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REVIEW ARTICLE

A Systematic Review on the Pharmacological Prospectives of *Vitex negundo* in *Ayurveda* Drug Research

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Abstract

In *Ayurveda*, there are many formulations containing *Vitex negundo* which is indicated in specific pathogenic and non-pathogenic condition. Hence this review attempted to explore the data of research at a glimpse. Peer reviewed and published data from PubMed had been selected for the same. The PubMed database (2009 to 2019) were searched using the keyword '*Vitex negundo*', '*Ayurveda*' with Boolean operator 'AND'. A total of 45 free full text articles were retrieved from 57 articles with specific inclusion criteria. All the 45 studies were categorized into in silico studies, analytical studies, *in vitro* studies, *in vivo* studies and clinical studies. Then the data such as the type and design of research, the techniques used, and sample size were categorized into tables and the results were attained through thorough reading and analysis. A descriptive method is adopted for the review and quality aspects of the data were not taken into consideration. Compounds namely, acerosin and thymol were studied in *in silico* as an inhibitor for mTOR and on skin diseases respectively. Analytical studies explored the presence of compounds such as estriol, angnuside, phydroxybenzoicacid etc. Pharmacological activities such as anti-bacterial, anti-cancerous, anti-tubercular were studied *in vitro* and *in vivo*. A clinical study on *Nirgundi khanavati* in *gridrasi* (sciatica) is found to be effective in reducing the pain threshold. The phytochemical research of the drug were also proven against carcinoma. There is a high scope for research for more clinical studies and standardization with advanced analytical procedures and marker compounds.

Keywords: *Ayurveda*, Pharmacology, Research, *Vitex negundo*

1. Introduction

Ayurveda describes *Vitex negundo* both as a single drug and a poly herbal formulation. Its roots and leaves are mainly used for preparing different formulations. *Vitex negundo* possess pharmacological activities even from the cellular events to the cancer signalling pathways. Apart from therapeutic activities, it can act as bio-control agents such as a *Vitex* repellent, larvicidal and pesticidal agent. *Vitex negundo* is mainly indicated in cough and arthritic conditions of high prevalence. The plant also acts as a heavy metal scavenger from the soil and water[1]. If the tissue culture possibilities of the drug are explored, it will be a great boon to *Ayurveda* as well as society.

Leaves constitute the most important part owing to the presence of a majority of sesquiterpenes, monoterpene, alcohols, ester, aromatic compounds, ether[2]. Besides the above-mentioned, the presence of high amounts of flavonoids and phenolic compounds also makes its leaves a vital part[3].

Vitex negundo as a single herbal formulation is indicated in chronic cough, otitis media and goitre. The key aetiological factors of chronic cough are Chronic

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Obstructive Pulmonary Disease (COPD), chronic lung infections and Gastro-oesophageal reflux disease. The commonest type of chronic cough is due to GERD, various inflammatory processes occurring in the airways can also cause the same[4]. The active compound Tris(2,4-di-tert-butylphenyl) phosphate from the leaves[5] and the leaf oil[6] shows anti-inflammatory properties owing to the protective action. Another notable base for chronic cough is Eosinophilic bronchitis[7]. The anti-eosinophilic activity of *Vitex negundo* is validated and it even reduces the hyperresponsiveness of bronchitis[8]. Even in a dose-dependent manner, the extract of *Vitex negundo* has showed inhibition of cough reflux which highlights its antitussive property[9].

Bacterial infections like streptococcus pneumonia, and staphylococcus aureus are the major causes of otitis media. Otitis media can be caused as a complication of the upper respiratory tract[10]. The methanolic extract of *Vitex negundo* leaves, various compounds such as terpenoids, flavonoids, steam-distilled oil, globulol, sterols and steam-distilled oils are proven to have antibacterial potential[11-13]. Together with the anti-inflammatory property of Tris(2,4-di-tert-butylphenyl) *phosphate* (TDTBPP)[14] the drug can also perform better in reducing inflammation and controlling further progress of disease.

Goitre is a disease caused by the faulty assimilation and absorption of iodine[15]. It has been proved that flavonoids and phenolic compounds in the roots have the capacity to increase the iodine uptake and thereby decrease goitre[16].

The polyherbal formulations containing *Vitex negundo* are indicated in diseases such as gout, Rheumatoid arthritis, osteoarthritis, pyrexia, oedema and skin diseases[17].

The deposition of uric acid crystals in joints cause an inflammatory process which in turn leads to gouti arthritis[18]. The active compound from the leaves of *Vitex negundo* Tris(2,4-di-tert-butylphenyl) phosphate and two new chromone derivatives, namely, 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl)benzoic acid and methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoate (1) have shown the features of anti-inflammatory effects[19]. High levels of phenolic compounds have been isolated from roots[20] and these phenolic compounds along with tannins, terpenoids have the capacity to act as an inhibitor for Xanthine oxidase and thereby blocking the biosynthesis of uric acid from purine metabolism[21].

The preparations from *Vitex negundo* are mentioned as effective for Rheumatoid arthritis and osteoarthritis. Leukotrine B4(LTB4) is a pro-inflammatory lipid mediator which when deposited in synovial fluid can increase the level of Rheumatoid arthritis, so the inhibition of biosynthesis of LTB4 can reduce the progress of the disease[22]. A compound *agnuside* which has been isolated from *Vitex negundo* showing properties capable of decreasing the level of ESR, leukotriene B4, cytokines and TNF-alpha[23]. So the compound *agnuside* reduces the level of LTB4 thereby reducing the production of pro-inflammatory cytokines and lessening RA. The alkaloid extract of *Vitex negundo* shows analgesic potential and various isolated flavonoid compounds have shown anti-inflammatory potential. Rheumatoid arthritis is an inflammatory progressive disease[24] therefore the inflammatory potential can be utilized in arthritis conditions. For the treatment of pain, inflammation and fever the same protocol of treatment can be proceeded. In all such cases, Non-Steroidal Anti-inflammatory Drugs (NSAIDs) can be advised[25].

