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Review Article

Phytochemistry and Pharmacological Potential of Vitex Negundo Linn.: A Critical Review of Traditional Uses, Bioactive Constituents, Quality Control and Future Direction

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ABSTRACT

Vitex negundo Linn., known as Nirgundi, is a fragrant medicinal shrub that features prominently in the folk medicine of South and Southeast Asia. Researchers have taken an interest in the plant because of its chemical variety and the many biological effects described for its extracts and purified compounds. The literature records flavonoids, iridoid glycosides, lignans, terpenoids, triterpenoids, phenolics and volatile components across different parts of the plant. Laboratory work has looked at anti-inflammatory, pain-relieving, antioxidant, antimicrobial, liver-protective, heart-protective, metabolic and neurological actions. The results, however, shift with the identity of the plant, where it was grown, the part used, the extraction solvent, the dose, the assay and the model, which makes side-by-side comparison hard and restricts extrapolation from one preparation to another. Recent reviews point to better chromatographic and spectrometric characterization, including HPLC, UHPLC, LC–MS/MS and NMR, yet they also stress shortfalls in biomarker-based standardization, pharmacokinetics, toxicology and clinical confirmation. This review offers a critical discussion of botanical identity, ethnomedicinal background, phytochemical groups, pharmacological evidence, analytical quality control, safety issues and future research needs. Special emphasis is placed on separating traditional use, preclinical activity and benefit actually demonstrated in people. The available data justify further study of authenticated, chemically characterized preparations, but they do not support the assumption that every traditional preparation is safe and effective. Moving toward reproducible phytopharmaceutical products will demand sound study design, validated analytical methods, uniform raw material, transparent reporting, exposure assessment and well-designed clinical studies.

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INTRODUCTION

Traditional healthcare has relied on medicinal plants for centuries, and they still matter to modern pharmacognosy, natural-product chemistry and drug-discovery research [10–13]. Plants supply intricate mixtures of primary and secondary metabolites, a few of which may act on biological targets or serve as starting scaffolds for further work. Even so, a record of traditional use or a favourable laboratory finding cannot by itself prove clinical efficacy. Any evidence has to be judged against the kind of preparation, the experimental model, the methodological quality and whether the effect can be reproduced. *Vitex negundo* Linn., called Nirgundi in India, has been documented in the ethnomedicinal literature of several Asian regions. In traditional practice it has been linked with pain, swelling, inflammatory complaints, certain skin conditions and other indications [2,4]. Such records give research a valuable starting point, yet the indications differ by locality and preparation, so they should be reported with their context and not presented as universal or clinically proven effects. Phytochemical studies of the species have reported several broad chemical groups: flavonoids, iridoid glycosides, lignans, terpenoids, triterpenoids, phenolic compounds and volatile constituents [1,3,45].

Vitexin and isovitexin are commonly cited flavone C-glycosides, whereas negundoside and agnuside are among the iridoid glycosides noted in the literature. The precise chemical profile is governed by the plant part, growing conditions, harvest stage, drying, storage and extraction method. For that reason, an activity found for one extract cannot simply be attributed to every extract of the species. Reviews from recent years have summarized the traditional uses and broad pharmacological potential, and one 2026 review specifically covers progress in phytochemistry, pharmacological mechanisms, analytical standardization and biotechnological strategies [3–6,8,9]. A second 2026 review addresses pharmacological attributes together with molecular docking [1]. Together these publications show that a wide catalogue of activities already exists; a worthwhile review should therefore organize the evidence critically, make its limitations clear and point out the practical steps that would improve reproducibility, instead of merely repeating lists of activities. A key concern in medicinal-plant research is keeping the levels of evidence apart. Chemical antioxidant capacity, cell-based activity, efficacy in animal models and clinical outcomes in humans are not interchangeable. Molecular docking can hint at a possible interaction but cannot confirm that a target is engaged in a living organism. Likewise, an anti-inflammatory effect in a cell model may support a hypothesis without showing that an oral preparation can reach the exposure humans would need. Drawing such distinctions clearly is necessary to prevent overstating the therapeutic importance of early findings. This review sets out to bring together the botanical and ethnomedicinal background of *V. negundo*, outline the phytochemical groups reported for it, discuss selected pharmacological activities, describe analytical and quality-control approaches, and identify gaps in safety and translational evidence. The focus is on academic interpretation and on the standards that further development requires.

1.1 Rationale and relevance to pharmacy

For pharmacy students, *V. negundo* is a handy case study of how a traditional medicinal plant is evaluated through pharmacognosy, phytochemistry, pharmacology, analytical chemistry and quality assurance. One plant can yield extracts of differing chemical composition, so pharmacological results mean something only when the tested material is adequately described. Pharmacists and researchers must also weigh contamination, adulteration, dosage form, stability, interactions and the strength of the evidence behind therapeutic claims.

