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Vitex negundo: A comprehensive review of phytoconstituents, their derivatives, and therapeutic applications

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Abstract

Currently, there is an increasing interest in herbs all over the world. It has led to an increase in the demand for medicines the production of these medicines has been commercialized to meet the increasing demand. The use of medicinal plants for the treatment of various ailments and as the basis of modern medicine is gaining increasing attention. *Vitex negundo*, a medicinal plant with a history in traditional medicine, has attracted the interest of many researchers. *V. negundo* is found Malaysia, India, China and Sri Lanka. Nirgundi, also known as Vitex Fragrance Leaf, is a plant of the Verbenaceae family. Historically, it has been used to treat a variety of conditions including depression, malaria, sexually transmitted diseases, asthma, wounds, skin diseases, painkillers, anti-inflammatory agents, ulcers and snake bites. This study investigated the plant and the active ingredients of *V. negundo*. Natural and synthetic derivatives of nagundo have concentrated biological activity.

Keywords: Antioxidant, derivative, pharmacological activity, phytoconstituent, *Vitex negundo*

1. Introduction

Vitex negundo L. (Verbenaceae) [3] is a tall, aromatic shrub or occasionally a delicate little tree, ranging from 30-45 cm to as high as 4.5 meters, widely found across numerous regions of India. This plant offers a multitude of advantages including: cancer suppressant, anti-inflammatory, antiseptic, fever reducer, diuretic, antihistamine, antioxidant, antibacterial, central nervous system relaxant, antifungal, venom antidote, mosquito deterrent, insect repellent, larvicide, pain reliever, antiandrogen, liver protector, reproductive regulator, skin rejuvenator, and anti-dopaminergic effects [4].

The Sanskrit term for *V. negundo*, nirgundi, can be interpreted as 'the protector of the body against ailments.' It is an herb that is cited in all Ayurvedic Samhitas. Most of the plant's components possess medicinal properties and are employed for healing purposes in different traditional medical practices like Ayurveda, Chinese medicine, Siddha, and Unani [5]. Phytochemical compounds found in medicinal plants are seen as essential for uncovering effective treatments for different diseases in ayurvedic and nutraceutical studies. The medicinal advantages of a plant are dictated by the presence of phytochemicals, dietary components, and trace elements. The efficacy of these healing plants in addressing various health conditions is associated with their therapeutic strength, which is influenced by their elemental concentrations. Similarly, it is important to conduct phytochemical screening, elemental and functional group analysis of plant extract in order to assess the medicinal properties of *Vitex negundo* [6].

Investigators have identified a range of compounds in *V. negundo* via phytochemical investigations, such as aromatic oils, lignans, flavonoids, iridoids, terpenes (triterpenes, diterpenes, sesquiterpenes), and steroids. [7]

The main active ingredient has been recognized, and its molecular configuration has been elucidated through a range of spectroscopic techniques including mass spectrometry, UV, IR, ¹H NMR, ¹³C NMR, as well as two-dimensional NMR methods like correlation spectroscopy and heteronuclear multiple bond correlation approaches [8].

48 compounds were identified, with 19 compounds making up 88.65% of the oil. Epiglobulol was discovered to make up 30.31% of the total concentration. Several other compounds identified in significant concentrations included delta-iraleine (10.34%), terpinen-4-ol (9.42%), gamma-elemene (5.72%), manool (5.16%), beta-iso-methyl ion (4.46%), beyerene (3.79%), and phytol (2.33%) [9].

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2. Taxonomic classification ^[10]

Table 1: Taxonomic classification of *Vitex negundo*

Kingdom	Plantae
Subkingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class Sub	Magnoliopsida
Class	Asteridae
Order	Lamiales
Family	Verbenaceae
Genus	<i>Vitex</i>
Species	<i>Negundo</i>



Fig 1: *Vitex negundo* L. ^[11]

3. Phytochemical constituents

3.1 Leaves

Aucubinaginside, Nishindine, Hydrocotylene, Glyoflavonoids, Orientin, Isoorientin, and 5-Hydroxy, 3, 6, 7, 31, 4 1 pentamethoxy flavone are present in the aqueous extract of *Vitex negundo* leaves ^[12]. 5-Hydroxy-7, 4'-dimethoxy flavone, 5,7-Dihydroxy-6,4'-dimethoxy flavone, Luteolin, 4 Luteolin-7-O-β-D-glucoside, 7, 8-Dimethyl herbacetin-3-rhamnoside, Vitegnoside, Iso-orientin, 5, 3'-Hydroxy-6, 7, 4'-trimethoxy flavone, Acerosin-5-O-glucoside, Corymbosin, 5-Hydroxy-6, 7, 8, 3', 4'-pentamethoxyflavone, 5, 6, 7, 8, 3', 4', 5'-Heptamethoxy flavone, 5-Hydroxy-6, 7, 8, 4'-tetramethoxy flavone, 5-Hydroxy-3,6,7,3',4'-pentamethoxy flavone, Vitexicarpin, 3,4,5,7,3',4',5'-Hexahydroxy-6,8-dimethoxyflavone, 4,5-Dihydroxy-3',4'-dimethoxyflavone 6-O-rhamnoglucoside, Betulinic acid, Ursolic acid, 3β-Acetoxyolean-12-en-27-oic acid, 2α,3α-Dihydroxyoleana-5, 12-dien-28-oic acid, 2β,3α-Diacetoxyoleana-5, 12-dien-28-oic acid, 2α,3β-Diacetoxy-18-hydroxyoleana-5, 12-dien-28-oic acid, β-Amyrin, 3-Acetyloxy-11-en-28-oic acid propyl ester, 3-Acetyloxy-11-oxo-olean-12-en-28- oic acid butyl ester, Oleanolic acid, 2α,3α, 23-Trihydroxyolean12-en-28-oic acid methyl ester, 2α, 3α-Dihydroxyolean-12-en-28- oic-acid, 2α,3α,23-Trihydroxyolean-12-en28-oic acid, Agnuside, Negundoside, 1,2-Di-substituted idopyranose, 1,4α,5,7α-

Tetrahydro-1-β-D-glucosyl7-(3',4'-dihydroxybenzoyloxymethyl)- 5-ketocyclopenta[c]pyran-4-carboxylic acid, Nishindaside, 6'-p-Hydroxybenzoylmussaenosidic acid, β-Sitosterone, β-Sitosterone acetate, Stigmasterone, β-Sitosterol, 22,23-Dihydro-α-spinasterol-β-Dglucoside ^[13, 14].

