



LOCAL ANAESTHETIC AND ANTIDEPRESSANT ACTIVITIES OF ETHANOL EXTRACT OF *VITEX NEGUNDO* LEAVES

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ABSTRACT

The objective of the present study was to evaluate the local anaesthetic and antidepressant activities of ethanol extract of *Vitex negundo* (VN) leaves as well its interaction with conventional local anaesthetic and antidepressant drugs using surface anaesthesia and locomotor activity in guinea pig and albino rats. The animals were divided in to four groups and administered the standard drug, control (saline) and test extract of *vitex negundo*. In surface anaesthesia, the guinea pig is used as the animal choice by using pricking method and administering the standard drug (Lignocaine 0.2%) by intradermal route. In antidepressant test of extract of VN (low dose-200 mg/kg; high dose-400 mg/kg), activity, the albino rat is used as the animal choice by

using actophotometer for locomotor activity and administering the standard drug CPZ by intraperitoneal route.

KEYWORDS: VN-*Vitex negundo*; CPZ-Chlorpromazine.

Medicinal Plants-Introduction

Morgenstern^[1] defines ethnobotany as the study of the indigenous uses of plants and the relationship between people and plants. Folk medicines of almost all civilizations of the world abound in herbal remedies. Majority of the traditional medicines used in healthcare are obtained from plants.^[2] In spite of several advancements in the field of synthetic drug chemistry and antibiotics, plants continue to be one of the major raw materials for drugs

treating various ailments of humans. Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action in human and animal systems.^[3] The world is gradually turning to herbal formulations which are known to be effective against a large repertoire of diseases and ailments. More importantly, they are not known to cause any notable derogatory effects,^[4] and are readily available at affordable prices.^[5] Prajapati *et al.*^[6], however, add a note of caution stating that plant remedies are effective and without side-effects, provided they are selected properly and taken under proper medical supervision. The active component, most often a secondary metabolite, varies in quality and quantity for a given plant species growing in different locations. The market value of such plants depends on their active content rather than merely their luxuriant growth. Purohit and Vyas^[7] reckon that close to 70,000 species of the plant kingdom have been used as herbal medicine at one time or other.

***Vitex negundo* Linn.- Introduction**

Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub growing to a small tree. It commonly bears tri- or penta-foliolate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. 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 the West Indies.^[8] Though *V. negundo* (will henceforth be referred to as Vn for sake of convenience) also finds use as a food crop (Facciola, 1990) and a source of timber^[9], this review deals only with the medicinal importance and other related attributes of the plant.

Literature Review

1. Anti-inflammatory and analgesic activity

Yunos *et al.*^[10] and Jana *et al.*^[11] established anti-inflammatory properties of *Vitex Negundo* extracts in acute and sub- acute inflammation. Anti-inflammatory and pain suppressing activities of fresh leaves of *Vitex Negundo* are attributed to prostaglandin synthesis inhibition^[12], antihistamine, membranestabilising and antioxidant activities.^[13]

2. Effect on oxidative stress

Leaf extracts of *Vitex Negundo* were determined to possess anti-oxidant potential by.^[14] The extracts were useful in decreasing levels of superoxide dismutase, catalase and glutathione

peroxidase in Freund's adjuvant induced arthritic-rats.^[15] The extracts also possess the ability to combat oxidative stress by reducing lipid peroxidation owing to the presence of flavones, vitamin C and carotene.^[16] Rooban et al.^[17] evaluated the antioxidant and therapeutic potential of Vitex Negundo flavonoids in modulating solenoid-induced cataract and found it to be effective.

3. Enzyme-inhibitory activity

Root extracts of Vitex Negundo showed inhibitory activity against enzymes such as lipoxygenase and butyryl-cholinesterase^[18]; α -chymotrypsin^[19]; xanthine-oxidase^[20] and tyrosinase.^[21] Woradulayapinij et al.^[22] reported the HIV type 1 reverse transcriptase inhibitory activity of the water extract of the aerial parts of Vitex Negundo.

