2. REVIEW OF LITERATURE

_Vitex negundo_ is one of the widely distributed and abundant species regarding which very less work has been done on quantification of secondary metabolites. So far, no work has been reported in this respect from Himachal Pradesh. However, some of the literature citing important medicinal and other beneficial properties of _Vitex negundo_ and its two phytochemicals _viz._ Vitexin and Quercetin is reviewed here under following headings:

2.1 Anti-inflammatory activity
2.2 Anti-oxidant activity
2.3 Anti-diabetic potential
2.4 Anti-microbial activity
2.5 Enzyme-inhibitory activity
2.6 Effect on reproductive potential
2.7 Histomorphological and cytotoxic effects
2.8 Drug potentiating ability
2.9 Other attributes
2.10 Literature on Vitexin
2.11 Literature on Quercetin

2.1 Anti-inflammatory activity

Yunos _et al._ (2005) and Jana _et al._ (1999) established the anti-inflammatory properties of _V. negundo_ extracts in acute and sub-acute inflammation. Anti-inflammatory and pain suppressing activities of fresh leaves of _V. negundo_ are...
attributed to prostaglandin synthesis inhibition (Telang et al. 1999), antihistamine, membrane stabilising and antioxidant activities (Dharmasiri et al. 2003). Kulkarni et al. (2008) standardized the total methanolic extract of the plant in terms of total polyphenols and evaluated its anti-inflammatory activity by carrageenan-induced rat paw edema method. They suggested that radical quenching may be one of the mechanisms responsible for anti-inflammatory activity of Vitex negundo.

2.2 Anti-oxidant activity

The polar fractions of V. negundo possess potent antioxidant properties which may be mediated through direct trapping of the free radicals and also through metal chelation (Tiwari and Tripathi, 2007). They confirmed the observations of Kulkarni et al. (2008) that the anti-inflammatory property of Vitex negundo could be through the down regulation of the free radical mediated pathway of inflammation. Devi et al. (2007) reported that the extracts were useful in decreasing levels of superoxide dismutase, catalase and glutathione peroxidase in Freund’s adjuvant induced arthritic-rats. The extracts also possess the ability to combat oxidative stress by reducing lipid peroxidation owing to the presence of flavones, vitamin C and carotene (Vishal and Gupta, 2005). Rooban et al. (2009) evaluated the antioxidant and therapeutic potential of Vitex negundo flavonoids in modulating solenoid-induced cataract and found it to be effective. Nagarsekar et al. (2011) extracted ethanolic and super critical fluid extract from leaves of Vitex negundo and studied the Free radical scavenging activity of both extracts by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. From the results they observed that the ethanolic extract had stronger reducing potential and ability to scavenge free radicals as compared to the supercritical fluid extract. Guha et al. (2010) investigated the phenolic constituents of methanolic and aqueous extracts of Vitex negundo in context of antioxidant potential and inhibition of oxidative stress-induced cytotoxicity. They concluded that both V. negundo extracts hold considerable potential as antitheses of free radical toxicity by virtue of their polyphenolic constituents, and might have significant clinical roles in prospect.
2.3 Anti-diabetic potential

Villasenor and Lamadrid (2006) validated the ethnobotanical use of the leaves of six medicinal plants including *Vitex negundo* as anti-diabetic agents using the oral glucose tolerance test. They observed that only the bark of *Syzygium cumini*, leaves of *Vitex negundo* and *Eucalyptus tereticornis* exhibited antihyperglycemic activities when fed simultaneously with glucose. *Vitex negundo* leaf extract exhibited greater anti-hyperglycemic activity than *Eucalyptus tereticornis* in *Syzygium cumini*-treated mice attributed to a significant decrease in blood glucose levels. Sundaram *et al.* (2012) isolated an active compound of iridoid glucoside from *V. negundo* leaves and investigated its efficacy in streptozotocin induced diabetic rats with special reference to carbohydrate metabolizing enzymes. A significant decrease was observed in the activities of aspartate aminotransferase, alanine aminotransferase and decrease in the levels of serum urea and creatinine in diabetic treated rats when compared to diabetic untreated rats. Treatment of iridoid glucoside alleviated body weight loss in diabetic rats. The effect produced by iridoid glucoside on various parameters was comparable to that of glibenclamide. They concluded that iridoid glucoside possess antihyperlipidemic effect in addition to its antidiabetic effect.

