

Impact of Vitex Nigunda Linn on the treatment of Rheumatoid Arthritis

Gauri Bhivshet¹, Dr. Jitendra Banweer²

¹Research Scholar, SIRT-Pharmacy, Faculty of Pharmaceutical Sciences, Sanjeev Agrawal Global Educational University, Bhopal

²Professor, SIRT-Pharmacy, Faculty of Pharmaceutical Sciences, Sanjeev Agrawal Global Educational University, Bhopal

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ABSTRACT

Vitex negundo Linn (family Verbenaceae), commonly known as Nirgundi, is a medicinal plant extensively used in traditional Ayurvedic systems for treating inflammatory and rheumatic disorders. This review combines recent scientific data on its pharmacological, biochemical, and clinical potential in the management of Rheumatoid Arthritis (RA). This plant has a wide variety of therapeutic effects such as anti-inflammatory, anti-oxidant, analgesic, and immunomodulatory effects mainly inhibited by COX, LOX, TNF- α , IL-1 β and NF- κ B signaling pathways. The therapeutic benefits are attributed to bioactive compounds such as casticin, negundoside and luteolin contributing to the multi-targeted therapeutic paradigm. The pre-clinical applicability is attractive and is associated with a clinically meaningful beneficial impact on joint inflammation, oxidative stress, and cytokine balance and safety confirmed with positive toxicology. The limitations are in clinical validation and standardization. The way forward would be to focus on multi-centric clinical studies and mechanistic studies using medical-omics approaches to better understand the favorable outcomes of V. negundo, including innovative formulations to optimize bioavailability and reproducibility. Overall, V. negundo Linn is defined as a multi-faceted complementary or adjunct treatment option for the management of RA, bridging traditional knowledge with contemporary pharmacology.

Keywords: Vitex negundo Linn, Rheumatoid Arthritis, Anti-inflammatory, Immunomodulatory, Phytochemicals, Clinical Validation.

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INTRODUCTION

1.1 Overview of Rheumatoid Arthritis (RA)

RA is an inflammatory disease that lasts a long time and impacts the whole body. It is mostly characterised by ongoing swelling in the synovium or damage to the joints that makes it impossible to do any activity [1]. Whereas osteoarthritis is mostly degenerative, RA is an inflammatory arthritis that is primarily autoimmune in nature that mainly acts on the synovium to generate a leader, called pannus, that damages destroyed cartilage, erodes bone structures and affects adjacent ligaments and tendons [2]. The disease mainly affects small joints, for example: RA can affect the joints in the hands, wrists, as well as feet, but it can also show up in other tissues or systems, such as the skin, eyes, lungs, circulatory system, and vascular system. This shows that RA has effects on the whole body [3]. People around the world are thought to have RA between 0.5 and 1 percent of the time. People ages 30 to 60 have been most inclined to have it, and women are more probable to have it than men. Most recently, the World Health Organization (WHO) reported the differences in prevalence by geographic regions; the highest prevalence appearing in Northern Europe and North America [4]. In

India, population studies estimate a prevalence rate of about ~0.75% prevalence rate imposing a burden of public health in India due to the chronic nature of the disease and socio-economic burden of long-term disability [5]. The suspected genetic predisposition to RA are mostly associated with the presence some proteins, specifically HLA-DRB1 alleles while the likely environmental triggers of the disease are smoking, infections, and hormonal influences [6].

1.2 Pathophysiology of Rheumatoid Arthritis

In rheumatoid arthritis (RA), genetic, environmental, and immune system factors all work together in a complicated way. It starts with an immune reaction that doesn't work right, which causes the joint membrane to stay inflamed [7]. Antigen-presenting cell types (APCs) are triggered, which makes CD4+ T lymphocytes work. After that, these cells let out cytokines that make inflammation happen. The ones in this group are tumour necrosis factor alpha (TNF-), interleukin-1, and interleukin-6. They bring in B cells and macrophages and make joint cells divide[8]. They also help new blood vessels grow. This makes pannus, an engorged tissue that hurts bone and cartilage, appear [9]. Antibodies against rheumatoid factors (RF) or conjugated proteins (ACPAs) are generated by B cells that have been

*Author for Correspondence: Gauri Bhivshet

stimulated. Inflammation in the joints and all of its effects are made worse by these antibodies, which are part of immune complexes [10]. A substance called RANKL turns on osteoclasts, which break down bone. At the same time, synoviocytes that are fibroblast-like (FLS) release substance called matrix metalloproteinase (MMPs), which breaks down cartilage. Oxidative stress happens when there is a lot of ongoing inflammation [11]. This means that ROS, or reactive oxygen species, are high and hurt the tissue. So, in the case of rheumatoid arthritis, the body's immune system is out of whack, which leads to too many cytokines being made and an imbalance at oxidative stress, which makes the inflammation worse [12].

Fig. 1 Joint destruction in rheumatoid arthritis (RA) and hemophilic arthropathy (HA)

Source: [13]

This schematic comparison illustrates the key pathogenic mechanisms of There is a form of arthritis called rheumatoid (RA) and haemophilic arthritis (HA). On the left, RA is shown as an autoimmune joint disease. In this condition, autoreactive T cells or autoreactive B cells are turned on, which make autoantibodies like rheumatoid factor and ACPAs [14]. These defence cells send out cytokines that cause inflammation, like TNF- α , IL-6, and IL-1 β . Through the RANKL pathway, these cytokines turn on macrophages and help osteoclasts grow. This damages the synovium and causes bone loss. On the right side, hemophilic arthropathy is driven primarily by recurrent intra-articular bleeding and iron deposition in the joint cavity [15]. The deposited iron induces oxidative stress and promotes macrophage activation, leading to the release of similar inflammatory cytokines (TNF- α , IL-6, IL-1 β) and osteoclast activation [16]. Despite distinct etiologies autoimmunity in RA and bleeding in HA both conditions converge on common mechanisms of inflammation, osteoclast-mediated bone resorption, and joint destruction [17].

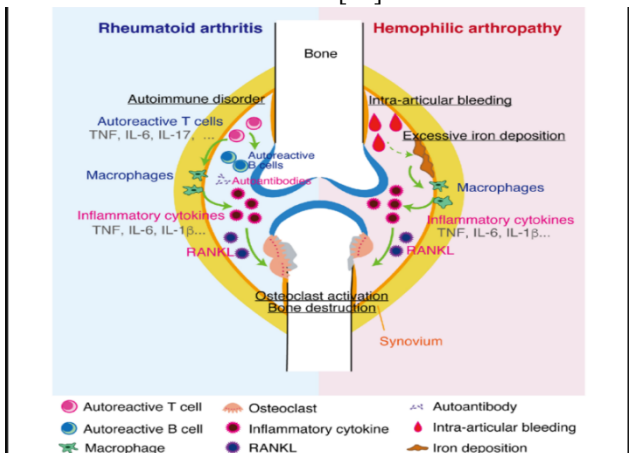


Table 1. Comparison between rheumatoid arthritis (RA) and hemophilic arthropathy (HA)

RA or HA are not the same, but they are similar in some ways. There are big differences at the reasons why the damage starts at different ages and in different places. In some ways, cytokines that trigger inflammation are the same.