4. Effect on reproductive potential

The flavonoid rich fraction of seeds of Vitex Negundo caused disruption of the latter stages of spermatogenesis in dogs^[23] and interfered with male reproductive function in rats.^[24] It must however be noted that these findings are in sharp contrast with the traditional use of Vitex Negundo as aphrodisiac.^[25] Hu et al.^[26] determined that ethanolic extracts of Vitex Negundo showed estrogen-like activity and propounded its use in hormone replacement therapy.

5. Histomorphological and cytotoxic effects

Tandon and Gupta^[27] 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.^[28] On one hand, Diaz et al.^[29] 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.^[10] reported that Vitex Negundo extracts were non-cytotoxic on mammary and genito-urinary cells of mice.

6. Drug potentiating ability

Administration of Vitex Negundo extracts potentiated the effect of commonly used anti-inflammatory drugs such as ibuprofen and phenylbutazone^[30]; analgesics such as meperidine, aspirin^[31], morphine and pethidine; sedative-hypnotic drugs like pentobarbitone, diazepam^[32] and chlorpromazine^[33]; anti-convulsive agents such diphenylhydantoin and valporic acid.^[34]

7. 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 Vitex Negundo were found to possess hepato-protective activity against liver damage induced by d- galactosamine^[35], commonly used tubercular drugs^[36] and carbon tetrachloride.^[37,38] Villasenor and Lamadrid^[39] have provided an account of the anti-hyperglycemic activity of Vitex Negundo leaf extracts.

Laxative activity of Vitex Negundo leaf extracts was exhibited in rats by Adnaik *et al.*^[40] Methanolic root extracts of Vitex Negundo showed antagonization of the lethal activity induced by venom of *Viperarussellii* and *Najakaouthia*.^[41] Immunomodulatory effect of Vitex Negundo extracts has been reported by Ravishankar and Shukla.^[42]

AIM OF WORK

The whole plant of Vitex negundo traditionally used for many diseases like stimulant, sialagogue, tooth ache and rubifacient properties. It is used the treatment of paralysis, hemiplegia, epilepsy and chronic ophthalmia. Our present study is to carryout phytochemical evaluation of the Ethanolic extract and also aimed to carry out the Local anasethetic and Anti-depressant activities of ethanolic extract of Vitex negundo.

SCHEME OF WORK

To extract the whole plant of Vitex negundo by using ethanol and identify components of various extract by means of phytochemical test and further evaluate the Local anasethetic and Anti-depressant activities of ethanolic extract by animal studies.

Plant Introduction

The plant of Vitex negundo is widely distributed in south and southeast Asia. The leaves are medically used.

Botanical Name : Vitex negundo.

Synonym : Vitex cannabifoliasiebold & Zucc, Vitex incise Lam.

Family : Lamiaceae.

Scientific Classification

Kingdom : Plantae.

Class : Eudicots.

Subclass : Asteridae.

Order	: Lamiales.
Family	: Verbenaceae.
Genus	: Vitex.
Species	: V.negundo.

Vernacular Names

Marathi	: Nirgunda.
Hindi	: Nirgundi.
Tamil	: Nochchi.
English	: Vitex, Five-leaved chaste tree.
Telugu	: Sindhuvara.

PLANT COLLECTION^[43]

The fresh leaves of *Vitex negundo* (Lamiaceae) were collected at the flowering stage in august from Anaikuttam, viradhunagar district, Tamilnadustate, India. It was authenticated and taxonomically identified by Dr.K. Linga Kumar, Associate Professor and Post Graduate Department of Botany, Ayya Nadar Janaki Ammal College, Sivakasi. The selected parts of the plant (leaves) were then dried in shade at temperature between 21-30° for 15 to 30 days, after which these parts were chopped and ground. Finally extraction was carried out by following procedure.