3.2 Seeds

Vitex negundo Linn seeds contain various chemical components such as n-Tritriacontane, n-hentriacontanol, nhentricontane, npentatricontane, n-nonacosane, β-sitosterol, phydroxybenzoic acid, 5-oxyisophthalic acid, 3, 4-dihydroxybenzoic acid, artemetin, 3β-acetoxyolean-12-en-27-oic acid, 5β-hydro8,11,13-abietatrien- 6α-ol, 2α,3α-dihydroxyoleana-5,12-dien28-oicacid, 2β,3α-diacetoxyoleana-5,12-dien-28-oicacid, 2α,3β- diacetoxy-18-hydroxyoleana-5,12-dien-28-oic acid, vitedoin A, vitedoamine A, vitedoin B, 5,7,3'- trihydroxy 6,8,4'-trimethoxy, and 6-hydroxy-4-(4-hydroxy-3- methoxyphenyl)-3-hydroxymethyl-7-methoxy-3, 4- dihydro-2- naphthaldehyde ^[15, 16].

3.3 Roots

Vitexoside, agnuside, R-dalbergiphenol, negundin A, negundin B, 6-hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaledehyde, vitrofolal E, (+)-lyoniresinol, (+)-lyoniresinol-3α-O-β-dglucoside, (+)-(-)-pinoresinol, and (+) diasyringaresinol, 2β,3α-diacetoxyoleana-5,12-dien-28-oic acid; 2α,3αdihydroxyoleana-5,12-dien-28-oic acid, 2α,3β - diacetoxy-18- hydroxyoleana-5,12-dien-28-oic acid, vitexin and isovitexin, acetyl oleanolic acid, sitosterol, 3-formyl-4.5-dimethyl-8- oxo-5H-6,7-dihydronaphtho (2,3-b) furan ^[15].

3.4 Stem and bark

The stem and bark contain a variety of chemical components including Pentamethoxy-5 Oglucopyranosyl rhamnoside, vitexin cafeate, 4'-O-methyl myricetin-3-O-[4'O-β-D-galactosyl]-β-D-galactopyranoside, β-amyrin, epifriedelinol, oleanolic acid, Hepta methyl-phenylcyclotetra siloxane, Cyclo heptasiloxane, tetra decamethylNona methyl, phenyl-cyclopenta siloxane, Cyclo octa siloxane, hexadeca methyl, Borazine, 2,4,6-tripheny-11, 3, 5-tryophl, Nonamethyl, phenyl-cyclopenta siloxane, Tetracosamethylcyclododeca siloxane, penta methyl phenylDisilane, Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,- tetradeca methyl, 3a,3a'- Dichloro-2α,3α - ethano-3β-methylcholestan-2a-one, Octadecamethyl, cyclonona siloxanes, Cyclo octa siloxane, hexadeca methyl, p-hydroxy benzoic acid, β-sitosterol, 5-hydroxy-3,6,7,3'4'-pentamethoxy flavone, 5-hydroxy-3'dihydroxy-7,8,4'-trimethoxy flavanone, 3βacetoxy-olean-12-en-27-oic acid, 3β-hydroxy-olean-5, 12-dien-28-oic acid ^[12].

3.5 Flowers and dried fruits

δ-guaiene; guaia-3,7-dienecaryophyllene epoxide; ethylhexadecenoate; α-selinene; germacren-4-ol; caryophyllene epoxide; (E)-nerolidol; β-selinene; α-cedrene; germacrene D; hexadecanoic acid; p-cymene; and valencene ^[17].

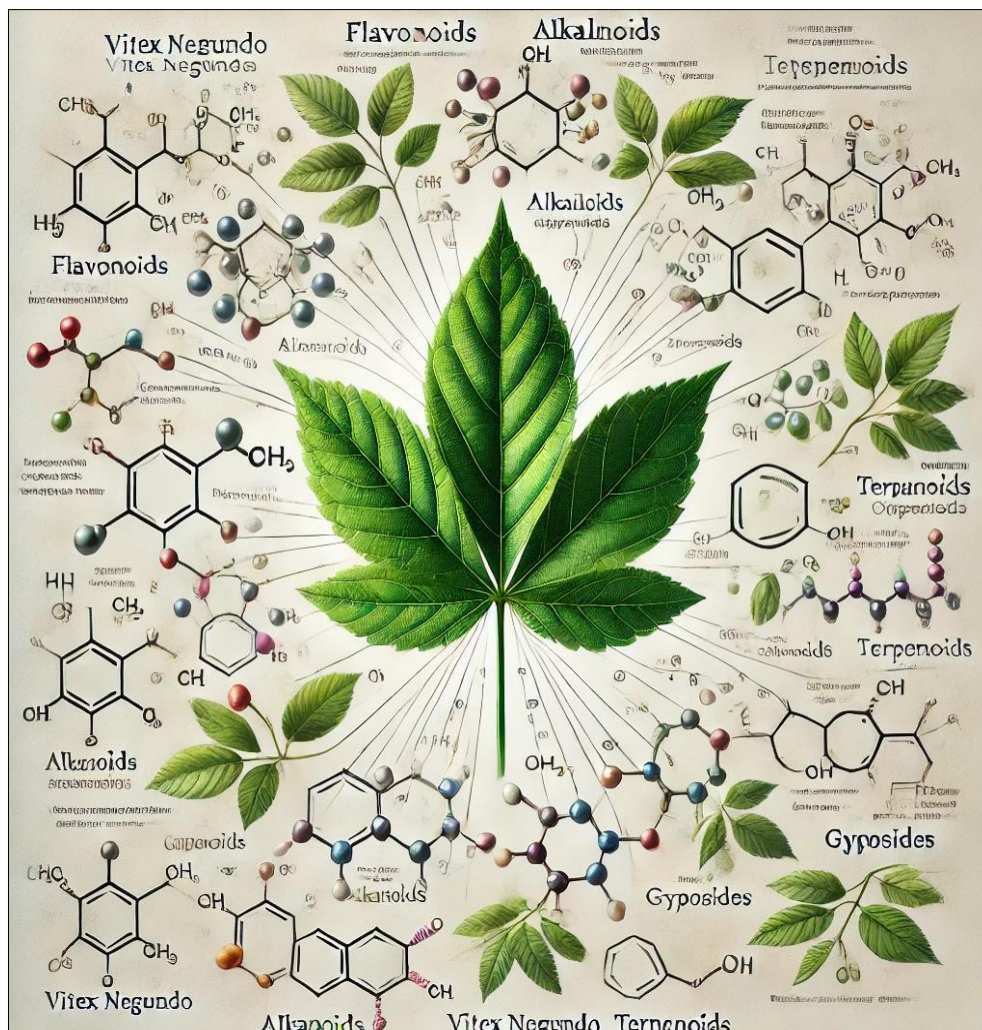


Fig 2: Phytochemical constituents of *Vitex negundo* leaves

4. Phytoconstituent and their derivative

4.1 Nirgundoside

Nirgundoside, found in the leaves of *Vitex nirgundo* Linn, is the active ingredient. (Family Verbenaceae) is utilized in folk medicine for its effects in reducing inflammation and

preventing seizures. Nirgundoside, also known as 2'-phydroxybenzoylmussaenosidic acid, is a type of iridoids O-glycoside^[18]. Different lipid-modified esters of Negundoside were identified through NMR and mass analysis^[19].

