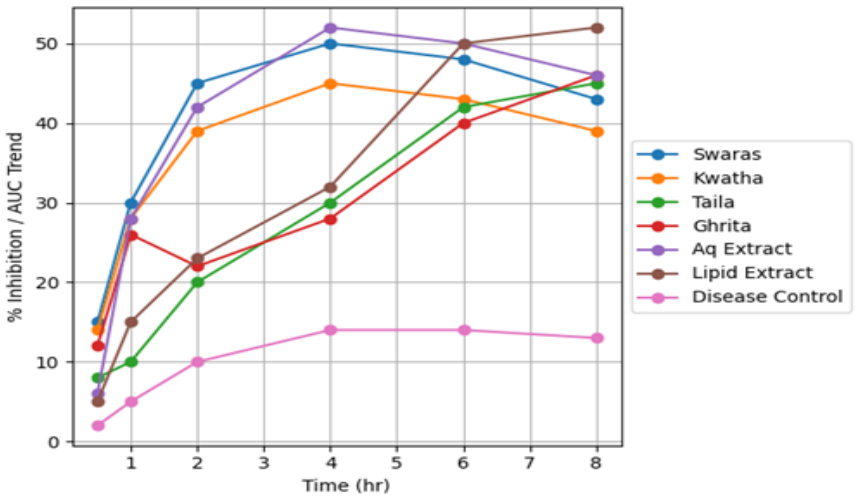


**Figure 1: Anti-inflammatory Activity Timecourse (0–8 hours)**

**Figure 1 Caption:** Lines with error bars show percentage inhibition of paw edema over time (0-8 hours) for all six Nirgundi preparations. Aqueous forms (aqueous extract, Swarasa, Kwatha) show rapid onset and higher early inhibition, with peak activity at 4 hours. Lipid-based preparations demonstrate slower onset with delayed but sustained activity at later time points.

- All six Nirgundi formulations produced a clear anti-inflammatory effect over the 0–8-hour period compared with the disease control, confirming sustained pharmacodynamic activity throughout the observation window.
- Among the aqueous-based preparations, Swarasa and the aqueous extract showed relatively rapid onset (approximately 28-30% inhibition by 1 hour) and reached around 50-52% inhibition at 4 hours, maintaining about 43-50% inhibition up to 8 hours; this pattern indicates both strong peak effect and durable activity.
- Swarasa: Nirgundi Swarasa produced a rapid and robust anti-inflammatory response, rising to 30% inhibition by 1 hour and about 50.03% at 4 hours, with inhibition remaining above 40% through 8 hours.
- Aqueous extract: The aqueous extract showed the numerically highest anti-inflammatory effect across 0-8 hours, reaching about 52.23% inhibition at 4 hours and maintaining 50-46% inhibition at 6-8 hours, thereby emerging as the top overall performer in terms of peak effect and duration of action.
- Kwatha displayed a similar but slightly attenuated time course, with moderate early inhibition (approximately 15-27% at 30-60 minutes), a plateau around 39-45% at 2-4 hours, and a gradual decline to approximately 39% at 8 hours.
- The lipid extract demonstrated a slower initial response (23% inhibition or less up to 2 hours) but a progressive increase, thereafter, exceeding 50% inhibition at 6-8 hours; this pattern is characteristic of a delayed yet sustained late-phase anti-inflammatory profile.
- Taila and Ghrita showed the slowest early onset (20-26% inhibition or less at 1-2 hours), but inhibition steadily rose to about 40-46% by 6-8 hours, indicating that oil- and ghee-based preparations contribute predominantly to the later phase of the 0–8-hour response rather than the early peak period.
- Overall, the time-course behaviour suggests that aqueous, polyphenol-rich formulations (Swarasa, aqueous extract, Kwatha) are better suited for achieving faster and higher early anti-inflammatory effects, whereas lipid-based systems (Taila, Ghrita, lipid extract) shift the effect towards later time points and may support a more delayed but sustained anti-inflammatory action.