LEAVES OF VITEX NEGUNDO

Preparation of the extract^[44]

For the preparation of the extract about 200 g of air dried, powdered leaves were charged in to soxhlet's apparatus and successively extracted with 95% ethanol at room temperature for 7 days. Ethanol was removed from the extract and a semisolid mass was obtained. The yield of

ethanolic extract was subjected to preliminary phytochemical tests and pharmacological screening by using animal models like guinea pig and albino rat.

PRELIMINARY PHYTOCHEMICAL STUDIES^[45]

The ethanolic extract of whole plant of *Vitex negundo* were subjected to qualitative analysis to identify the presence of various phytochemical constituents like carbohydrate, glycosides, flavonoids, steroids, triterpenoids, tannins etc., in the crude extract.

PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACT OF VITEX NEGUNDO LEAVES

S.No	Phyto chemical constituents	DIFFERENT SOLVENTS		
		Petroleum Ether	Dichloromethane	Ethanol
1.	Alkaloids	-	+	+
2.	Steroids	+	+	+
3.	Triterpenoids	+	-	+
4.	Coumarins	-	-	-
5.	Flavonoids	+	+	+
6.	Amino acids	+	+	+
7.	Carbohydrates	+	+	+
8.	Phenols	+	+	+
9.	Starch	+	-	+
10.	Anthraquinone	+	+	+

PHARMACOLOGICAL EVALUATION-ANIMAL SCREENING^[46]

Animal studies

Albino rats (250-400 g) was used. The animals were maintained on the standard laboratory conditions (eight period of 12 h/day and temperature 27°C with access to food and water ad libitum. The experimental procedures were carried out in strict compliance with the IAEC regulations. The experiments were carried out according to CPCSEA guidelines. The animals were fasted overnight and then were administered with the ethanolic extract of *Vitex negundo* at the following doses: 200 mg/kg and 400 mg/kg by oral route. The locomotor activity can be easily studied by actophotometer by using albino rats and the local anaesthetic activity measured by pricking method by using guinea pig.

Pharmacological Screening of Local Anaesthetics

A local anesthetic (LA) is a medication that causes reversible absence of pain sensation, although other senses are often affected, as well. Also, when it is used on specific nerve pathways (local anesthetic nerve block), paralysis (loss of muscle power) also can be achieved.

Types of Local Anaesthetics^[47]

1. Conduction Anaesthesia.
2. Infiltration Anaesthesia.
3. Surface Anaesthesia.
4. Epidural Anaesthesia.
5. Spinal Anaesthesia.

LOCAL ANAESTHETIC ACTIVITY OF ETHANOL EXTRACT OF VITEX NEGUNDO LEAVES BY SURFACE ANAESTHESIA

ANIMALS

Male guinea pig weight ranging from 250-400gm were chosen. The animals were provided with standard pellet diet with free access to water ad libitum.

PREPARATION OF SAMPLE

Control : Saline.

Standard : Lignocaine (0.2%).

Test drugs

Ethanol extract of Vitex negundo: 200 mg/kg (low dose) and 400 mg/kg (high dose).

METHOD: PRICKING IN GUINEA PIG WHEELS (INTRA DERMAL).

Principle

Pricking on the skin produces a twitch or movement indicating that the pain is produced. Intradermal injection of local anaesthetic in the skin inhibits this response in the area where the injection is given.

Procedure

On the day preceding the experiment, two areas of 4.5 cm diameter are shaved. The sensitivity of the skin is greatest in the midline and slightly more so in the front than in the

back area. For this reason each concentration of a local anaesthetic must be tested in both areas. The doses (200 mg/kg and 400 mg/kg) of local anaesthetics are injected intracutaneously in 0.1 ml saline. The size of the wheal is marked with ink. One side is used for the test preparation, the other side for the standard. The reaction to pin prick is tested after 5 minutes after injection in the following way. After observing the animal's normal reaction to a prick applied outside the wheal, 6 pricks are applied inside the wheal and the number of pricks is counted to which the guinea pig fails to react. The pricks applied at intervals of about 3-5 seconds. 6 pricks are applied every 5 minutes for 30 minutes.