Parameter	Rheumatoid arthritis (RA)	Hemophilic arthropathy (HA)	Reference (Author & Year)
Estimated number of patients in Japan	Estimated 800,000	Estimated 5,000	Smolen et al., 2018; Nationwide Survey on Coagulation Disorders, 2022
Male-to-female ratio	1 : 3	The majority are male (since hemophilia is an X-linked disease, the ratio is unbalanced)	Nakajima et al., 2020; Nationwide Survey on Coagulation Disorders, 2022
Typical age of joint pain onset	Late 60s	Under 30s	Kojima et al., 2020; Kulkarni and Soucie, 2011
Affected joints	Symmetric arthritis in various joints	Mainly in the elbow, knee, and ankle joints	Smolen et al., 2018; Kulkarni and Soucie, 2011
Causes	Autoimmune disorder	Intra-articular bleeding caused by coagulation disorders	Smolen et al., 2018; Kulkarni and Soucie, 2011
Highly associated cytokines	TNF, IL-6, IL-1 β , IL-17, IL-23, etc.	IL-6, TNF	Okamoto et al., 2017; Narazaki et al., 2017; Steiner et al., 1999; Mori et al., 1996; Tanaka et al., 2018; Narkbunnam et al., 2013; Handa et al., 2019; Zhou et al., 2018

Parameter	Rheumatoid arthritis (RA)	Hemophilic arthropathy (HA)	Reference (Author & Year)
Highly associated genes	HLA-DRB1, PTPN22, TNFAIP3, CCR6, IRF5, etc.	Blood clotting factors (F8, F9, etc.)	Okada et al., 2014; Begovich et al., 2004; Elsby et al., 2010; Kochi et al., 2010; Shimane et al., 2009; Castaman and Matino, 2019

Table 1 highlights the comparative characteristics of rheumatoid arthritis (RA) and hemophilic arthropathy (HA), emphasizing both their distinct etiologies and shared mechanisms of joint and bone destruction [18]. Despite differences in causative factors autoimmune reactions in RA and recurrent intra-articular bleeding in HA both diseases exhibit similar inflammatory pathways leading to osteoclast activation and bone erosion. The RANKL receptor stimulator in NF- κ B ligand has significance for the growth of osteoclasts at both situations. Cytokines that cause inflammation, such as IL-6, TNF, and IL-1², help the process of osteoclastogenesis [19]. The high levels of RANK and RANKL seen in HA synovium reflects the osteoclastic activity in RA. Thus, both diseases are associated with periarticular bone damage and overall reductions in bone mineral density (BMD) and some patients have osteopenia or osteoporosis [20]. The similarities suggest that therapies that target RANKL and pro-inflammatory cytokines such as denosumab or bisphosphonates would be of benefit to patients with either RA or HA [21].

1.3 Current Treatment Modalities and Their Limitations

Over the past several decades, the pharmacologic approach to the management of rheumatoid arthritis (RA) has shifted from the simplicity of symptomatic relief to a disease modifying approach that is more aimed at remission or low disease activity [22]. The recognition that there can be a "preclinical" stage of RA, detectable autoantibodies and/or subclinical inflammation, combined with advances in our understanding of its immunopathogenesis, has led to changing therapeutic paradigms to incorporate earlier and more aggressive therapy [23]. Meta-analyses suggest that delayed initiation of DMARD therapy increases the risk of radiographic evidence of joint damage, such as joint space narrowing and bony erosions which are predictive of disability. Therefore, early rheumatologic assessment and early initiation of DMARD therapy are paramount to optimizing disease outcomes [24]. Management of rheumatoid arthritis (RA) has significantly changed over the last several decades from the simplicity of symptomatic relief to the complex, early, aggressive, and targeted disease-modifying strategies with the goal of achieving remission and preventing irreversible joint damage [25]. The recognition of preclinical stages of RA—abnormal autoantibodies and/or subclinical inflammation—have prompted earlier therapeutic interventions aimed at halting disease progression.

Traditionally, practitioners manage RA by trying to treat inflammation, relieve pain, avert joint destruction, and

permit long-term maintenance of the use of one's hands and feet. Some of the medicines used to treat Some popular synthetic disease-modifying antirheumatic medicines (csDMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat RA are methotrexate, sulfasalazine, and hydroxychloroquine [26]. In recent years, biologic therapies that block specific immune mediators like cytokines have also become available. Methotrexate (MTX) has long been considered the standard for drug therapy for RA, typically using a low-dose weekly dose interval [27]. MTX acts as a folate antagonist by irreversibly binding to dihydrofolate reductase and also modulating adenosine signaling, oxidative stress, and cytokine generation. Clinical trials have demonstrated an important effect in slowing radiographic progression and improving clinical symptoms in RA, however side effects such as hepatotoxicity and myelosuppression and gastrointestinal intolerance require blood monitoring regularly, in particular while being on the drug [28]. The administration of subcutaneously MTX improved the effectiveness of use as it was associated with better tolerability and bioavailability when compared to the oral route. Genomic papers have reported variants in genes such as GGH, ATIC, SLC19A1 in association with MTX, but ultimately there is still not enough evidence reported on rather or not variants are significant expectations for effects of MTX therapy [29]. When monotherapy of Methotrexate is not effective enough to manage RA, Lack of evidence outweighs a combination of DMARD or escalation of a DMARD, or biologic DMARD therapy, or targeted synthetic DMARD (tsDMARD) [30]. Biologics (for example, adalimumab, etanercept, tocilizumab) have changed the treatment of rheumatics disease because they selectively inhibit single immune mediators; for example, TNF-alpha, IL-6, or other noted specific cytokines to reduce systemic inflammation and to advance to erode joint damage to enhance function as noted by literature [31]. In addition, Janus Kinase (JAK) inhibitors such as tofacitinib and baricitinib have added options as pharmacotherapy for RA as they more specifically address to inhibit IL-6. Despite these advancements, there are still significant limitations. NSAIDs and corticosteroids are only symptomatic relief, and can have gastrointestinal irritation, cardiovascular risk, and bone demineralization with prolonged use [32]. Conventional DMARDs are effective, but must be taken over an extended period of time with follow-up testing to monitor for hepatotoxic and teratogenic risk. Biologic agents are very specific, but can be expensive, can lead to serious infections or infusion reactions, and may lose efficacy with time due to immunogenicity [33]. In