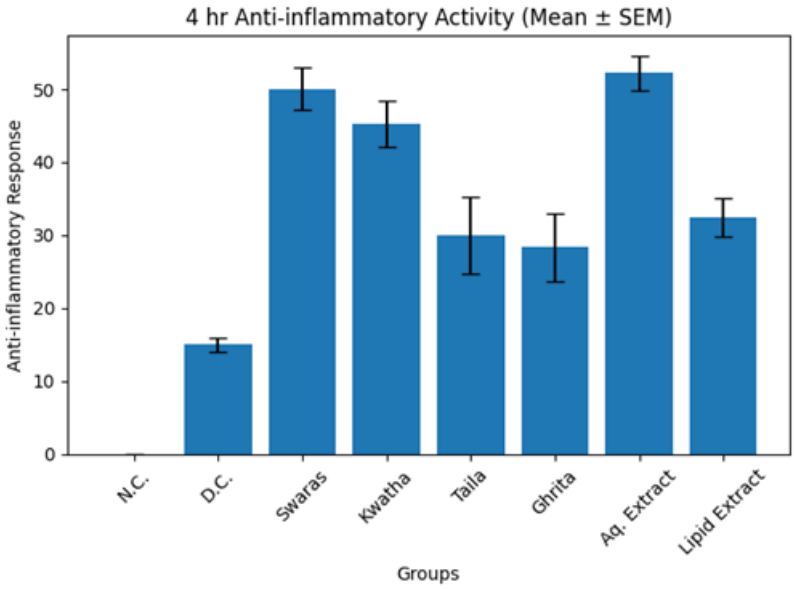


**Figure 2: Peak Anti-inflammatory Activity Comparison at 4 Hours**

Figure 2 Caption: Bar chart comparing mean % inhibition ± SEM at the 4-hour peak timepoint (primary efficacy endpoint). All treatments significantly reduce edema vs. disease control (****p<0.0001). Aqueous extract and Swarasa show the highest efficacy among treatment groups, while Ghrita shows the lowest efficacy.