Prostaglandins mainly help in resolving inflammation through receptor-dependent and independent mechanisms[26]. *Vitex negundo* leaf oil (500 microlitre/kg) was proved to be efficacious in inhibiting the COX-2 pathway and it was measured by using a COX inhibitor screening assay kit. Various phenyl naphthalene–type lignans were isolated from the seed extract of *Vitex negundo*[27] which have been shown to reduce serum inflammatory factors like TNF, and IL-6 and inhibit COX-2 pathway[28] thereby reducing arthritis, inflammation and pain.

Swelling can occur due to a multitude of reasons and in general, the treatment can vary from anti-inflammatory medicines to surgical procedures[29]. COX inhibition property of *Vitex negundo* can be considered as a rationale for the reduction of swelling. Along with that, an antioxidant compound Tris(2,4-di-tert-butyl-phen-yl)phosphate has been isolated from ethyl acetate extract of leaves which helps in reducing inflammation and thereby ultimately reducing the swelling in different parts of the body.

Fever can be brought down by inhibiting the COX pathway and here the *Vitex negundo* leaf oil was proved

to be beneficial in inhibiting the COX pathway. Hence from all these studies, it is evident and understood that *Vitex negundo* has various properties capable of lowering fever. Two chrome derivatives - methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl)ethyl)benzoic acid were isolated and proved to have analgesic and anti-inflammatory properties. A phenolic compound *Chrysosplenol-D* was isolated from the aerial parts which showed antiseptic potential[30] which further helps in controlling the infections and hence the fever caused by pathogens.

From the methanolic leaf extract of *Vitex negundo* a flavonoid named luteolin was isolated[31]. Luteolin has proved to decrease keratinocyte proliferation thereby diminishing the skin condition namely, psoriasis. It also inhibits the secretion of inflammatory mediators from human keratinocytes[32]. From the essential oil of *Vitex negundo* a compound named thymol has been isolated[33]. The anti-fungal activity of thymol against candida species is known[34], so *Vitex negundo* could act as an anti-fungal agent as well. Additionally, the antioxidant potential of *Vitex negundo* owing to the presence of high levels of phenolic compounds limits the production of free radicals thereby preventing skin diseases.

In many herbo-mineral preparations *Vitex negundo* is used as a wet triturating agent for the preparation of medicines, it has been already proved that the drugs used for trituration always have an impact on the physicochemical and biological properties of a formulation[35]. High levels of phenolics and flavonoids such as luteolin have been isolated from *Vitex negundo* and these flavonoids and phenolic compounds in turn act as a potential bioactive agent[36]. A compound Tris(2,4-di-tert-butyl-phen-yl)phosphate has been isolated from *Vitex negundo* and it is proven to have antidote activity so this may help in counteracting the impurities present in the formulations thereby revealing its action as a triturating agent.

2. Ayurvedic Prospectives

Vitex negundo commonly known as *Nirgundi,* implicit that the one which protects from a number of diseases, belonging to Verbanaceae family, is found throughout wastelands, banks of streams or in moist places. It is an aromatic large shrub or small tree with quadrangular branches. The various synonyms found in classics are *sinduvara, shephali, sugandhika, bhutakeshi* and it implies that the one that checks the accumulation of fluid in the body, which is liked by insects, and the one with aromatic leaves, eliminates evil organisms respectively[37].

Vitex negundo belongs to the class of *krimigna* (anti-microbial) drugs. *Vitex negundo* is classified based on the difference in colours of flowers such as white and blue[38]. The most commonly accepted ones are *nirgundi* and *sinduvara based* on the difference in the flowers with a similar botanical name - *Vitex negundo*. There are about 19 formulations containing *Vitex negundo*. Based on the routes of administration there are 5 formulations which are used externally, and 14 for internal administration. Based on the nature of preparations we can classify accordingly as aqueous soluble and lipid soluble. Among the 14 internally administered preparations, lipid extract was used in 6 of them and on the other hand the aqueous extract in 8 preparations. Hence the probable mode of internally administered aqueous soluble substances can be explained by understanding the aqueous soluble components identified from *Vitex negundo*. There are various isolated compounds from *Vitex negundo* which possess anti-inflammatory properties. It has been reported that these compounds with anti-inflammatory properties impart better relief from pain and avert the possibility of harmful effects[39]. The aqueous extract of the plant is used in a few formulations, which may be because of the presence of various constituents such as luteolin and various phenolic compounds which are soluble in water and thereby exhibiting its anti-oxidant, anti-inflammatory properties[40]. There are single drug formulations prepared with ghee and oil highlighting the drug carrier properties of ghee. The entrapment potency and the biocompatibility attribute of ghee have been proved making it a potential drug delivery applicator[41]. As a result, various lipid-soluble active constituents such as ursolic acid present in *Vitex negundo* can be easily engrossed in formulations prepared with ghee and oil. Ursolic Acid (UA) is a pentacyclic triterpenoid compound which has the ability to attune multiple molecular signalling pathways in multiple extra and intracellular targets that play a significant role in

metastasis, apoptosis and inflammatory procedure[42]. So, when UA is extracted with lipid substances, these lipid substances may act as a carrier and help in exploiting the properties of UA.

The potential external use of *Vitex negundo* may be attributed to the presence of phenolic compounds such as luteolin and vitexin. Generally, the skin offers some sort of defence mechanism against ROS but most of the time it may become insufficient. Hence the defence mechanism of skin can be enhanced by the addition of potential natural antioxidants that can inhibit ROS. In that case, the application of *Vitex negundo* is found effective externally[43].

