



## *Vitex negundo* and its medicinal value

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Received: 3 July 2018 / Accepted: 4 October 2018 / Published online: 11 October 2018  
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### Abstract

Natural products are rich in several potent bioactive compounds, targeting complex network of proteins involved in various diseases. *Vitex negundo* (VN), commonly known as “chaste tree”, is an ethnobotanically important plant with enormous medicinal properties. Different species of *Vitex* vary in chemical composition, thus producing different phytochemicals. Several bioactive compounds have been extracted from leaves, seeds, roots in form of volatile oils, flavonoids, lignans, iridoids, terpenes, and steroids. These bioactive compounds exhibit anti-inflammatory, antioxidant, antidiabetic, anticancer, antimicrobial. VN is typically known for its role in the modulation of cellular events like apoptosis, cell cycle, motility of sperms, polycystic ovary disease, and menstrual cycle. VN, reportedly, perturbs many cancer-signaling pathways involving p-p38, p-ERK1/2, and p-JNK in LPS-elicited cells, N-terminal kinase (JNK), COX-1 pathways, MAPK, NF-κB, tumor necrosis factor α (TNF-α), Akt, mTOR, vascular endothelial growth factor, hypoxia-inducible factor (HIF-1α). Several bioactive compounds obtained from VN have been commercialized and others are under investigation. This is the first review presenting up-to-date information about the VN, its bioactive constituents and their mode of action.

**Keywords** *Vitex negundo* · Signaling · Reactive oxygen species · Cancer · Apoptosis

### Introduction

Natural products are endowed with myriad therapeutics effects and used as traditional medicines, since ancient times. Conventional plants are rich in various bioactive compounds that stimulate the immune system and protect against many ailments. Many of these compounds have passed

clinical trials, and several others still remain unexplored [1]. Natural products may be of plant or animal origin, albeit plant products form the majority of natural products-derived drugs, thus increasing the demand for systematic evaluation of novel bioactive compounds [2]. One such plant is *Vitex negundo* L. (Verbenaceae) commonly known as five leaf chase tree, and nirgundi in India. The genus *Vitex* has around 270 known species, ranging from shrubs to trees in the tropical, sub-tropical regions and temperate zones [3]. The *Vitex* is used as a folk medicine in Bangladesh, India, China, Indo-China, Indonesia, Nepal, Pakistan, Philippines, and Sri Lanka [4].

VN is a slender tree with quadrangular branches, reaching up to 5 m in height and usually grows in wastelands. In ancient times, all parts of the plant were used as medicine, but leaves and bark extracts were more effective [5]. The leaves are lanceolate, with 3–5 leaflets, growing up to 4–10 cm in length, have glabrous upper surface and bear numerous short hairs at ventral surface. The flowers are bluish-purple in color, small in size, arranged as pedunculated tomentose cymes, and grow up to 10–20 cm in length. The fruit is succulent, round and black in color, when ripened and about 4 mm in diameter. The mature seeds, when sown in nursery beds, normally germinate within 2–3 weeks, and

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develop strong and deep roots. Leaves are mostly used for the treatment of eye diseases, inflammation, leucoderma, toothache, spleen enlargement, skin-ulcers, gonorrhea, rheumatoid arthritis, and bronchitis. Along with these, leaves are used as a vermifuge, lactagogue, tonics, antibacterial, anti-pyretic and antihistaminic agents [6, 7]. Dry VN leaves are placed with stored woolen garments, as the leaves prevent wool-destroying insects and worms. Indigenous leaves of *V. trifolia* L. were used as mosquito repellent against *Aedes aegypti* [8], and are considered antiparasitic [9]. Young shoots of *Vitex* are employed in basketry, stems as firewood, and the plant ash is used as an alkali in dyeing. *Vitex* also has a significant impact on the reproductive systems of both men and women. There are different species of *Vitex* such as *Vitex agnus-castus*, *V. rotundifolia*, *V. negundo* and others, which have pharmacological importance.