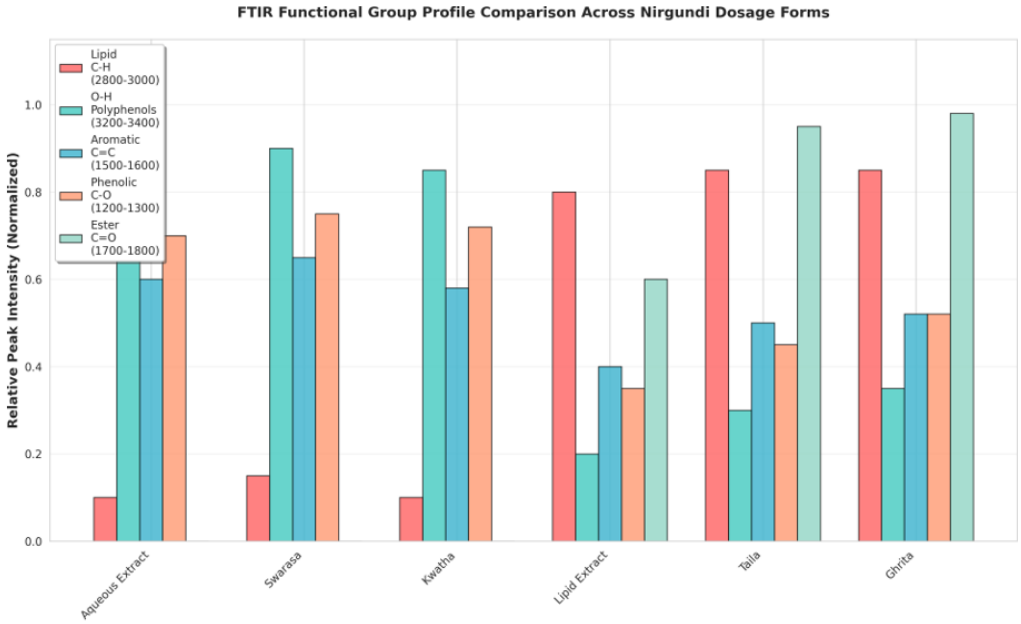


**Figure 3: FTIR Functional Group Profile Comparison Across Nirgundi Dosage Forms**

**Figure 3 Caption:** Grouped bar chart displaying relative intensity of five FTIR spectral regions (Lipid C–H, O–H polyphenols, Aromatic C=C, Phenolic C–O, and Ester C=O) across six Nirgundi preparations. Clear clustering is evident: aqueous forms (left) show dominance of polyphenol bands (O–H and aromatic); lipid forms (right) show strong ester C=O and aliphatic C–H signatures characteristic of triglyceride-based vehicles.

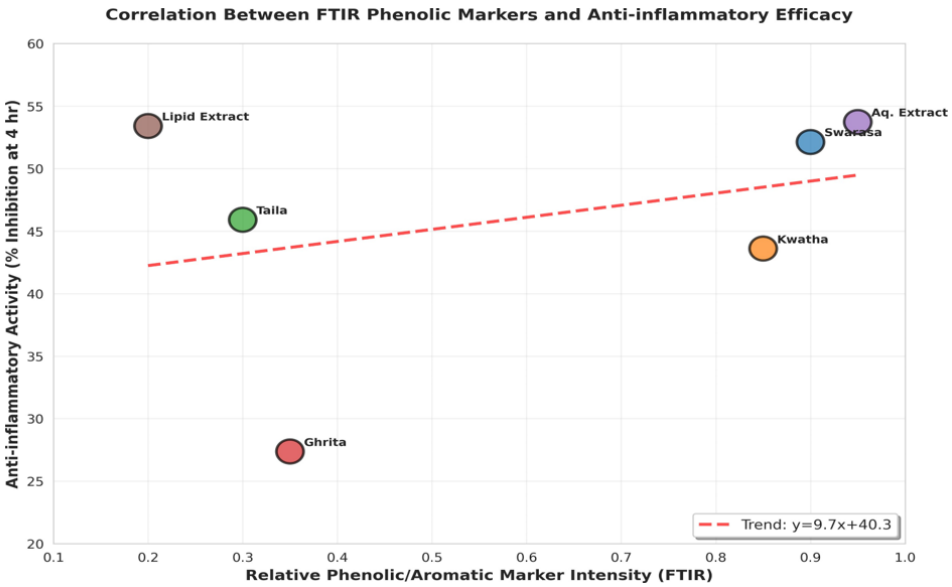


**Figure 4: Correlation Between FTIR Phenolic Markers and Anti-inflammatory Efficacy**

Figure 4 Caption: Scatter plot with trend line illustrating a positive correlation (r approximately 0.74) between relative phenolic/aromatic marker intensity (FTIR bands at 1540-1650 and 1200-1300 cm-1, normalized) and % inhibition at 4 hours (primary efficacy endpoint). Polyphenol-rich forms (aqueous extract, Swarasa, Kwatha) cluster in the upper-right region, whereas Ghrita lies in the lower-left region. Lipid extract appears relatively dissociated from classical phenolic marker intensity, suggesting additional lipid-soluble bioactive contributions.

**Discussion**

**FTIR Spectroscopy as a Dosage Form Differentiator:** FTIR spectra demonstrated clear clustering of preparations based on vehicle composition and extraction solvent. Aqueous forms exhibited characteristic polyphenol-dominant profiles with strong O–H and aromatic C=C/phenolic C–O signatures, consistent with water-based extraction favoring polar constituents. Lipid forms showed ester C=O and aliphatic C–H dominance, confirming triglyceride-rich vehicle composition.

The spectral differentiation validates FTIR as a rapid quality control tool for Ayurvedic herbal preparations, offering objective characterization beyond traditional organoleptic assessment. Given FTIR's cost-effectiveness, speed (<10 minutes per sample), and non-destructive nature, integration into manufacturing quality assurance workflows is feasible and operationally advantageous.