1.2 Objectives of the review

The review has the following objectives: (i) to describe botanical identity and traditional context; (ii) to summarize the main phytochemical classes reported; (iii) to discuss selected pharmacological findings critically; (iv) to outline authentication, standardization and analytical quality control; (v) to examine safety and translational limitations; and (vi) to propose research priorities that could make future investigations more reliable.

2. LITERATURE SEARCH AND REVIEW METHODOLOGY

Setting out a clear search strategy matters even in a narrative review, because it lets readers see how the literature was located and why certain studies received emphasis [37–39]. Suitable databases for this topic include PubMed, Scopus, Web of Science, Google Scholar and publisher platforms, while regional ethnobotanical and pharmacognosy journals may hold further relevant records. The date of the search should be stated, since the evidence base keeps changing. Search terms should pair the botanical name with topic-specific words, for example "*Vitex negundo*" AND phytochemistry; "*Vitex negundo*" AND anti-inflammatory; "*Vitex negundo*" AND analgesic; "*Vitex negundo*" AND antioxidant; "*Vitex negundo*" AND toxicity; "*Vitex negundo*" AND HPLC; and "*Vitex negundo*" AND clinical. Searching for "Nirgundi" can turn up regional material, but each record must be checked to confirm that the species concerned really is *V. negundo*, because a common name is not enough for taxonomic inclusion. Records should be screened first by title and abstract and then read in full where possible. Primary studies carry particular weight for claims about isolated compounds, doses, biological endpoints and toxicity. Reviews help in locating earlier work and following the historical development of the field, but claims should be traced back to the original study whenever practicable. Where the full text is unavailable, the review should not suggest that its methods or results were independently verified. Data should be extracted using a consistent framework. For phytochemical papers, note the plant part, origin, authentication, extraction solvent, analytical technique, confidence of identification and whether compound concentrations were measured. For pharmacology papers, note the model, sample size, dose or concentration, controls, endpoint, statistical analysis and principal limitations. For toxicology studies, record the route, duration, exposure, clinical observations, laboratory measurements and histopathology. Structured extraction of this kind lowers the chance of treating dissimilar studies as directly comparable. A hierarchy of evidence can help in arranging the findings. Well-designed human clinical studies bear most directly on clinical outcomes. Animal studies can reveal biological activity and effects on the whole organism, and cell-based assays can probe cellular responses, whereas chemical assays and in-silico predictions are generally exploratory. Every type of evidence has its place, but claims should be worded in proportion to it; in particular, an in-silico binding prediction must not be described as proof of therapeutic efficacy. The present document is a narrative academic draft and not a finished systematic review. It supplies no verified flow diagram, no exhaustive record counts and no formal risk-of-bias assessment. Should the supervisor or the target journal require a systematic review, the authors would need to set up a protocol, document database-specific queries, define inclusion criteria beforehand, report how many records were screened and excluded, and apply a suitable critical-appraisal method. PRISMA reporting guidance can assist with transparent presentation, although it does not in itself guarantee methodological quality.

2.1 Suggested inclusion and exclusion criteria

Peer-reviewed original studies should be included if they clearly identify *V. negundo* and report botanical, chemical, pharmacological, analytical or safety data; reviews may be included for background and citation tracking. Records on other Vitex species should be excluded unless they are used expressly for comparison, and studies whose species identity is uncertain should be flagged and not treated as definitive. Unreferenced commercial web pages should not be relied upon for pharmacological claims.

2.2 Plagiarism-conscious writing practice

A manuscript should combine ideas from several sources within the author's own structure and wording, and should not simply swap a few words in sentences lifted from an article. Direct quotations ought to be uncommon in a scientific review. Each factual statement that rests on a source needs a suitable citation, and every in-text citation must match a complete reference. A similarity score alone cannot decide whether plagiarism has occurred: common scientific terms may legitimately match, whereas uncited paraphrasing can still be improper. Before submission, the document should be run through the institution's approved similarity-

checking process and any unattributed overlap corrected.

