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PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF *VITEX NEGUNDO* LINN: A REVIEW

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ABSTRACT

Vitex negundo Linn. belong to family Verbenaceae. It is an important medicinal plant. Literature survey of *V. negundo* revealed the presence of different classes of natural products including essential oil, triterpenes, diterpenes, sesquiterpenes, lignan, flavonoids, flavones glycosides, iridoid glycosides, and stilbene derivative. The plant is traditionally reported for its use for the treatment of cough, asthma, fever, eye disease, inflammation, intestinal worms, skin diseases, nervous disorders, leprosy and rheumatism. Roots are tonic, anodyne, febrifuge, bechic, expectorant and diuretic. This review is short review of last two years reporting the natural products isolated and biological potential of *Vitex negundo* Linn.

INTRODUCTION: *Vitex negundo* Linn. (Verbenaceae), locally known as 'Nirgundi' an important medicinal plant¹, *Vitex negundo* Linn. is a woody, aromatic deciduous shrub growing to a small tree. It is an erect, 2-5 m in height, slender tree with quadrangular branchlets. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4-10 cm long, hairy beneath and pointed at both ends^{2, 3}. It thrives in humid places or along water courses in wastelands and mixed open forests and has been reported to occur in Afghanistan, India, Pakistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar. It is grown commercially as a crop in parts of Asia, Europe, North America and West Indies, also finds use as a food crop and a source of timber⁴.

- Class - *Magnoliopsida* - Dicotyledons
- Subclass - *Asteridea*
- Order - *Lamiales*
- Family - *Verbenaceae*
- Genus - *Vitex* Linn.
- Species - *Vitex negundo* Linn. (Chaste tree)⁵.

Plant Anatomy:

- Kingdom - *Plantae* - Plants
- Sub Kingdom - *Tracheobionta* - Vascular plants
- Super division - *Spermatophyta* - Seed plant
- Division - *Magnoliophyta* - Flowering plants



PHOTOGRAPHS OF *VITEX NEGUNDO* LINN.

Medicinal Plants: Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity^{6,8}.

Literature survey of *V. negundo* revealed the presence of volatile oil, triterpenes, diterpenes, sesquiterpenes, lignan, flavonoids, flavones glycosides, iridoid glycosides, and stilbene derivative. Though almost all parts of *V. negundo* are used, the extract from leaves and the roots is the most important in the field of phytomedicine and is sold as drugs. The leaf extract is used in Ayurvedic and Unani system of medicine. Water extract of mature fresh leaves exhibited anti-inflammatory, analgesic and antihistamine properties. Lignans, one class of natural compounds present in *V. negundo*, showed anti-cholinesterase activity in-vitro. However no studies were conducted to explore the effect of *V. negundo* extract against memory impairment in-vivo^{9,10}.

The leaf extract of *Vitex negundo* are generally used as a grain preserving material to protect the pulses against insects³. The leaves are the most potent for medicinal use. It is used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic and antihistaminic agents. Oil

prepared with it, is applied to sinuses and scrofulous sores. Its extract has also shown anticancer activity against *Ehrlich ascites* tumour cells¹¹.

The roots are used in rheumatism, dyspepsia, dysentery, piles and considered as tonic, febrifuge, expectorant, antihelmintic and diuretic. The flowers are astringent and are employed in fever, diarrhoea and liver complaints. The dried fruits are vermifuge and the bark is used in toothache. The chemical constituents of the essential oil of *V. negundo* leaves have been reported which indicated viridifloral to be its chief constituents^{12,16}.

The plant has been reported to exhibit medicinal properties including the curing of rheumatic pains and reducing swellings of the joints. In Chinese traditional medicine, it has been used for the treatment of chronic bronchitis. An infusion of the twigs is considered to be an effective therapy for headaches, dizziness, convulsions, coughs, mental unrest and is said to promote wakefulness¹⁷.

Its leaves and seeds are widely used externally for rheumatism and inflammations of joints and are also reported to have insecticidal properties. Internally, decoction of its leaves is taken as diuretic, expectorant, vermifuge, tonic and febrifuge. The chemical components of the essential oil of leaf isolated from *V. negundo* and other *Vitex* species have been reported by several researchers in the past. It's essential oil is found to be useful for sloughing wounds and ulcers. The leaves of *V. negundo* are reported to possess pesticidal, antifungal and antibacterial properties¹⁸⁻²⁰.

