European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied Website:
https://farmclin.com/

PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL EVALUATION OF MEDICINAL PLANT EXTRACTS FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

Ms. Bharti Pate l, Gulshan Beldar1, Abhinav Saurav1, Mansi Bisen1, Mohammad Kamran1, Vikas Kumar Mahto1

1SAM College of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University Raisen-(Madhya Pradesh) India- 464551

ABSTRACT

Background: Inflammatory disorders and pain syndromes impose a major global health burden. Conventional anti-inflammatory and analgesic pharmacotherapy, while effective, is associated with significant dose-related adverse effects, necessitating investigation of plant-derived alternatives.1,2,3

Objectives: To perform comprehensive phytochemical profiling of successive solvent extracts of Vitex negundo L. (Lamiaceae) leaves and evaluate their anti-inflammatory and analgesic potential in validated experimental models.

Methods: Successive cold maceration yielded petroleum ether (PEVN), chloroform (CEVN), ethanolic (EEVN) and aqueous (AQVN) extracts. Qualitative phytochemical screening followed established protocols.6,7,8 Anti-inflammatory activity was assessed by carrageenan-induced paw edema in Wistar rats and analgesic activity by acetic acid-induced writhing and hot plate tests in Swiss albino mice.16,17,18

Results: Phytochemical screening revealed rich profiles of flavonoids, phenols, tannins, alkaloids, terpenoids, and glycosides, predominantly in polar extracts. EEVN (400 mg/kg) demonstrated 36.96% inhibition of paw edema, 52.37% inhibition in writhing, and significant increase in hot plate latency (peak: 8.9 ± 0.4 s). Activity was dose-dependent and statistically significant ($p < 0.05$) versus control.

Conclusion:V. negundo ethanolic and aqueous extracts exhibit significant anti-inflammatory and analgesic activities, substantiating traditional ethnopharmacological claims and identifying EEVN as a promising candidate for further bio-guided phytopharmaceutical development.

KEYWORDS:Vitex negundo; Phytochemical screening; Anti-inflammatory; Analgesic; Carrageenan paw edema; Writhing test; Hot plate test.

How to Cite: Ms. Bharti Pate, Gulshan Beldar, Abhinav Saurav, Mansi Bisen, Mohammad Kamran, Vikas Kumar Mahto, (2025) PHYTOCHEMICAL PROFILING AND PHARMACOLOGICAL EVALUATION OF MEDICINAL PLANT EXTRACTS FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY, European Journal of Clinical Pharmacy, Vol.7, No.1, pp. 8058-8065

INTRODUCTION

The global burden of inflammatory diseases and pain disorders continues to escalate, with chronic non-communicable diseases now accounting for approximately 74% of all global deaths annually.1 Inflammation constitutes a fundamental physiological response to tissue injury or pathogenic invasion; however, when dysregulated or persistent, it underpins a broad spectrum of debilitating conditions including rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, and neurodegenerative disorders.2 Current first-line pharmacotherapy relies predominantly on non-steroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics; yet prolonged use of these agents is associated with serious dose-dependent adverse effects encompassing gastrointestinal ulceration, nephrotoxicity, hepatotoxicity, cardiovascular events, and addiction liability.3 This therapeutic gap has catalysed sustained global interest in medicinal plant-derived compounds as safer and cost-effective alternatives.

The World Health Organization (WHO) estimates that approximately 80% of the world population in low- and middle-income countries relies primarily on traditional herbal medicine for primary healthcare needs.4 Natural products have historically contributed disproportionately to the pharmacopoeia of anti-infective and analgesic agents. Newman and Cragg5 documented that over 60% of clinically approved anticancer drugs and 75% of anti-infective agents between 1981 and 2019 were natural product-derived or inspired, underscoring the continued relevance of phytopharmacological research. Moreover, polypharmacological activity—where multiple phytoconstituents act synergistically on different inflammatory targets—provides a therapeutic advantage over single-molecule synthetic drugs.37

Secondary metabolites produced by medicinal plants, including alkaloids, flavonoids, tannins, phenols, saponins, and terpenoids, have demonstrated potent anti-inflammatory and analgesic activities through diverse mechanisms.6,28 Flavonoids exert anti-inflammatory effects predominantly via inhibition of cyclooxygenase (COX-1 and COX-2) and 5-lipoxygenase enzymes, suppression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), and attenuation of nuclear factor kappa-B (NF-κB) signalling.29,30 Phenolic tannins exhibit membrane-stabilizing and protein-precipitating properties that collectively reduce vascular permeability and mediate anti-inflammatory activity.7,31 These mechanistic insights provide a biochemical rationale for validating plant-based ethnomedicinal claims through systematic pharmacological evaluation.