### Bioactive compounds in *V. negundo*

The bioactive constituents of *V. negundo* comprise volatile oils, flavonoids, lignans, iridoids, terpenes (triterpenes, diterpenes, sesquiterpenes) [5], and steroids [10] (Table 1). The potential of the extracts depends on the composition

and extraction method (e.g. temperature, pH, solvent system or any other mode of extraction), that in turn determines polarity, stability, and efficacy of the extract. The chemical composition varies within species, resulting in variation of phytochemicals, type, and extent of bioactivity [11]. This step leads to extraction of various compounds such as volatile oils, lignans, polyphenolic compounds, terpenes, glycosidic iridoids, flavonoids, alkaloids, and steroids, which can further be isolated, purified and assayed for several activities. Dutta and group isolated two nishindaside (2a) and negundoside (3a), glucosidic iridoids, from the leaves of *V. negundo* [12]. *V. negundo* leaves grown in Dehra Dun (India) were analyzed by gas chromatography–mass spectrometry (GC–MS) and revealed presence of 65 volatile compounds, of which 35 compounds exhibited 74.96% of the oil. The major isolated compounds present in *V. negundo* are viridiflorol, beta-caryophyllene, sabinene, 4-terpineol, gamma-terpinene, and various phenols [13, 14]. Methanolic extract of *V. negundo* was also reported to contain various flavonoids and related compounds with antimicrobial activities [15]. Additionally, methanolic extracts of roots of VN contain, negundin A and negundin B having tyrosinase inhibitory activity that is suggested for its putative role in the treatment of melanocytes-associated hyperpigmentation

**Table 1** Chemical constituents of *V. negundo*

Plant part	Bio constituents	References
Leaves	Hydroxy-3,6,7,3',4'-pentamethoxyflavone	[26]
	2'- <i>p</i> -Hydroxybenzoyl mussaenosidic acid, 6'- <i>p</i> hydroxy benzoyl mussaenosidic acid	[27]
	Nishindaside (2a), negundoside (3a), glucosidic iridoids	[12]
	Viridiflorol, $\beta$ -caryophyllene, sabinene, 4-terpineol, gamma-terpinene, caryophyllene oxide, 1-octen-3-ol; globulol	[13]
	Casticin, sitosterol, vitamin-C nishindine, <i>p</i> -hydroxybenzoic acid, gluco-nonitol	[28]
	Protocatechuic acid, oleanolic acid, flavonoids	[29]
	Negundoside, agnuside, vitegnoside, 7,8 dimethyl herbacetin 3-rhamnoside, 5,3'-dihydroxy-7,8,4'-trimethoxy flavanone, 5-hydroxy-3,6,7,3',4'-pentamethoxy flavone, 5,7 dihydroxy-6,4' dimethoxy flavanone, and 5 hydroxy-7,4' dimethoxyflavone	[15]
	Betulinic acid $3\beta$ -hydroxylup-20-(29)-en-28-oic acid; ursolic acid ( $2\beta$ -hydroxyurs-12-en-28-oic acid); <i>n</i> -hentriacontanol; $\beta$ -sitosterol; <i>p</i> -hydroxybenzoic acid	[5]
	Salviaplebeiaside, $\gamma$ -tocopherol, chryso-splenol-d, and isovitexin, with $\alpha$ -tocoquinone and $\beta$ -sitosterol	[14]
	Seeds	$3\beta$ -Acetoxylean-12-en-27-oic acid; $2\alpha$ , $3\alpha$ -dihydroxyoleana-5,12-dien-28-oic acid; $2\beta$ , $3\alpha$ diacetoxyleana-5,12-dien-28-oic acid; $2\alpha$ , $3\beta$ -diacetoxylean-18-hydroxyoleana-5,12-dien-28-oic acid
6-Hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde		[30]
$\beta$ -Sitosterol, <i>p</i> -hydroxybenzoic acid, <i>n</i> -tritriacontane, n-hentriacontane, <i>n</i> -pentatriacontane, and <i>n</i> -nonacosane		[28]
Roots	$2\beta$ , $3\alpha$ -Diacetoxyleana-5,12-dien-28-oic acid; $2\alpha$ , $3\alpha$ -dihydroxyoleana-5,12-dien-28-oic acid; $2\alpha$ , $3\beta$ -diacetoxylean-18-hydroxyoleana-5,12-dien-28-oic acid; vitexin and isovitexin	[30]
	Negundin-A; negundin-B; (+)-diasyringaresinol; (+)-lyoniresinol; vitrofolal-E and vitrofolal-F	[17]
	Acetyl oleanolic acid, sitosterol, 3-formyl-4,5-dimethyl-8-oxo-5H-6, and 7-dihydronaphtho (2,3-b)furan	[31]
	Negundin A, negundin B, 6-hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde, vitrofolal E, (+)-lyoniresinol, (+)-lyoniresinol-3- $\alpha$ - <i>O</i> - $\beta$ -D-glucoside, (+)-(-)-pinoresinol, and (+)-diasyringaresinol	[16]
	Negundins A and B were isolated along with (+)-diasyringaresinol, (+)-lyoniresinol, vitrofolal E	[17]
Essential oil	$\delta$ -Guaiene, guaia-3,7-dienecaryophyllene epoxide, ethyl-hexadecenoate, $\alpha$ -selinene, germacren-4-ol; caryophyllene epoxide, (E)-nerolidol, $\beta$ -selinene, $\alpha$ -cedrene, germacrene D, hexadecanoic acid, <i>p</i> -cymene and valencene	[32]