Nirgundi is said to posess *tikta* (bitter), *katu (*pungent), *kashaya* (astringet) taste, *laghu* (light) in nature, *ushna veerya* (hot potency) and *katu (*alkaline) *vipaka (*digestion and metabolism). Mainly it is *kaphavata shaamaka* (the pharmacodynamics action is to reduce *kapha* and *vata*)[44].

The drug is being indicated in various diseases. Based on their specific action, the four most common diseases have been explained in detail. *Cough is predominant with vatha and kapha in doshik composition*. Its pathogenesis starts with vitiation of *kapha dosha* in *pranavaha srotas* (channel which carries external air into the body) which leads to obstruction of *vata*[45]. *Nirgundi* may act due to its *deepana* (appetizer), *anulomana* (carminative) properties thereby correcting the *agni (*digestive fire) and symptoms of *aama*. Since it reduces *kapha* and *vata*, it can cure the disease by correcting the obstruction caused by *kapha dosha*. The *tikta rasa* (bitter taste) generally decreases *kapha dosha*[46]. This property of the drug can be utilized for preventing obstruction due to *kapha*. Even the *vrishya* (rejuvenating) and *rasayana* (strengthening) property of *Nirgundi* helps in the restoration of *normal* colour, strength and vigour besides regaining immunity.

Shotha(swelling) is caused by vitiation of all three *doshas - vata, pitta* and *kapha*. *Vata dos*a gets vitiated and causes obstruction of *srotas (*channels which transport metabolized tissues in the body)[47]. The *tikta* and *katu* rasa (bitter and pungent taste) and *laghu* (light), *ruksha* (dryness) qualities help in controlling *kapha dosha* and thereby preventing obstruction. The *tiktarasa* (bitter taste) has the property of increasing urine production[48] and this produced urine can be excreted out with the help of *ushna veerya* (hot potency). The *ushna veerya* (hot potency) of the drug can alleviate the vitiated *vata* and enhance perspiration. By removing the obstruction in channels and eliminating the excess *kleda* (moistness)[49] through perspiration, *ushna veerya* acts in the pathogenesis of *sotha*. Hence swelling can be resolved by the combined action of *ushna veerya* (hot potency), *tikta* and *katu rasa* (bitter and pungent taste), *laghu* (light), *rooksha* (dryness) qualities of *Nirgundi*.

Vataroga (arthritis), a disease that minimizes the activities of different joints is caused by vitiation of only *vata dosha* or *vata* associated with *ama*. The pathophysiology mainly includes aggravation of *vata dosha* and this aggravated *vatadosha* is accumulated in the joints. *Gridhrasi* (sciatica) is a condition in which *ama* is associated with vitiation of *vata*. The *tikta rasa* (bitter taste) present in *Nirgundi* is mainly composed of *akasha* and *vayu mahabhoota* (elements of space and air)[50], hence it got an affinity towards *asthi dhatu (*bone tissue). In the context of treatment of *asthikshaya* (decrease in bone density), it is advised to administer milk and ghee processed with *tiktarasa* (bitter) drugs[51]. So, it is evident that *tiktarasa* (bitter taste) of *Nirgundi* plays an important role in *vatha roga*. *Tiktarasa (bittertaste)* also possess digestant properties thereby improving the metabolism. *Tikta rasa* (bitter taste) can reduce the burning sensation caused by *pitta dosha*. It can also reduce the swelling by increasing the urine output and sweat formation. The *ushna veerya* (hot potency) of the drug helps in controlling *vaata*. Thus these qualities of *nirgundi* can alleviate the pain and swelling in arthritis. The *rasayana* (strengthening) property of the plant controls the degenerative changes caused by *vaata*.

Kushta (skin disease) is a common disease which is caused due to faulty habits which leads to aggravation of all three *doshas - vata,pitta* and *kapha* and cause vitiation of *rasa, rakta (blood)* and *mamsa*[52]. *Tikta rasa* (bitter taste) has the property of dessicating or eliminating *kleda* (moistness) which is the prime factor in the aetiopathogenesis of skin disease. *Nirgundi* can be administered as external applicant in skin disease with *kapha* association. *Laghu* (light) and *ruksha* (dryness) properties are supportive for removing *kleda* and thereby enhancing its therapeutic action on the skin.

3. Aims and Objectives

To study the present status of research of *Vitex negundo* in PubMed database.

4. Materials and Methods

4.1 Search Strategy

All published research articles of *Vitex negundo* from 10 years (2009 to 2019) were searched in the PubMed database. All the 45 published research articles of *Vitex negundo* were taken into study and 45 articles included 2 *in silico* studies, 11 analytical, 19 *in vitro*, 11 *in vivo* and 2 clinical studies (Tables 1 to 3). A detailed schematic representation of the search strategy was included as Figure 1.

5. Result and Discussion

5.1 *In Silico* Studies

In-silico activity of the plant has been studied in two compounds namely, acerosin and thymol. Acerosin (which is a trihydroxyflavone) extracted from *Vitex negundo* was studied and proved to be a potent lead candidate for mTOR protein inhibitor against Leigh syndrome (neurological disorder which leads to loss of mental and movement abilities)[53]. mTOR is a protein kinase belonging to phosphoinositide 3-kinase family which can interact with proteins to form complexes such as mTOR C1 and mTORC 2 and these pathway exhibits function such as growth, stress, protein; lipid synthesis cellular response to hypoxia and autophagy[54]. A molecule named rapamycin has been identified which can target mTOR and form complexes thereby inhibiting its activity and these complexes can control the organization of actin cytoskeleton and cell survival. The mTOR and rapamycin have been shown to have specific roles in disease and health conditions[55]. Leigh syndrome is a disease that disrupts mitochondrial function. It is shown that by inhibiting mTOR the curability of Leigh syndrome can be improved[56], so acerosin from *Vitex negundo* was proved as an inhibitor for mTOR thereby improving the mitochondrial functions. Thymol content in essential natural oil showed the best docking score by inhibiting activities of *Pseudomonas elastase*. *Pseudomonas* infections usually include external otitis, ecthyma gangrenosum, and infections in puncture wounds. The isolated thymol can be utilized in various skin diseases as it has been proved effective in various fungal infections.