3. BOTANICAL IDENTITY, DISTRIBUTION AND ETHNOMEDICINAL

CONTEXT *Vitex negundo* Linn. is placed in the family Liliaceae by current taxonomic systems, although older publications assign the genus to Verbenaceae. The difference comes from changes in botanical classification and should not be read as disagreement at species level. The accepted name and family should be checked against a current botanical database or regional flora when the manuscript is finalized. The plant is usually described as an aromatic shrub bearing palmately divided leaves and small flowers that can look bluish or purple [2,3]. Details of leaf arrangement, leaflet number, flower colour, fruit and other morphological traits help in preliminary identification, but morphology can change with developmental stage and growing conditions. Identification resting only on a photograph or a vernacular name is inadequate for plant material meant for chemical or pharmacological testing. *V. negundo* has been reported from India and other parts of South and Southeast Asia. Local abundance, environmental conditions and cultivation practices can influence both how much plant material is available and its chemical profile. Soil, rainfall, temperature, season, plant age and stress may affect the accumulation of secondary metabolites, so geographical origin and collection conditions should be recorded whenever results from different studies are compared. Traditional medicinal systems describe using various plant parts, particularly the leaves, in preparations for pain, swelling and inflammatory complaints [2,4]. Other reported uses differ between regions and sources. Ethnomedicinal information of this kind can help researchers prioritize hypotheses, but it should be presented as a record of traditional practice and not as evidence of efficacy. Wherever available, the preparation method, route of use, region and source should be reported. Plant parts must not be regarded as chemically equivalent: leaves, roots, fruits, seeds and aerial parts may differ in their content of volatile constituents, flavonoids, glycosides and terpenoids, and the choice of solvent further alters which constituents are extracted. A hydroalcoholic leaf extract, an essential oil and an isolated molecule are different test materials and may show different pharmacological and toxicological profiles. Botanical authentication is an essential quality step. Studies should state who authenticated the specimen, the place and date of collection, the plant part, and the voucher specimen number together with the herbarium where it is kept. A voucher lets later investigators re-examine the taxonomic identity. A reproducible supply chain should likewise document drying, storage, transport and processing, as degradation or contamination can occur before laboratory analysis. Related species may share common names, and commercial herbal material can be substituted, deliberately or by accident. Other Vitex species may look like *V. negundo*, and powdered or processed material can be hard to identify macroscopically. Microscopy, chromatographic fingerprints and, if necessary, DNA-based methods can complement botanical expertise, although DNA methods can support species identity without establishing chemical quality or therapeutic potency. Ethnomedicinal evidence is strongest when its source is specific and transparent. A review should steer clear of sweeping statements such as “the plant cures” a disease unless reliable clinical evidence backs them. Better wording includes “has been traditionally used for,” “has been investigated in experimental models,” or “has shown activity under the conditions tested.” Such careful phrasing respects traditional knowledge while keeping it distinct from modern clinical conclusions.

3.1 Implications for pharmacognosy

Identity, purity and quality form the foundation of pharmacognostic evaluation [15–17,19]. Depending on the material and the applicable monograph, relevant measures may include macroscopic and microscopic characters, foreign matter, moisture content, ash values and extractive values. Such measurements do not establish pharmacological efficacy, yet they help detect poor-quality material and promote consistency. Analytical fingerprinting can add evidence that a batch resembles an authenticated reference material.

3.2 Traditional knowledge and responsible interpretation

Traditional knowledge can steer the choice of plant parts, preparation methods and possible indications for study. Moving into modern formulations, however, calls for respect for the original context and careful weighing of benefit and risk. Traditional preparation methods may not be chemically equivalent to laboratory extracts prepared with concentrated organic solvents, and the final article should acknowledge this limitation whenever traditional use is compared with experimental findings.

4. PHYTOCHEMICAL CONSTITUENTS

Phytochemical studies depict a chemically diverse profile for *V. negundo*. The classes reported are flavonoids, iridoid glycosides, lignans, terpenoids, triterpenoids, phenolic compounds and volatile constituents, which differ in polarity, stability, biological activity and ease of analytical detection. Detecting a class in a qualitative screening test is only preliminary evidence, and structural identification calls for more specific analytical data. Flavonoids are among the groups most often discussed for this species [3,45]. Vitexin and isovitexin are C-glycosyl flavones often cited in reviews, together with luteolin-related compounds and other flavonoid derivatives, and their possible biological relevance has been explored in various experimental settings. Even so, activity reported for an isolated flavonoid should not be assumed to account for the activity of a crude extract unless its concentration, contribution and interaction with other constituents have been examined. Iridoid glycosides, including negundoside and agnuside in the published literature, are another group of interest. Glycosides may differ from their aglycones in solubility, stability and metabolism, and their measured concentration can depend on extraction solvent, pH, temperature and sample preparation. Reliable identification should rest on a traceable analytical method, ideally backed by reference standards or orthogonal structural evidence. Lignans and terpenoid constituents have also been reported [1,45]. Terpenoids cover chemically varied molecules, from volatile constituents to less volatile diterpenoid or triterpenoid structures. Essential-oil profiles can change with plant part and distillation conditions, while non-volatile constituents may need different extraction and chromatographic procedures. The word “terpenoids” should therefore not be used as though it denoted one chemical entity or a uniform pharmacological mechanism. Recent work has examined triterpenoids and triterpenoid saponins isolated from aerial parts, assessing selected compounds in an LPS-stimulated BV-2 microglial-cell model. In that setting, some compounds were reported to lower nitric-oxide release. A study of this type links phytochemical isolation to biological testing, but its interpretation is limited by the model, the concentration range, compound exposure and the need for replication in other systems, and it does not establish a clinical anti-inflammatory effect. Phenolic compounds are frequently discussed with regard to antioxidant capacity. A high total phenolic content or a strong result in a chemical radical-scavenging assay, however, does not suffice to show a beneficial effect in vivo, since phenolics differ in absorption, metabolism, protein binding and tissue distribution. A compound that reacts strongly in a test tube may not reach the relevant tissues in active form after oral administration. Chemical characterization should pair qualitative profiling with quantitative measurement [14,17]. Thin-layer chromatography and high-performance thin-layer chromatography offer practical fingerprints. HPLC or UHPLC with UV or photodiode-array detection can quantify selected markers, and LC–MS/MS adds selectivity and supports profiling of complex mixtures. NMR can contribute to structure elucidation, especially when an isolated compound needs confirmation. FTIR is useful for certain characterization tasks but generally cannot, by itself, establish the identities of individual molecules in a complex botanical extract. Confidence in compound identification should be stated openly. A peak that matches an expected retention time or mass-to-charge ratio may support a tentative assignment but is not always conclusive. Stronger identification can draw on an authentic standard, diagnostic fragmentation, high-resolution mass spectrometry and NMR, as appropriate, and quantification should use validated calibration and quality-control procedures. The final manuscript should separate “reported,” “tentatively identified,” “isolated,” and “quantified” constituents instead of using these terms interchangeably.