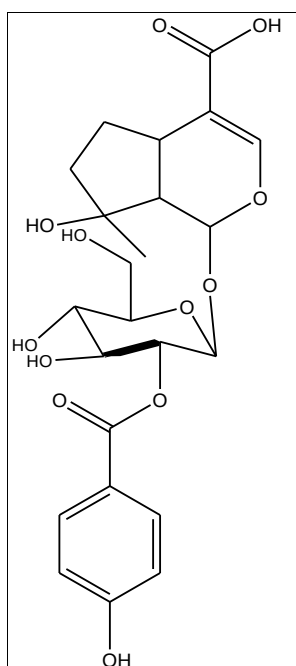
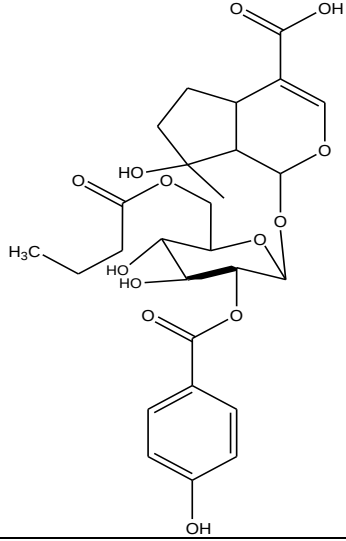
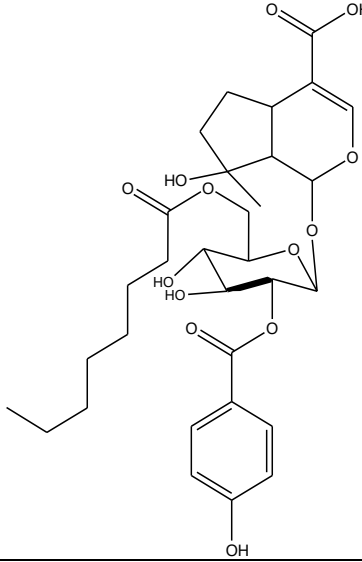
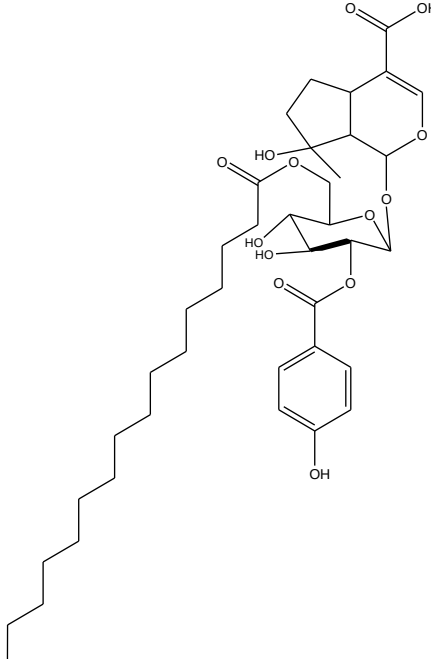


Fig 3: Structure of nirgundoside

Table 2: Derivative of negundoside

Name of compound	Structure
6'-O-Butanoyl-Negundoside	
6'-O-Octanoyl-Negundoside	
6'-O-Palmitoyl-Negundoside	

4.2 Agnuside

Iridoids consist of a cyclopentane ring, secondary monoterpenoids are being produced, metabolic compounds in

plants and possess numerous therapeutic effects. Various types of Vitex plants working in conjunction with other chemical components demonstrates the existence of various

iridoids such as agnuside, negundoside, nishindaside, and aucubin are substances that in charge of the various activities related to drugs or medications. In the midst of these in the family of iridoids, agnuside plays a significant role. a chemotaxonomic indicator suitable for applying in standardizing Vitex extract and making it uniform. Formulations that include it. Agnuside is additionally discovered compound, Buddhlejoside, from Vitex plant. Different types of Rhinanthus plants. It is a compound derived from aucubin. and p-hydroxybenzoic acid. It exposes pharmacological effects such as anti-inflammatory for arthritis. anti-inflammatory properties, before the menstrual cycle condition and so on ^[20]. Due to the widespread use of crude extracts of *V. negundo* in traditional Chinese, Ayurvedic, and Unani medicine for many years, we focused on modifying Agnuside by adding a suitable lipophilic component to the hydroxyl groups on the aglycan part of the molecule. This study aims to explore the impact of these modifications on the adjuvanticity of the new analogues.

Different lipid-modified forms of Agnuside were identified through NMR and mass study ^[19].

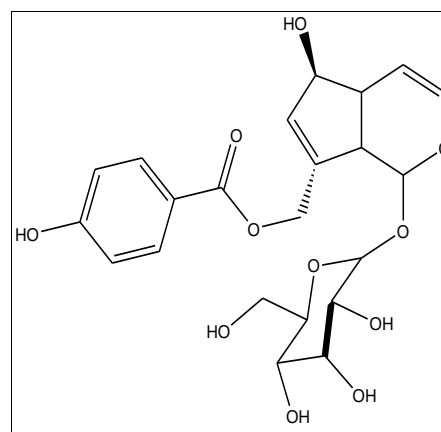


Fig 4: Structure of agnuside

Table 3: Derivative of agnuside

Name of compound	Structure
6-O-Butanoyl-Agnuside	
6-O-Octanoyl-Agnuside	
6-O-Palmitoyl-Agnuside	

4.3 Vitexin

Vitexin is found in the leaf extract of *Vitex negundo*, where it occurs naturally and is also synthesized. This flavonoid has strong antitumor effects in preclinical models of breast,

prostate, liver, and cervical cancer. Vitexin can be effectively extracted and identified from *V. negundo* with low % RSDs, indicating it is a suitable marker for the plant's leaf extract.^[4]

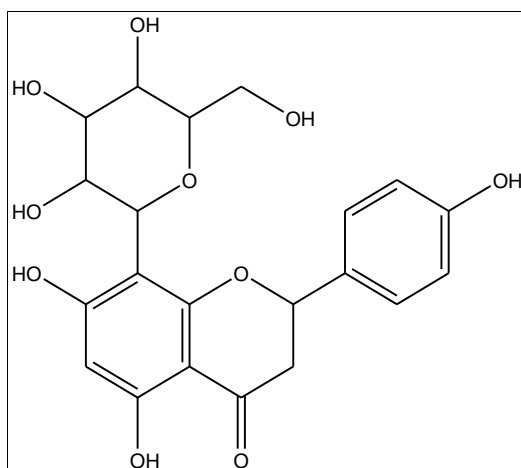


Fig 5: Vitexin

Table 4: Derivative of vitexin

Name	Structure
Vitexin2-o-rhamnoside	
Vitexin "4 -o-rhamnoside	
isovitexin	

Vitexin-2-o-xyloside	
3-o- acetvitexin	
Vitexin 2''-o-P-cumarate	
2''-o-galloylvitexin	
Vitexin-2''-glucoside	
Vitexin-4''-o-glucoside	