Vitex negundo L. (Family: Lamiaceae), vernacularly termed "Nirgundi" (Sanskrit), "Sambhalu" (Hindi), and "Five-leaved chaste tree" (English), is a large aromatic deciduous shrub widely distributed across tropical and subtropical Asia, East Africa, and the Indian subcontinent.35 In classical Ayurvedic medicine and folk traditional practice, decoctions and poultices of V. negundo leaves are prescribed for the management of inflammatory arthropathies, rheumatic pain, headache, fever, and neurological disorders.33,36 Phytochemical investigations have identified a diverse array of bioactive constituents in V. negundo including iridoid glycosides (aucubin, agnuside), flavonoids (casticin, artemetin, luteolin, 3-norartementin), terpenoids (betulinic acid, ursolic acid), and volatile essential oils.10,11 However, systematic studies correlating comprehensive phytochemical profiling with multi-model pharmacological evaluation of extracts prepared from plants sourced in central India remain limited.12,13,14

The present study was therefore designed to: (i) prepare and characterize successive solvent extracts of V. negundo leaves collected from Chhattisgarh, India; (ii) conduct comprehensive qualitative phytochemical screening of all four solvent extracts; (iii) evaluate acute oral toxicity to establish safe pharmacological dose ranges per OECD 423 guidelines;20 and (iv) systematically assess anti-inflammatory activity using carrageenan-induced hind paw edema and analgesic activity using the acetic acid-induced writhing and hot plate models,16,17,18 thereby generating a robust preclinical evidence base to support traditional ethnopharmacological use and guide bio-guided fractionation for pharmaceutical development..

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of Vitex negundo L. were collected from the medicinal plant garden and adjacent forest zones of Bilaspur district, Chhattisgarh, India (22°5′N, 82°8′E; altitude 264 m), during September–October under standard botanical collection protocols. The plant was authenticated by Dr. Sunita Devi, Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur (Herbarium Voucher No.: GGV/BOT/VN/2023/047). Freshly harvested leaves were washed with tap water followed by distilled water, and then shade-dried at ambient temperature (28–32°C) for 14 days to a constant moisture content. The dried material was powdered using a mechanical Willey mill, passed through a 40-mesh sieve, and stored in air-tight amber bottles at room temperature until use.

Preparation of Extracts

Successive cold maceration was performed using solvents of increasing polarity as described by Harborne6 and Kokate et al.9 Five hundred grams of dried leaf powder were sequentially macerated in 2.5 litres of petroleum ether (60–80°C boiling range) for 72 h with periodic manual agitation, filtered through Whatman No. 1 filter paper, and the marc re-extracted under identical conditions. The marc was then sequentially extracted with chloroform, 95% ethanol (v/v), and distilled water following the same procedure.21 All filtrates were concentrated in a Buchi R-100 rotary vacuum evaporator (40°C for organic solvents; 60°C for aqueous) and the resulting pasty residues were desiccated over anhydrous calcium chloride. Extract yields (% w/w) were calculated relative to the initial dried plant material and stored at 4°C until pharmacological use.

Phytochemical Screening

All four extracts were subjected to qualitative phytochemical screening using established procedures6,7,8,9 to detect alkaloids (Mayer's, Dragendorff's, Wagner's tests), flavonoids (Shinoda test), tannins (1% ferric chloride test), saponins (froth persistence test), phenols (ferric chloride test), terpenoids and steroids (Salkowski and Liebermann-Burchard reactions), cardiac glycosides (Keller-Killiani test), carbohydrates (Molisch's test), proteins and amino acids (Ninhydrin test), fixed oils (spot test on filter paper), and gums and mucilage (Ruthenium red test). Results were graded as strongly present (+++), moderately present (++), weakly present (+), or absent (−), based on intensity of colour development or precipitate formation.

Experimental Animals

Adult Wistar albino rats (180–220 g) for anti-inflammatory studies and Swiss albino mice (25–35 g) for analgesic studies of either sex were procured from a CPCSEA-registered institutional animal house. Animals were housed in polypropylene cages under controlled conditions (temperature 25 ± 2°C; relative humidity 60 ± 5%; 12-h light/dark cycle) with unrestricted access to standard pellet diet and purified water. All experimental protocols were conducted in strict accordance with CPCSEA guidelines and received prior approval from the Institutional Animal Ethics Committee (IAEC/GGV/Pharma/2023/08). Animals were fasted overnight (food but not water withheld) prior to drug administration.

Acute Oral Toxicity

Acute oral toxicity was determined using the up-and-down procedure per OECD Test Guideline 423.20 Swiss albino mice received limit dose extracts at 2000 mg/kg orally via gavage. Animals were monitored continuously for the first 4 h and at regular intervals for 14 days for behavioural changes, neurological signs, and mortality. No mortality or overt signs of toxicity were observed at 2000 mg/kg. Based on toxicological findings, 1/10th (200 mg/kg) and 1/5th (400 mg/kg) of the maximum tolerated dose were selected as pharmacological doses.