4.1 Factors affecting the phytochemical profile

Genotype, soil, climate, altitude, seasonal conditions, plant maturity, drying temperature, light exposure, storage duration and solvent composition can all influence the chemical profile [14,18]. These variables may account for the different yields or relative abundances reported for the same class of constituents. To make studies more comparable, investigators should report collection and processing details and consider a validated fingerprint as a baseline for characterizing batches.

4.2 From constituents to biological effects

A phytochemical and an observed effect are not always directly related. Extracts hold many constituents that may act additively, synergistically or antagonistically, and in-vitro experiments run at concentrations far above plausible systemic exposure can exaggerate relevance. A robust investigation should measure constituent levels in the tested extract, assess concentration –response relationships and, where possible, tie the biological response to chemical exposure and target engagement.

5. PHARMACOLOGICAL ACTIVITIES

5.1 Anti-inflammatory activity

Anti-inflammatory activity is a leading theme in the *V. negundo* literature. Studies have used crude extracts, fractions and isolated constituents in biochemical assays, cell-based systems and animal models [1,3,6]. A recent study of triterpenoids and triterpenoid saponins found that selected compounds inhibited nitric-oxide release in LPS-stimulated BV-2 microglial cells. Other reviews discuss flavonoids and related constituents in connection with inflammatory mediators and signalling pathways [3,4,45]. These findings supply a rationale for further study but must be described as experimental evidence and not as proof of clinical benefit. Inflammatory responses involve many mediators and regulatory networks. Nitric oxide, prostaglandins, cytokines and transcriptional pathways can serve as experimental endpoints, yet a change in a single endpoint does not necessarily indicate broad anti-inflammatory efficacy; a drop in mediator release may, for example, come from cytotoxicity, assay interference or altered cell number. Good experimental design therefore includes viability measurements, suitable positive and vehicle controls, independent replicates and a concentration range wide enough to capture both activity and toxicity. Mechanistic claims should rest on direct measurements. When a paper proposes an effect on NF-κB, MAPK, COX-2 or another pathway, the review should state whether the pathway was measured directly, inferred from downstream biomarkers or predicted computationally. Docking alone does not establish binding under biological conditions, and a change in gene or protein expression does not automatically prove that the pathway causes the phenotype. Stronger evidence may include target engagement, pathway inhibition or rescue experiments.

5.2 Analgesic and antinociceptive potential

The traditional use of Nirgundi for pain-related complaints has led to studies of analgesic or antinociceptive effects [2–4]. Experimental work may use thermal, mechanical or chemically induced pain models, each of which captures a different aspect of nociception. Results from these models are not interchangeable, and a reduced behavioural response in animals does not on its own establish an analgesic effect in humans. Route of administration, dose, comparator and possible sedative or motor effects all matter for interpretation. The quality of preclinical analgesic research rests on randomization, blinding, justification of sample size, appropriate controls and transparent reporting of exclusions. If the test extract causes sedation or reduced movement, behavioural outcomes may be confounded. Studies should, where possible, separate peripheral anti-inflammatory effects from central nervous system effects and should characterize the extract well enough to permit replication. Clinical translation would need evidence on dose, formulation, safety, interactions and patient-relevant outcomes.