Isovitexin-7-o-glucoside	
Isovitexin-6''-o-glucoside	

4.4 Vitexicarpin

Fractionation of the chloroform-soluble extract from *Vitex negundo* leaves was guided by bioassays, resulting in the discovery of the flavone vitexicarpin, which demonstrated wide cytotoxicity in various human cancer cell lines. In order to enhance the cytotoxic effectiveness of Vitexincarpin, various acylation reactions were conducted on the compound resulting in its methylated form (3,5,6,7,3',4'-Hexamethoxyflavone), acetylated form (5,3'-Diacetoxy-3,6,7,4'-tetramethoxyflavone), and six additional acylated derivatives [21].

Vitexincarpin, a compound found in the leaves of the *Vitex negundo* plant, is utilized for its various medicinal properties such as antibacterial, antitumor, astringent, acne-fighting, eczema-relieving, antipyretic, sedative, liver disorder treating,

vermifuge, anti-parasitical, insect repellent, anti-inflammatory, catarrhal fever-relieving, cough remedy, anti-ulcer, skin disease treatment, and hair growth promoting effects [22].

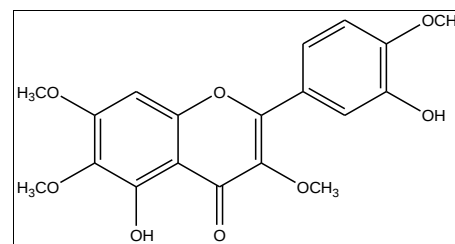
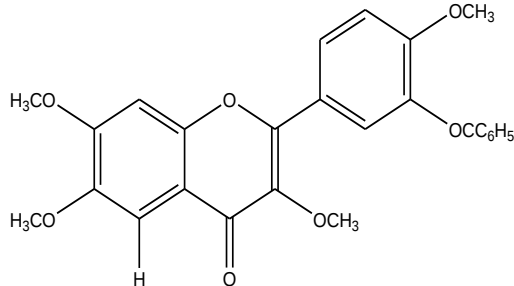
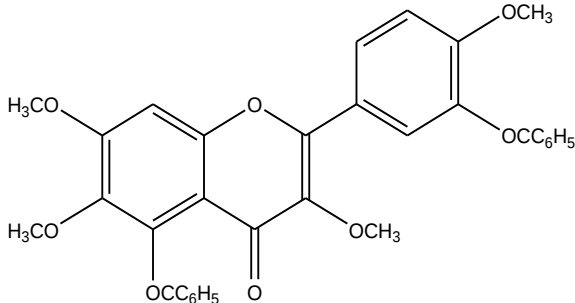
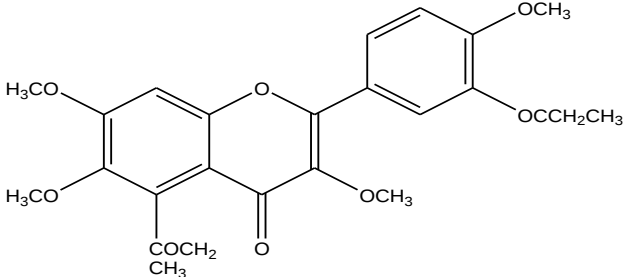
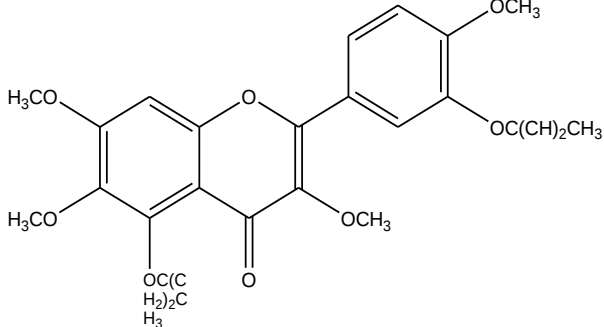
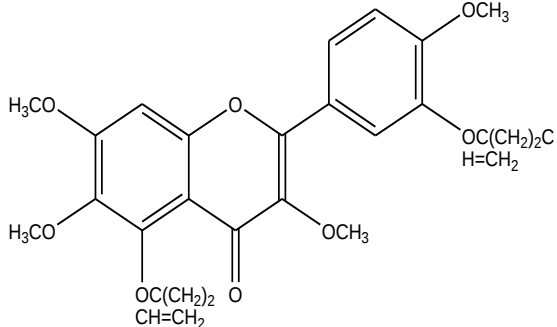
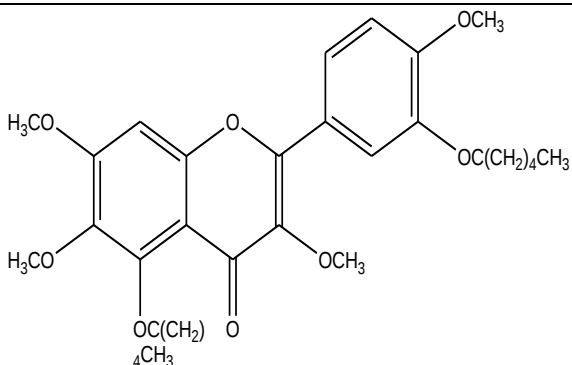


Fig 6: Structure of vitexicarpin

Table 5: Derivative of vitexicarpin

Name of compound	Structure
3,5,6,7,3',4'-Hexamethoxyflavone	
5,3'-Diacetoxy-3,6,7,4'-tetramethoxyflavone	

3'-Benzoyloxy-5-hydroxy-3,6,7,4'-tetramethoxyflavone	
5,3'-Dibenzoyloxy-3,6,7,4'-tetramethoxyflavone	
5,3'-Dipropanoyloxy-3,6,7,4'-tetramethoxyflavone	
5,3'-Dibutanoyloxy-3,6,7,4'-tetramethoxyflavone	
5,3'-Dipent-4-enoyloxy-3,6,7,4'-tetramethoxyflavone	
5,3'-Dihexanoyloxy-3,6,7,4'-tetramethoxyflavone	

4.5 Luteolin:(3', 4', 5, 7-tetrahydroxyflavone)

Luteolin is the flavonoid that can be found in the leaves of the Nirgundi tree. There is a high demand for luteolin, leading to its supplementation as nutraceuticals. Luteolin possesses antioxidant and anti-inflammatory properties that aid in combating inflammation in different important organs and tissues. According to literature, Luteolin is also involved in multiple functions like fighting ulcers, microbes, and cancer, as well as treating fever, diarrhea, wounds, and acting as an insecticide and repellent^[23].