2.4 Anti-microbial activity

vulgaris NCTC 8313, P. aeruginosa ATCC 27853, Psuedomonas putida ATCC 12842, Salmonella typhimurium ATCC 23564. They observed that extracts of root and bark gave intermediate anti-microbial activity when compared with high anti-microbial activity of flowers and leaves. The most susceptible gram positive bacteria was Bacillus cereus, while the most susceptible gram negative bacteria were K. pneumoniae, P. aeruginosa, P. putida. Among these bacteria P. vulgaris was resistant against all extracts of leaves and flowers. Panda et al. (2009) investigated the anti-microbial activity of petroleum ether, chloroform, ethanol, methanol and aqueous extracts from the leaves and bark of Vitex negundo against three gram positive bacteria, viz., B. subtilis, S. epidermidis, S. aureus and five gram negative bacteria, viz., E. coli, S. typhimurium, P. aeruginosa, Vibrio cholerae and Vibrio alginolyteus. Results showed promising anti-bacterial activity of all the extracts of both leaf and bark against E. coli, followed by S. aureus. Ethanolic and methanolic extracts of the leaves showed inhibitory action against both gram positive and gram negative bacteria, whereas petroleum ether and chloroform extracts of bark had better anti-bacterial activity against gram positive bacteria. Sathiamoorthy et al. (2007) isolated a new flavone i.e. glycoside from ethanolic extract of the leaves of V. negundo and found it to have significant anti-fungal activity against Trichophytone mentagrophytes and Cryptococcus neoformans.

2.5 Enzyme-inhibitory activity

Root extracts of Vitex negundo showed inhibitory activity against enzymes such as lipoxygenase and butyryl-cholinesterase (Azahar et al. 2004); α-chymotrypsin (Lodhi et al. 2008); xanthine-oxidase (Umamaheswari et al. 2007) and tyrosinase (Azhar et al. 2006). Woradulayapinij et al. (2005) reported the HIV type 1 reverse transcriptase inhibitory activity of the aqueous extract from aerial parts of V. negundo.
2.6 Effect on reproductive potential

The flavonoid rich fraction of seeds from *V. negundo* caused disruption of the latter stages of spermatogenesis in dogs (Bhargava, 1989) and interfered with male reproductive function in rats (Das *et al.* 2004). It must however be noted that these findings are in sharp contrast with the traditional use of *V. negundo* as aphrodisiac (Khare, 2004). Hu *et al.* (2007) determined that ethanolic extracts of *V. negundo* showed estrogen-like activity and propounded its use in hormone replacement therapy.

2.7 Histomorphological and cytotoxic effects

Tandon and Gupta (2004) studied the histomorphological effect of *Vitex negundo* extracts in rats and found the stomach tissue to be unaffected even by toxic doses; of *V. negundo* extract. while dose-dependent changes were observed in the heart, liver and lung tissues. Cytotoxic effect of leaf extracts of *V. negundo* was tested and affirmed using COLO-320 tumour cells (Smit *et al.* 1995). On one hand, Diaz *et al.* (2003) found chloroform extracts of *V. negundo* leaves to be toxic to a human cancer cell line panel while on the other; Yunos *et al.* (2005) reported that *V. negundo* extracts were non-cytotoxic on mammary and genito-urinary cells of mice.

2.8 Drug potentiating ability

Administration of *Vitex negundo* extracts potentiated the effect of commonly used sedative-hypnotic drugs like pentobarbitone, diazepam (Gupta *et al.* 1997) and chlorpromazine (Gupta *et al.* 1999). Tandon and Gupta (2005) studied interaction of *Vitex negundo* leaf extract with standard anti-inflammatory drugs in sub-effective doses to evaluate its potential role as an adjuvant therapy. They used carrageenin-induced hind paw oedema and cotton pellet granuloma test in albino rats.
for the study and found that the sub-effective dose of *Vitex negundo* leaf extract potentiated anti-inflammatory activity of phenylbutazone and ibuprofen significantly.