5.3 Antioxidant activity

Antioxidant capacity has been examined with chemical assays such as DPPH and ABTS radical scavenging, reducing-power methods and total phenolic measurements [26–29]. Such assays are useful for screening and comparison under defined conditions but do not directly measure antioxidant action in a clinical

sense. Results vary with solvent, reaction time, concentration, colour interference and the particular radical system, and different assays can rank samples differently because they work through different chemical mechanisms. More biologically relevant studies may assess oxidative injury in cells or tissues, antioxidant enzymes, lipid peroxidation and markers of redox balance. Even these outcomes need careful interpretation, since a biomarker can shift without any improvement in disease outcome. The review should therefore avoid stating that antioxidant activity means the plant prevents or treats a particular disease. A more defensible conclusion is that antioxidant findings offer a hypothesis for further mechanistic investigation.

5.4 Antimicrobial and antifungal activity

Extracts and fractions of *V. negundo* have been tested against selected microorganisms. Some studies look at phenolic constituents and antifungal effects against Candida albicans, using both experimental and in-silico approaches [7]. Antimicrobial screening is sensitive to the extraction method, inoculum, medium, incubation conditions and endpoint [40,41]. Diffusion-zone measurements suit preliminary screening but cannot replace standardized broth-dilution measurements when potency is compared [31,32]. A well-reported antimicrobial study should give the identity and source of the microorganism, inoculum standardization, extract concentration, solvent control, positive comparator and replicate data. Minimum inhibitory concentration and, where relevant, minimum bactericidal or fungicidal concentration should be determined by recognized methods. Selectivity also counts: an extract that inhibits microbial growth but is equally toxic to mammalian cells may have limited therapeutic use.

Predictions made computationally ought to be offered as hypothesis-generating evidence.

5.5 Metabolic and organ-protective activities

Reviews describe studies of *V. negundo* in metabolic, hepatic, renal, ocular, cardiovascular and other experimental settings. Because these fields rely on different models and outcome measures, the evidence should be appraised separately for each [3,4,6,8]. For every claim, the final review should name the exact plant material, experimental model, dose, comparator, duration, endpoint and main limitations. A result in an induced animal model should not be called a proven treatment for the matching human disease. Organ-protective studies should, where possible, include more than one type of outcome. Biochemical markers can be informative but may be influenced by non-specific factors, while histopathology, functional measurements and exposure data can strengthen interpretation. Adequate controls are essential for deciding whether an apparent protective effect arises from the test material or from differences in the model. Independent replication is especially valuable when a finding is used to support a broad therapeutic claim.

5.6 Neurobiological and neuroprotective relevance

Interest in neuroprotective potential stems from the contribution of oxidative stress and inflammatory signalling to several forms of neuronal injury. Still, general antioxidant activity, or suppression of an inflammatory marker in a non-neural cell model, is not enough to establish neuroprotection. A focused review should separate studies that use neuronal cells, microglia or disease-relevant animal models from those that give only indirect mechanistic clues. Relevant neurobiological studies should report the cell type or animal model, the injury or disease paradigm, the dose and timing, behavioural outcomes where applicable, histological endpoints and measures of neuronal survival. Brain exposure matters for compounds meant to act within the central nervous system; without pharmacokinetic evidence, it may be unclear whether a constituent reaches the target tissue at an effective concentration. Safety, motor function and sedation should also be considered when behavioural outcomes are used. Molecular docking and pathway diagrams can help generate hypotheses about possible targets, but they do not show that a compound reaches or modulates those targets in vivo. Claims concerning Nrf2/HO-1, NF-κB, MAPK or other pathways should be tied to the measurements the original study actually reported. Future work should give priority to reproducible disease-relevant models, target engagement, pharmacokinetic characterization and independent confirmation before strong neuroprotective claims are made.

5.7 Cardiovascular effects

A dedicated review has discussed possible cardioprotective effects of *V. negundo* extracts and constituents. Cardiovascular evidence should be read in light of the specific model, the exposure, the haemodynamic and biochemical endpoints, and possible effects on blood pressure, heart rate, coagulation or other physiological processes. A positive result in one model cannot be generalized to all cardiovascular conditions. Should future studies aim to develop a cardiovascular preparation, they should establish reproducible chemistry, relevant dose–response relationships, safety pharmacology and interaction potential. Since herbal preparations may contain several active compounds, the absence of interactions with conventional medicines should not be assumed. Clinical relevance can be judged only after adequate nonclinical evidence and appropriately designed human research.