addition, no available pharmacologic treatment can provide a definitive cure or universal remission. The burden of lifelong therapy can also present economic and psychological stress to patients. The enduring challenges of RA have contributed and fueled increasing interest in natural, plant-derived therapeutic tools with anti-inflammatory, antioxidant, and immunomodulatory activity [34]. Among the natural plant-derived therapeutics is Vitex negundo Linn (family Verbenaceae) or Nirgundi in Ayurvedic medicine, which has a historical use in traditional and alternative medicine for treating

inflammatory and rheumatologic conditions. Vitex negundo has a complex mix of phytochemical compositions and multi-target pharmacological behaviors making it an attractive candidate to potentially be adjunct or alternative therapy in RA [35]. Subsequent sections in this review will discuss the phytochemical compounds that comprise Vitex negundo Linn, their mechanistic pathways, available preclinical studies, and clinical evidence supporting the potential therapeutic activity of Vitex negundo Linn in rheumatoid arthritis Table 2. Present Modern Pharmacologic.

Table 2: Modern Pharmacologic Therapies for Rheumatoid Arthritis

Classification	Name	Mechanism of Action	Potential Mechanisms	Common Side Effects
Conventional Synthetic DMARDs	Methotrexate	Analog of folic acid	Interferes with folate-dependent processes, adenosine signaling, and cytokine modulation	Elevated liver enzymes, pulmonary toxicity
	Leflunomide / Teriflunomide	Pyrimidine synthesis inhibitor	Inhibits DHODH pathway; affects leukocyte adhesion, NF-κB and kinase signaling	Hypertension, nausea, hepatotoxicity
	Sulfasalazine	Anti-inflammatory and immunosuppressive	Inhibits cyclooxygenase, leukotriene synthesis, and cytokine production	Gastrointestinal and hematologic effects
	Chloroquine / Hydroxychloroquine	Immunomodulatory	Affects toll-like receptors, lysosomal pH, and T-cell responses	Skin, CNS, and retinal toxicity
Biological DMARDs (Antibody-based)	TNF-α inhibitors (Infliximab, Adalimumab, Etanercept, Golimumab, Certolizumab pegol)	TNF-α blockade	Suppresses pro-inflammatory cytokines, chemokines, and osteoclast function	Infection (pneumonia, tuberculosis), injection reactions
	B-cell targeted (Rituximab, Ofatumumab, Belimumab, Atacicept, Tabalumab)	B-cell depletion or inhibition	Induces B-cell apoptosis, inhibits cytokine release	Hypogammaglobulinemia, infection, neutropenia
	T-cell targeted (Abatacept, Belatacept)	Co-stimulation blockade (CD28/CTLA-4, CD80/CD86)	Reduces T-cell activation and infiltration	Infection, malignancy

Classification	Name	Mechanism of Action	Potential Mechanisms	Common Side Effects
	Interleukin targeted (Tocilizumab, Anakinra, Canakinumab, Rilonacept, Secukinumab, Ixekizumab)	IL-6, IL-1, or IL-17 inhibition	Regulates acute-phase proteins and cytokine activity	Infections, neutropenia, liver dysfunction
	Growth and differentiation factors (Denosumab, Mavrilimumab)	RANKL or GM-CSF inhibition	Reduces osteoclast activation and macrophage differentiation	Hypocalcemia, muscle cramps, cellulitis
Small Molecules (Targeted Synthetic DMARDs)	Tofacitinib	JAK1/3 inhibitor	Inhibits cytokine-mediated inflammation and T-cell activation	Zoster infection, cytopenia
	Baricitinib	JAK1/2 inhibitor	Reduces synovial inflammation and joint damage	Infections, lipid changes
	Filgotinib	JAK1 inhibitor	Blocks pro-inflammatory cytokine signaling	Mild infection risk

2. METHODOLOGY OF LITERATURE REVIEW

The following review has been performed using a structured and systematic method to cover and critically evaluate the available literature related to Vitex negundo Linn as a potential therapeutic option for Rheumatoid Arthritis (RA). A number of reputable scientific databases were searched for high-quality peer-reviewed publications discussing the pharmacological, biochemical and therapeutic dimensions of the medicinal plant. The sites PubMed, Scopus, ScienceDirect, SpringerLink, and Google Scholar were all searched in a planned way for works. The search strategy included a wide-ranging diversity of evidence from phytochemical studies, from pre-clinical studies on animals to clinical proof in humans, Vitex negundo Linn is becoming better understood as a possible anti-inflammatory and immune-modulating drug. The book search was restricted to studies that came out between 2000 and 2025 so that it would include both old and new changes in the field of study, ensuring balanced coverage of older salient and newer advances in the fields of rheumatology and herbal therapeutics.

2.2 Search Strategy

We used terms to create a thorough, all-encompassing search strategy to find the relevant literature in Vitex negundo Linn and its chemical effects in the treatment of Rheumatoid Arthritis (RA). We used both Medical Subjects headings (MeSH) keywords or free-text terms to make sure

our searches were accurate and to contain studies that used different language. We strategically employed “AND” and “OR” Boolean operators to link related terms and to narrow the findings to their best sensitivity and specificity. In our core search, we included key constructs related to Vitex negundo Linn (also referred to Nirgundi) and its pharmacological properties which were anti-inflammatory, immunomodulatory, phytoconstituents, and flavonoids. We also included disease-associated terminology such as Rheumatoid arthritis, RA and autoimmune inflammation, which focused on the therapeutic pertinence of the plant in inflammatory issues. A number of internet sources were searched: PubMed, Scopus, ScienceDirect, SpringerLink, and Google Scholar, each of which required minor variations to its syntax in order to follow the database-specific search rules. In addition, we screened the bibliographies of some relevant studies to uncover articles that might not be indexed in the more commonly used databases. This multi-tiered strategy enabled the collection of all potentially relevant literature, reduced uncertainty for omissions, and increased reliability and reproducibility of the whole review process.