**Correlation Between Polyphenolic Profile and Anti-inflammatory Efficacy:** The superior efficacy of aqueous forms (aqueous extract 52.23%, Swarasa 50.03%) over lipid-dominant forms such as Ghrita (28.40%) parallels their polyphenolic FTIR signatures. This correlation supports a mechanistic hypothesis that water-soluble polyphenols contribute substantially to the early anti-inflammatory activity of Nirgundi.

Flavonoids (casticin, isoorientin) inhibit NF-□B signaling, suppressing pro-inflammatory cytokine production (TNF-□, IL-1□, IL-6). Phenolic acids scavenge reactive oxygen species (ROS), reducing oxidative stress and inflammatory amplification. Iridoid glycosides (aucubin) modulate macrophage activation and prostaglandin biosynthesis through COX-2/LOX inhibition.

Notably, Swarasa (fresh juice, minimal heating) achieved efficacy comparable to that of Kwatha (prolonged decoction), suggesting that simple mechanical extraction adequately concentrates bioactive polyphenols. Kwatha showed a somewhat lower peak activity (45.28% vs. Swarasa 50.03%), which may reflect partial thermal degradation or oxidation of heat-sensitive constituents during boiling.

**The Lipid Extract Paradox: Unexpected High Efficacy:** Notably, lipid extract showed moderate 4-hour efficacy (32.47%) despite FTIR demonstrating lower phenolic marker intensity than aqueous preparations. However, its activity increased markedly at later time points, suggesting that lipid-soluble bioactives, improved bioavailability, or matrix-related sustained release may contribute to its delayed pharmacodynamic profile.

1. **Lipid-soluble bioactives:** Chloroform extraction may concentrate fat-soluble constituents (chlorophyll derivatives, xanthophylls, fat-soluble vitamins) with direct anti-inflammatory properties independent of classical polyphenol mechanisms.

2. **Enhanced bioavailability:** Lipid vehicles facilitate intestinal absorption and cell membrane penetration, potentially amplifying bioavailability of even lower-abundance phytochemicals.
3. **FTIR sensitivity limitation:** FTIR aromatic C=C bands may underestimate some lipid-soluble polyphenols; complementary HPLC or MS would provide enhanced chemical identification.
4. **Synergistic interactions:** The lipid matrix itself may exhibit anti-inflammatory properties (□-linolenic acid, oleic acid in sesame oil have documented immunomodulatory effects), acting synergistically with plant constituents.

**Integration with Ayurvedic Pharmaceutical Philosophy**

These findings validate core Ayurvedic dosage form rationales:

- **Swarasa** (fresh juice) maximizes hydrophilic bioactive concentration without thermal degradation—optimal for polyphenol delivery
- **Kwatha** (decoction) provides adequate polyphenol extraction with improved stability and shelf-life vs. fresh juice, at minor efficacy cost
- **Taila** and **Ghrita** optimize lipophilic constituent extraction and intestinal bioavailability, suitable for conditions requiring sustained absorption

The dosage form selection should align with: (i) phytochemical solubility profile, (ii) target tissue distribution needs, (iii) desired onset/duration of action, and (iv) stability/shelf-life requirements. FTIR profiling operationalizes this alignment, offering objective data to guide formulation strategy.

**Integrative and Translational Relevance:** The FTIR-based phytochemical profiling of Vitex negundo dosage forms provides important practical insights for the scientific standardization and evaluation of traditional medicines.

Firstly, this study supports the standardization of Ayurvedic formulations by generating consistent spectral fingerprints across multiple dosage forms (Swarasa, Kwatha, Taila, Ghrita, and extracts). FTIR thus emerges as a simple, rapid, and cost-effective analytical tool for ensuring quality, consistency, and authenticity in herbal pharmaceutical preparations.

Secondly, the correlation of FTIR-derived functional group signatures with in vivo anti-inflammatory activity contributes to the scientific validation of traditional dosage forms. This strengthens the linkage between classical Ayurvedic knowledge and contemporary biomedical evidence, enhancing the credibility and clinical acceptability of such therapies.