[16]. Furthermore, Malik and team found the cytotoxicity of a crude methanolic extract of *V. negundo* against shrimp [17]. On the other hand, insecticidal, ovicidal, growth inhibitory activity of acetone extract of VN against a noxious lepidopteron insect-pest was demonstrated by Prajapati et al. [18]. In vivo studies have demonstrated xanthine oxidase inhibitory activity and hypouricemic activity of the methanolic and water extracts against potassium oxonate-induced hyperuricemic in mice [19]. Antigenotoxic, antihistamine, and CNS depressant potential was also reported from leaves of *V. negundo*. Moreover, silver nanoparticles (Ag-NPs) were synthesized and used as reductant and stabilizing agents extracted from the leaf extract of *V. negundo* [20]. The medicinal value of different extracts of *V. trifolia* L. comprises of methanolic root extract with anti-snake venom tendency [21]. Ethanolic extracts of leaves of *V. negundo* is reported to induce apoptosis in human breast cancer cell line (MCF-7) [22] and revealed presence of flavone glycoside with antimicrobial activity, and antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* [23]. In another study, petroleum ether and ethanol extracts of *V. trifolia* leaves showed inhibitory activity against both Gram+ and Gram– bacteria [24]. The water and ethanol extracts of *Vitex* sp. are reported to have a role in the treatment of AIDS with ~90% inhibitory activity against HIV type 1 reverse transcriptase [25].

### Role of bioactive constituents of *V. negundo*

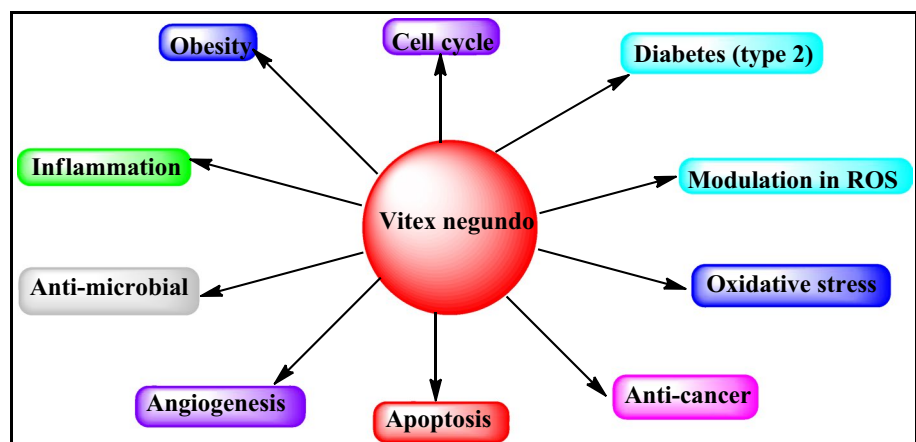
The multi-dimensional role of ingredients present in *Vitex* makes it a versatile natural product in the signaling pathways (Fig. 1). However, their target molecules and intrinsic role in specific signaling pathways remains unexplored. Current chemotherapies and drug approaches are facing pitfalls like resistance, selectivity, and toxicity and urgently necessitate new scaffolds and new drug designing approaches. In this context, *V. negundo* may provide a new and suitable