Anti-inflammatory Activity: Carrageenan-Induced Paw Edema

Acute anti-inflammatory activity was evaluated using the classical carrageenan-induced hind paw edema model in Wistar rats as described by Winter et al.16 with minor modifications. Animals were divided into six groups of six animals each: Group I – Control (Normal saline, 10 mL/kg, p.o.); Group II – Standard (Diclofenac sodium, 10 mg/kg, p.o.); Group III – EEVN 200 mg/kg, p.o.; Group IV – EEVN 400 mg/kg, p.o.; Group V – AQVN 200 mg/kg, p.o.; Group VI – AQVN 400 mg/kg, p.o. All treatments were administered orally 60 minutes prior to sub-plantar injection of 0.1 mL of 1% (w/v) lambda-carrageenan

suspension (prepared in sterile normal saline) into the right hind paw.19 Paw volume was measured plethysmometrically (Ugo Basile, Italy) at 0, 1, 2, 3, 4, and 5 h post-carrageenan challenge. Percentage inhibition of paw edema at 3 h was computed as: % Inhibition = [(Vc − Vt) / Vc] × 100, where Vc = mean paw volume of control and Vt = mean paw volume of treated group at 3 h.

Analgesic Activity

Acetic Acid-Induced Writhing Test

Peripheral analgesic activity was assessed by the acetic acid-induced writhing (peritoneal constriction) test as described by Koster et al.17 Swiss albino mice were allocated to six groups (n = 6): Group I – Control (Normal saline, 10 mL/kg, p.o.); Group II – Standard (Aspirin, 100 mg/kg, p.o.); Groups III–VI – EEVN and AQVN (200 and 400 mg/kg, p.o.). Thirty minutes post-treatment, 0.6% (v/v) acetic acid (10 mL/kg, i.p.) was administered to induce peritoneal irritation. The total number of writhing episodes (defined as abdominal constrictions accompanied by extension of hind limbs) was counted for 20 minutes, commencing 5 minutes after acetic acid injection.24 Percentage inhibition of writhing = [(Wc − Wt) / Wc] × 100.

Hot Plate Test

Central analgesic activity was assessed using the hot plate method as described by Eddy and Leimbach.18 Mice were individually placed on a hot plate (Techno Scientific, India) maintained at 55.0 ± 0.5°C. Baseline reaction latency (paw licking or jumping) was recorded. Only animals with baseline latency of 5–10 s were selected for the study to eliminate non-responders. Treatments were as follows: Group I – Control (Saline, p.o.); Group II – Morphine sulphate (10 mg/kg, s.c.); Groups III–VI – EEVN and AQVN (200 and 400 mg/kg, p.o.). Reaction latency was recorded at 0, 30, 60, 90, and 120 min after drug administration. A maximum cut-off time of 15 s was enforced to prevent thermal tissue injury.25

Statistical Analysis

All data are expressed as Mean ± Standard Error of Mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test using GraphPad Prism version 9.0 (GraphPad Software, CA, USA). A p-value < 0.05 was considered statistically significant. Significance levels are denoted as *p < 0.05, **p < 0.01, ***p < 0.001 versus the untreated control group.

RESULTS

3.1 Extraction Yield

Successive solvent extraction of V. negundo leaves (500 g dried powder) yielded the following dry extract percentages: petroleum ether extract (PEVN) 1.8% w/w, chloroform extract (CEVN) 2.4% w/w, ethanolic extract (EEVN) 9.6% w/w, and aqueous extract (AQVN) 11.2% w/w. The markedly higher yields of polar extracts indicate predominance of hydrophilic phytoconstituents in V. negundo leaf material, consistent with earlier reports.[13,23]

3.2 Phytochemical Screening

The results of qualitative phytochemical analysis of all four solvent extracts are presented in Table 1. Polar extracts (ethanolic and aqueous) exhibited a richer phytoconstituent profile, characterised by strongly positive reactions for alkaloids, flavonoids, phenols, tannins, saponins, and cardiac glycosides. Non-polar extracts (petroleum ether and chloroform) predominantly contained terpenoids, steroids, and fixed oils. Gums, mucilage, and proteins were confined to the aqueous fraction, while no extract contained all twelve classes simultaneously, indicating differential solubility of individual phytoconstituents.