STATISTICAL ANALYSIS

The statistical analysis were carried out by student "t" test considered as significant.

LOCAL ANAESTHETIC ACTIVITY OF ETHANOL EXTRACT OF VITEX NEGUNDO LEAVES BY PRICKING METHOD											
CHOICE OF ANIMAL USED: GUINEA PIG											
ROUTE OF ADMINISTRATION: INTRA DERMAL											
Group	Drug	Weight of the Animal (g)	Dose Loaded Mg/kg	5 mts	10 mts	15 mts	20 mts	20 mts 25 mts	30 mts	Total No. of Failure (out of 36)	% Response
I	Standard Drug Lignocaine (0.2%)	250	0.5ml	0	0	0	0	1	2	3	91.67
		300	0.6ml	0	0	0	0	1	1	2	94.44
		325	0.65ml	0	0	0	1	1	1	3	91.67
		275	0.55ml	0	0	0	0	0	1	1	97.22
II	Normal Saline	500	1ml	6	6	6	6	6	6	36	0
		450	0.9ml	6	5	6	6	5	5	33	8.33
		300	0.6ml	6	6	5	5	6	6	34	5.56
		325	0.65ml	6	5	6	6	5	6	34	5.56
III	Vitex Negundo Test-200 mg/kg	275	0.55ml	2	2	1	0	0	0	5	86.11
		325	0.65ml	2	1	1	1	0	0	5	86.11
		350	0.7ml	2	2	1	1	1	0	6	83.33
		300	0.6ml	2	2	1	1	0	0	6	83.33
IV	Vitex Negundo Test 400mg/kg	250	1ml	3	3	2	1	1	1	11	69.44
		350	1.4ml	3	3	1	1	1	1	10	72.22
		450	1.8ml	3	2	2	2	1	1	11	69.44
		400	1.6ml	3	3	2	1	1	1	11	69.44

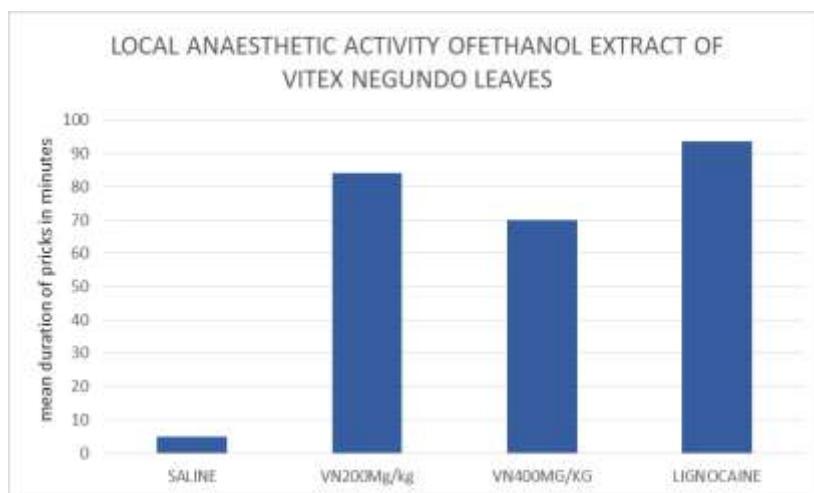


FIG.1: Effects of *VITEX NEGUNDO* (200, 400 mg/kg). Lignocaine (0.2%) on mean duration of pricks in minute's *sintra dermal surface anaesthesia*. Values are expressed as mean \pm n=4*as compared to vitex negundo (200 mg/kg) treated group as compared to vitex negundo (400mg/kg) treated group using Student T test.

Pharmacological evaluation of Anti-depressants^[46]

Depression is a major affective or mood disorder. It is characterized by extreme exaggerations and disturbances of mood. It may adversely affect cognition or psychomotor function. Antidepressants are drugs used for the treatment of major depressive disorder and other conditions, including dysthymia, anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, neuropathic pain and, in some cases, dysmenorrhea, snoring, migraine, attention-deficit hyperactivity disorder (ADHD), addiction, dependence, and sleep disorders.