structural moiety as a suitable scaffold to the pharmaceutical industry [33]. The various bioactive compounds play important role in the functioning of the cell.

### Anti-inflammatory activity

Reactive oxygen species (ROS) and intrinsic anti-oxidants, are produced as a response to normal cell metabolism and a balance between the both are maintained in a normal state, but an imbalance resulting from the high concentration of ROS leads to oxidative stress [34]. VN has demonstrated commendable anti-oxidant activity and scavenges free radicals, as a consequence of altered gene expression in stress conditions. Oral administration of leaf extract of *V. negundo* claimed to be anti-inflammatory, analgesic, and antihistamine properties [35]. The fruit of VN has been proved to be effective in the treatment of reddened, painful, puffy eyes, and arthritic joints [36]. An oral intake of 100–150 ml juice in the empty stomach for 15 days is effective against pile diseases [37]. Vitexdoin A is a phenyl dihydro naphthalene-type lignin compound isolated from leaves, vitedoamine B (phenyl naphthalene-type lignan alkaloid) and phenyl naphthalene-type lignans; vitexdoins B-E were isolated from seeds of *V. negundo*. These compounds exhibit nitric oxide (NO) inhibiting activity in LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner [38]. Vitexin, flavonoids regulate anti-inflammatory effect by controlling the expression of recruitment of neutrophil and activation of macrophages. Vitexin reduces the leukocyte migration in vivo in RAW 264.7, reduce TNF- $\alpha$ , IL-1 $\beta$  and NO release in the peritoneal cavity of lipopolysaccharide-challenged mice. Vitexin also regulates the expression of transcriptional factors for pro-inflammatory mediators, reducing the expression of p-p38, p-ERK1/2 and p-JNK in LPS-elicited cells [39]. Lignin found in *V. negundi* has anti-inflammatory activity [40]. Leaf oil of VN is a powerful anti-inflammatory agent and inhibits COX-2 without interfering with COX-1

**Fig. 1** *Vitex negundo* and its various roles



pathways [41]. Casticin isolated from *V. rotundifolia* and *V. agnus-castus* exhibits an anti-inflammatory effect in vivo. C57BL/6 mice were (whole-body) exposed to mainstream cigarette smoke (CS) or fresh air for 2 weeks. Treatment with casticin inhibits neutrophils, macrophages, and lymphocytes and reduces the levels of proinflammatory cytokines and chemokines in the bronchoalveolar lavage fluid (BALF). Furthermore, casticin decreased epithelium thickness and the infiltration of peribronchial and perivascular inflammatory cells. This study indicates the importance of casticin in the lung inflammation induced by CS in a mouse model and thus provides an opportunity for the researcher to work in various lung diseases [42].

## Antioxidant

Antioxidants are the molecules which control free radical and their damage caused by them. Oxidative stress due to abnormal induction of ROS molecules is believed to be involved in the etiology of many diseases [43]. In a recent study, two coumarin glycosides vitexnegheteroins I-J were isolated along with two iridoid glycosides, vitexnegheteroins K-L, that exhibit strong antioxidant potential [44]. VN extract acts as a potent antioxidant to prevent ongoing thioacetamide (TAA) induced nephrotoxicity in rats, both biochemically and morphologically [45].