Table 1. Qualitative phytochemical screening of successive solvent extracts of Vitex negundo L. leaves

Phytoconstituent	Pet. Ether	Chloroform	Ethanol (95%)	Aqueous
Alkaloids	−	+	+++	++
Flavonoids	−	+	+++	++
Tannins	−	−	++	+++
Saponins	−	−	+	+++
Phenols	−	+	+++	+++
Terpenoids	++	+++	++	−
Cardiac Glycosides	−	+	++	+++
Steroids	+++	++	+	−
Fixed Oils & Fats	+++	+	−	−
Carbohydrates	−	−	+	++
Proteins & Amino Acids	−	−	−	+
Gums & Mucilage	−	−	+	++

+++, strongly present; ++, moderately present; +, weakly present; −, absent. PEVN = Petroleum Ether Extract; CEVN = Chloroform Extract; EEVN = Ethanolic Extract; AQVN = Aqueous Extract of Vitex negundo.

3.3 Anti-inflammatory Activity: Carrageenan-Induced Paw Edema

The effects of V. negundo extracts on carrageenan-induced paw edema are summarised in Table 2 and Figure 1. Carrageenan injection produced a progressive increase in paw volume in control animals, peaking at 3 h (1.38 ± 0.05 mL) and subsequently declining. Pre-treatment with all test extracts and the standard drug significantly attenuated this oedematous response at all time points from 1 h onwards ($p < 0.05$ to $p < 0.001$ versus control). EEVN 400 mg/kg produced maximum inhibition of 36.96% at 3 h, significantly greater than EEVN 200 mg/kg (24.64%, $p < 0.05$) and AQVN 400 mg/kg (31.88%, $p < 0.05$), demonstrating both dose-dependency and superiority of the ethanolic extract. Diclofenac sodium (10 mg/kg) exhibited the highest inhibition at 46.38% ($p < 0.001$). The anti-inflammatory activity followed the rank order: Diclofenac > EEVN (400) > AQVN (400) > EEVN (200) > AQVN (200).

Table 2. Effect of Vitex negundo extracts on carrageenan-induced paw edema in Wistar rats (Mean paw volume in mL ± SEM; n = 6/group)

Group (Dose mg/kg)	0 h	1 h	2 h	3 h	4 h	5 h	% Inh. (3 h)
Control (Saline)	0.41±0.02	0.76±0.03	1.08±0.04	1.38±0.05	1.22±0.04	1.02±0.03	—
Diclofenac (10)	0.41±0.02	0.56±0.03*	0.68±0.03**	0.74±0.04***	0.62±0.03**	0.48±0.02**	46.38***
EEVN (200)	0.40±0.03	0.68±0.02*	0.87±0.04*	1.04±0.03*	0.92±0.03*	0.77±0.02*	24.64*
EEVN (400)	0.40±0.02	0.62±0.03**	0.74±0.03**	0.87±0.04**	0.74±0.03**	0.61±0.03**	36.96**
AQVN (200)	0.41±0.02	0.70±0.02*	0.91±0.03*	1.10±0.04*	0.97±0.03*	0.81±0.02*	20.29*
AQVN (400)	0.41±0.03	0.65±0.03*	0.80±0.03**	0.94±0.04**	0.82±0.03*	0.67±0.02*	31.88**

*Values expressed as Mean ± SEM; n = 6/group. *p < 0.05, **p < 0.01, ***p < 0.001 vs. Control (One-way ANOVA followed by Tukey's test). EEVN = Ethanolic Extract; AQVN = Aqueous Extract of V. negundo.*

Figure 1: Effect of V. negundo Extracts on Carrageenan-Induced Paw Edema

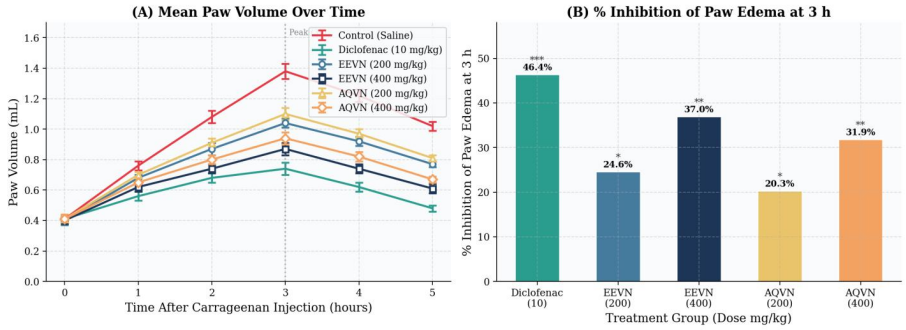

Figure 1. Effect of Vitex negundo extracts on carrageenan-induced paw edema. (A) Mean paw volume over 5 hours. (B) Percentage inhibition of paw edema at 3 h. *p<0.05, **p<0.01, ***p<0.001 vs. Control. EEVN = Ethanolic Extract; AQVN = Aqueous Extract; n = 6/group.