Table 3. Search Strings and Keywords Used in the Literature Review

Search Strings	Keywords
Plant-related terms	“Vitex negundo Linn”, “Vitex negundo”, “Nirgundi”, “Verbenaceae”, “medicinal plant”, “herbal extract”, “plant-based therapy”
Disease-related terms	“Rheumatoid arthritis”, “RA”, “autoimmune disorder”, “inflammatory joint disease”, “chronic inflammation”, “arthritic models”
Pharmacological activity terms	“Anti-inflammatory”, “immunomodulatory”, “analgesic”, “antioxidant”, “disease-modifying”, “cytokine inhibition”, “cartilage protection”
Phytochemical composition terms	“Phytoconstituents”, “flavonoids”, “alkaloids”, “iridoid glycosides”, “terpenoids”, “lignans”, “essential oils”
Experimental and clinical context	“In vitro”, “in vivo”, “animal models”, “clinical trials”, “toxicity studies”, “pharmacodynamics”, “pharmacokinetics”
Search combination examples	(“Vitex negundo Linn” OR “Nirgundi”) AND (“Rheumatoid arthritis” OR “RA”) AND (“anti-inflammatory” OR “immunomodulatory” OR “phytoconstituents” OR “flavonoids”)

The table presents search strings and keywords combining plant, disease, and pharmacological terms to comprehensively identify studies on Vitex negundo Linn and its therapeutic role in Rheumatoid Arthritis.

2.3 Inclusion and Exclusion Criteria

Specific criteria for what to include and what not to include were used in the process of screening to make sure that the literature review stayed relevant, credible, and scientifically sound. The following conditions had to be met for a study to be included: (i) it focused on Vitex negundo Linn or its bioactive chemical constituents concerning Rheumatoid Arthritis (RA) or relevant inflammatory and autoimmune disorders; (ii) it was an original research study, reviews, or a clinical/preclinical study published in a peer-reviewed journal; and (iii) the literature was available in English and published between 2000 and 2025 to ensure inclusion of high-quality and relevant contemporary evidence.

Publications were excluded if they (i) lacked primary evidence on the anti-inflammatory or immunomodulatory effects of Vitex negundo Linn; (ii) were a conference abstract, editorial, letter or non-peer-reviewed literature; or (iii) conversely, reported only extraneous interest on pharmacological effects such as antimicrobial or antioxidant effects unrelated to RA; or (iv) were insufficient in methodological details and/or were examining previously registered datasets. The inclusion and exclusion process mitigated the potential to include lower quality less scientifically credible and thematically relevant publications, providing a robust treatment to examine Vitex negundo Linn in treatment of Rheumatoid Arthritis.

2.4 Data Extraction and Analysis

The data gathering was done in a planned way using a uniform script. Information that was useful was gathered, like the author, the year of release, the study methodology, and the group number, experimental model, phytochemical constituent, dosage, outcomes, and conclusion. Studies included both preclinical and clinical to examine the anti-

inflammatory, ant oxidative, and immunomodulatory effects of Vitex negundo Linn in rheumatoid arthritis. Data extraction was organized both by theme and chronologically to evaluate trends, methodological strengths, and gaps in research. Tables were used to summarise the quantitative data, and stories were used to show the emotional data. Comparative data were drawn to inform on the overall effect of V. negundo in comparison to standard therapy and the feasibility of V. negundo in adjunct therapy or as an alternative therapy for rheumatoid arthritis.

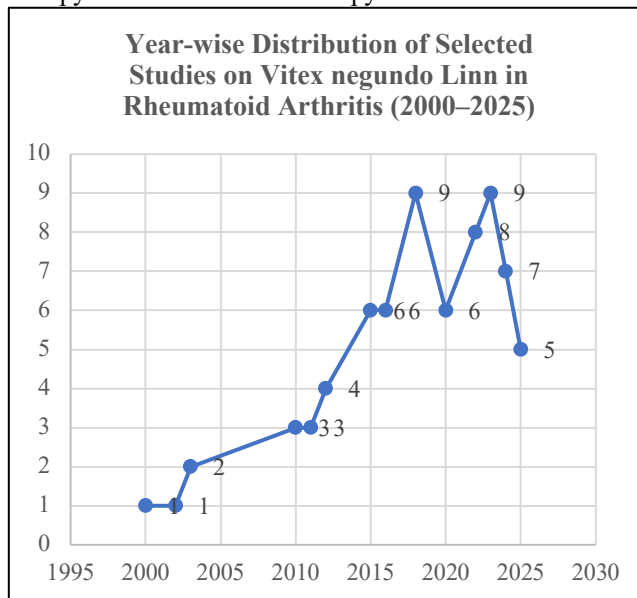


Figure 2: Flowchart of the articles under Vitex negundo Linn in Rheumatoid Arthritis

Following 2010, research on adaptive robotic grippers started to increase, notably from 2015 onward there was a notable rise in publications. The peak of research in the field appeared between 2018 and 2023, indicating significant progress in robotic adaptability, materials and control systems, while reduced output in 2024-2025, may indicate

the maturity of robotics research or simply a shift toward technologies that fully integrate automations.