ANTI DEPRESSANT ACTIVITY OF ETHANOL EXTRACT OF VITEX NEGUNDO LEAVES BY LOCOMOTOR ACTIVITY USING ACCTOPHOTOMETER

ANIMALS

Male albino rat's weight ranging from 250-400gm were chosen. The animals were provided with standard pellet diet with free access to water ad libitum.

PREPARATION OF SAMPLE

Control : Saline.

Standard : Chlorpromazine.

Test drugs

Ethanol extract of *Vitex negundo*: 200 mg/kg (low dose) and 400 mg/kg (high dose).

METHOD: LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER**Principle**

Most of the central nervous system acting drugs influence the locomotor activities in man and animals. The CNS depressant drugs such as barbiturates and alcohol reduce the motor activity while the stimulants such as caffeine and amphetamines increase the activity. In other words, the locomotor activity can be an index of wakefulness (alertness) of mental activity. The locomotor activity (horizontal activity) can be easily measured using an actophotometer which operates on photoelectric cells which are connected in circuit with a counter. When the beam of light falling on the photocell is cut off by the animal, a count is recorded. An actophotometer could have either circular or square arena in which the animal moves. Both rats and mice may be used for testing in this equipment.

Procedure

Weigh the animals and number them. Turn on the equipment (check and make sure that all the photo cells are working for accurate reading) and place individually each rat in the activity cage for 10 minutes. Note the basal activity score of all the animals. Inject chlorpromazine (1 ml/100 g), and after 30 min re-test each rat for activity scores for 10 min. Note the difference in the activity, before and after chlorpromazine. Calculate percent decrease in motor activity indicates CNS depressant property of the drug.

ANTI DEPRESSANT ACTIVITY OF ETHANOL EXTRACT OF VITEX NEGUNDO LEAVES BY USING ACTOPHOTOMETER CHOICE OF ANIMAL USED: ALBINO RAT ROUTE OF ADMINISTRATION: STANDARD (I.P) & TEST (ORAL)						
Group	Body weight	Drug	Dose (mg/kg)	Before treatment	After treatment	% change in activity
I						
1	80	SALINE (I.P)	0.8	72	70	2.78
2	75		0.75	74	72	2.78
3	125		1.25	80	82	2.5
4	150		1.50	81	80	1.23
II						
1	100	Chlorpromazine (I.P)	0.12	109	25	77.06
2	110		0.13	120	24	80
3	120		0.144	130	23	82.31
4	150		0.18	125	20	84
III						
1	100	Vitex Negundo Test-200mg/kg (ORAL)	0.5	157	117	25.48
2	100		0.5	160	120	25
3	100		0.5	170	130	23.53
4	120		0.6	127	101	20.47

IV						
1	125	Vitex Negundo Test-400 mg/kg (ORAL)	1.25	132	90	31.82
2	125		1.25	140	92	34.29
3	110		1.10	170	80	52.94
4	120		1.20	120	70	41.67