### 2.9 Other attributes

In addition to the above mentioned activities *Vitex negundo* extracts have also been tested for a range of other systemic effects. Leaf extracts of *V. negundo* were found to possess hepatoprotective activity against liver damage induced by d-galactosamine (Yang *et al.* 1987); Tandon *et al.* (2008) and Tasduq *et al.* (2008) reported that negundoside exerts a protective effect on CYP2E1-dependent CCl₄ toxicity via inhibition of lipid peroxidation, followed by an improved intracellular calcium homeostasis and inhibition of Ca²⁺-dependent proteases. Laxative activity of *Vitex negundo* leaf extracts was exhibited in rats by Adnaik *et al.* (2008). Immunomodulatory effect of *V. negundo* extracts has been reported by Ravishankar and Shukla (2007). Huang *et al.* (2012) conducted a study aimed at investigating the anti-melanogenic and antioxidative properties of the essential oil extracted from leaves of *V. negundo* and reported that essential oil decreased melanin production in B16F10 melanoma cells and showed potent antioxidant activities. *In vitro* osteogenic activity of *Allophysus serratus, Cissus quadrangularis* and *Vitex negundo* was reported by Kumar *et al.* (2010), they found that five out of the fourteen compounds isolated led to increase in osteoblast differentiation and mineralization. It was reported by Sahare *et al.* (2008) that root extract of *Vitex negundo* and leaf extract of *Aegle marmelos* at 100 ng/mL concentration showed complete loss of motility of *Brugia malayi* microfilariae after 48 hr of incubation. Zhenga *et al.* (2009) reported the analgesic properties of *Vitex negundo* seeds which are most likely to be mediated by its anti-inflammatory activity rather than through opioid receptor system. Mohd Abd Razak *et al.* (2014) studied the effect of selected local medicinal plants on the asexual blood stage of chloroquine resistant *Plasmodium falciparum*. They determined the antiplasmodial activities of 54 plant extracts from 14 species by *Plasmodium falciparum* Histidine Rich Protein II ELISA technique. In their study, they found that
twenty three extracts derived from *Curcuma zedoaria* (rhizome), *Curcuma aeruginosa* (rhizome), *Alpinia galanga* (rhizome), *Morinda elliptica* (leaf), *Curcuma mangga* (rhizome), *Elephantopus scaber* (leaf), *Vitex negundo* (leaf), *Brucea javanica* (leaf, root and seed), *Annona muricata* (leaf), *Cinnamomum iners* (leaf) and *Vernonia amygdalina* (leaf) showed promising antiplasmodial activities against the blood stage chloroquine resistant *P. falciparum* (*EC_{50} < 10 \mu g/ml*). Khan et al. (2015) explored the mechanisms underlying the effectiveness of *Vitex negundo* in hyperactive respiratory disorders. They found that crude extract of *V. negundo* leaves possesses a combination of papaverine-like PDE inhibitor and diltiazem-like Ca(++) entry blocking constituents, which partly explain its bronchodilatory effect.

### 2.10 Literature on Vitexin

Pharmacological properties of Vitexin had been reported from other plants but not from *Vitex negundo*. In order to develop a new anti-photoaging agent, Kim et al. (2005) focused on the antioxidant effects of the extract of tinged autumnal leaves of *Acer palmatum*. They isolated a compound from an ethyl acetate soluble fraction of the *A. palmatum* extract using silica gel column chromatography and identified the chemical structure as apigenin-8-C-beta-D-glucopyranoside, more commonly known as vitexin, by spectral analysis including LC-MS, FT-IR, UV, 1H-, and 13C-NMR. The biological activities of vitexin was investigated for the potential application of its anti-aging effects in the cosmetic field. Kim et al. (2005) found that Vitexin inhibited superoxide radicals by about 70% at a concentration of 100 \mu g/mL and DPPH radicals by about 60% at a concentration of 100 \mu g/mL. Intracellular reactive oxygen species (ROS) scavenging activity was indicated by increases in dichlorofluorescein (DCF) fluorescence upon exposure to UVB 20 mJ/cm² in cultured human dermal fibroblasts (HDFs) after the treatment of vitexin. Their results showed that oxidation of 5-(6-)chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) is inhibited by vitexin effectively and that vitexin has a potent free radical scavenging activity in UVB-irradiated HDFs. In ROS imaging using a confocal microscope they
visualized DCF fluorescence in HDFs directly. Kim et al. (2005) have suggested that vitexin can be effectively used for the prevention of UV-induced adverse skin reactions such as free radical production and skin cell damage.