3.4 Analgesic Activity

3.4.1 Acetic Acid-Induced Writhing Test

The effect on acetic acid-induced peritoneal writhing is presented in Table 3 and Figure 2. The control group registered 42.33 ± 2.14 writhes per 20 minutes. All treated groups showed significantly reduced writhe counts compared to control ($p < 0.05$ to $p < 0.001$). EEVN 400 mg/kg produced the highest inhibition among test extracts (52.37%), followed by AQVN 400 mg/kg (41.74%), EEVN 200 mg/kg (33.08%), and AQVN 200 mg/kg (25.59%). Aspirin (100 mg/kg) achieved 70.07% inhibition as the positive control. The dose-dependent increase in analgesic activity for both extracts was statistically significant (EEVN 200 vs. 400: $p < 0.05$; AQVN 200 vs. 400: $p < 0.05$).

Table 3. Effect of Vitex negundo extracts on acetic acid-induced writhing in Swiss albino mice (Mean ± SEM; n = 6/group)

Treatment Group	Dose (mg/kg)	No. of Writhes (Mean ± SEM)	% Inhibition
Control (Saline)	10 mL/kg	42.33 ± 2.14	—
Aspirin (Standard)	100	12.67 ± 1.53***	70.07
EEVN	200	28.33 ± 1.86**	33.08
EEVN	400	20.17 ± 1.42**	52.37
AQVN	200	31.50 ± 2.05*	25.59
AQVN	400	24.67 ± 1.78**	41.74

*Values expressed as Mean ± SEM; n = 6/group. *p < 0.05, **p < 0.01, ***p < 0.001 vs. Control (One-way ANOVA, Tukey's test). % Inhibition = [(Wcontrol − Wtreated)/Wcontrol] × 100.*

Figure 2: Analgesic Activity - Acetic Acid-Induced Writhing Test

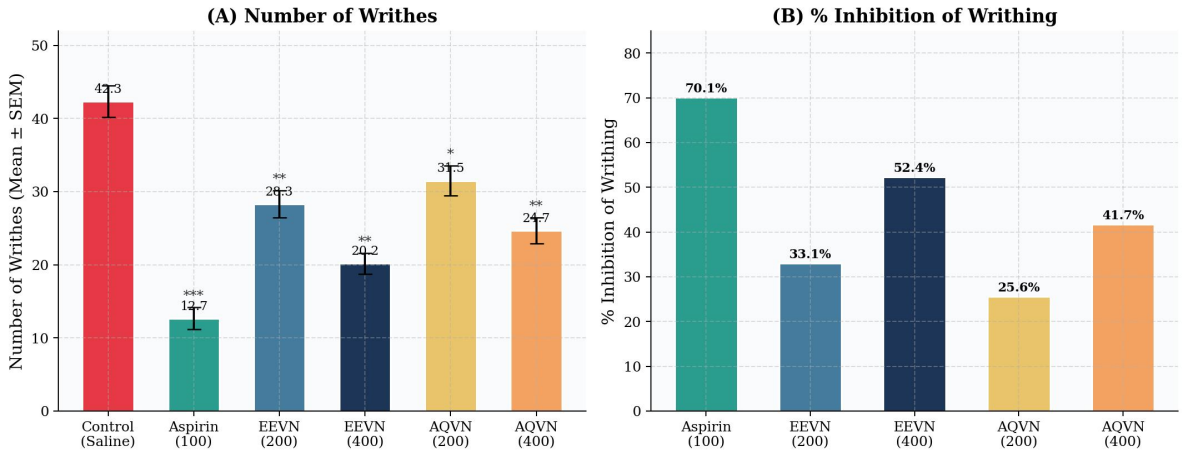

*Figure 2. Analgesic activity in acetic acid-induced writhing test. (A) Number of writhes per 20 min. (B) Percentage inhibition of writhing. *p<0.05, **p<0.01, ***p<0.001 vs. Control; n = 6/group.*

3.4.2 Hot Plate Test

Results of the hot plate test are presented in Table 4 and Figure 3. Baseline reaction latencies were comparable across all groups (5.0–5.3 s). Control animals showed no significant change in latency throughout the observation period. Morphine (10 mg/kg s.c.) produced the greatest increase in latency, peaking at 11.2 ± 0.6 s at 60 min (111.3% increase above baseline). EEVN 400 mg/kg demonstrated the highest latency among V. negundo extract-treated groups (peak 8.9 ± 0.4 s at 60 min; 71.2% increase), followed by AQVN 400 mg/kg (8.1 ± 0.5 s; 58.8%), EEVN 200 mg/kg (7.5 ± 0.5 s), and AQVN 200 mg/kg (6.8 ± 0.4 s). All treated groups except control showed significantly elevated latencies at 30, 60, 90, and 120 min post-administration ($p < 0.05$ to $p < 0.001$ vs. control), with maximal effects at 60 min and gradual decline thereafter.