Leaves of this plant have been shown mosquito repellent effects as well as antiulcerogenic, antiparasitic, antimicrobial and hepatoprotective potentials. The methanolic root extract possessed potent snake venom neutralizing capacity The acetone extract of *V. negundo* was found to possess insecticidal, ovicidal, growth inhibition and morphogenetic effects against various life stages of a noxious lepidoteron insect-pest⁶.

Petroleum ether extract of *Vitex negundo* leaves has shown significant analgesic activity and the anticonvulsant activity against strychnine and leptazole. Dried leaves powder of *Vitex negundo* showed anti-arthritis activity in rats²¹.

V. negundo have diverse medicinal uses in the folk medicinal system of Bangladesh. Along with the utilization in traditional medicine by local practitioners and healers, this plant also reportedly showed diverse pharmacological properties including analgesic, antinociceptive, anti-inflammatory, anti-fertility, anti-feedant, anti-oxidant, antihyperglycemic effect, cytotoxicity for human cancer cell line, hepatoprotective activity against liver damage induced by d-galactosamine, commonly used tubercular drugs and carbon tetrachloride, laxative activity, immunomodulatory effect, and mosquito repellent effect.

The plant parts are reported to have anti-microfilarial, anti-viral, anti-bacterial, anti-fungal, insecticidal, larvicidal, as well as significant effect on antagonizing the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity in both *in vitro* and *in vivo*. The plant is reported to contain potent and novel therapeutic agents for scavenging of NO and the regulation of pathological conditions caused by excessive generation of NO and its oxidation product, peroxynitrite. Administration of *V. negundo* extracts also potentiated the effect of commonly used anti-inflammatory drugs sedative-hypnotic drugs (and anti convulsive agents. Inhibitory effect of *V. negundo* against active enzymes has also been observed for lipoxygenase and butyrylcholinesterase α -chymotrypsin xanthine-oxidase and tyrosinase²².

Medicinal herb and various parts of the plant have been employed in the folklore systems of medicine in Asia including India, China and Malaysia for various diseases. Many ethno botanical and pharmacological activities of *V. negundo* have been reported such as: analgesic and anti-inflammatory activity, antioxidant activity, enzyme inhibitions, nitric oxide scavenging activity, antiradical and antilipoperoxidative activity, CNS activity, hepatoprotective activity, anti-bacterial activity, antifungal activity, larvicidal activity, antiandrogenic effects and mosquito repellent activity. *V. negundo* leaves were found to have NSAIDs like activity²³.

The plant is traditionally reported for its use for the treatment of cough, asthma, fever, eye disease, inflammation, intestinal worms, skin diseases, nervous disorders, leprosy and rheumatism. Roots are tonic,

anodyne, febrifuge, bechic, expectorant and diuretic. The decoction of leaves is given as a drink to reduce phlegm in coughs, chronic bronchitis and asthma. Drugs currently used to treat cough are among the most widely used over-the-counter drugs in the world, despite a recent analysis suggesting that there is a little evidence to suggest that such drugs produce any meaningful efficacy.

The primary action of currently available cough suppressants (opiates, dextromethorphan etc.) is on the central cough pathway. The significant side effects of these agents such as constipation, respiratory depression, dependence, drowsiness and death limit their uses in humans and thus highly unsatisfactory. No peripherally acting antitussives, apart from local anesthetics such as lignocaine and possibly benzonatate, are currently established and available for use in patients. There is a current need for the development of safe and effective antitussive therapeutic options in the treatment of persistent cough as alternative to existing medications¹.

Biological Activities:

1. **Anti-amnesic activity:** Anti-amnesic effect of *V. negundo* aqueous extract on scopolamine administered at different stages of active avoidance learning in rats. An automatic reflex conditioner with two-way shuttle box (Ugo Basile, Italy). The rats were treated orally with the standard drug through an intragastric feeding tube. Similarly the plant extract were administered for 14 days. For this purpose each rat is placed in a compartment separated from the other one by a guillotine door in the shuttle box.

Exploration period of 2 min is given initially. Thereafter, the trial start, in each trial the animal is subjected to a light for 30 s followed by a sound stimulus for 10s. Immediately after the sound stimulus, the rat receives a single low intensity foot shock (0.5 mA; 3 s) from 10th day to 14th through the floor grid if it does not transfer to the other shock free compartment. Infrared sensors monitor the transfer time from one compartment to another, which is recorded as avoid (after the stimulus of either light alone or both light and sound) and escape (after the foot shock) response.