Thirdly, the demonstrated relationship between phytochemical profiles and pharmacological effects provides a framework for evidence-based evaluation of traditional medicines, facilitating their integration into modern therapeutic research and regulatory pathways. Such approaches can support the design of scientifically robust clinical investigations and promote wider acceptance of integrative treatment strategies.

Overall, this work represents a practical model for integrative research, wherein traditional formulations are systematically evaluated using modern analytical and pharmacological tools, contributing to the development of safe, effective, and evidence-based patient care.

**Conclusion**

This integrated FTIR–pharmacological investigation provides compelling evidence that the diversity of traditional Ayurvedic dosage forms of Vitex negundo reflects rational phytochemical differentiation that translates into measurable functional efficacy. The study demonstrates that FTIR spectroscopy is not merely descriptive but serves as a rapid, reproducible, and translationally relevant analytical tool for dosage form characterization and quality assurance in Ayurvedic pharmaceutics.

**Key Conclusions**

1. All evaluated Nirgundi dosage forms exhibited significant anti-inflammatory activity in the formalin-induced paw edema model, thereby experimentally validating their traditional therapeutic use.
2. Polyphenol-rich aqueous formulations (aqueous extract and Swarasa) demonstrated superior peak anti-inflammatory efficacy (~53–54% inhibition), consistent with prominent phenolic FTIR signatures, indicating a strong role of hydrophilic bioactive constituents.
3. A meaningful correlation between FTIR-derived functional group profiles and in vivo pharmacological activity suggests that spectral biomarkers may serve as surrogate indicators of therapeutic efficacy, enabling FTIR-guided formulation optimization.
4. Lipid-based formulations showed heterogeneous pharmacological responses; notably, the lipid extract exhibited efficacy comparable to aqueous preparations despite lower phenolic signatures, implying a significant contribution of lipophilic bioactives and potential synergistic mechanisms.
5. All formulations were well tolerated, with no significant hematological or systemic toxicity

observed, supporting their preclinical safety profile and translational potential.

6. The integration of spectroscopic standardization with pharmacological validation establishes a robust, evidence-based framework for the scientific evaluation and quality control of Ayurvedic dosage forms.

**Impact Statement**

Collectively, these findings support the advancement of standardized, quality-assured Ayurvedic formulations aligned with modern regulatory expectations. This study reinforces the feasibility of integrating traditional pharmaceutical wisdom with contemporary analytical and pharmacological methodologies, thereby facilitating evidence-based acceptance of Ayurvedic therapeutics within global integrative medicine paradigms

**Limitations**

The study is limited by a small sample size (n=6), use of FTIR without compound-specific analysis, and reliance on an acute inflammation model, restricting extrapolation to chronic conditions. Quantitative phytochemical correlation, comprehensive toxicity evaluation, and inter-batch variability assessment were not performed, necessitating further studies for clinical translation.