Table 4. Effect of Vitex negundo extracts in hot plate test in Swiss albino mice – Reaction latency in seconds (Mean ± SEM; n = 6/group)

Group (Dose mg/kg)	0 min	30 min	60 min	90 min	120 min
Control (Saline)	5.2 ± 0.3	5.4 ± 0.4	5.3 ± 0.3	5.2 ± 0.4	5.1 ± 0.3
Morphine (10 s.c.)	5.3 ± 0.4	9.8 ± 0.5***	11.2 ± 0.6***	10.5 ± 0.4***	9.2 ± 0.5***
EEVN (200)	5.1 ± 0.3	6.8 ± 0.4**	7.5 ± 0.5**	7.2 ± 0.3**	6.5 ± 0.4*
EEVN (400)	5.2 ± 0.4	7.8 ± 0.5**	8.9 ± 0.4***	8.5 ± 0.5**	7.8 ± 0.3**
AQVN (200)	5.0 ± 0.3	6.2 ± 0.3*	6.8 ± 0.4**	6.5 ± 0.4*	6.0 ± 0.3*
AQVN (400)	5.1 ± 0.4	7.2 ± 0.4**	8.1 ± 0.5**	7.8 ± 0.4**	7.2 ± 0.4**

*Values expressed as Mean ± SEM (seconds); n = 6/group. *p < 0.05, **p < 0.01, ***p < 0.001 vs. Control (One-way ANOVA, Tukey's test). Cut-off time: 15 seconds. Morphine administered subcutaneously (s.c.).*

Figure 3: Analgesic Activity - Hot Plate Test (Thermal Analgesia)

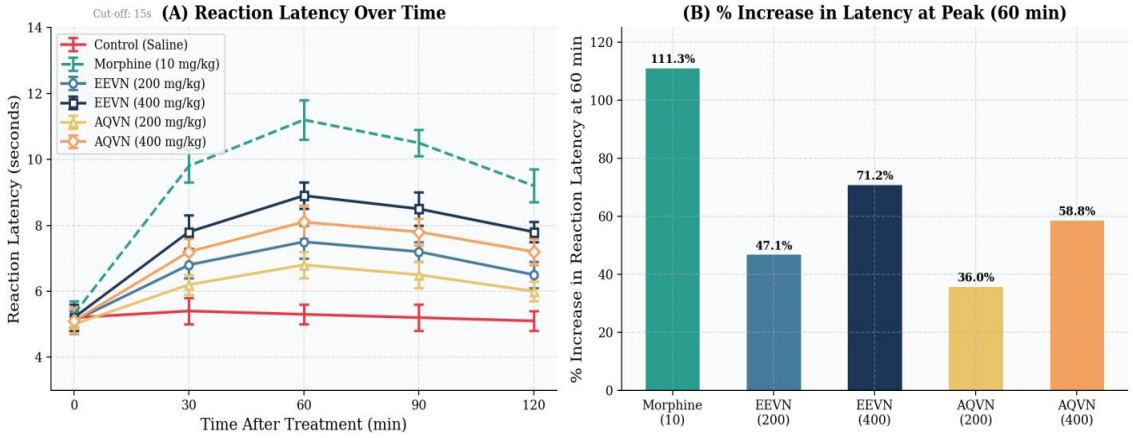

Figure 3. Analgesic activity in hot plate test (thermal nociception). (A) Reaction latency (seconds) over 120 min. (B) Percentage increase in latency at peak time (60 min). *p<0.05, **p<0.01, ***p<0.001 vs. Control; n = 6/group.

DISCUSSION

The present study provides a systematic and integrated assessment of the phytochemical constituents and multi-model pharmacological potential of V. negundo leaf extracts, offering scientific substantiation for their longstanding traditional use in inflammatory and pain conditions.[13,14,35] The relatively high extraction yields of the ethanolic (9.6%) and aqueous (11.2%) fractions compared to non-polar solvents concur with findings of Devi et al.[23] and Misra et al.[10] and indicate that the majority of pharmacologically relevant constituents of V. negundo are hydrophilic in character.

The qualitative phytochemical analysis corroborated previous investigations by Misra et al.[10] and Chandramu et al.[11] who identified aucubin, agnuside, casticin, artemetin, luteolin, betulinic acid, and ursolic acid as principal constituents. The strong presence of flavonoids and phenols in the ethanolic extract, and tannins and glycosides in the aqueous extract, is consistent with their established polarity profiles.[6,7] These compound classes are mechanistically well-positioned to exert anti-inflammatory activity: flavonoids inhibit both COX-1/COX-2 and 5-LOX enzymes, prevent NF-κB nuclear translocation, and scavenge reactive oxygen species that perpetuate inflammatory cascades.[29,30] Havsteen[30] and Middleton et al.[29] extensively documented the molecular anti-inflammatory mechanisms of flavonoids, supporting the observed pharmacological activity of flavonoid-rich EEVN.