5.2 *In Vitro* Studies on *Vitex* Species

After going through the full-text articles a total of 18 invitro studies have been identified. Among these, there are 4 studies on which the results are further established by conducting *in vivo* studies. Compound isolation and its anticancer efficacy have been proven in 5 papers. The majority of the papers highlighted the anti-microbial property of *Vitex negundo*. Its antibacterial activity has been highlighted in 8 papers, anti-filarial in 3 papers, and anti brucella, anti-tubercular and anti-plasmodial activity in one paper each.

An endophytic fungus named colletotrichum gloeosporioides was isolated from *Vitex negundo* leaves and its methanolic extract has been proven as a source against multidrug-resistant *S. aureus* strain[57]. The supercritical fluid extract, steam distilled oil and petroleum ether extract of leaves showed potent antibacterial activity against *Bacillus subtilis*[58]. The methanolic extracts from leaves of *Vitex* species were found effective against *Bacillus aureus, B. subtilis*, *B. pumilus*, *Micrococcus luteus* and *Staphylococcus aureus* and also against all pathogenic enteric bacteria such as *Vibrio cholera, Vibrio parahaemolytics, Vibrio mimicus, E. coli*, *Shigella* sps. and *Aeromonas* sps., even the methanolic extract of bark showed significant antimicrobial activity against *V. cholerae, B. cereus, S. aureus, E. coli, S. typhimurium* and *S. sonnei*[59]. The ethanolic extract of leaves showed activity against *Mycobacterium smegmatis*[60]. The silver nanoparticles (10-30 nm) which were synthesized using methanolic extract of leaves of *Vitex negundo* showed an antibacterial effect on *E. coli* and *Staphylococcus aureus*[61]. Chrysosplenol-D isolated from the aerial parts of the plant showed antibacterial activity against *E. coli*, *Bacillus subtilis*, *Micrococcus tetragens* and *Pseudomonas fluorescens*. From above mentioned various articles, it is evident that the various extracts of *Vitex negundo* show anti-bacterial properties against a multitude of strains. The anti-bacterial property can be further utilized for controlling infections such as fever, upper respiratory tract infection and otitis media.

Table 1. *In vitro* and *in silico* studies

Sl.No.	Title	Journal name	Author name	Publishing year	Part used	Compound isolated	Result
1	Purified vitexin compound 1, a new neolignan isolated compound, promotes PUMA- dependent apoptosis in colorectal cancer	Cancer medicine	Jingfei C. *et al.,*	2018	seeds	VB-1	Suppress proliferation of cells, induced apoptosis
2	Proliferation and apoptosis of choriocarcinoma cells JEG-3 induced by VB2 and its invitro mechanism	Journal of central south university(medical sciences)	Deng J. *et al.,*	2013	Seeds	VB-2	Repress proliferation of choriocarcinoma cells
3	Novel bioactive metabolites producing endophytic fungus colletotrichum gloeosporioides against multidrug-resistant staphylococcus aureus	FEMS Immunol med microbial	Arivudainambi U. S. *et al.,*	2011	leaves	Colletotrichum gloeosporioides	Action against multidrug-resistant *S. aureus*
4	Detection of antituberculosis activity in some folklore plants by radiometric BACTEC assay	Letters in Applied Microbiology	Gupta V. K. *et al.,*	2010	leaves	Ethanolic extract	Showed activity against *M. smegmatics*
5	Large-scale screening of ethnomedicinal plants for identification of potential antibacterial compounds	Molecules	Panda S. K. *et al.,*	2016	bark	Methanolic extract	Showed antimicrobial activity
6	Vitexin compound 1, a novel extraction from a Chinese herb, suppresses melanoma cell growth through DNA damage by increasing ROS levels	Journal of Experimental and Clinical Cancer research	Nian Liu *et al.,*	2018	seeds	VB1	Suppress melanoma cell growth
7	Evaluation of composition and antimicrobial activity of supercritical fluid extract of leaves of *V. negundo*	Indian journal of pharmaceutical sciences	Nagarsekar K. S. *et al.,*	2010	leaves	Supercritical fluid extract, petroleum ether extract	Activity against *Bacillus subtilis*
8	*In vitro* antibacterial potential of some V*itex* species against human pathogenic bacteria	Asian pacific journal of tropical medicine	Kannathasan K. *et al.,*	2011	leaves	Methanolic extract	Effective against *Bacillus aureus*, *B. subtilis, B. pumilus*, *Micrococcus luteus* and *Staphylococcus aureus.*