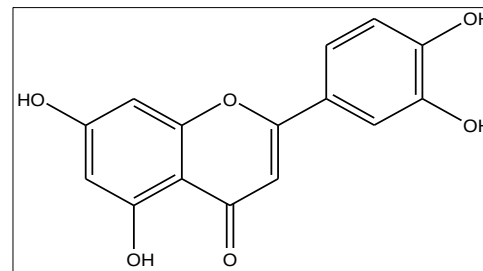


Fig 7: Structure of luteolin

Table 6: Derivative of luteolin

Name of compound	Structure
Chloro- luteolin derivative	
Leuteolin-7-o-glucoside	
Luteolin-8-c-β-glucopyranoside	
Luteolin-7-o-β rutinocide	

4.6 Stigmasterol

Various therapeutic plants contain stigmasterol, an unsaturated plant sterol also known as Stigmasterin or Wulzen anti-stiffness factor. Numerous synthetic and semi-synthetic chemicals for the pharmaceutical sector are made using stigmasterol in a variety of chemical procedures. The potential health benefits of stigmasterol, such as its antiosteoarthritic, antihypercholesterolemic, cytotoxic, antitumor, hypoglycemic, antimutagenic, antioxidant, anti-inflammatory, and central nervous system properties, have been investigated^[24].

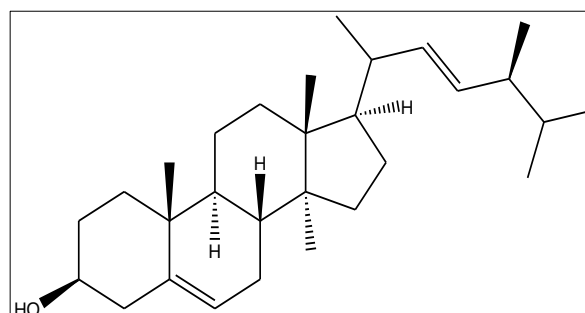
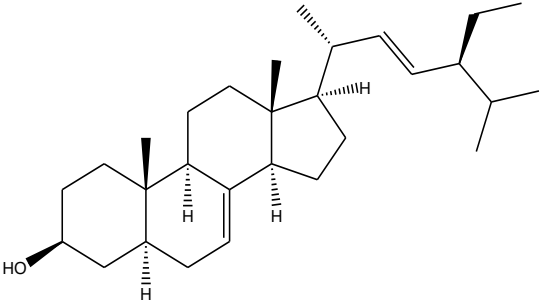
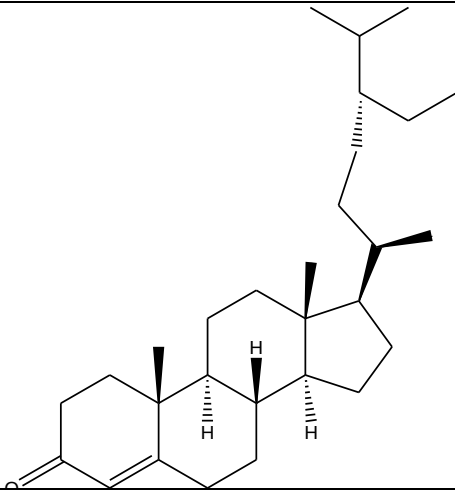
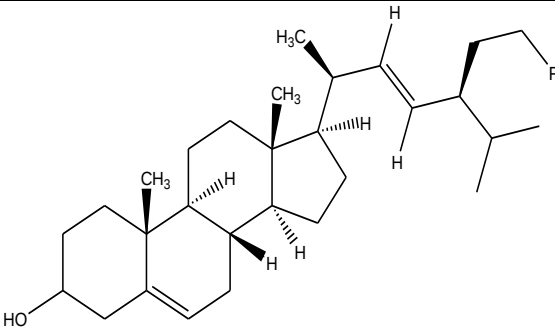
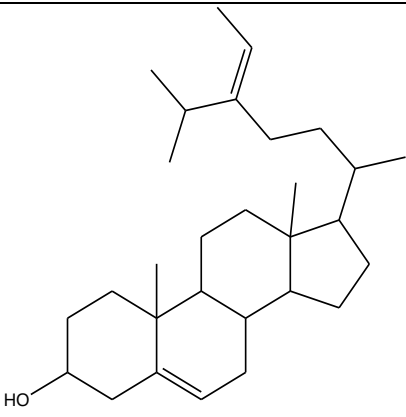
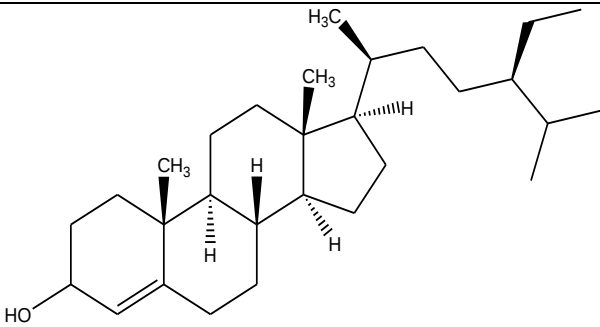
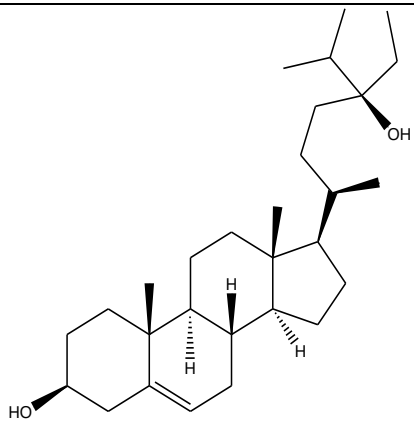
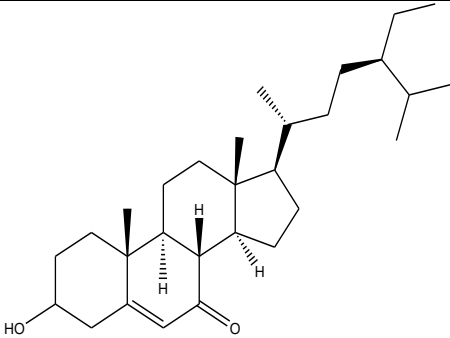
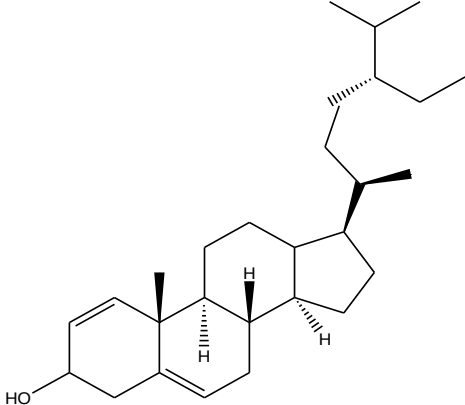