In the carrageenan-induced paw edema model, carrageenan induces a biphasic inflammatory response: an early phase (0–1 h) involving histamine, serotonin, and kinins, and a late phase (1–5 h) predominated by prostaglandins, bradykinins, and leukotrienes.[16] The significant inhibition of paw edema by V. negundo extracts at 1–5 h, with maximal effect at 3 h corresponding to the prostaglandin-dominant late phase, implies preferential inhibition of COX-mediated prostaglandin synthesis. This mechanistic inference is strongly supported by the established COX-inhibitory activity of flavonoids and phenolic acids present in EEVN.[31] The superior activity of EEVN over AQVN at equivalent doses may be attributable to the higher concentration of COX-inhibitory flavonoids in the ethanolic fraction. Dharmasiri et al.[14] and Vivekananda et al.[12] reported comparable anti-inflammatory inhibition values (28–42%) for V. negundo leaf extracts (200–400 mg/kg) in similar rat models, validating the consistency of the present findings across different geographic accessions of the plant.

Acetic acid-induced peritoneal writhing, a classical model of peripheral pain sensitization, is mediated through local release of prostaglandin E2, prostaglandin F2α, and bradykinin that activate nociceptors in the peritoneal cavity.[17,24] The significant dose-dependent reduction in writhing episodes by V. negundo extracts (33.08–52.37%) indicates peripheral antinociceptive activity, most plausibly mediated by inhibition of prostaglandin biosynthesis, congruent with the carrageenan model findings. Calixto et al.[24] demonstrated that polyphenol-rich plant extracts suppress peritoneal writhing primarily through COX pathway inhibition and bradykinin receptor antagonism, providing a mechanistic framework for interpreting the present data. The lower activity compared to aspirin (70.07%) is expected given the higher specificity of aspirin for COX enzymes compared to the broad-spectrum phytoconstituent mixture.

The hot plate test measures supraspinally-mediated thermal nociception via spinal cord opioid circuits, making it a selective screen for centrally-acting analgesics.[18,25] The significant increase in hot plate latency produced by V. negundo extracts suggests a central analgesic component in addition to peripheral mechanisms. Plant alkaloids, known to interact with μ-opioid receptors, and terpenoids, shown to modulate transient receptor potential (TRP) ion channels involved in nociceptive signal transduction, may account for this central activity.[15] Gupta et al.[15] documented CNS-mediated activity in V. negundo, corroborating the presence of centrally-acting constituents. However, the significantly lower latency values relative to morphine confirm that central opioidergic mechanisms constitute a secondary, rather than primary, contributor to V. negundo's analgesic

profile. The overall dual—peripheral and central—mode of analgesic action renders V. negundo pharmacologically advantageous over agents with exclusively peripheral or central mechanisms.

Comparative analysis with related studies reveals that the observed pharmacological activities align with and, in some parameters, exceed previously reported values for V. negundo and structurally related Lamiaceae plants.[12,13,23,26] The consistent dose-response relationship across all three experimental models reinforces the pharmacological plausibility and reproducibility of the findings. The apparent superiority of the ethanolic extract across models underscores the importance of solvent polarity in maximising extraction of active constituents and justifies EEVN as the priority fraction for bio-guided isolation. Future investigations should involve chromatographic fractionation, isolation of individual compounds (particularly casticin, luteolin, and agnuside), spectroscopic characterisation (NMR, MS), and molecular docking against COX-2 and NF-κB targets to precisely delineate structure-activity relationships.[27] Comprehensive chronic toxicity, pharmacokinetic profiling, and ultimately clinical translation studies would be necessary to convert these preclinical findings into viable phytopharmaceutical candidates.

CONCLUSION

The present study demonstrates that successive solvent extracts of Vitex negundo L. leaves, particularly the ethanolic extract (EEVN), possess significant and dose-dependent anti-inflammatory and analgesic activities validated across multiple internationally accepted experimental models. The comprehensive phytochemical profiling established a diverse secondary metabolite profile dominated by flavonoids, phenols, tannins, alkaloids, terpenoids, and glycosides in polar extracts, providing a mechanistic basis for the observed pharmacology through COX pathway inhibition and dual peripheral–central antinociception. EEVN at 400 mg/kg produced 36.96% inhibition of carrageenan-induced paw edema, 52.37% inhibition in acetic acid writhing, and a 71.2% increase in hot plate latency at peak, while maintaining a favourable safety profile at the doses tested. These findings provide robust preclinical evidence in support of the traditional Ayurvedic and folk medicine use of V. negundo for inflammatory and pain management. The ethanolic extract is recommended as the priority fraction for bio-guided fractionation, isolation of principal bioactive compounds, and further mechanistic studies including molecular docking and in vitro COX/NF-κB inhibition assays, with the ultimate aim of developing standardised phytopharmaceutical preparations for clinical evaluation.