## Anti-microbial

Flavonoids were extracted from different parts of the selected plants *V. negundo*, and *Andrographis paniculata* (Nees) were effective against the late III or early IV instar larvae of *Aedes aegypti* and *Anopheles stephensi* [46]. In vitro and in vivo studies of water and methanol extracts exhibit the antibacterial potential of VN leaf extracts against diverse enteric pathogens. The methanolic extract of *V. negundo* leaves exhibits strong vibriocidal activity both in vitro and in vivo and may turn a good alternative to treat cholera [47]. Likewise, *V. simplicifolia* has been reported to possess trypanocidal activity as assayed on rat skeletal myoblast (L6) cells [48].

## Apoptosis

Natural products and their ingredients advance medical facilities by discovering and targeting new genes [49]. One of the important processes is apoptosis, comprising of various events leading to cell death and little aberration in its functioning consequences in dreadful diseases. In this respect, Vitex critically regulates apoptosis in any event of tumorigenesis, since cancer cells possess the innate ability

to control signal in such a way that enhances the probability of their survival and development. The unique morphology and various biochemical changes in the functioning of the cell culminates in apoptosis which is vital in cancer signaling [33]. Vitexin EVn-50 and its pure compound 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde induce apoptosis. It cleaves the poly ADP ribose polymerase protein and modulates the signaling cascade by upregulating Bax and downregulating Bcl-2 level. These events of induction of apoptosis are mediated by the activation of caspases, thus have a cytotoxic effect on breast, prostate, and ovarian cancer cells [50]. Vitexin 6 (VB6) also induces apoptosis and autophagy in cancer cells by cleavage of poly (ADP-ribose) polymerase and caspase-3. VB6 enhances the Jun N-terminal kinase (JNK) phosphorylation with upregulation of Bax and downregulation of Bcl-2 expression in time and concentration-dependent manner [51].

## Anticancer

Cancer is a multifactorial disease with uncontrolled cell growth and proliferation targeting immune system [52]. Dynamic nature and gene network indulged in cancer renders the researchers with the challenging task of designing therapeutic drugs [33]. Screening of several phytochemicals led to the isolation of flavonoids, schrysofenetin and chrysofenol D exhibiting effect against human pancreatic cancer (PANC-1). Furthermore, Chrysofenetin proved effective against 25 cancer cell lines of lung, breast, colon, melanoma, ovarian, prostate and stomach at the submicromolar range [53]. The chloroform-soluble extract of the leaves of *V. negundo* isolated the flavone vitexicarpin also exhibits anticancer property [54]. 5,3'-Dihydroxy-6,7,4'-trimethoxyflavanone (DHTMF), constituents of *V. rotundifolia* suppressed growth and induced apoptosis by decreasing expression of Bcl-2 and increasing Bax level and cleaved caspase-3 in lung carcinoma cells, in a dose-dependent manner. DHTMF treatment reduced the phosphorylation of Akt and mammalian target of rapamycin (mTOR), vascular endothelial growth factor (VEGF), hypoxia-inducible factor (HIF-1 $\alpha$ ) which are key proteins in angiogenesis. Along with this, DHTMF reduced CD34 expression, tube formation and migration in human umbilical vein endothelial cells (HUVECs). This study highlights the role of DHTMF in inhibition of angiogenesis as apoptosis via the Akt/mTOR pathway [55]. Similarly, vitexin compound 2 (VB2) inhibits proliferation and apoptosis in human choriocarcinoma JEG-3 cell lines by inhibiting the expression of mTOR and 4E-BP1 [56]. Even-50 also inhibits cell growth and induce apoptosis in MCF-7 and MCF-7/TAM-R cells by down-regulating

ERK1/2 phosphorylation in MAPK signal pathway and Akt signal pathway [57].

Casticin targets and suppresses NF- $\kappa$ B and MAPK signaling in lipopolysaccharide-stimulated mouse macrophages by inhibiting COX-2 and iNOS expression. Casticin from fruits of *V. rotundifolia* inhibits the nitric oxide and PGE<sub>2</sub>, decreased the level of interleukin, IL-6, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and thus, induces apoptosis. Along with this, casticin suppresses the expression of iNOS and COX-2 and increased HO-1 and Nrf2 production. Furthermore, casticin significantly inhibited NF- $\kappa$ B subunit p65 proteins in the nucleus and decreased Akt and MAPK activation [58].