6. SAFETY, TOXICITY AND TRANSLATIONAL LIMITATIONS

Safety is a core requirement for any candidate medicinal product. A plant's traditional use does not guarantee that every extract, dose, route or duration is safe. Concentrated extracts can differ markedly from customary preparations, and extraction solvents may enrich constituents that occur only at low levels in traditional formulations. Safety conclusions must therefore refer to the exact test material and exposure studied. Published toxicity evidence should be assessed for dose, route, duration, species, sample size, clinical observations, laboratory parameters and histopathology [4]. Acute toxicity testing can reveal some immediate hazards but cannot establish safety in repeated or long-term use, whereas repeated-dose studies may uncover effects on organs, blood chemistry or body weight that a single exposure does not show. Where the proposed use justifies it, genotoxicity, reproductive and developmental toxicity, safety pharmacology and other studies may be needed [22–24,33,34]. Chemical characterization is essential for meaningful toxicology. If the composition of the test material is not reported, another laboratory may be unable to reproduce the exposure. A study on an aqueous leaf preparation cannot automatically establish the safety of a concentrated ethanolic extract, an essential oil or a purified constituent, and safety findings for one plant part should not be extended to the whole species without evidence. Possible risks arise from contamination, substitution, pesticide residues, heavy metals, microbial load, mycotoxins or residual solvents [17,18,43]. These are not peculiar to *V. negundo*, but they are important quality considerations for herbal products. Quality systems should cover supplier qualification, raw-material authentication, validated testing and traceability from collection to finished product. Drug interactions deserve attention because constituents may influence enzymes, transporters, coagulation, blood pressure or other physiological processes. The current evidence may be too limited to characterize these interactions fully, and a responsible review should not claim that interactions are absent merely because none have been reported; it should acknowledge the uncertainty and recommend evaluation where the pharmacology or intended use makes it relevant. Pharmacokinetic data are needed to link in-vitro activity to achievable exposure. Absorption, distribution, metabolism and excretion determine whether a compound reaches a relevant tissue, how long it stays there and which metabolites form. If the active constituent is quickly metabolized or poorly absorbed, the activity seen in a cell assay may not be attainable after administration, so studies should quantify parent compounds and relevant metabolites when validated methods exist. Translation also depends on formulation, since solubility, stability, particle size, excipients, storage conditions and packaging can all affect product performance. A standardized botanical preparation requires more than a botanical name and a nominal extract dose; it should be defined by a reproducible chemical profile and justified acceptance limits. Clinical investigations must use material sufficiently consistent with the preparation characterized in preclinical studies. Clinical claims should rest on appropriately designed trials, whose important features include a clearly defined indication, prespecified outcomes, adequate sample size, suitable comparators, blinding where feasible, transparent adverse-event reporting and registration when required. Observational or uncontrolled reports can help generate hypotheses but are not enough to establish efficacy. Until high-quality clinical

evidence is available, therapeutic statements should stay cautious.

6.1 Benefit–risk interpretation

The potential benefits of a botanical preparation should be set against uncertainty about its composition, exposure and safety. A plant may contain compounds with promising activity and still lack a reproducible preparation suitable for clinical use. Risk assessment should take into account the intended population, route, dose, duration, co-medications and the consequences of delaying established treatment. Herbal products should not be offered as substitutes for evidence-based care without adequate clinical support.

6.2 Recommendations for future safety research

Future work should characterize test materials chemically, use exposure ranges relevant to the intended use, follow suitable toxicology guidance and report both positive and negative findings [22,23,33,34]. Studies should look into possible organ toxicity, genotoxicity and interactions where the proposed product warrants it. Adverse-event monitoring should be built into human research, and product batches should be traceable to the material used in supporting studies [25].

7. STANDARDIZATION, ANALYTICAL METHODS AND QUALITY

CONTROL Standardization is needed because medicinal plants vary biologically and the same species can give chemically different preparations [17,18,43,44]. A suitable quality system starts with correct botanical identity, a documented source, a defined plant part and controlled post-harvest handling. The next stage establishes physicochemical quality and a reproducible chemical fingerprint, and finally marker compounds or groups of markers are quantified with validated analytical methods. Macroscopic and microscopic examination can help authenticate intact or cut plant material [15,17,19]. Pharmacognostic parameters such as foreign matter, moisture content, total ash, acid-insoluble ash and extractive values may be useful where appropriate to the material and the applicable monograph. These tests inform on identity and general quality but do not prove that the material contains a particular active constituent or has a specific pharmacological effect. TLC and HPTLC offer economical fingerprinting and can help compare raw materials or extracts [14,17]. HPLC and UHPLC give greater separating power and can quantify selected constituents when suitable reference standards and validated procedures exist. LC–MS/MS can support selective measurement in complex matrices and aid metabolite profiling, while NMR supplies structural information and can help confirm isolated molecules. The method should be chosen according to the analytical question and not simply because an instrument is at hand. Marker selection needs scientific justification. Vitexin and isovitexin are often proposed as potential markers, but a single marker may not represent all the chemical or pharmacological properties of a multi-component extract. A combined fingerprint with several quantitative markers may give a more informative quality profile, and the final method should define the intended purpose of the marker, the acceptance criteria and the relationship between the marker and product consistency. Analytical method validation should suit the method's intended use [20,21]. Relevant characteristics may include specificity, linearity, range, accuracy, precision, detection limit, quantification limit, robustness and system suitability, and calibration standards, quality-control samples and system checks should be documented. Validation terminology must be used correctly; an assay developed for identification, for instance, may need different validation characteristics from a quantitative assay used for batch release. Sample preparation can introduce considerable bias. Drying temperature, grinding, extraction time, solvent-to-material ratio, filtration and storage may alter the measured concentration of compounds, so analysts should report these details and show that analytes remain stable during preparation and analysis. Recovery experiments and matrix-effect assessment can be especially important for LC–MS methods. Controls for contamination and adulteration should form part of quality assurance. Testing may cover microbial limits, pesticide residues, heavy metals, residual solvents and other contaminants as required by the product and the relevant standards [17,43,44]. Botanical identity can be supported by microscopy, chromatography or DNA methods as appropriate, but