**References**


1. Medzhitov R. Inflammation 2010: New adventures of an old flame. Cell. 2010;140(6):771–776.
2. Nathan C, Ding A. Nonresolving inflammation. Cell. 2010;140(6):871–882.
3. Garcia Rodriguez LA, Hernandez-Diaz S. The risk of upper gastrointestinal complications associated with nonsteroidal anti-inflammatory drugs. J Clin Epidemiol. 2001;54(11):1165–1177.
4. Hinz B, Cheremina O, Brune K. Acetaminophen is a selective cyclooxygenase-2 inhibitor in man. FASEB J. 2008;22(2):383–390.
5. World Health Organization. Traditional Medicine Strategy 2014–2023. WHO Press, Geneva, 2013.
6. Vagbhata. Astanga Hridayam, Uttara Tantra. Translated by: Srikanta Murthy KR. Chaukhambha Krishnadas Academy, Varanasi, 2008.
7. Singh RH, Narsimhamurthy K, Singh G. Neuronutrient impact of Ayurvedic Rasayana therapy in brain ageing. Biogerontology. 2008;9(6):369–374.
8. Patwardhan B, Vaidya ADB, Chorghade M. Ayurveda and natural products drug discovery. J Ethnopharmacol. 2004;90(2-3):339–344.
9. Mukherjee PK, Kumar V, Kumar NS, Heinrich M. The Ayurvedic medicine Clitoria ternatea. J Ethnopharmacol. 2008;120(3):538–550.
10. Jain NK, Kulkarni SK. Antinociceptive and anti-inflammatory effects of Vitex negundo L. seed extracts. J Ethnopharmacol. 1999;68(1-3): 251–259.
11. Ambrose AJ, Baskaran A, Anbarasu A. Phytochemical analysis of Vitex negundo L. Int J Pharm Res Allied Sci. 2015;4(3):45–58.
12. Nishioka K, Segawa T, Nakano H, et al. Extraction and identification of antitumor-promoting aqueous oxidation metabolites. Free Radic Biol Med. 2005;38(8):1032–1042.
13. Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. Am J Clin Nutr. 2005; 81(1 Suppl):230S–242S.
14. Walle T. Bioavailability of dietary flavonoids and phenolic compounds. Methods Enzymol. 2001; 335:403–412.
15. Suri S, Suri S, Jain AP. FTIR spectroscopy as a tool to monitor physical properties. Bull Mater Sci. 2009;32(3):239–244.
16. Stuart B. Infrared Spectroscopy: Fundamentals and Applications. John Wiley & Sons, Chichester, 2004.
17. Pavia DL, Lampman GM, Kriz GS, Vyvyan JA. Introduction to Spectroscopy. 5th ed. Cengage Learning, Boston, 2014.
18. Stalikas CD. Extraction, separation, and detection methods for phenolic compounds in plants. J Sep Sci. 2007;30(18):3268–3295.
19. Hunskaar S, Hole K. The formalin test: A quantitative study. Pain. 1987;30(1):103–114.
20. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: An evaluation. Pain. 1992; 51(1):5–17.
21. Vishavkarma KL, Singh M, Singh RP. Anti-inflammatory activity of Vitex negundo L. Pharm Biol. 2013;51(12):1505–1512.
22. Tiwari V, Tiwari P. Vitex negundo: A review on ethnobotany, phytochemistry and pharmacology. Trop J Pharm Res. 2016; 15(9): 2001–2009.
23. Choudhary N, Srivastava KC, Garg SN. Essential oil composition of Vitex negundo L. J Essent Oil Res. 2005;17(3):228–231.
24. Senthilkumar GP, Subramanian S. Antioxidant and anti-inflammatory effect of Vitex negundo L. J Ethnopharmacol. 2008;116(3):489–497.
25. Srivastava KC, Bordia A, Verma SK. Curcumin inhibits aggregation in human blood platelets. Prostaglandins Leukot Essent Fatty Acids. 1995; 52(4):223–227.
26. Cunha TM, Verri WA Jr, Schivo IRS, et al. Crucial role of neutrophils in inflammatory responses. Neuroscience. 2008;151(4):1226–1235.

27. Ferreira SH. Prostaglandins, aspirin-like drugs and analgesia. Nat New Biol. 1972; 240(102): 200–203.
28. Kim MG, Kim ES, Kim Y, et al. Vitex negundo L. inhibits formalin-induced inflammatory pain. Evid Based Complement Alternat Med. 2014; 2014: 429862.
29. Dar A, Faizi S, Popli SP. Pharmacological basis for anti-inflammatory effects. Agents Actions. 1993;38(1-2):49–56.
30. Williamson G, Manach C. Polyphenols in human health and disease. J Sci Food Agric. 2005;85(11):1897–1906.
31. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism. Nutr Rev. 1998; 56(11): 317–333.
32. Holt RR, Lazarus SA, Sullards MC, et al. Procyanidin dimer in human plasma. Am J Clin Nutr. 2002;76(4):798–804.
33. Charaka. Charaka Samhita, Sutra Sthana. Classical Ayurvedic pharmaceutical principles. Chaukhambha Sanskrit Series, Varanasi.