Each animal received a daily session of 15 trials with an inter-trial duration of 15 s for 5 days *i.e.*, a maximum of 75 trials. The rats were evaluated on the basis of their performance in the last session *i.e.*, in the 5th session for their decrease in amnesic activity and increased learning and memory. The criterion for improved cognitive activity was taken as significant increase in the avoidance response on 5th session (retention) compared to 1st session ²⁴.

2. **Antioxidant activity:** Preliminary studies showed that *V. negundo* leaf exhibited antioxidant properties and contain natural antioxidants. Thus, the objective of this study was to analyze the antioxidant activity of methanol and hexane extract and essential oil from *V. negundo* leaf using different *in vitro* antioxidant assays. In addition, total phenolic contents, flavonoids, tocopherol and carotenoids content of leaf of *V. negundo* were also quantified using high performance liquid chromatography (HPLC).
3. **1, 1-diphenyl-2-picrylhydrazyl (DPPH) method:** DPPH method measured the ability of antioxidant in scavenging free radicals present. Antioxidant activity of *V. negundo* leaf was expressed as the concentration that inhibits 50% DPPH free radical (IC₅₀). Results obtained in the study showed that the IC₅₀ of methanol extract of *V. negundo* (138±11.68 µg/ml) was significantly ($p < 0.05$) lower than that of both essential oil (432±12.65 µg/ml) and hexane extract (567±17.37 µg/ml), revealing its higher antioxidant activity than those of hexane extract and essential oil.
4. **FRAP method:** The FRAP test measures the ability of samples to reduce ferric ion to the ferrous form of TPTZ (2, 4, 6-tripyridylstriazine). Arbitrarily, one FRAP unit is defined as the reduction of 1 mol of Fe³⁺ to Fe²⁺. Similarly, result of the study showed that the antioxidant capacity of methanol extract (44.6±7.8 µM TE/g) was significantly ($p < 0.05$) higher than that of hexane (11.30±1.3 µM TE/g) and essential oil (11.53±1.35 µM TE/g) of leaves of *V. negundo* (Figure 2). However, there was no significant ($p < 0.05$) difference on the antioxidant capacity between hexane extract and essential oil. The antioxidant capacity of methanol extract was noted to be four times higher than that of hexane

extract and essential oil. It is interesting to note that the trend of antioxidant activity obtained from FRAP assay was similar to that obtained in DPPH assay ¹⁷.

5. **Antibacterial activity:** The bacteria used for antibacterial tests were Gram (+) *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 0121) and Gram (-) *Escherichia coli* (MTCC 0051), *Pseudomonas aeruginosa* (MTCC 0741). All the strains used for these studies were procured from MTCC, IMTECH, Chandigarh, India. Antibacterial potential of all three samples of essential oils and successive extracts was evaluated by agar well diffusion method. Nutrient agar plates were swabbed with the broth culture of the respective microorganisms (diluted to 0.5 McFarland Standard) and were kept at room temperature for 15 min for absorption to take place.

Wells of 8 mm diameter were punched into the agar medium and filled with 100 µl each of the essential oils and extracts. DMSO, DMF and hexane were taken as solvent blank and Ciprofloxacin was used as positive control. The inoculated agar plates were incubated for 24 h at 37°. All the tests were made in triplicate and diameter of the inhibition zones was calculated in mm. The average of diameter of the inhibition zones of each sample was taken called clearing zone (CZ) and the antimicrobial index (AI) was computed as the clearing zone (CZ) minus the diameter of the hole divided by the diameter of the hole.

All the extracts and essential oils were found to be highly effective in inhibiting the growth of bacteria at a minimum concentration of 30 and 60 µg/100 µl, respectively. Each of the essential oil and extracts were found to be active against *B. subtilis* and *E. coli* with antimicrobial index (AI) ranging from 0.3 to 1.8. Leaf essential oil inhibited *S. aureus* with maximum AI of 1.5 while fruit essential oil showed its inhibition against *E. coli* and *B. subtilis* with AI of 1.3 and 1.0, respectively. Flower oil did not show any activity against *S. aureus* while leaf and fruit oils were ineffective against *P. aeruginosa*. Ethyl acetate extract was found to be most potent among all the extracts tested.

Petroleum ether and aqueous extracts did not show any activity against *P. aeruginosa* while all the extracts were found potent against *S. aureus*. Ciprofloxacin was used as positive standard control and the results of tested samples were very promising in comparison to standard drug ciprofloxacin¹⁸.