each technique has limits; DNA authentication, for example, may identify a species yet cannot quantify active constituents or show whether the material has degraded. Batch-to-batch consistency should be demonstrated with multiple lots and not a single sample. Fingerprints can be compared using defined similarity criteria, and marker concentrations can be monitored against justified limits. If biological activity is used as a quality attribute, the assay must be validated and reproducible. A good manufacturing system should link raw-material specifications, in-process controls, finished-product testing, stability data and traceability.

7.1 Stability and storage

Stability studies should assess whether relevant constituents stay within justified limits during storage and whether the product changes in appearance, odour, moisture, microbial quality or performance. Light, heat, oxygen and humidity may drive degradation. Packaging and storage recommendations should rest on data and not on assumption. If a finished formulation is developed, stability should be tested on that formulation and not only on the crude plant material.

7.2 Quality control as a bridge to pharmacology

Chemical standardization makes biological experiments easier to interpret. When the same fingerprint and marker range are kept across batches, investigators can better tell whether a difference in activity reflects experimental conditions or a change in the material. Standardization also supports reproducibility between laboratories and is an important prerequisite for pharmacokinetic, toxicological and clinical research.

8. RESEARCH GAPS AND FUTURE DIRECTIONS

The literature shows a wide range of experimental interests, but several gaps still hinder translation. The first is inconsistency in plant authentication and in the reporting of preparations. Studies may use different plant parts, solvents, extraction ratios and collection conditions, which makes comparison difficult. Future research should routinely provide voucher specimens, complete extraction details and chemical fingerprints. The second gap is the limited use of biomarker-based standardization across studies. Identifying a compound in one sample does not show that it is consistently present in commercial or experimental preparations. Researchers should develop validated assays for justified markers, examine multiple batches and report quantitative results, and where no single marker adequately represents the preparation, multi-marker strategies and fingerprints should be considered. The third gap is mechanistic certainty. Many biological effects are described with broad labels such as antioxidant or anti-inflammatory even when only one assay or endpoint has been measured. Mechanistic studies should combine complementary approaches, include target-engagement or pathway-rescue experiments where appropriate, and exclude cytotoxicity and assay interference, and findings should be independently replicated before strong causal claims are made. The fourth gap is pharmacokinetics. A compound can act in a tissue only if it arrives there at sufficient concentration and for an adequate time. Future work should characterize absorption, distribution, metabolism and excretion of key constituents and metabolites and determine whether in-vitro concentrations are plausible in vivo, which is especially important for claims involving the central nervous system or other tissues with specific access barriers. The fifth gap is the need for a fuller safety profile. Toxicity studies should use chemically characterized preparations and relevant exposure ranges, and depending on the intended use they may include repeated-dose toxicity, safety pharmacology, genotoxicity, reproductive toxicity and drug-interaction potential. A lack of published toxicity data should be described as uncertainty and not as evidence that the plant is safe. The sixth gap is limited clinical validation. Traditional use and preclinical activity may justify further research but do not establish a treatment's benefit–risk balance. Clinical studies should follow a clearly defined research question, use standardized material, choose meaningful endpoints and report adverse events, and trial registration, transparent reporting and independent replication can raise confidence in the findings. Biotechnology may help with supply and consistency problems. Plant tissue culture, elicitation, controlled growth conditions and other approaches could influence secondary-metabolite production, but a rise in a marker compound under laboratory conditions

does not automatically produce a clinically useful product; the chemical profile, biological activity, stability, scalability and cost of the resulting material must all be assessed. Research priorities should be tied to intended applications. For a preparation considered for topical use, skin exposure, local tolerability, formulation stability and relevant microbial or inflammatory endpoints may matter most. For a proposed oral product, oral bioavailability, systemic exposure, metabolism and interactions become central. If a neuroprotective application is hypothesized, evidence of brain exposure and disease-relevant outcomes is required. A generic claim that the plant is useful for many conditions says less than a focused, testable development plan.