Choi et al. (2006) carried out their study to investigate the effect of Vitexin on hypoxia-inducible factor-1a (HIF-1a) in rat pheochromacytoma (PC12), human osteosarcoma (HOS) and human hepatoma (HepG2) cells. They reported that Vitexin inhibited HIF-1a in PC12 cells, but not in HOS or HepG2 cells. In addition, it diminished the mRNA levels of hypoxia-inducible genes such as vascular endothelial growth factor (VEGF), smad3, aldolase A, enolase 1, and collagen type III in the PC12 cells. They also found that vitexin inhibited the migration of PC12 cells as well as their invasion rates, and it inhibited tube formation by human umbilical vein endothelium cells. Choi et al. (2006) suggested the potential use of vitexin as a treatment for diseases such as cancer.

The extracts and powder of Commelina communis L. are important food materials for prophylaxis against type 2 diabetes. Shibano et al. (2008) identified eleven flavonoid glycosides including vitexin from the aerial parts of C. communis. Their antioxidant activities were measured using in vitro assays employing the DPPH and superoxide radical-scavenging assays. Their results suggest that isoquercitrin, isorhamnetine-3-O-rutinoside, vitexin, and swertisin inhibited the activity of alpha-glucosidase from rat intestine.

Kim et al. (2010) investigated the adipogenesis inhibitory effect of Spirodela polyrhiza. The flavonoids were isolated from ethanolic extract of S. polyrhiza and their chemical structures were identified as chrysoeriol, apigenin, luteolin, vitexin, cosmosin, orientin and luteolin-7-O-β-d-glucoside by spectroscopic analysis. Studies on the adipogenesis and intracellular triglyceride accumulation inhibitory effect showed that vitexin and ornitin had the highest adipogenic activity in 3T3-L1 cells.
Choo et al. (2012) conducted a study to identify and evaluate bioactive constituents in the leaves of *Ficus deltoidea* with *in vivo* α-glucosidase inhibition. They subjected the partitioned extracts, subfractions and pure bioactive constituents to α-glucosidase inhibition assay. The identified bio-active constituents were administered orally to sucrose loaded normoglycemic mice and induced diabetic rats. The percentage of postprandial blood glucose reduction was highest in sucrose loaded induced diabetic rats administered orally with 200 mg/kg of vitexin or 100 mg/kg of isovitexin. Both vitexin and isovitexin did not exert any signs of toxicity at the highest dose of 2 g/kg administered orally to normoglycemic mice and induced diabetic rats. Choo et al. (2012) concluded that both the C-glycosyl bioflavonoids, namely, vitexin and isovitexin exhibited *in vivo* α-glucosidase inhibition.

Lee et al. (2012) determined the anticancer effects and molecular mechanisms of vitexin on U937 leukemia cells. They showed that vitexin can potently induce programmed cell death in U937 leukemia cell growth as well as morphological changes that were examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and phase contrast microscopy, respectively. The DNA content and the levels of mitochondrial membrane potential (∆Ψm) were determined by flow cytometric analysis. The cell cycle arrest-regulated and apoptosis-associated protein levels were measured by western blotting. Lee et al. (2012) observed that vitexin-triggered apoptosis was accompanied by a decrease of the level of mitochondrial membrane potential and the percentage of viability and provoked apoptosis in U937 cells. The downregulation of the protein level for Bcl-2 with the simultaneous upregulation of caspase-3 and -9 protein expression in U937 cells were observed after treatment with vitexin. They suggested their data provides a potential mechanism for the chemopreventive activity of vitexin, and vitexin may serve as a therapeutic agent for the treatment of human leukemia.