Fig 8: Structure of stigmasterol

Table 7: Derivatives of stigmasterol

Name of compound	Structure
Spinsterol	
Stigmastan-4EN-3one	
29-Fluoro stigmasterol	
Fucosterol	
12-Hydroxystigmast-4en-3-one	

3- Hydroxystigmast-5en-7-one	
Fucosterol epoxide	
Cyasterone	
Stigmasterol glycoside	
6-chloro stigmasterol	

Stigmast-5-EN-3 β , 24-diol	
3-hydroxystigmast-5-en-7-one	
24R) stigmast-1, 5-dien-3 β -ol	

5. Pharmacological activity



Fig 9: Pharmacological activity

5.1 Anti-oxidant activity^[25]

Oxidation is integral to the aging process in humans and the emergence of various ailments. Research has shown that VN seeds exhibit a remarkable capacity to counteract lipid peroxidation due to their potent antioxidant properties. Isofraxidin and 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde are identified as compounds that contribute to the antioxidant prowess of VN seeds. The methanol extract from VN leaves also demonstrated significant free radical scavenging abilities in the 1, 1-diphenyl-2-picrylhydrazyl assay and notably diminished the production of thiobarbituric acid reactive substances, underscoring exceptional antioxidant efficacy against lipid peroxidation. Vitedoin A, vitedoamine A, 6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde, vitrofolalE, vitrofolalF, and detetrahydroconidendrin exhibited enhanced antioxidant activity when compared to alpha-tocopherol in the ferric thiocyanate method, and revealed a superior ability to scavenge the stable free radical, 1,1-diphenyl-2-picrylhydrazyl, over L-cysteine. Negundin B has shown impressive ability to inhibit lipoxygenase enzyme activity, potentially impacting diseases like arthritis, cancer, and inflammation. suggested that the antioxidant characteristics of VN essential oil can help in preventing melanogenesis, leading to the possibility of its use in skincare products^[13].

DPPH radical scavenging assays

The fact that the spare electron delocalizes over the entire molecule, preventing the molecule from dimerizing as most other free radicals do, makes the molecule 1, 1-diphenyl-2-picrylhydrazyl (DPPH) a stable free radical. The reduced form, which loses its violet hue, is created when a DPPH solution is combined with one of a substrate (AH) that can donate a hydrogen atom^[26]. The percentage of inhibition was then determined using the specified formula:
Calculate scavenging as:

$$\% \text{ Scavenging} = A-B / (A) \times 100$$

Vitex negundo leaf methanolic extract was assessed for its antioxidant activity by contrasting its DPPH free radical scavenging capacity with that of ascorbic acid, and the results of comparing the DPPH radical scavenging capacity of the plant extract and the standard were determined as half maximum Inhibitory Concentration (IC₅₀) values^[27].

5.2 Bronchodilator activity

Extracts from the leaves of *V. negundo* have shown to be effective in treating asthma, cough, and bronchitis, based on their traditional medicinal use for hyperactive respiratory disorders. We employed direct methods and evaluated the *V. negundo* extract for potential bronchodilator effects in normotensive anesthetized rats.

To explore the bronchodilatory impact of *V. negundo*, a comprehensive analysis was conducted on the tracheal smooth muscles. Guinea pig tracheal strips were first contracted using CCh (1 mM) and high K⁺ before experimentation. The rough extract was subsequently introduced incrementally to the stimulated contractions and was discovered to be more effective against CCh compared to high K⁺ precontractions, resembling papaverine, which inhibits both phosphodiesterase (PDE) and Ca⁺⁺ channels. Diltiazem showed greater effectiveness in inhibiting high K⁺ induced contractions compared to CCh, unlike the raw extract

and papaverine, indicating typical Ca⁺⁺ channel blocker properties. This discovery indicates that the raw extract of *V. negundo* may contain substances that inhibit PDE similar to papaverine. Flavonoids and tannins in the *V. negundo* extract could be the cause of its PDE and calcium channel blocking effects^[28].

5.3 Anti-inflammatory Activity

The leaves of *Vitex negundo* possess anti-inflammatory activity. Experimental investigations revealed that the mature fresh leaf of *Vitex negundo* have dose-dependent activity against inflammation as revealed in the carrageenan and formaldehyde models. Mature fresh leaf extract of *Vitex negundo* also demonstrated a dose-dependent Prostaglandin (PG) synthesis inhibition, membrane stabilising and antihistamine activities^[29].

Dried leaves powder of *V. negundo* showed anti-inflammatory in rats

The dehydrated leaves of *V. negundo* were finely crushed and soaked in a cold aqueous methanol solution (50:50) overnight. After that, the substance was extracted multiple times with hot methanol solution. The Vapor-Liquid Equilibrium (VLE) was utilized for the investigation. *In vivo* experiments involved giving VLE orally after mixing it in a 0.3% carboxymethyl cellulose suspension (1 g VLE/5 ml). Flavonoid compounds such as 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone and 3,5-dihydroxy-3',4',6,7-tetramethoxy flavonol were found in the leaves of *V. negundo*. 1.30 % of flavonoid compounds were found in the *V. negundo* leaf on a crude basis. Flavonoids have been identified as inhibitors of the enzyme prostaglandin synthetase, particularly the endoperoxidase17, and are known to exhibit a notable anti-inflammatory impact^[21].

5.4 Antimicrobial Activity^[30]

Medicinal plants can produce numerous bioactive secondary metabolites such as alkaloids, terpenoids, glycosides, saponins, flavonoids, steroids, tannins, quinones, and coumarins. Plant-derived antimicrobial substances are derived from these biomolecules. screening hydro alcoholic extracts of *Vitex negundo* Linn leaves to identify potential antimicrobial agents from new sources by extracting active molecules^[31].

Nirgundi was extracted using ethanol. Solutions of 10mg/ml, 20mg/ml, and 30mg/ml of extracts were evaluated for their inhibitory effects using the Agar well diffusion technique and compared to Ciprofloxacin at equivalent concentrations. The ethanolic extract of *Vitex negundo* demonstrated notable antimicrobial effectiveness against *Pseudomonas aeruginosa* bacterial populations. *Vitex negundo* shows strong antibacterial effects against specific hospital pathogens, with its greatest impact seen on *Pseudomonas aeruginosa*, producing a 20mm zone of inhibition with ethanol extract compared to Ciprofloxacin^[5].