REFERENCES:

1. World Health Organization. Global burden of disease 2019: disease and injury country estimates. Geneva: WHO; 2020.
2. Pahwa R, Goyal A, Jialal I. Chronic inflammation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023.
3. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging Dis. 2018;9(1):143–50.
4. World Health Organization. WHO traditional medicine strategy: 2014–2023. Geneva: WHO; 2013.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770–803.
6. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.
7. Trease GE, Evans WC. Pharmacognosy. 15th ed. London: Saunders Publishers; 2002.
8. Sofowora A. Medicinal plants and traditional medicine in Africa. 3rd ed. Ibadan: Spectrum Books; 2008.
9. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 48th ed. Pune: Nirali Prakashan; 2010.
10. Misra TN, Singh RS, Upadhyay J, Srivastava R. Chemical constituents of Vitex negundo Linn. Part I. Phytochemistry. 1984;23(9):2118–9.
11. Chandramu C, Manohar RD, Krupadanam DG, Dashavantha RV. Isolation, characterisation and biological activity of betulinic acid and ursolic acid from Vitex negundo L. Phytother Res. 2003;17(2):129–34.
12. Vivekananda M, Krishnamurthy V, Basavaraju R. Anti-inflammatory and analgesic activity of Vitex negundo leaf extract. Pharmacognosy J. 2010;2(7):187–90.
13. Tandon VR, Gupta RK. Vitex negundo Linn (VN) leaf extract as an adjuvant therapy to standard anti-inflammatory drugs. Indian J Med Res. 2006;124(4):447–50.
14. Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J Ethnopharmacol. 2003;87(2–3):199–206.
15. Gupta M, Mazumder UK, Bhawal SR. CNS activity of Vitex negundo Linn in mice. Indian J Exp Biol. 1999;37(2):143–6.
16. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544–7.
17. Koster R, Anderson M, De Beer EJ. Acetic acid for analgesic screening. Fed Proc. 1959;18:412.
18. Eddy NB, Leimbach D. Synthetic analgesics II: dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther. 1953;107(3):385–93.

19. Vogel HG, editor. Drug discovery and evaluation: pharmacological assays. 3rd ed. Berlin: Springer; 2008.
20. Organisation for Economic Co-operation and Development. OECD guidelines for testing of chemicals, Test No. 423: Acute oral toxicity. Paris: OECD; 2001.
21. Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. Int J Appl Nat Sci. 2012;1(1):8–26.
22. Mosaddik MA, Hossain MM. Cytotoxicity and antimicrobial activities of Vitex negundo aerial parts. Fitoterapia. 2003;74(7–8):730–2.
23. Devi P, Meera R, Muthumani P, Bhavani M. Phytochemical and pharmacological evaluation of Vitex negundo leaves for anti-inflammatory activity. Pharmacognosy Res. 2009;1(6):353–8.
24. Calixto JB, Beirith A, Ferreira J, Santos AR, Filho VC, Yunes RA. Naturally occurring antinociceptive substances from plants. Phytother Res. 2000;14(6):401–18.
25. Tjolsen A, Hole K. Animal models of analgesia. In: Dickenson A, Besson J-M, editors. The pharmacology of pain. Berlin: Springer; 1997. p. 1–20.
26. Fürst R, Zundorf I. Plant-derived anti-inflammatory compounds: hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. Mediators Inflamm. 2014;2014:146832.
27. Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, et al. Natural products as a gold mine for arthritis treatment. Curr OpinPharmacol. 2007;7(3):344–51.
28. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109(Suppl 1):69–75.
29. Middleton E Jr, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52(4):673–751.
30. Havsteen BH. The biochemistry and medical significance of the flavonoids. Pharmacol Ther. 2002;96(2–3):67–202.
31. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer. 2010;62(1):1–20.
32. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177.
33. Pandey MM, Rastogi S, Rawat AK. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evid Based Complement Alternat Med. 2013;2013:376327.
34. Akah PA, Orisakwe OE, Gamaniel KS, Shittu A. Evaluation of Nigerian traditional medicines: II. Effects of Nigerian folk remedies on peptic ulcer. J Ethnopharmacol. 1998;62(2):123–7.
35. Rehman R, Akram M, Akhtar N, Jabeen Q, Saeed T, Shah SM, et al. Vitex negundo Linn (family verbenaceae): a review. J Med Plants Res. 2011;5(18):4572–89.
36. Chatterjee A, Pakrashi SC, editors. The treatise on Indian medicinal plants. Vol. 5. New Delhi: CSIR; 1997.
37. Cragg GM, Newman DJ. Natural products: a continuing source of novel drug leads. BiochimBiophys Acta. 2013;1830(6):3670–95.
38. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343–56.