- From the study, the zones of inhibition produced by the methanol extract, petroleum ether and carbon tetrachloride fractions were found to be 07-16 mm, 07-11 mm and 06-11 mm respectively at a concentration of 200 g/disc in case of 09 bacterial strains and 02 fungal strains where standard kanamycin (30µg/disc) showed zone of inhibition of 08-19 mm. Prominent activity was found against *Bacillus subtilis* (13-16 mm) by all of the fractions. Methanol extract showed significant inhibition (09-10 mm) against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi*. Petroleum ether and carbon tetrachloride fractions showed most prominent inhibitory action (zone of inhibition 11-18 mm) against *Bacillus megaterium*, *Bacillus subtilis*, *Salmonella typhi* and *Vibrio mimicus* in comparison to standard antibiotic (kanamycin, 30µg/disc). All the fractions of *Vitex negundo* were also tested for antifungal activity against 03 fungi. The extracts had inhibitory effect against all the test pathogens in different degree. The methanol extract and petroleum ether fraction showed profound activity against *Aspergillus niger* and *Candida albicans* respectively⁶.
- Volatile oil of *Vitex negundo* is reported to contain β-caryophyllene, sabinene, linalool, terpinen-4-ol, α-guaiene and globulol as major constituents along with sesquiterpenes, monoterpenes, terpenoids and sterols. A wide variety of essential oils are known to possess the antimicrobial properties and in many cases this activity is due to the presence of monoterpene constituents which exerts membrane damaging effects and stimulate leakage of cellular potassium ions which provides evidence of lethal action related to cytoplasmic membrane damage. Presence of terpenoids in supercritical fluid extract as evident by TLC pattern explains its stronger antibacterial potential²⁵.

6. **Phytopathogenic antibacterial activity:** There is a worldwide interest in searching for the safe and effective novel antibacterial compounds of plant origin for the control of plant pathogenic bacteria which is responsible for the great impact on the growth and productivity of agriculture crops. In this study an attempt was made to determine the in vitro antibacterial activity of sequentially extracted different solvent (dichloromethane, ethyl acetate, ethanol, methanol and water) extracts of leaf, flower and fruit of *Vitex negundo* L. and bulb of *Allium sativum* L. (Garlic) against phytopathogens namely *Pseudomonas solanacearum* and *Xanthomonas axonopodis* pv. citri.

The preliminary antibacterial activity was performed by agar well diffusion method and the minimum inhibitory concentration (MIC) values were determined by agar dilution method. The test samples were also subjected to qualitative phytochemical analysis. One way analysis of variance (ANOVA) followed by least significant difference (LSD) test were done for the statistical analysis of the data. All the test samples showed inhibitory effect on both of the test pathogens and the diameter of inhibition zone ranged from 9.9 ± 0.5 mm to 48.5 ± 1.3 mm and the inhibitory effect differed significantly ($P < 0.05$) among the samples. Ethyl acetate extract of flower of *Vitex negundo* L. showed significantly ($P < 0.05$) higher inhibition on *Pseudomonas solanacearum* and *Xanthomonas axonopodis* pv. citri.

The MIC values of ethyl acetate extracts of fruit and flower of *Vitex negundo* L. and *Allium sativum* and ethanol extract of flower of *Vitex negundo* L. ranged from 2.5mg/ml to 40mg/ml. Phytochemical analysis of above extracts revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides and terpenoids. Further studies are being carried out to elucidate the active principles responsible for the inhibitory effect of these pathogens and to determine their activity in vivo. This is the first report that reveals the inhibitory effect of *Vitex negundo* L. on *Pseudomonas solanacearum* and *Xanthomonas axonopodis* pv. Citri²⁶.

7. **Antibacterial effect of silver nanoparticles using *Vitex negundo* Linn.:** Different biological methods are gaining recognition for the production of silver nanoparticles (Ag-NPs) due to their multiple applications. One of the most important applications of Ag-NPs is their use as an anti-bacterial agent. The use of plants in the synthesis of nanoparticles emerges as a cost effective and eco-friendly approach. In this study the biosynthesis of silver nanoparticles using *Vitex negundo* L. extract and its antimicrobial properties has been reported. The resulting silver particles are characterized using transmission electron microscopy (TEM), X-ray diffraction (XRD) and UV-Visible (UV-Vis) spectroscopic techniques.