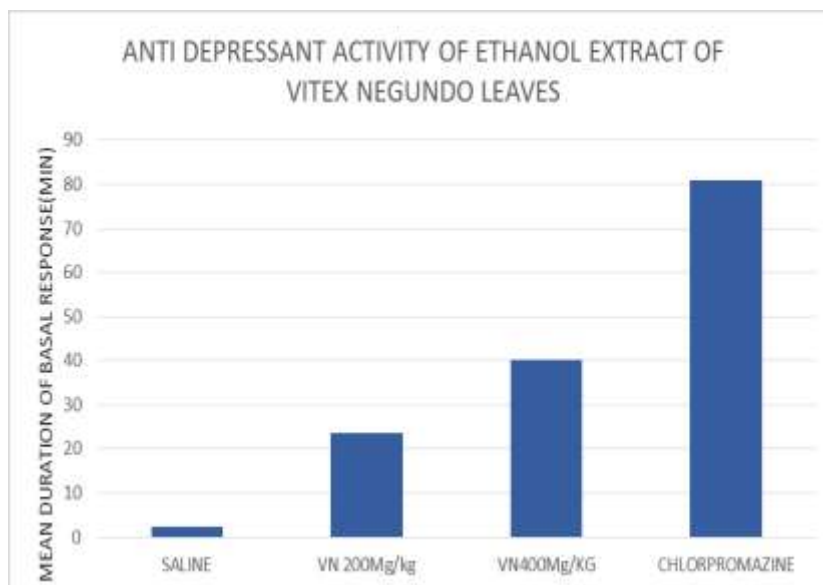


FIG.2: Effects of *VITEX NEGUNDO* (200, 400 mg/kg). Chlorpromazine (3mg/kg) on mean duration of basal response (in minutes) in Actophotometer. Values are expressed as mean \pm as compared to vitex negundo (200mg/kg) treated group as compared to vitex negundo (400mg/kg) treated group using Student T test.

CONCLUSION

The preliminary phytochemical analysis of ethanolic extract of leaves of *VITEX NEGUNDO* revealed the presence of carbohydrates, alkaloids and flavonoids.

Results of the present study showed that ethanolic extract of leaves of vitex negundo at the dose of 200mg/kg reflects significant local anaesthetic activity when compared to high dose (400 mg/kg) by using pricking technique in guinea pig by intradermal method. The depressant activity of vitex negundo showed at a dose of 400 mg/kg when compared to low dose (200 mg/kg). Finally there is a reduction in depressant activity when compared to standard. Hence Vitex negundo may be served as a potential resource for anaesthesia and depression.

REFERENCES

1. Morgenstern, K. (2001) 'What is ethnobotany?', 1st February, 2009.
2. Kala, C.P., Farooquee, N.A. and Dhar, U. 'Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India', *Biodiversity and Conservation*, 2004; 13: 453-469.
3. Dutta, S.C. *Medicinal Plants*, National Council for Education Research and Training, New Delhi, 1973.
4. Kirtikar, K.R. and Basu, B.D. *Indian Medicinal Plants*, Bishen Singh Mahendra Pal Singh, Dehradun, 1984.
5. Sharma, A., Shanker, C., Tyagi, L.K., Singh, M. and Rao, C.V. 'Herbal medicine for market potential in India: An overview', *Academic Journal of Plant Sciences*, 2008; 1: 26-36.
6. Prajapati, D.S., Purohit, S.S., Sharma, A.K. and Kumar, T. *A Handbook of medicinal plants*, Agrobios India, Jodhpur, 2004.
7. Purohit, S. and Vyas, S. 'Medicinal plant cultivation: A scientific approach', Agrobios, India, 2004.
8. de Padua, L.S., Bunyaphatsara, N. and Lemmens, R.H.M.J. *Medicinal and Poisonous Plants, Plant Resources of South East Asia*, Backhuys Publishers, Leiden, 1999.
9. Jabeen, A., Khan, M., Ahmad, M., Zafar, M. and Ahmad, F. 'Indigenous uses of economically important flora of Margallah Hills National Park, Islamabad, Pakistan', *African Journal of Biotechnology*, 2009; 8: 763-784.
10. Yunus, N.M., Mat Ali, R., Kean, O.B. and Abas, R. 'Cytotoxicity Evaluations on Vitex negundo Anti-inflammatory Extracts', *Malaysian Journal of Science*, 2005; 24: 213-217.
11. Jana, U., Chattopadhyay, R.N. and Shaw, B.P. 'Preliminary studies on anti-inflammatory activity of *Zingiber officinale* Rosc., *Vitex negundo* Linn. And *Tinosporacordifolia* (Willid) Miers in albino rats', *Indian journal of pharmacology*, 1999; 31: 232-233.
12. Telang, R.S., Chatterjee, S. and Varshneya, C. 'Studies on analgesic and anti-inflammatory activities of *Vitex negundo* Linn.', *Indian journal of pharmacology*, 1999; 31: 363-366.
13. Dharmasiri, M.G., Jayakody, J.R.A.C., Galhena, G., Liyanage, S.S.P. and Ratnasooriya, W.D. 'Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*', *Journal of Ethnopharmacology*, 2003; 87: 199-206.
14. Tiwari, O.P. and Tripathi, Y.B. 'Antioxidant properties of different fractions of *Vitex negundo* Linn', *Food Chemistry*, 2007; 100: 1170-1176.