Table 1. to be Continued…

Sl.No.	Title	Journal name	Author name	Publishing year	Part used	Compound isolated	Result
9	Green synthesis and antibacterial effect of silver nanoparticles using *Vitex negundo* L.	molecules	Zargar M. *et al.,*	2011	leaves	Methanolic extract	Synthesized silver nanoparticles
10	Anti-septic activity and phenolic constituents of aerial parts of *Vitex negundo* var. *cannabifolia*	molecules	Ling TJ *et al.,*	2010	Aerial part	Chrysosplenol-D	Activity against *E. coli*, *Bacillus subtilis*, *Micrococcus tetragens* and *Pseudomonas fluorescens*
11	Anti-filarial activity of ethyl acetate extract of *Vitex negundo* leaves *in vitro*	Asian pacific journal of tropical medicine	Sahare K N *et al.,*	2013	Leaves	Ethyl acetate extract	Effective against *Setaria cervia*
12	*In vitro* and *in vivo* bactericidal activity of *Vitex negundo* leaf extract against diverse multidrug-resistant enteric bacterial pathogens	Asian pacific journal of tropical medicine	Kamruzzaman M *et al.,*	2013	Leaves	Methanolic extract	Effective against enteric pathogenic bacteria
13	Antitubercular activity and isolation of chemical constituents from plant *Vitex negundo* Linn	Iranian journal of pharmaceutical research	Lada PL *et al.,*	2018	leaves	Ursolic acid	Antitubercular activity
14	PASS- predicted *Vitex negundo* activity: Antioxidant and antiproliferative properties on human hepatoma cells- an *in vitro* study	BMC Complementary and Alternative Medicine	Kadir FA *et al.,*	2013	leaves	Ethanolic extract	Antioxidant and antiproliferative properties on hepatocarcinoma cells
15	In vitro assay for the anti –brucella activity of medicinal plants against tetracycline-resistant Brucella melitensis	Journal of Zhejiang University. Science B	Motamedi H *et al.,*	2010	seeds	Ethanolic and methanolic extracts	Antibrucella activity
16	Vitexins, nature–derived lignan compounds, induce apoptosis and suppress tumor growth.	Clinical Cancer Research	Yingjun Zhou *et al.,*	2009	seeds	Mixture of vitexin lignan compounds	Anti-tumour activity
17	Possible implication of oxidative stress in anti-filarial effect of certain traditionally used medicinal plants invitro against Brugia malayi microfilariae	Pharmacognosy Research	Sharma RD *et al.,*	2010	Root	extract	Antifilarial activity
18	Inhibition efficiency of a newly isolated flavonoid compound from Vitex negundo L.leaves against cattle- endosymbiont Setarvi cervi : phytomedicine for lymphatic filariasis	Parasite epidemiology and control	Rana G	2018	leaves	4,5-diethyl-3'-ethoxy-pyroflavone	Antifilarial activity

Table 1. to be Continued…

Sl.No.	Title	Journal name	Author name	Publishing year	Part used	Compound isolated	Result
19	Molecular docking analysis of essential oil constituents as elastase inhibitors	Bioinformation Discovery at the interface of physical and biological sciences	Periyaswamy S *et al.,*	2012		Thymol from essential oil	Inhibitory action against pseudomonas elastase
20	Effect of selected local medicinal plants on the asexual blood stage of chloroquine-resistant plasmodium falciparum	BMC Complementary and Alternative Medicine	Mohd Abd Razak MR *et al.,*	2014	leaves	Dichloromethane, methanol and water extract	Antiplasmodial activity
21	In silico investigation of potential mTOR inhibitors from traditional Chinese medicine for treatment of Leigh syndrome	Biomed research international	ChenKC,LeeWY *et al.,*	2014		acerosin	Act as mTOR protein inhibitors

mTOR –mammalian target of rapamycin

The anticancerous activity has been proved and compound isolation has also been done. A compound named VB-1 has been purified from a mixture of neolignan compounds named EVn-50 from the seeds of *vitex* and has been proven to suppress the proliferation of cells and induced apoptosis in colorectal cancer both *in vitro* and *in vivo*, it even induced apoptosis through p53 upregulated modulator[62] and antitumour activity on seven xenograft models such as breast, prostate, liver and cervical cancers has been proved[63]. It also increases the Reactive Oxygen Species (ROS) levels in BRAFi-resistant melanoma cells and causes DNA damage thereby suppressing melanoma cell growth[64]. A compound VB2 isolated from acetoacetate extract of seeds of *Vitex negundo* has been proven to repress the proliferation of choriocarcinoma cells, JEG-3 and instigate apoptosis[65], anti-oxidant and anti-proliferative properties of ethanolic extract of leaves of *Vitex negundo* has been proved on human hepatocarcinoma cells[66]. These isolated compounds have anti-inflammatory properties owing to their protective action and various antioxidants present in the plant inhibit ROS and arrest the proliferation of carcinoma cells.

A compound 4,5-diethyl-3'-ethoxy-pyroflavone has been isolated from the methanolic extract of *V. negundo* leaves and its effect on the parasite *Setaria cervia* has been proved[67,68] highlighting its ant filarial effect. Apart from that the root extract also showed an antifilarial effect associated with oxidative stress[69].

The antitubercular property of *Vitex negundo* has been proved using petroleum ether and chloroform fraction of ethanolic extracts of leaves by nitrate reduction assay and the structure with significant antitubercular potential was identified as ursolic acid[70]. The ethanolic and methanolic extracts of seeds showed anti-brucella activity[71]. The dichloromethane extract, methanol and water extract of leaves of *V. negundo* showed anti-plasmodial activity against *Plasmodium falciparum*[72].

The anti-bacterial, anti-cancerous, anti-tubercular, anti-filarial and anti-plasmodic activity of various extracts of *Vitex negundo* has been proved effectively through the *in vitro* method.