The TEM study showed the formation of silver nanoparticles in the 10–30 nm range and average 18.2 nm in size. The XRD study showed that the particles are crystalline in nature, with a face centered cubic (fcc) structure. The silver nanoparticles showed the antimicrobial activity against Gram positive and Gram negative bacteria. *Vitex negundo* L. was found to display strong potential for the synthesis of silver nanoparticles as antimicrobial agents by rapid reduction of silver ions (Ag⁺ to Ag⁰)²⁷.

8. **Antifungal activity:** Sathiamoorthy *et al.*, (2007) isolated six compounds from the powdered leaf extracts of *Vitex negundo*. The isolated compounds were evaluated for antifungal and anti-bacterial activity. From the isolated compounds two possess potent anti-fungal activities and very active when compared to other isolated compounds. Significant antifungal activity in ethanolic extract against *Cryptococcus neoformans* and *Trichophyton mentagrophytes* was offered by two compounds isolated from the leaf extract of *Vitex negundo*²⁸.

9. **Anti-inflammatory and analgesic activities of *Vitex negundo*:** Inflammation may start in every part of our body. Any time when the word describing a disease ends with - it is, it's an inflammatory disease. Dermatitis means an inflammation of the skin, arthritis an inflammation of joints, an othitis an inflammation of the ear. Thus anti-inflammatory activity of a compound is considered to be a valuable feature. 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. The inverse dose-response relationship shown by acute anti-inflammatory, antihistamine, PG synthesis inhibition and membrane stabilising activities may be due to reduction of the effectiveness of the active principle at its high concentrations.

Sedatives and stress are responsible for producing analgesia. There was no sign of stress observed in the rats treated with the mature fresh leaves extract of *Vitex negundo*. Mature fresh leaves extract of *Vitex negundo* is effective against the establishment of chronic inflammation which happens at the later stage of acute inflammation. Moreover treatment with the mature leaf extracts of *Vitex negundo* in rats did not show a gastric lesion which is an advantage when compared with the use of modern NSAIDs. Treatment of Mature fresh leaves extract of *Vitex negundo* for 14 days in rats orally did not produce detectable toxic effect in terms of body weight, serum concentrations of urea, creatinine, glucose and serum activity of ALT.

This is a very important criterion that favours the use of this extract for medicinal purposes. The anti-inflammatory and analgesic activities of the leaves did not disappear after the flowering of the tree in contrast to *Anisomeles indica* which lost these activities after flowering of the plant. These studies provide evidence for the anti-inflammatory and analgesic properties of mature fresh leaves of *Vitex negundo* claimed in Ayurveda medicine²⁸.

10. **Enzyme-inhibitory activity:** Root extracts of *Vitex negundo* showed inhibitory activity against enzymes such as lipoxygenase and butyryl-cholinesterase, α -chymotrypsin, xanthine-oxidase and tyrosinase. Woradulayapinij *et al.* reported the HIV type 1 reverse transcriptase inhibitor activity of the water extract of the aerial parts of *Vitex negundo*⁴.

11. **Effect on Reproductive Potential:** The flavonoid rich fraction of seeds of *Vitex negundo* caused disruption of the latter stages of spermatogenesis in dogs and interfered with male reproductive function in rats. It must however be noted that these findings are in sharp contrast with the traditional use of *Vitex negundo* as aphrodisiac. Hu *et al.* determined that ethanolic extracts of *Vitex negundo* showed estrogen-like activity and propounded its use in hormone replacement therapy⁴.
12. **Histomorphological and Cytotoxic Effects:** Tandon and Gupta studied the histomorphological effect of *Vitex negundo* extracts in rats and found the stomach tissue to be unaffected even by toxic doses; while dose-dependent changes were observed in the heart, liver and lung tissues. Cytotoxic effect of leaf extracts of *Vitex negundo* was tested and affirmed using COLO-320 tumour cells. On one hand, Diaz *et al.*, found the chloroform extracts of *Vitex negundo* leaves to be toxic to a human cancer cell line panel while on the other; Yunos *et al.* reported that *Vitex negundo* extracts were non-cytotoxic on mammary and genito-urinary cells of mice⁴.
13. **Anticonvulsant Activity:** The plant has been studied for its anticonvulsant activity. The petroleum and butanol leaf extracts have shown protection, whereas, none of root extract has shown protection against maximal electro shock (MES) seizures. Petroleum root extract could only provide protection against Leptazole induced convulsions whereas methanolic leaf extract showed significant protection against Strychnine and Leptazole induced convulsions. Gupta and Tandon (2002) not only suggested anticonvulsant activity of ethanolic leaf extract of this plant but also indicated that it can potentiate the effects of standard anticonvulsants, which may help to reduce dose and dose related side effects of standard anticonvulsants²⁹.
14. **Anxiolytic Activity:** Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger. It may be regarded as a particular form of behavioral inhibition that occurs

in response to environmental events that are novel. Anxiety affects one-eighth of the total population worldwide and has become a very important area of research interest in psychopharmacology during this decade. There are various ways of explaining the mechanisms of action of anti-anxiety agents because of the involvement of many CNS chemical mediators.