15. Devi, P.R., Kumari, S.K. and Kokilavani, C. 'Effect of Vitex negundo leaf extract on the free radicals scavengers in complete Freund's adjuvant induced arthritic rats', *Indian Journal of Clinical Biochemistry*, 2007; 22: 143-147.
16. Vishal, T. and Gupta, R.K. 'Effect of Vitex negundo on oxidative stress', *Indian journal of pharmacology*, 2005; 37.
17. Rooban, B., Lija, Y., Biju, P., Sasikala, V., Sahasranamam, V. and Abraham, A. 'Vitex negundo attenuates calpain activation and cataractogenesis in selenite models', *Experimental Eye Research*, 2009; 88: 575.
18. Lodhi, A., Choudhary, I., Malik, A. and Ahmad, S. ' α -Chymotrypsin inhibition studies on the lignans from Vitex negundo Linn', *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2008; 23: 400-405.
19. Azhar-Ul-Haq, Malik, A., Anis, I., Khan, S.B., Ahmed, E., Ahmed, Z., Nawaz, S.A. and I., C.M. 'Enzyme inhibiting lignans from Vitex negundo', *Chemical and Pharmaceutical Bulletin*, 2004; 52: 1269-1272.
20. Umamaheswari, M., AsokKumar, K., Somasundaram, A., Sivashanmugam, T., Subhadradevi, V. and Ravi, T.K. 'Xanthine oxidase inhibitory activity of some Indian medical plants', *Journal of Ethnopharmacology*, 2007; 109: 547-551.
21. Azhar-Ul-Haq, Malik, A., Khan, M.T.H., Khan, S.B., Anwar-Ul-Haq, Ahmad, A. and Choudhary, M.I. 'Tyrosinase inhibitory lignans from the methanol extract of the roots of Vitex negundo Linn. and their structure-activity relationship', *Phytomedicine*, 2006; 13: 255-260.
22. Woradulayapinij, W., Soonthornchareonnon, N. and Wiwat, C. 'In vitro HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and Canna indica L. rhizomes', *Journal of Ethnopharmacology*, 2005; 101: 84-89.
23. Bhargava, S. 'Antiandrogenic effects of a flavonoid-rich fraction of Vitex negundo seeds: A histological and biochemical study in dogs', *Journal of Ethnopharmacology*, 1989; 27: 327-339.
24. Das, S., Parveen, S., Kundra, C.P. and Pereira, B.M.J. 'Reproduction in male rats is vulnerable to treatment with the flavonoid-rich seed extracts of Vitex negundo', *Phytotherapy Research*, 2004; 18: 8-13.
25. Hu, Y., Zhang, Q., Hou, T., Xin, H., Zheng, H., Rahman, K. and Qin, L. 'Estrogen-like activities in Vitex species from China determined by a cell based proliferation assay', *Pharmazie*, 2007; 62: 872-875.
26. Khare, C.P. *Encyclopedia of Indian Medicinal Plants*, Springer, Berlin, 2004.