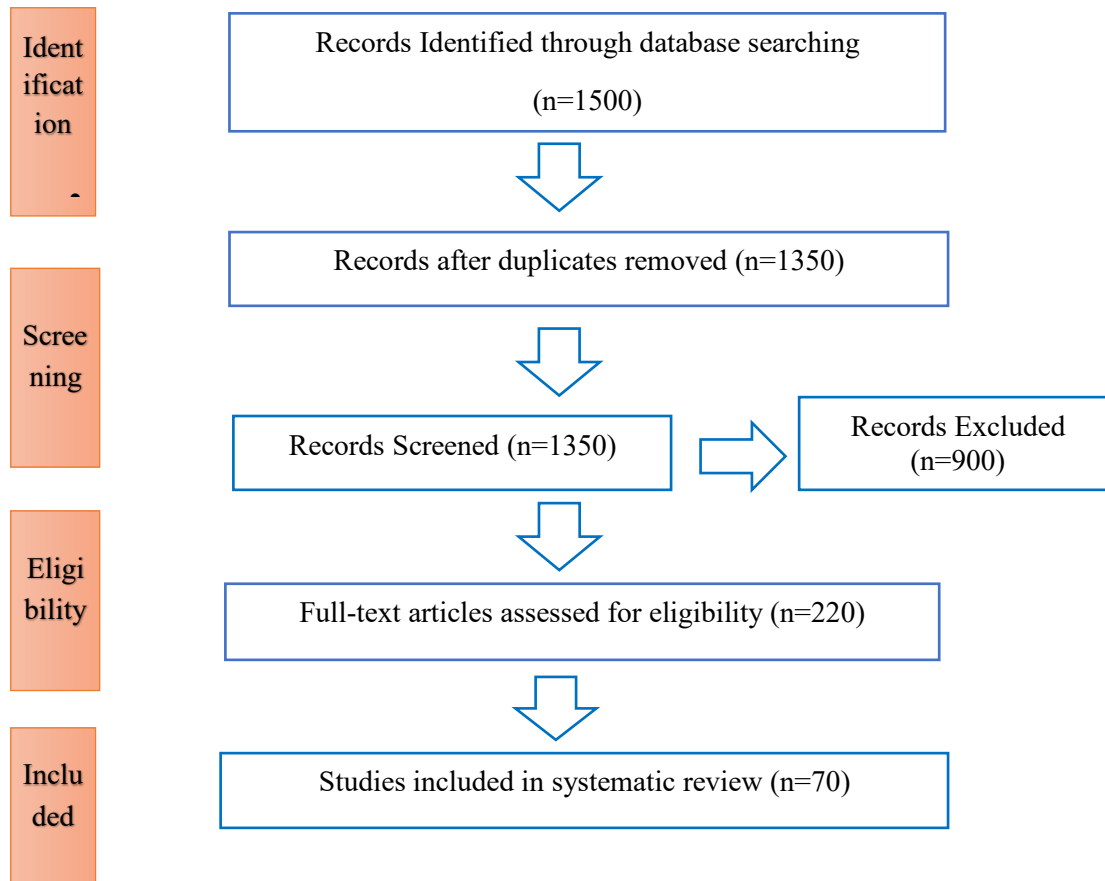


Figure 3: PRISMA Model

Source: Author

The PRISMA flow plan for choosing studies can be seen in Figure 1. After we determined an initial total of 1,500 records, and subsequently removed duplicates, we were left with a total of 1,350 records. After a screening process and application of eligibility criteria, we were able to review the full text of 220 articles, and we finally included a total of 70 high quality studies in the systematic review.

3. BOTANICAL AND ETHNOPHARMACOLOGICAL PROFILE OF VITEX NEGUNDO LINN

3.1 Taxonomy and Botanical Description

Vitex negundo Linn. It is a big bush or as small tree that gets to be 2–5 m tall and smells nice. It's also called the "Chinese holy tree" and "Nirgundi" in Ayurveda. It is part of the Verbenaceae family (name changed to Lamiaceae not long ago) [36]. It has quadrangular grayish stems that are hairy, and the leaves are compound palmate with five lanceolate leaflets. The leaf surfaces are rough green above and whitish with dense hairy pubescence beneath. The blue-purple flowers are grouped in simple or twisted panicles at the ends (and along the sides) of the stems. They bloom in the summer. The fruits are small and globose drupes that are black when ripe and each fruit contains four seeds. *Vitex*

negundo is known for the aromatic smell originating from its volatile essential oils.

3.2 Geographical Distribution and Cultivation

A lot of *Vitex negundo* Linn. can be found in the warm and subtropical parts of Asia, Africa, or the Pacific countries. It grows along roadsides, riverbanks, or empty places in India, up to an elevation of 1500 meters, in both flat and hilly areas [37]. This plant grows best in full sun and loamy soil that drains well. It can be grown at a number of different agroclimatic zones. It usually propagates through stem cuttings or seeds, and it is not hard to maintain. This plant is resistant to pest pressure and drought, and this has led to its widespread cultivation for medicinal and commercial use. The plant has a great adaptability and wide ecological range, making it widely utilized within numerous systems of traditional medicine.

3.3 Traditional Uses

Vitex negundo Linn. is used in Ayurveda or traditional medicine for its ability to reduce inflammation, ease pain, and fight rheumatism. Classical Ayurveda texts refer to Nirgundi as an important drug for joint pain, arthritis, muscle stiffness, and various inflammatory conditions [38]. Typically, the leaves can be infused into a poultice applied to the area of concern, or taken internally as a decoction for its benefit in alleviating swelling and pain. It may also be

used to treat fever, cough, and lung diseases. The Unani and Siddha systems also recommend Nirgundi for neuralgia, headache, wounds, and menstrual conditions. Some rural folk practitioners in India have also used Nirgundi to treat rheumatic pain, sprains, and insect bites [39]. The smoke produced from burning the plant material may be traditionally associated with the repellent qualities of the plants as it is fragrant.

3.4 Parts Used

All parts of *Vitex negundo* Linn. are medicinally valuable, though leaves are the most widely used.

Leaves: *Vitex negundo* Linn leaves have many beneficial substances in them, such as antioxidants, terpenoids, phenolic acid, and vital oils. Major constituents include flavonoids (casticin, artemetin, vitexicarpin, gardenin A and B, luteolin derivatives), terpenes (α -pinene, β -pinene, caryophyllene, farnesol, camphor, nerolidol, squalene), phenolic compounds (friedelin, vitamin C, carotene), and glycosides (agnuside, vitenoside, aucubin) [40]. These phytochemicals contribute to the plant's anti-inflammatory, antioxidant, analgesic, and immunomodulatory activities, supporting its traditional and pharmacological use in managing inflammatory disorders such as rheumatoid arthritis [41].

Roots: Historically, *Vitex negundo* Linn roots have been used to treat rheumatism, fever and dyspepsia. The *Vitex negundo* Linn roots contain a wide range of bioactive phytochemicals, such as lignans, flavonoids, terpenoids, and sterols. These include vitexoside, agnuside, R-dalbergiphenol, negundin A as well as B, vitrofolal E, (+)-lyoniresinol, (+)-pinoresinol, (+)-diasyringaresinol, oleanane-type triterpenoids, and negundin A and B. These chemicals are called 2²,3[±]-diacetoxyoleana-5,12-dien-28-oic acids, 2 α ,3 \pm -dihydroxyoleana-5,12-dien-28-oic acid, as well as 2³² diacetoxy-18 hydroxyoleana-5 acid [42]. Molecular names for these substances include vitexin, isovitexin, acetic oleanolic acid, sitosterol, and 3-formyl-4,5-dimethyl-8-oxo-5H-6,7-dihydronaphthofuran. The latter is a new furanoeremophilane compound. Phytochemicals support beneficial anti-inflammatory, antioxidant, or hepatoprotective efficacy and therefore reinforce the therapeutic relevance of the plant in traditional health and modern health paradigms [43].

Bark: Utilized for its tonic and febrifuge effects in traditional medicine. The stem and bark of *Vitex negundo* Linn contain a rich mixture of flavonoids, triterpenoids, phenolic acids, and siloxane derivatives. Some of the main parts are vitexin, caffeine β -amyryn, epifriedelinol, oleanolic acid, and 3,6,7,3²,4²-pentamethoxy-5-O-glucopyranosylrhannoside. These chemicals are known to reduce inflammation and protect cells from damage. A number of siloxanes and disilane compounds were also found, including tetradecamethyl, phenyl-cyclopentasiloxane, cyclooctasiloxane, tetracosamethylcyclododecasiloxane, cycloheptasiloxane tetradecamethyl, phenyl-cyclopentasiloxane, cyclooctasiloxane, as well as tetracosamethylcyclododecasiloxane. These chemicals add to the chemical complexity of the plant [44]. β -sitosterol,

p-hydroxybenzoic acid, 5-hydroxy-3,6,7,3ⁱ,4ⁱ-pentamethoxyflavone, 3 - The bark also has acetoxy-olean-12-en- acid and 3 -hydroxy-olean-5,12-dien acid. These bioactive compounds collectively support the anti-arthritis, antimicrobial, and hepatoprotective activities of *Vitex negundo*, making its stem and bark valuable components in traditional and modern medicinal formulations [45].