Lignans (agnucastaside A, B, C and aucubin, agnuside, mussaenosidic) with Tyrosinase inhibitory, Antimicrobial, and Cytotoxic activities are extracted from the flowering stems using methanol^[32].

5.5 Antifungal activity

The antifungal capabilities of aqueous, ethanol, methanol, and chloroform extracts derived from dried *V. negundo* leaf powder were evaluated against clinical isolates of *Candida albicans* and *Candida tropicalis*. Significant obstacles faced in antifungal treatment include a restricted variety of potent

antifungal medications, toxicity, resistance, and expense. The ethanol extract displayed a robust inhibitory effect on *C. albicans*, while no inhibitory effect was noted against *C. tropicalis* fungal isolates. The methanol extract showed strong suppression of *C. albicans* but only slight inhibition of *C. tropicalis* fungal isolates. The chloroform extract completely inhibited both tested fungal isolates of *C. albicans* and *C. tropicalis* [33].

The antifungal properties of *Vitex negundo* leaf extract were evaluated against *Alternaria alternata*, *Cucurbitaria lunata*, and *Sclerotinia sclerotiorum*, which are known to cause significant damage to various valuable crop. The findings indicated that the higher the concentration of the extract, the greater the antifungal activity [34].

5.6 Antifeedant activity

Antifeedants derived from plants offer the extra benefit of rapid biodegradability and are safe for non-target organisms. Therefore, insect antifeedants have caught the attention of many people [35].

The antifeedant activity of *Vitex negundo*'s active constituents like betulinic acid, ursolic acid, n-hentriacontanol, β -sitosterol, and p-hydroxybenzoic acid was tested against castor semilooper larvae in a laboratory experiment with no other food choices available. Ursolic acid exhibited greater antifeedant activity compared to betulinic acid. Nevertheless, both of these substances have displayed a slight antibacterial effect [36].

5.7 Effect on reproductive potential

The seeds of *Vitex negundo*, packed with flavonoids, were found to disrupt the final phases of sperm production in dogs and also hindered male fertility in rats. The plant is believed to possess anti-androgenic properties. It is important to point out that these results contradict the traditional belief in the aphrodisiac effects of *Vitex negundo* as stated by Khare (2004). The ethanolic extracts of *Vitex negundo* exhibited estrogenic properties and could potentially be utilized in hormone replacement therapy [37].

5.8 Anti-HIV activity

The ethanolic extract derived from the leaves of *Vitex negundo* L. exhibited the greatest activity of 92.8% against HIV-1 reverse transcriptase at a concentration of 200 μ g/ml. The inhibition of HIV-1 reverse transcriptase by the leaf extract of *Vitex negundo* L. was analyzed, alongside the measurement of flavonoid concentrations in relation to its anti-HIV efficacy. The potency of the ethanolic leaf extract from *Vitex negundo* Linn against HIV-1 reverse transcriptase was examined. The effectiveness of a non-radioactive colorimetric Enzyme Linked Immunoassay kit for HIV-RT utilizing recombinant HIV-1 enzyme was evaluated in a controlled laboratory environment. The research indicates that the ethanol extract shows anti-HIV effects and flavonoids act as antiviral agents [15].

5.9 Anti-androgenic activity [25]

The infusion derived from *V. negundo* leaves acts as an effective antioxidant and a potent antiandrogenic compound when processed in ethanol. Consequently, it is ideal for treating hyperandrogenic conditions such as polycystic ovary syndrome. Utilizing a polar solvent like ethanol for extraction yielded the maximum quantity of polyphenols, flavonoids, and alkaloids, demonstrating notable antioxidant and anti-steroidogenic properties. The leaf of *V. negundo* is a more

suitable option for use as an antiandrogenic agent compared to the seed because a larger quantity of seed oil is known to be harmful. The inhibitory effect of various solvent extracts of *V. negundo* leaves on ovarian steroidogenic enzyme activity was measured by using the 3 β - and 17 β - hydroxysteroid dehydrogenase (HSDH) enzymes [38].

S.K. Bhargava (1984, 1986) and R.P. Samy *et al.* (1998) both documented the antiandrogenic properties of different flavonoids found in *Vitex negundo* seeds. The substances with estrogenic effects and anti-implantation properties are 5, 7, 3'-trihydroxy and 6, 8, 4'-trihydroxy flavones [39].

5.10 Anti-hyperglycemic activity

Multiple researches have demonstrated that VN has substantial anti-hyperglycemic effects. Villasenor and Lamadrid discovered that mice treated with VN leaves exhibited a noteworthy reduction in blood glucose levels (BGLs) at the 60-minute mark ($\alpha = 0.05$) during the oral glucose tolerance test. The use of 1,2-di-substituted idopyranose (100) from VN leaves showed promise in treating diabetes, providing protection for liver, kidney, and pancreatic cells likely through its anti-inflammatory effects on NF- κ B and iNOS in streptozotocin-induced diabetes. Sundaram *et al.* recently announced the antihyperglycemic impact of negundoside, an iridoid glucoside found in VN leaves, which is connected to its function in controlling carbohydrate metabolic enzymes and glycoprotein elements [13].

5.11 Anticonvulsant activity

It is possible that *Vitex negundo* is exerting anticonvulsant effects by elevating the concentration of (GABA), a neurotransmitter that inhibits activity in the central nervous system. Nonetheless, while various processes contribute to the anti-seizure effects, it is too early in the research to conclude that the anticonvulsant impact is linked to elevated GABA levels. Therefore, this aspect/theory needs to be further examined in the future [40]. The leaf extract from petroleum ether and butanol has been found to provide protection against seizures induced by electroshock, the sample has demonstrated minimal impact. The root's petroleum ether extract could only offer defense against leptazole. The leaf extract offered significant protection against both strychnine and induced convulsions, while the methanolic extract caused convulsions.

Convulsions were triggered by leptazole. The leaf's ethanolic extract exhibits anticonvulsant properties, but also has the ability to enhance the effects of 10 common anticonvulsants, potentially leading to lower doses and fewer side effects. Adverse reactions of typical antiepileptic medications [17].

5.12 Hepatoprotective activity

Study the hepatoprotective effects of the methanol extract from *Vitex negundo* leaves on CCl₄-induced toxicity in rat hepatocytes, HepG2 cells, and animal models. Collected mature *Vitex negundo* leaves were authenticated and underwent methanolic extraction. Recently purified rat liver cells were exposed to carbon tetrachloride (1%) alongside varying doses of the methanolic extract of *Vitex negundo* (50-250 μ g/ml) to evaluate specific liver enzyme activities. This investigation examined the protective effects of *Vitex negundo* methanolic extract (spanning concentrations from 50 to 250 μ g/ml) through MTT assay utilizing HepG2 cells, illustrating its ability to shield against CCl₄-induced injury. The protective properties against CCl₄-triggered liver toxicity

were further confirmed by the alcoholic seed extract of *V. negundo* [12].