The effect of most of the anxiolytic agents is to enhance the response to GABA, by facilitating the opening of GABA-activated chloride channels. GABAA receptors were involved in anxiety and their direct activation would have an anxiolytic effect. Anti-anxiety drugs have also been shown to act on limbic system, hypothalamus, and the brain stem reticular system. Benzodiazepines are still the most frequently used drugs for the treatment of generalized anxiety disorder despite their undesirable side effects such as muscle relaxation, sedation, physical dependence, memory disturbance, and interaction with other drugs.

However, the realization that benzodiazepines present a narrow safety margin between the anxiolytic effect and those causing unwanted side effects has prompted many researchers to evaluate new compounds in the hope that other anxiolytic drugs will have less undesirable effects. In recent years, the development of new anxiolytics has been an area of interest.

It has been established that there are lot of secondary plant metabolites being employed in the treatment of psychotic disorders especially for anxiety in traditional medicine practice, most of which directly or indirectly affect the central nervous system such as noradrenaline, serotonin, gamma-aminobutyric acid (GABA) and benzodiazepine (BDZ) neurotransmitters' activities. On the basis of these considerations, it was the purpose of this study to characterize the anxiolytic-like activity of an ethanolic extract prepared from the roots of *Vitex negundo* Linn (VN)²¹.

15. **Antipyretic Activity:** The literature reveals that the leaf extract of *Vitex negundo* causes a significant antipyretic effect in yeast provoked elevation of body temperature. In the cases, the methanol

extract caused a significant lowering of body temperature, with the effect being comparable to that of paracetamol. Thus the present pharmacological evidence provides support for the folklore claim as an antipyretic agent. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation, pyrexia and pain perception. Flavonoids reduce lipid peroxidation by preventing or slowing the onset of cell necrosis and by increasing the vascularity. Hence the presence of flavonoids in the methanol extract of *Vitex negundo* may be contributory to its antipyretic activity³⁰.

16. **Cytotoxic agent against PANC-1 and a Panel of 39 Human Cancer Cell:** Recently it was observed that the methanol extract of *Vitex negundo* from Myanmar displayed potent cytotoxic activity preferentially in the nutrient starvation condition with 100% cell death at a concentration of 10µg/mL. Therefore, it was subjected to phytochemical analysis to identify active constituents. The active constituent was further tested against PANC-1 cells as well as a panel of 39 human cancer cell lines (JFCR-39) at the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research.

The ethanol extract of *V. negundo* showed potent preferential cytotoxicity against PANC-1 human pancreatic cancer cells. Bioassay-guided fractionation and purification of *Vitex negundo* led to the isolation of chrysoplenetin (**1**) and chrysosplenol D (**2**) as the most active constituents with a PC₅₀ value of 3.4µg/mL and 4.6µg/mL, respectively, against the PANC-1 human pancreatic cancer cell line. They showed characteristic apoptosis-like morphological changes of PANC-1 cells in NDM.

The most active compound, chrysoplenetin was further evaluated against a panel of 39 human cancer cell lines at the Japanese Foundation for Cancer Research. Chrysoplenetin exhibited differential cell growth inhibition at submicromolar concentration against most of the cell lines. *Vitex negundo* and its active constituents might have possible beneficial effects for patients suffering from cancer in a real clinical situation³¹.

17. **Testing for Cellular Toxicity:** The cellular toxicity of extracts was determined against human erythrocytes adopting the procedure of He and Ursula. Eight fold serial dilutions of the extract were made in phosphate buffered saline and a total volume of 0.9 ml for each dilution was placed in an eppendorf tube. Fresh human erythrocytes were added to each tube to give a final volume of 1 ml.