Seeds: Acknowledged for their stimulant and vermifuge activity, they are used in treating respiratory and digestive problems. In *Vitex negundo* Linn seeds, there are many chemicals that may be useful for health. These include terpenoids, phenolic acids, flavonoids, and hydrocarbons. These are the important chemicals: β -sitosterol, p-hydroxybenzoic acids, n-hentriacontanol, n-hentricontane, n-nonacosane, and 5-oxyisophthalic acid are some of the chemicals that are found in plants. Free radicals and swelling can be stopped by them [46]. Different types of diterpenes, such as abietane-type diterpenes such as 5-hydro-8,11,13-abietatrien-6 \pm -ol and 3²-acetoxy-olean-12-en-27-oic acid, are also found in the seeds. More oleanane-type triterpenoids have been found, such as 2 α ,3 α -dihydroxyoleana-5,12-dien-28-oic acids and 2 β ,3 α -diacetoxyoleana-5,12-dien-28-oic acid, also known as 2 α ,3²-diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid. There are additional chemicals at the plant which ease arthritis, kill germs, and ease pain. Vitodoin A, vitamin B, vitedoamine A, as well as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde are some of these [47]. So, the seeds may be utilised in traditional as well as contemporary medicines.

In summary, the extensive use of *Vitex negundo* Linn. for ethnopharmacological purposes across different traditional healthcare systems demonstrates its therapeutic versatility, particularly in the management of inflammatory and rheumatic disorders, specifically rheumatoid arthritis [48].



Figure 4: *Vitex negundo* leaves

Table 4. Vernacular Names of *Vitex negundo* Linn

Language	Common Names
Assamese	Aslok, Pochatia
Bengali	Beguna, Nishinda
Hindi	Nirgundi, Sambhalu
Kannada	Karilakki, Lakkagida
Malayalam	Karunocci, Nochchi
Marathi	Nirgunda, Nengar
Oriya	Thingkhawilupa, Niligundi
Sanskrit	Nirgundi, Sindhuvara
Tamil	Nallanocci, Nochi
Telugu	Nallavavili, Sindhuvaruma
Urdu	Sambhalu, Tukhmsambhalu

4. PHYTOCHEMICAL COMPOSITION

The *Vitex negundo* Linn plant, which is also called the five-leaved chaste tree, is in the Verbenaceae family. It is a useful medical plant [49]. It is full of different bioactive phytochemical components that can be used for their many medical benefits, especially as anti-inflammatory or immunomodulatory agents to treat rheumatoid arthritis and other inflammatory and autoimmune diseases.

4.1 Major Bioactive Compounds

The phytochemical makeup of *Vitex negundo* shows that it has a lot of active substances, including flavonoids, iridium glycosides, lignans, triterpenoids, and volatile oils that are thought to have medical uses [50]. Interesting flavonoids are casticin, orientin, luteolin, and negundoside because they work well as pain relievers, antioxidants, and immune system boosters.

As a flavonoid glycoside, negundoside stops the production of inflammation molecules like TNF- α , IL-6, or COX-2 enzymes. Iridoid glycosides such as agnuside and negundoside B inhibit oxidative stress and stabilize lysosomal membranes minimizing damage to tissues. Volatile oils such as sabinene, 1,8-cineole, β -caryophyllene, and α -pinene have analgesic, anti-arthritis, and antimicrobial properties [51]. Lignans including phylligenin and vitedoin A–C, and triterpenoids such as oleanolic acid and ursolic acid act to increase their anti-inflammatory and hepatoprotective properties, achieving systemic health through chronic inflammation [52].

4.2 Extraction Methods

The extraction of phytochemicals from *Vitex negundo* varies depending on the polarity of solvents as well as the class of desired compounds. The extraction of polar compounds (flavonoids and glycosides) is usually achieved using solvents which includes ethanol and methanol as well as mixtures of ethanol with water. Methanol extracts are particularly favorable since they are a rich source of polyphenolic ingredients with effective antioxidant and anti-arthritis properties [53]. Ethanol extracts are preferred for iridoid glycosides and lignans the have immunomodulatory potential. Essential oils can be extracted primarily from leaves and seeds through hydrodistillation or steam distillation and are particularly volatile and made up of monoterpene and sulfur-containing sesquiterpenes. Soxhlet extraction for laboratory scale and cold maceration are commonly used method for isolations, and they include methods for industrial applications using supercritical CO₂ extraction [54]. These are efficient and provide high yield and purity with reduced degradation. Method used for extracting phytochemicals can affect the subsequent concentration and then biological activity of isolated phytochemical constituents, and could directly affect its pharmacology efficacy.

4.3 Phytochemical constituents of extract of Vn leaves

The leaves of *Vitex negundo* are abundant with a variety of phytochemicals that elicit a number of therapeutic and biological actions. The methanolic leaf extract prepared from Soxhlet extraction contains bioactive metabolites such as flavonoids, terpenoids, phenolic acids, steroids, and triterpenoids. These components possess strong antioxidant, anti-inflammatory, anti-microbial, protective hepatoprotective and anticancer effects [55]. Flavonoids such as casticin, hydroxy-pentamethoxyflavone and trimethoxy flavonoids enhance free radical scavenging activity. Monoterpenoids such as γ -terpinene and sabinene provide hepatoprotective and gastroprotective effects. Triterpenoids such as betulinic acid and ursolic acid have antibacterial, antiviral, and anti-tumor efficacy. Phenolic compounds such as protocatechuic acid and p-hydroxybenzoic acid promote cancer prevention and treatment. The presence of β -sitosterol enhances both the immune-modulatory and anti-tumor activities of *Vitex negundo* [56]. Together, the abundance of phytochemicals lends credence medicinal significance of *Vitex negundo* leaves in both traditional and modern-based formulations.