5.3 *In Vivo* Studies

A total of 11 studies were conducted on animals to prove the efficacy of the plant. Most of the studies

Table 2. *In vivo* studies and clinical studies

Sl.No.	Title	Journal	Publishing year	Author	Part Used	Type of Animals	Number	Dose	Result
1	Anti–nephrotoxic activity of some medicinal plants from tribal-rich pockets of Odisha	Pharmacognosy Research	2014	Mishra S *et al.,*	Methanolic extract of the root	Male wistar albino rats	36 rats-divided into 6 groups(6mic each	200 mg/kg once daily	Nephroprotective action
2	Scientific investigation of crude alkaloids from medicinal plants for the management of pain	BMC Complementary and Alternative Medicine	2016	Shoaib M *et al.,*	Alkaloid extract of aerial parts	BALLB/C mice(25-30g)	Each group contain 6 animals	50 mg /kg	Analgesic potential
3	Studies on wound healing potential of polyherbal formulation using invitro and *in vivo* assays	Journal of Ayurveda and Integrative Medicine	2017	Yogesh p Talekar *et al.,*	Aqueous extract of leaves	Wistar rats(150 - 200 g)	18`(3 groups-6 animals each)	Topical application	Wound healing potential with E.officinalis and Tridax procumbens
4	Analysis on the alterations of lens proteins by *Vitex negundo* in selenite cataract models	Molecular vision	2011	Rooban BN *et al.,*	Ehyl acetate extract-leaves	Sprague-Dawley rat pups(10 – 12 g)	3 groups(8 rats in each group)	1.0 mg/kg	Protective effect was proved
5	Antioxidant and antilipid peroxidation potential of supercritical fluid extract and ethanol extract of leaves of *Vitex negundo* L.	Indian Journal of Pharmaceutical Sciences	2011	Nagarsekar KS et al.,	Ethanolic extract and supercritical fluid extract-leaves	Females of Sprague Dawley strain(150 – 180 g)	4 group-6 animals each	500 mg/kg/d	Antilipid peroxidation potential
6	Active compound from the leaves of *V. negundo* L shows anti-inflammatory activity with evidence of inhibition for secretory phospholipase A(2) through molecular docking	Bioinformatics	2011	Vinuchakkaravarthy T *et al.,*	TDTBPP	Wistar albino rats(125 – 150 g)		50 mg/kg and 70 mg/kg	Anti-inflammatory activity

Table 2. to be Continued…

Sl.No.	Title	Journal	Publishing year	Author	Part Used	Type of Animals	Number	Dose	Result
7	Hepatoprotective role of ethanolic extract of *V. negundo* in thioacetamide induced liver fibrosis in Male rats	Evidence based Complementary and alternative medicine:e CAM	2013	Kadir FA *et al.,*	Ethanolic extract-leaves	Sprague Dawley rats (180-200g)	42 (7 groups of 6 rats each)	100 mg/kg, 300 mg/kg	Hepatoprotective effect
8	Antiestrogenic and anti-inflammatory potential of n-hexane fraction of *Vitex negundo* Linn leaf extract: A probable mechanism for blastocyst implantation failure in Mus musculus	International scholarly research notices	2014	Jivrajani M *et al.,*	n-hexane extract -leaves	Swiss albino strain, Mus musculus	5 groups	52 mg/kg body wt	Antiestrogenic potential
9	Effect of oral administration of ethanolic extract of *Vitex negundo* on thioacetamide induced nephrotoxicity in rats	BMC Complementary and Alternative Medicine	2013	Kadir FA *et al.,*	Ethanolic extract –leaves	Sprague dawley rats	24, 4 groups-each contain 6 rats	100 mg/kg and 300 mg/kg	Nephroprotective action
10	*Vitex negundo* inhibits cyclooxygenase -2 inflammatory cytokine-mediated inflammation on carrageenan-induced rat hind paw edema	Pharmacognosy research	2012	Chattopadhyay P *et al.,*	Leaf oil	Male wistar rats(200 – 250 g)	5 groups	200,1000 and 2000microlitre	Anti-inflammatory property
11	Bioactive chromone constituents from *Vitex negundo* alleviate pain and inflammation	Journal of Pain Research	2017	A.khan , S.Naz *et al.,*	methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo-4H-chromen-2-yl) ethyl)benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H- chromen-2-yl) ethyl)benzoic acid	BALB/c mice	6 groups(6 animals)	50 and 100 mg/kg	Analgesic and anti-inflammatory property

Table 2. to be Continued…

Sl.No.	Title	Journal	Publishing year	Author	Part Used	Type of Animals	Number	Dose	Result
12	A clinical study of nirgundi Ghana vati and matra basti in the management of Gridhrasi with special reference to sciatica	Ayu.	2010	Ali M *et al.,*	NGV and NGV+ Matra vasti with nirgundi taila		119 patients		Combination was found effective in gridhrasi
13	Repellent activity of some essential oils against Simulium species in India	Journal of Insect Science	2012	Hazarika S *et al.,*	Leaf essential oil (10% conc.)		6 volunteers		Repellent activity against Simulium species

focussed on the anti-inflammatory potential of the plant and it has been proved by isolating various components. A compound has been isolated from ethyl acetate extract of *Vitex negundo* leaves and its leaf oil showed anti-inflammatory activity on carrageenan-induced paw oedema model of albino rats of Wistar strains. Two chrome derivatives-methyl 3-(2-(5-hydroxy-6-methoxy-4-oxo- 4H-chromen-2-yl)ethyl)benzoate and 3-(1-hydroxy-2-(5-hydroxy-6-methoxy-4-oxo-4H- chromen-2-yl)ethyl)benzoic acid were isolated from ethyl acetate fraction and both given in the doses of 50 mg/kg and 100 mg/kg and has been proved to have analgesic and anti-inflammatory property by conducting carrageenan-induced paw oedema assay and abdominal constriction assay on BALB/C mice of either sex.

Methanolic extracts of the root of *Vitex negundo* (200 mg/kg, once daily) showed nephroprotective action on gentamicin-induced nephrotoxicity in male Wistar albino rats[73]. Crude alkaloid extract of aerial parts (50 mg/kg) showed analgesic potential on BALLB/C mice[74]. The wound healing potential of aqueous extract of leaves was proved when given along with extracts of bark of *E. officinalis* and whole plant of Tridax procumbens on either sex of Wistar rats[75]. The protective effect of ethyl acetate extract from leaves of *Vitex negundo* was proved by regulating selenite-induced cataracts through changes in lens proteins and by preventing the loss of cytoskeletal proteins[76].