27. Tandon, V. and Gupta, R.K. 'Histomorphological changes induced by *Vitex negundo* in albino rats', *Indian journal of pharmacology*, 2004; 36: 176-177.
28. Smit, H.F., Woerdenbag, H.J., Singh, R.H., Meulenbeld, G.J., Labadie, R.P. and Zwaving, J.H. 'Ayurvedic herbal drugs with possible cytostatic activity', *Journal of Ethnopharmacology*, 1995; 47: 75-84.
29. Diaz, F., Chavez, D., Lee, D., Mi, Q., Chai, H.B., Tan, G.T., Kardono, L.B.S., Riswan, S., Fairchild, C.R. and Wild, R. 'Cytotoxic flavone analogues of vitexicarpin, a constituent of the leaves of *Vitex negundo*', *Journal of Natural Products*, 2003; 66: 865-867.
30. Tandon, V.R. and Gupta, R.K. 'Anti-inflammatory Activity and Mechanism of Action of *Vitex negundo* Linn', *International Journal of Pharmacology*, 2006; 2: 303-308.
31. Gupta, R.K. and Tandon, V.R. 'An experimental evaluation of anticonvulsant activity of *Vitex negundo*', *Indian Journal of Physiology and Pharmacology*, 49: 163-172.
32. Gupta, M., Mazumder, U.K., Bhawal, S.R. and Swamy, S.M.K. 'CNS activity of petroleum ether extract of *Vitex negundo* Linn in mice', *Indian Journal of Pharmaceutical Sciences*, 1997; 59: 240-245.
33. Gupta, M., Mazumder, U.K. and Bhawal, S.R. 'CNS activity of *Vitex negundo* Linn. in mice', *Indian Journal of Experimental Biology*, 1999; 37: 143-146.
34. Tandon, V.R. and Gupta, R.K. 'An experimental evaluation of anticonvulsant activity of *Vitex- negundo*', *Indian Journal of Physiology and Pharmacology*, 2005; 49: 199-205.
35. Yang, L., Yen, K., Kiso, Y. and Hikino, H. 'Antihepatotoxic actions of formosan plant drugs', *Journal of Ethnopharmacology*, 1987; 19: 103-110.
36. Tandon, V.R., Khajuria, V., Kapoor, B., Kour, D. and Gupta, S. 'Hepatoprotective activity of *Vitex negundo* leaf extract against anti-tubercular drugs induced hepatotoxicity', *Fitoterapia*, 2008; 79: 533-538.
37. Tasduq, S.A., Kaiser, P.J., Gupta, B.D., Gupta, V.K. and Johri, R.K. 'Negundoside, an irridiod glycoside from leaves of *Vitex negundo*, protects human liver cells against calcium-mediated toxicity induced by carbon tetrachloride', *World Journal of Gastroenterology*, 2008; 14: 3693-3709.
38. Raj, P.V., Chandrasekhar, H.R., Vijayan, P., Dhanaraj, S.A., Rao, C.M., Rao, J.V. and Nitesh, K. 'In vitro and in vivo hepatoprotective effect of *Vitex negundo* leaves', *Pharmacology Online*, 2008; 3: 281-295.
39. Villaseñor, I.M. and Lamadrid, M.R.A. 'Comparative anti-hyperglycemic potentials of medicinal plants', *Journal of Ethnopharmacology*, 2006; 104: 129-131.

40. Adnaik, R.S., Pai, P.T., Mule, S.N., Naikwade, N.S. and Magdum, C.S. 'Laxative Activity of *Vitex negundo* Linn. Leaves', *Asian Journal of Experimental Sciences*, 2008; 22: 159-160.
41. Alam, M.I. and Gomes, A. 'Snake venom neutralization by Indian medicinal plants (*Vitex negundo* and *Emblicaofficinalis*) root extracts', *Journal of Ethnopharmacology*, 2003; 86: 75-80.
42. Ravishankar, B. and Shukla, V. 'Indian Systems of Medicine: A Brief Profile', *African Journal of Traditional, Complementary and Alternative Medicines*, 2007; 4: 319-337.
43. *Indian J. Physiol. Pharmacol*, 2010; 54(4): 318-328.
44. *Indian J. Physiol. Pharmacol*, 2010; 54(4): 318-328.
45. Harborne J.B., *Phytochemical methods*, 2ND Edition Chapman and Hale Publisher.
46. R.K. Goyal., *Practicals in pharmacology*, 94 and 131-133
47. *Drug discovery and evaluation; pharmacological assays*, 3rd edition by H.gerhardvogel.