5.13 Snake venom neutralization activity [41]

The root extracts of *Vitex negundo* Linn. in methanol displayed activity against snake venom. The extracts from *Vitex negundo* Linn. plant effectively countered the lethal effects induced by *Vipera russellii* and *Naja kaouthia* venom in both *in vitro* and *in vivo* experiments. The plant extracts effectively neutralized the hemorrhagic, coagulant, defibrinogenating, and inflammatory effects caused by *Vipera russellii* venom. There were no visible bands seen when the plant extract was mixed with snake venom [42].

5.14 Anti-osteoporotic activity

Recent findings have revealed that *V. negundo* possesses anti-osteoporotic properties. Osteoporosis undermines the integrity of bones, leading to fragility and brittleness. Research has shown that *V. negundo* can improve bone mineral density and fortitude, while also inhibiting bone loss in animal studies related to osteoporosis. *V. negundo* is thought to possess anti-osteoporotic properties due to its ability to enhance bone development and inhibit bone resorption. This plant is rich in diverse bioactive substances like flavonoids, terpenoids, and phenolic acids, all recognized for their protective benefits for skeletal health. Research indicates that *V. negundo* shows promise as a natural remedy for osteoporosis [13].

5.15 Antifilarial activity

The drug's impact on the worms' viability was confirmed through a MTT reduction assay, showing its cellular-level effect by decreasing mitochondrial enzyme levels that convert MTT to formazan. Antifilarial activity demonstrated at a lower concentration by reducing motility compared to controls suggests potential drug candidates, still requiring further confirmation through assessing parasite viability. As a result, the IC50 value was further determined. At a lower concentration, the ethyl acetate extracts from *V. negundo* leaves displayed encouraging outcomes. These findings suggest that the effectiveness of the therapeutic impact of certain active compounds in the plant extracts is dependent on their permeability properties [43].

5.16 Analgesic activity

VNE was examined for its pain-relieving capabilities in both peripheral (non-narcotic) and central (narcotic) pain models. The writhing reaction caused by acetic acid serves as a method to evaluate analgesic effects that act both peripherally and centrally [44]. VNE pretreatment significantly lessens the pain response induced by acetic acid, evident as writhing at the utilized dosages. In the acetic acid-induced writhing model, the contractions brought on by acetic acid in mice result from an acute inflammatory reaction characterized by the production of PGE2 and PGF2 α in the peritoneal fluid. Hence, it is probable that VNE may inhibit the synthesis of these compounds or counteract their effects to demonstrate analgesic efficacy [45].

5.17 Anticancer activity

Cancer stands as a principal factor in global morbidity and mortality. The therapeutic efficacy of four unique extracts (Petroleum ether, Ethyl acetate, Chloroform, and Methanol) derived from the stem and leaves of *Vitex negundo* was analyzed on A549 cell lines utilizing the MTT cell viability assay. The stem of *Vitex negundo* demonstrated significant

cytotoxic properties against the A549 cell line compared to the leaves. Specifically, the chloroform extract from the stem showed considerable cytotoxicity toward A549 cell lines, containing components like Vitamin C, carotene, glucanonal, benzoic acid, β -sitosterol, and C-glycoside. Seeds have hydrocarbons, β -sitosterol, benzoic acid and phthalic acid, anti-inflammatory diterpene, flavanoids, artemetin, and triterpenoids. extracted fatty acids, β -sitosterol, vanillic acid, p-hydroxybenzoic acid, and luteolin from the bark The bark of the stem is rich in leucoanthocyanidins, p-hydroxybenzoic acid, and β -sitosterol. Reports indicate that the ethanolic leaf infusion of *Vitex negundo* is packed with various metabolites like 5-hydroxy-3, 6, 7-trimethoxy-2-(3, 4-dimethoxyphenyl)-4H-chromen-4-one and 5, 7-dihydroxy-2-(3, 4-dihydroxyphenyl)-4H-chromen-4-one. Negundoside, Agnuside, and Vitegnoside are also found in the methanolic extract. This indicates the cytotoxic and apoptotic effects [46] Negunoside has demonstrated anticancer properties through both *in vitro* and *in silico* investigations. The IC50 value was determined to be 62.69 μ g/ml, and *in silico* analyses show it forms a higher number of hydrogen bonds with minimal binding and docking energy, suggesting its potential as an EGFR tyrosine kinase inhibitor [47].

5.18 Immunomodulation effect

The immunomodulatory effects of *Vitex negundo* (VN) Linn. Nirgudi stem and leaves at a dosage of 100 mg/kg resulted in a notable rise ($p < 0.05$) in the body weight of Wistar rats. A noteworthy rise in the lymphocyte and overall leukocyte count an increase in neutrophil adhesion to nylon fibers was also noticed. The present study sheds light on Potential of immunomodulation from the leaves of VN and extract from the stem have been shown to demonstrate the results Vietnam has the potential to be therapeutic in promoting. different blood-related measurements and were able to function as a potent immunomodulatory [48].

6. Conclusion

Herein, we documented the existing phytochemical and pharmacological researches on VN. The importance of collecting this information of VN lies in the fact that this herb possesses versatile and potent pharmacological properties and can form a practical base for further scientific research. Nearly all of the classical applications of VN have been substantiated by contemporary pharmacological research, emphasizing its pain-relieving, inflammation-reducing, tumor-fighting, antioxidant, insect-repelling, germicidal, anti-androgen, bone-strengthening, cataract-preventing, liver-protecting, and blood sugar-lowering properties, among others. Despite the growing body of research exploring the medicinal properties of VN, these studies remain insufficient. Additional pharmacological investigations into this species are necessary to confirm traditional uses and elucidate the pharmacological mechanisms at play, thereby enhancing our comprehension of the Traditional Chinese Medicine principles related to VN's application. This evaluation underscores the diverse compounds derived from *Vitex negundo*, accentuating their remarkable medicinal effects. The derivatives of this chemical component showcase an extensive array of biological activities, encompassing anti-inflammatory, analgesic, antimicrobial, antioxidant, and antitumor attributes. These results not only highlight the therapeutic importance of *V. negundo* but also reveal the promise of its chemical components for creating innovative treatment solutions.

The alterations in the structure of these components have resulted in derivatives with enhanced efficacy and superior pharmacokinetic characteristics, indicating that these substances may be vital in the innovation of forthcoming therapies. Ongoing investigation into their molecular action mechanisms, coupled with *in vivo* and clinical trials, is essential to convert these encouraging pharmacological outcomes into viable clinical uses. This review highlights the significance of delving into plant-derived chemical constituents and their derivatives due to their tremendous promise in contemporary medicine.

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