A negative control (containing saline only) and a positive control (containing test extracts 5 mg/ml) were also included in the analysis. Solutions were incubated at 37°C for 30 min and all tubes were centrifuged at 3000 rpm for 5-10 min and then observed for hemolysis. Complete hemolysis was indicated by a clear red solution without any deposits of erythrocytes. Hemolysis was also checked microscopically by presence or absence of intact RBCs. leaf and seeds extracts of *Vitex negundo* were also assayed for cellular toxicity to fresh human erythrocytes and found to have no cellular toxicity³².

18. **Antitussive and Toxicological Evaluation of *Vitex negundo* Linn:** Antitussive effects of the n-butanol fraction of *Vitex negundo* on sulphur dioxide-induced cough have been examined in mice. Cough is the most common respiratory symptom that has been experienced by every human. It is an essential protective and defensive act whose action secures the removal of mucus, noxious substances and infections from the larynx, trachea and larger bronchi. On the other hand, a number of patients have non-productive cough, which is not associated with mucus clearance and may have a different stimulation.

It may be first overt sign of disease of airways or lungs and may significantly contribute to the spread of airborne infections and in some instances, may result in severe functional and structural damage to the organism. There is an acute current need for the development of safe, effective antitussive therapeutic options for the treatment of persistent cough as alternative to the existing medications. *Vitex negundo* reflects potency to inhibit chemically induced cough.

From the experimental results, it was observed that extract of *Vitex negundo* demonstrated a significant inhibition of cough reflex in a dose dependent manner.

For instance, the activity possessed by 250 mg/kg at 30 min was lesser than other increased doses in all the experimental duration. The highest inhibition of cough reflex was achieved by the extract at the dose level 1000 mg/kg at 60 min of experiment. These results provide pharmacological evidence in support of folklore claims as an antitussive agent. The available antitussives have been derived from various compounds that have different pharmacological actions, such as analgesic, anticholinergic and antihistaminic.

Plant extracts tend to exhibit numerous pharmacological actions with multiple mechanisms, which are activated by several different types of compounds. There is considerable ethnomedical and pharmacological evidence that *Vitex negundo* possesses analgesic, antipyretic, antihistaminic, antiallergic, antibacterial, antioxidant and antiphlegmatic, potential.

This not only suggests that extract of *Vitex negundo* in improved formulation may inhibit coughing more effectively, but it may also provide interesting and meaningful efficacy in respiratory conditions such as asthma, bronchitis, tuberculosis and other lower respiratory tract infections which are accompanied with combined symptoms of fever, inflammation, phlegm and cough. The data received indicates that *Vitex negundo* possesses obvious antitussive activity against SO₂ induced cough in mice. The antitussive activity of this plant correlates with its various biological and ethnopharmacological properties which may justify its widespread use in various respiratory conditions in folk medicine. It was found a safer drug in the different toxicity assays performed ¹.

19. **Hepatoprotective activity:** The study was intended to evaluate the hepatoprotective activity on ethanolic extract of leaves of *Vitex negundo* was determined by using Wistar Albino rats in male sex. The *V. negundo* Linn is a natural plant product, in its the leaves are used with the added advantage to

revert Ibuprofen induced hepatotoxicity. Oral administration of ethanol extract of *V. negundo* (100 300 mg) produced a significant and dose dependent inhibition to the acute hepatotoxic induced rats and various parameters were analysed, when compared with negative control *V. negundo* showed that the significant activity in 300 mg/kg/b.wt. They exhibited a significant inhibition of hepatic toxicity by using various marker enzymes and the histopathological analysis.

The inhibitory effect of the *V. negundo* on hepatotoxicity was compared to that of positive control group. The various parameters such as glucose, protein, triglycerides, bilirubin, urea, creatinine, ALP, ACP, SGPT, SGOT and histopathological parameters was measured by dissection the rats. A significant index and values were observed in the acute assays; concluded that the *V. negundo* having the potential effectiveness at the dose of 300 mg/kg/b.wt, (p<0.01) significance in a dose dependent manner. These results suggest that leaves of *V. negundo* having the hepatoprotective activity, which support the hepatic cells protection ⁵.

20. **Anti snake Venom Activity:** The methanolic root extracts of *Vitex negundo* Linn and *Embllica officinalis* Gaertn were explored for the first time for antisnake venom activity. The plant (*V. negundo* and *E. officinalis*) extracts significantly antagonized the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity both in in vitro and in vivo studies. *V. russellii* venom-induced haemorrhage, coagulant, defibrinogenating and inflammatory activity was significantly neutralized by both plant extracts. No precipitating bands were observed between the plant extract and snake venom.