Table 5. Phytochemical Constituents of Extract of *Vitex negundo* Leaves

Secondary Metabolite	Compounds Identified	Biological / Pharmacological Uses
Flavonoids	Hydroxy-3,6,7,3',4'-pentamethoxyflavone, Casticin, 5,3'-Dihydroxy-6,7,4'-trimethoxyflavone	Antioxidant, Free Radical Scavenging (FRS) activity
Monoterpenoids	γ -Terpinene, Sabinene, 6'-p-hydroxybenzoyl mussaenosidic acid, 2'-p-hydroxybenzoyl mussaenosidic acid	Hepatoprotective, Gastroprotective
Sesquiterpenoids	Viridiflorol, β -Caryophyllene, Caryophyllene oxide, Globulol, 4-Terpeneol	Pharmaceutical agents

Secondary Metabolite	Compounds Identified	Biological / Pharmacological Uses
Triterpenoids	Betulinic acid, Ursolic acid	Antibacterial, Antiviral, Antitumor, Antioxidant
Steroids	β -Sitosterol, Sitosterol	Antitumor, Immunosuppressive
Phenolic Compounds	p-Hydroxybenzoic acid, Protocatechuic acid, Oleanolic acid, Vitamin C	Cancer prevention and treatment

5. PHARMACOLOGICAL PROPERTIES RELEVANT TO RA

Vitex negundo Linn may be able to help treat rheumatoid arthritis because it has many medical benefits, including being an anti-inflammatory, antioxidant, pain-relieving, and anti-arthritis, and immunomodulatory properties, among others, which, have been verified in multiple preclinical experiments, biochemical tests, and subsequent comparisons with typical anti-arthritis pharmaceutical drugs [57].

5.1 Anti-inflammatory Activity

Vitex negundo Linn exhibits strong anti-inflammatory effects by inhibiting classical pro-inflammatory mediators and signaling pathways. Its various bioactive constituents (i.e. casticin, negundoside, luteolin, and β -caryophyllene) can inhibit cyclooxygenase (COX) and lipoxygenase (LOX) enzymes responsible for prostaglandin and leukotriene production. *Vitex negundo* additionally reduces the release of cytokines, which are chemicals that cause inflammation in RA [58]. These include IL-6, IL-1, and TNF-. They are part of the defence system and cause inflammation. In general, it stops the production of genes that cause inflammation by changing the NF- κ B or MAPK signalling pathways. *V. negundo* is also shown to have a significant effect in experimental studies (using methanolic and ethanolic leaf extract) where adjuvant-induced animal models of arthritis showed a significant reduction in paw edema, myeloperoxidase activity and C-reactive protein levels [59]. Combined, these results highlight the potential of *V. negundo* as a novel natural anti-inflammatory agent that reduces and alleviates chronic inflammation associated with RA.

5.2 Antioxidant Activity

Oxidative stress plays a big role in the development in rheumatoid arthritis by damaging joint tissue, causing inflammation in the synovial membrane, and throwing off the immune system [60]. *Vitex negundo* Linn is a powerful antioxidant plant because it contains a lot of flavonoids or phenols. There are several types of antioxidants, and they all work by getting rid for reactive oxygen compounds (ROS) or reactive nitrogen species (RNS). This keeps cell walls and proteins from getting damaged by oxidation. Natural antimicrobial enzymes such as super oxide dismutase (SOD), cathode (CAT), and glutathione peroxidase (GPx) work better when the *V. negundo* plant is present. It also restores redox balance and lowers the breakdown of lipids in joint tissues [61]. Studying *V* in

living things. *negundo* in arthritic rats showed that levels of malondialdehyde (MDA), an oxidative stress biomarker, was reduced and activity of antioxidant enzymes was increased. Overall, there appeared to be a protective effect from *V. negundo* on oxidative related joint degeneration.

5.3 Analgesic and Anti-arthritis Effects

Vitex negundo Linn has been used for a long time to treat arthritis pain and swelling, and recent preclinical tests have shown that it is a powerful pain reliever and anti-arthritis. Both methanolic or ethanolic extracts of *V. negundo* root and leaves significantly reduced paw thickness, joint deformity, and cartilage erosion in models of adjuvant-induced or collagen-induced arthritis [62]. This was similar to the effects of the standard anti-inflammatory drugs indomethacin or methotrexate, but with less damage to the liver or digestive tract. The analgesic effects are mediated through inhibition of pain mediators such as prostaglandins and bradykinins, and the anti-arthritis effect primarily through inhibition of synovial hyperplasia and bone turnover. Histopathological examinations showed decreased infiltration of inflammatory cells and restoration of joint architecture post treatment with *V. negundo* suggesting a chondroprotective action [63].

5.4 Immunomodulatory Effects

Rheumatoid arthritis changes the immune system's reactions, which are controlled by T-cells, B-cells, or macrophages that are triggered. *Vitex negundo* Lin has a lot of immunomodulatory effects and manages the hormones that cause and stop inflammation [64]. Early research has shown that *V. negundo* extracts can lower levels of active Th-1 cytokines (IL-2, IFN- γ) whereas increasing levels of Th2 cytokines (IL-4, IL-10). They can also keep the immune system in balance. Moreover, extracts of *V. negundo* inhibit hyperactivation of macrophages, which leads to less nitric oxide and inflammatory cytokine release. This plant can also modulate B-cell antibody production, which is important in the context of RA, as this will reduce autoantibody-mediated damage to tissues [65]. These beneficial effects support that *V. negundo* will modulate both innate and adaptive immunity, and possibly decrease disease progression and reoccurrence in patients with rheumatoid arthritis.

5.5 Safety and Toxicity Profile

Safety evaluations of *Vitex negundo* Linn indicate a favorable therapeutic index and low toxic effects at pharmacologically active doses. Acute and subchronic toxicity studies conducted in rodents showed no signs of mortality, behavioral or blood parameters' abnormalities at

doses of 2000 mg/kg. It was found in research publications that LD₅₀ values of methanolic extracts were found to be above 3000 mg/kg, indicating that it is safe for use below that therapeutic dose [66]. In that regard, during a histopathological examination, no indication of acute damage to vital organs (liver, kidney and heart) were found even after chronic exposure. In vitro assays using human fibroblast and macrophage cell lines also demonstrated little cytotoxicity potential; thus, supporting biocompatibility [67]. With the existing research information noted here, *V. Negundo* may be a good way to treat RA and other inflammatory diseases, but only in a limited and regulated setting [68].

6. MECHANISM OF ACTION

Vitex negundo Linn operates via multiple mechanisms to target key pathological hallmarks of rheumatoid arthritis (RA), which are inflammation and oxidative stress, and immune dysfunction. The plant's phytoconstituents, including casticin, negundoside, and iridoid glycosides have a synergistic effect to mitigate the inflammatory cascades. The plant also stops pro-inflammatory substances from working, such as the enzyme cyclooxygenase-2 (COX-2), enzyme lipoxygenase (LOX), TNF- α , or interleukins (IL-1 β , IL-6), is a protein that helps fight tumours. This makes joint pain and stiffness go down [69]. At a molecular level, negundo virus slows down or entirely stops the NF- κ B or MAPK signalling pathways. This stops the production of genes that make inflammatory enzymes. Antioxidant components clear reactive oxygen and nitrogen species (ROS/RNS) to decrease oxidative damage of synovial tissues and cartilage. It also has immunomodulatory effects that normalize secretion of cytokines in T-cell responses, B-cell responses in RA and modulates macrophages from polarizing M1, pro-inflammatory, to M2, anti-inflammatory. Overall, these are important mechanisms creating the therapeutic effect of *V. negundo*, by: reducing inflammation, maintaining joint structure, and leaving the immune system in homeostasis [70].