## Motility and viability of sperms

Flavonoid-rich fraction (5,7,3'-trihydroxy, 6,8,4'-tri-methoxy flavones) isolated from the seeds of *V. negundo* antagonize the androgen action of exogenous testosterone of the male reproductive system in castrated prepubertal and intact adult dog. After treatment for 30 days (10 mg/kg), compound of interest causes disruption of spermatogenesis at latter stages. The content of protein, sialic acid, and RNA of the testes and epididymides were reduced and devoid of spermatozoa, while testicular cholesterol and phosphatase activity in testes and epididymides were elevated. Administration after treatment in intact adult and castrated prepubertal dogs increases the viability of spermatozoa, which kept the epididymal physiology normal and increased the cellular heights of epididymides [59]. The petroleum ether, chloroform, and methanol extracts in vitro studies reduce the motility and viability of sperms in male mice [60].

## Menstrual cycle

Female Guinea pigs were given a different treatment of *Vitex* tincture for 90 days. After treatment, *Vitex* reduces the level of estrogen and enhances progesterone levels in the pituitary gland. Along with this, the level of luteinizing hormone and prolactin hormones increases with a decrease in Follicle stimulating hormone (FSH) level secretion [68].

Traditionally, *Vitex* has been used to cure menstrual disorders and hormonal related problems. Irregularity in the functioning of menstrual cycle occurs due to inefficiency of corpus luteum and suboptimal ovarian function. This inefficiency may lead to the pre-menstrual syndrome, ovulatory cycle, polymenorrhea, secondary amenorrhoea, infertility and hyperprolactinemia [61]. A small amount of *Vitex* enhances the breast milk production in postpartum women, whereas high doses decrease the production of breast milk (Table 2). In addition, *Vitex* also affects the expression of neurotransmitter, dopamine [62]. Williamson and team reported in vitro dopaminergic activity of *V. negundo*, which modulates the effect of selegiline, amantadine, carbidopa, levodopa, pramipexole, ropinirole, bromocriptine, and pergolide medication. Thus scientific community can explore the *V. negundo* more in dopaminergic effects, which is responsible for prolactin-inhibition and role in numerous diseases [63]. Another species *V. agnus-castus* (VAC) targets the functioning of hypothalamus and pituitary gland, thus indirectly modulates the balance between different sex hormones. Mechanistically, in vivo studies find the evidence of interaction with dopaminergic receptors and subsequent reduction of prolactin secretion [64–66]. Different isolated compounds from *Vitex* named as vitexin due to structural similarity to the natural estrogen behaves like selective estrogen receptors modulators. Phytoestrogens found in the ethanolic extracts of *Vitex* species displayed the highest estrogenic-like activity and can be useful in hormone replacement therapy (HRT). This study provides the platform to the researcher to work more on alternative medicines for treating hormonal disorders such as hormone replacement therapy [67].

## Polycystic ovary syndrome (PCOS)

PCOS is a complex endocrine disorder with symptoms of polycystic ovaries, chronic anovulation, and hyperandrogenism which results in irregular menstrual cycles, acne, hirsutism, and infertility. Administration of extracts of *V. agnus-castus* results in reduced luteinizing hormone (LH), prolactin, fasting insulin and testosterone. Treatment

**Table 2** Effect of *Vitex negundo* on different diseases and symptoms

S. no	Disease	Symptoms	References
1	Premenstrual syndrome	Mood swings, sore breasts, fatigue in the body and restless	[68]
2	Breastfeeding	Increased milk flow and ease of milk release	[62]
3	Infertility	Disruption of spermatogenesis, epididymides elevated, reduction in androgen production	[59]
4	Hyperprolactinemia	Reduction of Prolactin release shortened luteal phases and deficits in progesterone production	[63]
5	Menopause	Hot flushes and irregularities in the menstrual cycle, mood savings depression	[67]
6	Acne	Acne	[69]

regulates the process of ovulation, improved metabolic hormone profile and fertility [70].