The above observations confirmed that the plant extracts possess potent snake venom neutralizing capacity and need further investigation ³³.

21. **Larvicidal Activity:** Mosquito control is facing a threat due to the emergence of resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. The acetone, chloroform, ethyl acetate, hexane, methanol and petroleum

ether extracts of leaf, flower and seed of *Cassia auriculata* L., *Leucas aspera* (Willd.), *Rhinacanthus nasutus* KURZ., *Solanum torvum* Swartz and *Vitex negundo* Linn. Were tested against fourth instar larvae of malaria vector, *Anopheles subpictus* Grassi and Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). The larval mortality was observed after 24 h of exposure.

All extracts showed moderate larvicidal effects; however, the highest mortality was found in leaf petroleum ether, flower methanol extracts of *C. auriculata*, flower methanol extracts of *L. aspera* and *R. nasutus*, leaf and seed methanol extracts of *S. torvum* and leaf hexane extract of *V. negundo* against the larvae of *A. subpictus* (LC₅₀=44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm; LC₉₀=187.31, 188.29, 233.18, 142.66, 151.60, 121.05 and 192.11 ppm, respectively) and against the larvae of *C. tritaeniorhynchus* (LC₅₀= 69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm; LC₉₀=335.26, 245.63, 300.45, 361.83, 185.09, 351.41 and 302.42 ppm, respectively).

These results suggest that the leaf petroleum ether, flower methanol extracts of *C. auriculata*, leaf and seed methanol extracts of *S. torvum* and leaf hexane extract of *V. negundo* have the potential to be used as an ideal eco-friendly approach for the control of the *A. subpictus* and *C. tritaeniorhynchus*. This is the first report on the mosquito larvicidal activity of the medicinal plant extracts³⁴.

22. Antihelmintic activity: Ethanolic extracts of *Moringa oleifera* and *Vitex negundo* were taken for anthelmintic activity against Indian earthworm *Pheritima posthuma*. Various concentrations of both extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as a control group. Dose dependent activity was observed in both plant extracts but *Moringa oleifera* shows more activity as compared to *Vitex negundo*³⁵.

Essential Oils: The chemical components of the essential oil from *V. negundo* have been reported. Its essential oil is found to be useful for sloughing wounds and ulcers. The essential oils from fresh leaves, flowers and dried fruits were extracted and analysis by GC/MS which may be responsible for the various medicinal properties of the plant.

- 1. From leaves:** The identified constituent- *p*-cymene, *cis*-ocimene, citronellal, β -curcumene, β -caryophyllene, α -guaiene, guaia-3,7-diene, δ -guaiene, valencene, caryophyllene epoxide, ethyl-9-hexadecenoate, palmitic acid, (E)-nerolidol, humulene epoxide 1, globulol, humulene epoxide 2, epi- α -cadinol, α -muurolol, α -cadinol and α -bisabolol acetate represented about 85.5% of total composition of the essential oil of leaf¹⁸.
- 2. From flowers:** Twelve identified constituent in flower essential oil were formic acid, n-heptane, *p*-cymene, β -caryophyllene, trans- α -bergamotene, valencene, α -selinene, β -selinene, germacren-4-ol, caryophyllene epoxide, (E)-nerolidol and *P*-(1,1-dimethylethyl) toluene represented about 65% of total composition of the oil, (Khokra et al., 2008) from the flower oil of *V. negundo*, the main constituents of the oil were sabinene, linalool, terpinen-4-ol, β -caryophyllene, α -guaiene and globulol constituting 61.8% of the oil as major constituents along with sesquiterpenes, monoterpenes, terpenoids and sterols^{36, 25}.
- 3. From fruits:** The thirteen constituents namely α -copaene, β -caryophyllene, α -cedrene, α -guaiene, guaia-3, 7-diene, α -humulene, aristolene, germacrene D, β -selinene, caryophyllene oxide, n-hexadecanoic acid, palmitic acid and traces of acetyl lactyl glycerate were identified in dried fruit oil¹⁸.

Proximate Analysis of *Vitex negundo* Linn.³⁷:

S.No	Parameters	Quantitative (%)
1	Ash	7.5-8.5
2	Moisture	15.00-18.70
3	Crude protein	12.22-15.23
4	Crude fiber	25.50-30.50
5	Fat	5.00-9.00
6	Carbohydrate	7.5-10.57
7	Alkaloids	0.5

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