Yang et al. (2013) investigated the possible existence of p53-dependent pathway underlying vitexin-induced metastasis and apoptosis in human oral cancer cells, OC2 cells. They reported that vitexin decreased cell viability significantly.
Meanwhile, the expression of tumor suppressor p53 and a small group of its downstream genes, p21(WAF1) and Bax, were upregulated.

2.11 Literature on Quercetin

Pharmacological properties of Quercetin had been reported from other plants but not from *Vitex negundo*. Thangasamy *et al.* (2010) have demonstrated that the bioflavonoid Quercetin promoted a p53-mediated response and sensitized melanoma to Dacarbazine (DTIC). In this study they demonstrated that quercetin also sensitizes cells to Temozolomide (TMZ) and proposed a mechanism that involves the modulation of a truncated p53 family member, ΔNp73. Quercetin treatment in combination with TMZ abolished drug insensitivity and caused a more than additive induction of apoptosis compared to either treatment alone. Treatment with quercetin caused redistribution of ΔNp73 into the cytoplasm and nucleus, which has been associated with increased p53 transcriptional activity.

Kim *et al.* (2013) reported that quercetin induces mitochondrial mediated apoptosis and protective autophagy in human glioblastoma U373MG cells. Quercetin is a dietary flavonoid with known antitumor effects against several types of cancers by promoting apoptotic cell death and inducing cell cycle arrest. However, U373MG malignant glioma cells expressing mutant p53 are resistant to a 24 h quercetin treatment. Kim *et al.* (2013) re-evaluated the anticancer effect of quercetin in U373MG cells, and they found that quercetin was significantly effective in inhibiting proliferation of U373MG cells in a concentration-dependent manner after 48 and 72 h of incubation.

Tangsaengvit *et al.* (2013) isolated quercetin for the first time from ethyl acetate extract of *Caesalpinia mimosoides* Lamk. They determined the antioxidant capacity in terms of radical scavenging activity of quercetin as IC50 of 3.18 ± 0.07 μg/mL, which was higher than that of Trolox and ascorbic acid (12.54 ± 0.89 and
10.52 ± 0.48 μg/mL, resp.). The suppressive effect of quercetin on both purified and cellular acetylcholinesterase (AChE) enzymes was found to be as IC50 56.84 ± 2.64 and 36.60 ± 2.78 μg/mL, respectively. They concluded that, quercetin at a very low dose of 1 nM enhanced survival and induced neurite outgrowth of P19-derived neurons and is a promising valuable drug candidate for the treatment of neurodegenerative disease.

Vidhya and Indira (2009) investigated the protective effects of quercetin on chronic ethanol-induced liver injury. They treated rats with ethanol at a dose of 4 g/100g/day for 90 days. Increased amounts of lipid peroxidation products viz. hydroperoxides, conjugated dienes and malodialdehyde were observed on ethanol intoxication. Ethanol administration resulted in significant decrease in liver glutathione content. Vidhya and Indira (2009) reported that the changes in enzyme activities as well as levels of lipid peroxidation products were reversed to a certain extent by quercetin. Quercetin supplementation resulted in increase of glutathione content to a significant level. This study shows the protective effect of quercetin against chronic ethanol induced hepatotoxicity.

Liu et al. (2012) evaluated the effect of quercetin on myocardial oxidative stress and immunity function impairment induced by isoproterenol in rats. To induce myocardial ischemia, Wistar rats were subcutaneously injected with isoproterenol (70 mg/kg). The rats administrated with isoproterenol showed the declines in myocardial antioxidant enzymes activities. Administration of quercetin significantly ameliorated myocardial oxidative injury and immunity function impairment induced by isoproterenol. Liu et al. (2012) reported that quercetin possesses activity against isoproterenol-induced myocardial oxidative injury and immunity function impairment, and that the mechanism of pharmacological action was related at least in part to the antioxidant activity of quercetin.