The ethanol extract (500 mg/kg/day) and supercritical fluid extract (500 mg/kg/day) of leaves of *Vitex negundo* when administered for 15 days in ethanol-induced oxidative stress model of Sprague Dawley strain showed anti-lipid peroxidation potential, even by TLC and GCMS analysis of extracts revealed the presence of polar compounds like flavonoids in ethanol extract and non-polar compounds like terpenes in supercritical fluid extract[77]. Ethanolic extract of leaves (at two different doses – 100 mg/kg and 300 mg/kg body weight) administered for 12 weeks showed hepatoprotective effect against thioacetamide-induced hepatic injury in Sprague Dawley rats[78,79]. n-hexane extract of leaves of *V. negundo* when administered for day 1-6 of pregnancy in female Swiss albino mice showed failure of blastocyte implantation highlighting its antiestrogenic potential[80].

Table 3. Analytical studies

Sl. No.	Title	Journal	Author	Publishing Year	Part	Compounds	Method
1	Inhibition of melanogenesis versus antioxidant properties of essential oil extracted from leaves of *Vitex negundo* L. and chemical composition analysis of GC-MS	Molecules	Huang HC *et al.,*	2012	Essential oil from leaves	sesquiterpenes, monoterpenes, alcohols, ester, aromatic compounds, ketone and ether	GC-MS
2	Quality assessment of medicinal products and dietary supplements containing *Vitex agnus-castus* by HPLC fingerprint and Quantitative analyses	Chemical and Pharmaceutical Bulletin	Sogame M *et al.,*	2019	extract	casticin to agnuside ratio -38.3, negundoside- helps in quality assessment	HPLC
3	Countercurrent separation assisted identification of two mammalian steroid hormones in *Vitex negundo*	Journal of chromatography.A.	FanQ *et al.,*	2018	Methanol extract leaves	Estriol and progesterone	CCS
4	Data on the inhibitory effect of traditional plants from Sri Lanka against tyrosinase and collagenase	Data in brief	Ito J *et al.,*	2018	Leaf extract	Inhibits tyrosinase and collagenase	Tyrosinase inhibition assay, Collagenase inhibition assay
5	Tris(2,4-di-tert-butyl-phen-yl)phosphate	Acta Crystallographica. Section E, Structure reports online	Vinuchakkaravarthy T *et al.,*	2010	Ethyl acetate extract -leaves	Tris(2,4-di-tert-butyl-phen-yl) phosphate	Silica gel chromatography
6	HPTLC analysis, Antioxidant and Antigout Activity of Indian plants	Iranian Journal of Pharmaceutical Research: IJPR	Nile SH *et al.,*	2014	Roots	High levels of phenolics	HPTLC
7	Validated HPLC method for identification and quantification of p-hydroxy benzoic acid and agnuside in *Vitex negundo* and *Vitex trifolia*	Journal of Pharmaceutical Sciences	Shah S *et al.,*	2013	Methanol extract of leaves, barks	p-hydroxy benzoic acid and agnuside	HPLC

Table 3. to be Continued…

Sl. No.	Title	Journal	Author	Publishing Year	Part	Compounds	Method
8	Comparative assessment of extraction methods and quantitative estimation of luteolin in the elaves of *V. negund* L. by HPLC	Asian Pacific Journal of Tropical Medicine	Abidin L *et al.,*	2014	Methanol extracts of leaves	luteolin	Reflux technique
9	Development and characterization of EST-SSR markers for Vitex negundo var.heterophylla (Lamiaceae)	Applications in plant sciences	Liu L *et al.,*	2019	leaves	Genomic DNA, 12,075 simple sequence repeat markers	Cetyl trimethyl ammonium bromide method
10	Larvicidal activity and GC-MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*	Journal of vector-borne diseases	Gautam K *et al.,*	2013	Flavonoid extract of flower buds	phenols,naphthalene,2,3-dihydrobenzofuran,5-hydroxy-3,6,7,3′,4′-pentamethoxy flavones, phenol-2,4-Bis flavones	GC-MS
11	Comparative evaluation of polyphenol contents and antioxidant activities between Ethanol extracts of *Vitex negundo* and *Vitex trifolia L.* leaves by Different methods	Plants	Sarla S *et al.,*	2017	Ethanolic extract leaves	High phenolics and flavanoids	Spectrophotometric method and aluminium chloride assay