Mechanism of Action of *Vitex negundo* Linn in Rheumatoid Arthritis

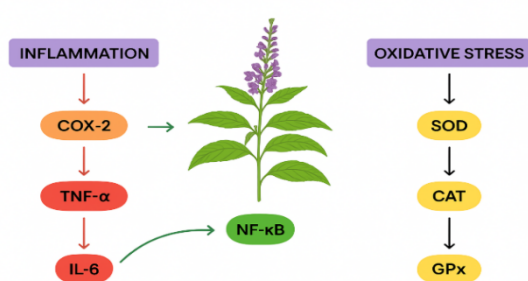


Figure 5. Mechanism of Action of *Vitex negundo* Linn in Rheumatoid Arthritis

The diagram illustrates the integrative molecular mechanism of *Vitex negundo* in rheumatoid arthritis. It shows inhibition of COX-2, LOX, TNF- α , IL-6, and NF- κ B pathways, enhancement of antioxidant enzymes (SOD,

CAT, GPx), and modulation of immune responses leading to reduced inflammation and oxidative stress.

7. FORMULATION AND DRUG DELIVERY ASPECTS

In recent years, the formulation and delivery methodologies for *Vitex negundo* Linn have made significant strides to enhance the bioavailability, stability, and therapeutic effect of its bioactive compounds. There are now nanoemulsion systems of essential oils or ethanolic extracts of *V. negundo* that have been developed aimed at improving solubility and development of targeted delivery to inflamed sites. These nanoformulations enable a slower release of the drug, which improves permeability and has antioxidants, all factors that may help improve the management of rheumatoid arthritis (RA). Topical formulations such as ointments, creams, and gels containing hydroalcoholic extracts have also been studied and demonstrated evidence preclinically and clinically of anti-inflammatory and analgesic activity. Topical delivery systems may offer localized outcomes in proximity to inflamed joints and eliminate exposure to effects in systemic circulation. In addition, polyherbal combinations using *V. negundo* with *Withania somnifera*, *Boswellia serrata*, and *Curcuma longa* are being studied for possible synergy with inflammatory mediators, such as TNF- α and IL-6, as one example. Additionally, we are studying unique delivery systems, including nanoparticles, liposomes, and transdermal delivery patches to obtain a consistent flow of the drug and pharmacokinetic parameters suitable for RA management. In summary, the literature indicates overwhelmingly that these types of new and emerging formulations support greater bioavailability, reduced first pass metabolism, and retention of plasma levels over time, which would lead to greater symptom management of RA and disease progression.

8. RESEARCH GAPS AND FUTURE DIRECTIONS

While extensive preclinical research has provided clarity regarding the pharmacological aspects of *Vitex negundo* Linn in reducing rheumatoid arthritis (RA), several research gaps remain which continue to impede clinical translation and larger-scale therapeutic applications. One important limitation is the lack of standardized extract or consistency in formulations which can provide a substantial challenge to clinical research around this plant's therapeutic use. Variability in extraction techniques, solvent polarities, parts of the plant, and botanical origins can result in varying phytochemical profiles, and this variability further limits comparisons and dose-response relationships across studies. Standardization procedures of bioactive compounds (e.g., casticin, negundoside, and iridoid glycosides) among the varied extract formulations is critical to ensure reproducibility and clinical significance. Additionally, a limitation observed is a lack of a robust clinical trial design. The majority have largely limited their studies to in vitro or animal-model studies that provide relevant, but only preliminary, support of efficacy and safety profiles. The rationale is to report the need for a large-scale, randomised double-blind clinical study with strong designs that look at the antioxidant, anti-inflammatory, and immunomodulatory effects of *V.*

negundo on RA patients. As well to provide a greater understanding of potential molecular targets and signaling networks moderated.

Additionally, future research should evaluate more new drug discovery, new formulations (for example, nanoformulations, osmotic transdermal systems, and phytosome complexes) to improve bioavailability and target delivery. Molecular docking and simulation studies will also support future studies to understand and identify specific phytoconstituents interactions with key drug targets in RA such as COX-2, TNF- α , and NF- κ B. Lastly, regulation and quality remains a challenge for the use of herbal medicine. Developing globally comparable pharmacopeial standards, Good Manufacturing Practices (GMP), and analytical methods with documented evidence is urgent to provide confidence in purity, potency, and safety of *Vitex negundo* Linn-based formulations. Addressing these barriers with the help of interdisciplinary teams that involve pharmacologists, biotechnologist researchers, and clinical researchers is an important part of allowing *V. negundo* to have a place as a scientifically validated and clinically endorsed adjunct therapy for rheumatoid arthritis.

9. CONCLUSION

Together, the data shows that *negundo* vitamin Linn may be useful as an extra or additional medicine for rheumatoid arthritis. The numerous bioactive phytoconstituents demonstrated to contribute to the anti-inflammatory, antioxidant, and immunoregulatory actions of *Vitex negundo* include flavonoids, iridoid glycosides, and terpenoids, which work in synergy. *V. negundo* modulates important molecular pathways associated with inflammatory processes, including NF- κ B, MAPK, and proinflammatory cytokines such as TNF- α , IL-6, and IL-1 β , to suppress inflammation, reduce oxidative injury, and restore immune function. Combined, these actions lead to alleviating joint pain, swelling, and tissue degeneration observed with RA. *V. negundo* has an edge over traditional pharmacologic therapies, such as methotrexate or biologics because it has low toxicity, biocompatibility, and cost, which is beneficial for low-resource healthcare settings. Moreover, pre-clinical studies suggest that methanol and hydroalcoholic extracts of *V. negundo* can obtain similar effects to conventional pharmacologic anti-inflammatories without side-effect profiles associated with conventional pharmacologic anti-inflammatory drugs. However, while it seems that *V. negundo* is effective based on the current totality of evidence, its clinical application is limited by a lack of standardized formulations, lack of validated biomarkers, and Phase III studies with a sizeable sample in human studies. Future research should standardize extraction methodologies, identify bioactive compounds, support multi-centric randomized clinical trials, and ultimately establish efficacy and safety. Ultimately, the future of *V. negundo* efficacy will also depend on studies utilizing omics technologies (genomics, transcriptomics, etc.) to better describe the pathways in which *V. negundo* efficacy occurs.

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