## Tyrosine inhibitor

Lyoniresinol, lignans purified from the methanol extract of VN have tyrosinase inhibitory activity. Lyoniresinol down-regulates the expression of microphthalmia-associated transcription factor (MITF) and tyrosinase protein expression levels. Furthermore, it activates the phosphorylation of extracellular receptor kinase (ERK), which results in MITF protein degradation and suppression of tyrosinase activity. Thus, lyoniresinol can be used in dermatologic use as a skin lightening agent [71].

## Cell cycle

The cell cycle is regulated by multiple molecular pathways and regulated critically by CDKs and cyclin proteins [33]. Ethanol extract of Vitex has antioxidant and antiproliferative activity in HepG2 cells [72]. The root extract of Vitex contains alkaloids, saponins; flavonoids exhibits anti-filarial effect against *Brugia malayi* microfilariae [73]. Casticin, a poly methoxy flavone induces apoptotic cell death, with activation of FOXO3a (transcription factor). Casticin decreases the expression levels of FoxM1, survivin, and polo-like kinase 1 (PLK1), whereas increases the expression of p27KIP1. Casticin silenced the FOXO3a expression using siRNA, which increased FoxM1 expression levels and thus attenuated the process of apoptosis [74]. Isolated diterpene, nishindanol exhibits cytotoxicity against cancer cell lines by targeting the Hedgehog (Hh) signaling pathway. Vitetrifolin D inhibits the production of Hh-related protein (PTCH and BCL2). Electrophoresis mobility shift assay revealed that vitetrifolin D disrupted GLI1 binding on its DNA binding domain [75]. Six new polyoxygenated triterpenoids, cannabifolins A-F isolated from the leaves of *V. negundo* var. *cannabifolia* and found to possess anti-inflammatory property [76]. The anticancer effect of Lignan mixture EVn-50 and pure compound VB1 was analyzed by the expression based study of different proteins involved in the cell cycle, in cancer cells. Treatment with EVn-50 or VB1 causes the arrest in the MDA-MB-435 and SMMC-7721 cells at G2/M phase. Along with this, it also increases phosphorylation of Histone 3 at Ser10, Cdk1 at Tyr15 phosphorylation, cyclin B1 expression, and decreases the expression of Cdc25c. This study shows broad-spectrum cytotoxic effect via arresting cancer cell cycle, which subsequently induces apoptosis [77].

## Oxidative stress

Various fractions of a hydroethanolic extract of the leaves of VN against ethanol-induced cerebral oxidative stress in rats. Administration of ethanol-induced stress elevated serum biochemical parameters like alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), triglycerides, uric acid, and lipoprotein levels. Along with this, there was an increase in malondialdehyde (MDA) and lipid hydroperoxide (LH) levels and a decrease in enzymatic and non-enzymatic antioxidants in the brain tissue. This study suggests that fractions of VN offer significant protection against ethanol toxicity in rat brain, which may be attributed to its anti-oxidant potential [78].

## Anti-diabetic effect

The leaves extract of *V. negundo* has a productive effect on glycoprotein metabolism in addition to its antidiabetic effect. The anti-hyperglycemic effect of iridoid glucoside is comparable with glibenclamide [79]. Vitexin from the seed of *V. negundo* is promising in combating hepatocellular carcinoma. Vitexin suppresses the proliferation of HepG2, Hep3B, Huh-7 cells and inhibits the anchorage-dependent and independent HepG2 cell growth by arresting cell cycle at G1/G0. Vitexin also reduces the secretion of VEGF which results in the inhibition of endothelial tube formation. Phosphorylation of Akt and FOXO3a were downregulated by treatment of vitexin in the HepG2 cells. Furthermore, knock-down of Akt1 by small interfering RNA (siRNA) enhanced growth inhibition, whereas silencing FOXO3a by siRNA decreases the level of expression [80]. Akt controls the downstream proteins such as mTOR which are a direct target of insulin signaling, thus forming the link between cancer and diabetes.