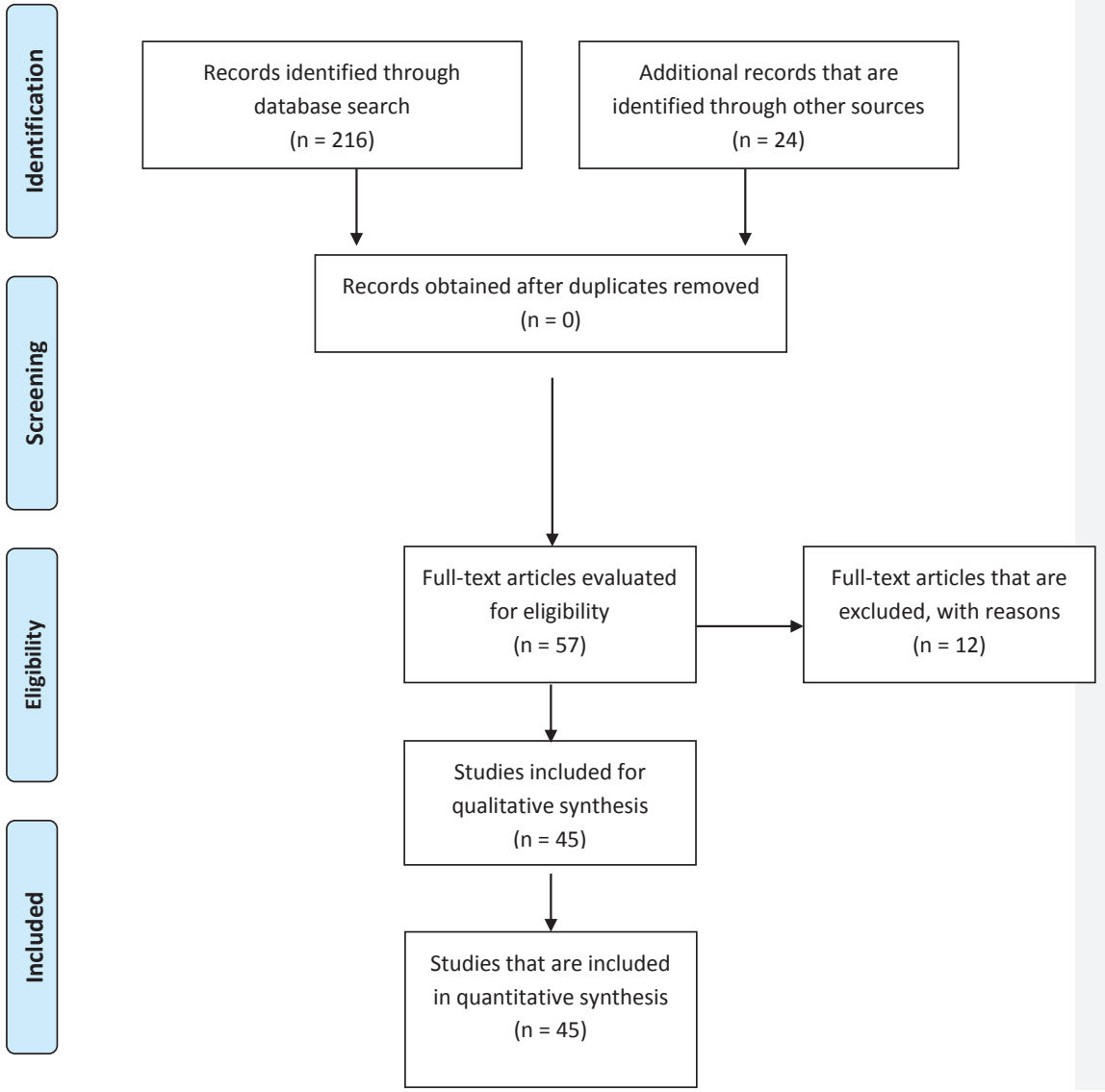

Figure 1. Schematic representation of search strategy.

5.4 Clinical Studies

Nirgundi Ghana vati (dried water extract) two tablets (each tablet – 500 mg) thrice daily and administration of 60 ml of *nirgundi taila* as *basti* was found effective in the management of *gridhrasi*[81]. Gridhrasi is mainly *vata kapha* predominant. The *ushna veerya* (hot potency) of the drug helps in controlling the *vata dosha, tikta rasa* (bitter taste) and *ruksha guna* (dryness) help in reducing *kapha dosha* and thereby reducing swelling. Essential oil extracted from leaves of *Vitex negundo* at 10% concentration showed repellent activity against Simulium species for 5 hours[82].

The essential oil of *Vitex negundo* was found efficacious in inhibiting COX-2 pathway and thereby reducing inflammation, pain and arthritis.

5.5 Analytical Studies

Identification and quantification using different types of analytical procedures have been reported.

In total, 11 studies have been conducted to assess the plants' quality as well as to isolate various compounds from it. The proper identification of *Vitex negundo* can be done by considering the casticin to agnuside ratio of -38.3 and considering the marker

compound negundoside[83]. From the methanol extract of leaves, estriol and progesterone was identified through high-speed counter current chromatography and agnuside and p-hydroxy benzoic acid through the HPLC method. Even the methanol extract of bark showed the presence of p-hydroxy benzoic acid[84,85]. *V. negundo* extract and essential oil from leaves showed an inhibitory effect on tyrosinase and collagenase, components of oil also decrease the amount of melanin production, components are identified as sesquiterpenes, monoterpenes, alcohols, ester, aromatic compounds, ketone and ether through GCMS[86]. A compound Tris(2,4-di-tert-butyl-phen-yl) phosphate was isolated from ethyl acetate extract of leaves by silica gel chromatography.

Using HPTLC analysis with ethylacetate: methanol: formic acid: water as mobile phase the roots of *Vitex negundo* and even ethanolic extract of leaves by spectrophotometric method showed the presence of high levels of phenolics. Through various extraction techniques as well as using different solvents it was estimated that the luteolin content in leaves can be attained to its maximum by reflux technique and using methanol as solvent. From the leaves genomic DNA was extracted using the cetyltrimethylammonium bromide method and 12,075 simple sequence repeat markers were detected[87]. The flavonoid extract of flower buds showed the presence of phenols, naphthalene, 2,3-dihydrobenzofuran, 5-hydroxy-3,6,7,3',4'-pentamethoxy flavones, phenol-2,4-Bis flavones through GCMS analysis[88].

Based on the polarity of the solvent selected various compounds can be isolated which was proven to be effective both externally and internally.

6. Limitations of Review

It is only a descriptive review without reporting standards, hence no quality assessment. The quantitative details were not assessed. There are various unpublished quality research in *Ayurveda*, and only PubMed database has been included.

7. Conclusion

Phytochemical research on *Vitex negundo* gives new insights about the drug. Still, it has to develop more to explore the pharmacological uses of the drug. *Vitex negundo* can be even utilized as a potential antidote for the most alarming disease even carcinoma. The further scope of the research lies in identifying the marker compounds and thereby standardising the formulations. Clinical pharmacological research is the need of the time for enabling and ratifying a specific action. With so much accessibility, *Vitex negundo* can truly be a propitious drug of choice for both the preventive and curative aspects of diseases.

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