8.1 Proposed staged development pathway

A practical development pathway starts with botanical authentication and controlled sourcing, followed by extraction optimization, chemical fingerprinting and marker quantification. The next stage applies validated biological assays with concentration–response analysis and suitable controls. Promising findings should be replicated independently and then followed by pharmacokinetic and toxicological studies. Only once a coherent preclinical package exists should a standardized product advance to a suitably designed clinical investigation.

8.2 Reporting priorities

Researchers should report negative and inconclusive findings alongside positive ones, because selective publication can distort the apparent strength of the evidence. Full reporting of sample sizes, exclusions, randomization, blinding, statistical methods and raw or supplementary data can improve reproducibility. Review authors should also disclose the scope of their search, the limitations arising from inaccessible papers and any uncertainty in bibliographic details.

Table 1: Evidence-Extraction Framework for Vitex Negundo L. Primary Studies

Domain	Information to extract	Critical questions for Vitex negundo L.
Botanical identity	Accepted name (Vitex negundo L.); family (Liliaceae; placed in Verbenaceae in older literature); plant part (leaf, root, stem, bark, flower or seed); collection site; season; authentication method; herbarium/voucher number.	Did a qualified botanist authenticate the plant? Does a voucher specimen exist? Is the accepted botanical name applied consistently?
Phytochemistry	Plant part and preparation; extraction solvent and method; extract yield; chromatographic or spectrometric methods (e.g., HPLC, GC–MS, LC–MS); reported constituents/marker compounds; confidence of identification; details of quantification and validation.	Were compounds identified and, where relevant, quantified by suitable validated methods? Is the extract characterized well enough for replication and comparison?
Anti-inflammatory activity	Experimental model (in vitro, ex vivo or in vivo); extract/fraction and concentration or dose; inflammatory stimulus; endpoints/mediators; positive and vehicle controls; cell viability; sample size and statistical analysis.	Might cytotoxicity, solvent effects or assay interference account for the results? Were suitable controls and reproducible outcome measures employed?
Analgesic activity	Pain model; animal species/strain or cell model; extract/fraction, dose and route; comparator; timing; outcome measures; randomization, blinding and sample size; checks for motor activity or sedation.	Were motor impairment or sedation considered as alternative explanations? Were randomization, blinding and suitable positive controls reported?
Antimicrobial activity	Test organism and strain; inoculum; extract/fraction and concentration; method; MIC/MBC/MFC where applicable; positive and negative controls; extract profile; number of replicates.	Were standardized methods and controls used? Was the activity confirmed with appropriate endpoints? Were selectivity and toxicity toward mammalian cells assessed?

Domain	Information to extract	Critical questions for Vitex negundo L.
Neurobiological activity	Neural/cellular or animal model; insult or disease model; extract/fraction, dose, route and exposure; behavioural, biochemical, histological or molecular outcomes; controls; brain-exposure data if available.	Is the evidence direct and relevant to the proposed neurological condition? Were behavioural confounders taken into account? Is the brain exposure of active constituents known?
Toxicology and safety	Test material and batch; dose range; route; single or repeated dosing; duration; clinical signs; body weight; haematology/clinical chemistry; organ weights and histopathology; adverse events; study guideline.	Does the tested material correspond to the preparation proposed for use? Were the dose, duration and safety endpoints adequate? Are limitations and adverse findings clearly reported?

CONCLUSION

Vitex negundo Linn. is a chemically diverse medicinal plant with a large body of traditional-use literature and a growing volume of experimental research. Published studies describe flavonoids, iridoid glycosides, lignans, terpenoids, triterpenoids, phenolic compounds and volatile constituents, and investigations have covered anti-inflammatory, analgesic, antioxidant, antimicrobial, metabolic, organ-protective and neurobiological effects. These findings make the species relevant to pharmacognosy and natural-product research, but the breadth of reported activity should not be mistaken for established clinical efficacy. Interpretation remains limited by differences in botanical identity, plant part, geographical source, extraction technique, chemical composition and experimental design. Many findings come from chemical assays, cell-based models or animal studies, which can help identify hypotheses and mechanisms but cannot by themselves establish benefit in humans. Molecular docking and pathway predictions are likewise supportive tools and not proof of therapeutic action. Future work should stress authenticated plant material, transparent sourcing, validated analytical methods, justified marker selection and reproducible batch quality. Pharmacological studies should use appropriate controls, concentration–response analysis, viability checks and independent replication, and pharmacokinetic, toxicological and interaction data are needed to judge whether experimental activity can translate into safe and effective use. Any clinical development should use a well-characterized preparation and appropriately designed trials. In conclusion, *V. negundo* remains a worthwhile subject for evidence-based investigation, but responsible development depends on matching the strength of each claim to the quality of the evidence. A standardized, critically evaluated research programme is more likely to yield useful results than an unqualified catalogue of traditional uses or laboratory activities.

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