## Commercial products

In recent times, numerous products of *V. negundo* and their byproducts have undergone clinical tests and some of them have been commercialized. Several manufacturers were using *Vitex* as a major component or as an adjuvant to other molecules e.g. V-Gel, Liv. 52, Pilex, Acne-n-Pimple Cream, and Muscle and Joint Rub, Massage oil, Nigundi ghrita, Nirgundi Churna, Vitex women health, Relief cream, syrup, and tablets. The oil from the leaves of *Vitex* is used for relieving pains (Table 3) [81]. Combinatorial effect of water extract of *V. negundo* and matra basti as 500 mg tablets showed relief in sciatica pain, weakness, numbness, comforts from low back

**Table 3** Various companies and their products of *V. negundo*

S. no	Manufacturer	Name of product	Use
1	Phlemex fort	Syrup (leaf)	Asthma, cough
2	Himalayan drug corporation, Bangalore	Joint care B cream	Rheumatic disorder
		V Gel	Cervicitis, vaginitis
		Liv-52	Detoxify liver
		Pilex tablet	Hemorrhoids (piles)
		Acne-n-pimple cream	Acne and skin disorders
3	Hamdard laboratory, New Delhi	Jigrine	Liver capacity
4	IndSwift Limited, Chandigarh	Massage oil	Joint pain, gout, frozen shoulders
5	VHCA herbal, Haryana, India	Nigundi ghrita	Traumatic weakness
6	Vindhya herbal, Bhopal	Nirgundi Churna	Kidney pain
7	NOW food	Vitex women health	Maintain hormone balance
8	Surya herbal Ltd, Noida	Relief cream	Pain in muscles and joints
9	Pascual Labs, Philippines	Syrup and tablet	Cough, asthma

pain, herniated disk, piriformis syndrome, spinal stenosis in 119 patients of the age group ranging from 20 to 60 year [82]. Dopaminergic diterpenes compound isolation from *V. aganus castus* was effective in prolactin suppressive effect by treating premenstrual symptoms such as mastodynia. Premenstrual mastodynia is due to high level of hyperlactatemia in the stressful conditions, due to the high release of prolactin as compared to serum prolactin [83].

### Side effect

*Vitex negundo* is a common herb used in the commercialization of various products in the pharmaceutical industry. The acute and sub-chronic toxicity were verified by guidelines (402, 411) Organization for Economic Cooperation and Development (OECD). Oil treatment of VN on both sexes of Wistar of 2000 mg/kg body weight for acute dermal toxicity. On the other hand, rats were exposed to VN oil 250, 500 and 1000 mg/kg body weight, respectively, five times a week for 90 d for dermal sub-chronic toxicity study. Studies demonstrate that all animals were normal without any behavioral, serum biochemistry, hematology, necroscopical and histopathological changes. The globally harmonized system categorizes VN oil in 5 with an LD50 value of over 2000 mg/kg [84]. People with hormone-dependent abnormalities should not take *Vitex* like as endometriosis, uterine fibroids, and cancers. *Vitex* is also not recommended during pregnancy [85]. Recent studies showed that bioactive chromosome present in *Vitex* alleviate pain and inflammation [86].

### Conclusion

The natural products have immense tendency to target specific protein without side effect. *V. negundo* is used as a remedial agent against various diseases and their products

get commercialized which increased their demand. Acute and sub-chronic toxicity side effects were also reported but their benefits nullify these ill effects. Various bioactive compounds from *Vitex* modulates the signaling pathway in different metabolic activities. In addition to above, numerous protein are still unexplored with *Vitex* and its compounds. Future prospective in VN need isolation of specific bioactive compounds, which assists in drug designing and may provide platform or scaffold for synthesis of the drug. The computational studies will be employed for screening of isolated bioactive compounds which targets particular gene in a signaling pathway and further clinical trials will be carried out. Our research team is engaged in analyzing the anticancer property of various natural products.

**Acknowledgements** The authors are thankful to Central University of Punjab, India, for providing the necessary facilities to carry out this work.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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