Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(2); 194-199

ISSN: 0975-4873

Research Article

Evaluation of Anti-Arthritic and Immunostimulatory Activities of Chloroform Extracts of Leaves of *Vitex negundo L.*

*Amrutha V. Audipudi[1], P.Hari Brahma Reddy[2], Madhuri Vajha[3], C.V.S Bhaskar[4]

[1,2]*Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur-522510, A.P., India.*
[3]*Departments of Biotechnology, Krishna University, Machilipatnum-521001 A.P India.*
[4]*Department of Botany V.R College, Nellore 523001A.P India.*

Available online: 1st June 2014

ABSTRACT

To search for anti-arthritic bioactive with immunostimulatory activities of chloroform extracts of *Vitex negundo L.* (VCE) were screened for the first time. Chloroform extracts of *V.negundo L.* leaves were screened for various photochemical constituents and in-vitro anti-arthritic activity along with other Immunostimulatory activities in terms of DPPH assay, %-5-Lipoxigenase assay, α-amylase assay and Brine Shrimp Lethality assay. Levels of Flavonoids and alkaloids of VCE were found to be significantly high. VCE showed 100% anti-arthritic activity at the concentration of100 µg/ml. Antioxidant activity was found to be significantly high (IC50 value of >100 µg/ml) when compared to Vitamin–C. Crude extracts showed greater value of anti-inflammatory activity (IC_{50} 19.26 µg/ml) and. α- amylase inhibitory activity (IC50 >100µg/ml) when compared to respective standards curcumin (IC50 9.38µg/ml) and Acarbose (IC50 9.88µg/ml). VCE showed moderate cytotoxicity activity (ED50 76.77µg/ml) when compared with standard Podophyllotoxin (ED 50 2.50µg/ml). Results of current investigation revealed that phytochemical constituents of VCE have strong anti-arthritic potential and further investigation may be advocated for the identification of lead molecule with pharmacological significance.

Key words: *Vitex negundo L.* anti-Arthritic activity, DPPH assay, α-amylase assay, Lipoxigenase, Cytotoxic activity

INTRODUCTION

Plants are reservoir of fairly innocuous and potentially useful chemical compounds which serve as drugs to cure all ailments of mankind. [1] Traditionally plants have been available as crude extracts, screening of such mixtures of substances as natural products provide newer leads and clues for modern drug design synthesis. Screening and isolation of active compound from the hit extract is the only practical alternative but considered to be uneconomical mainly because of high production costs. Moreover development of optimization technologies for refining compounds identified in such screen into successful drugs is in its infancy.[2,3] Such perfect example of medicinal plants credited with innumerable medicinal qualities and used since ancient time are *Vitex negundo L* . *Vitex negundo L.* commonly known as five-leaves chaste tree or monk's pepper (telugu–Vaavili). It is a woody aromatic shrub found throughout India [4]. One of the ancient uses of *Vitex negundo L.* documented in Ayurveda is to provide mental peace [5]. The plant has been reported to exhibit medicinal properties including the curing of rheumatic pains, reducing swelling of the joints, treatment of dengue, dyspepsia and diarrhea [6,7]. Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, antioxidant and hepatoprotective disorders[8,9] headaches, dizziness, convulsions, coughs, mental unrest and also said to promote wakefulness[10,11]. Leaves and roots possess anti-arthritic and analgesic activity [12]. Flowers are useful in cholera, fever, haemorrhages, hepatopathy and cardiac disorders [13, 14]. The dried fruit acts as a vermifuge, nervine, cephalic and emmenagogue [15, 16]. So far all the medical applications are in the form of Ayurveda formulations such as dried powder, bhasma, churna, khashaya (decoction) etc.

However very few investigation were carried out on chloroform extracts of *Vitex negundo L.* leaves for their biological activities such as anti-arthritic, antioxidant, anti-inflammatory, antimicrobial, anti- obesity and cytotoxicity. In present investigation Vitex *negundo L.* was focused for their phytochemical constituents and immunostimulating effects such as anti-arthritic and immunostimulatory (anti-oxidant, anti-inflammatory, anti-obesity) activities in order to search for possible new compound from chloroform extract. Leaves were used for extraction of compounds and variations in their bioactivity were compared.

MATERIALS AND METHODS

Plant collection and identification*:* Fresh leaves of the plants *Vitex negundo L* .were collected in the month August and September, 2012 from Acharya Nagarjuna University campus, Guntur district A.P. India. The taxonomic identity of the plant was confirmed by the Department of Botany, Acharya Nagarjuna University and

*Author for correspondence: E-mail: audipudi_amrita@yahoo.com

Table. 1: Phytochemical constituents of chloroform extracts of *V. negundo L.*

VCE	Color of extract	Alkaloids	Tannins	Phenols	Glycosides	Flavonoids	Saponins	Phytosterols
	green	-	-	++	-	+++	+	+

+++ = High amount, ++ = Relatively high amount, + = trace amount, - =Absent

Fig. 1: In-vitro anti-arthritic activity of chloroform extracts of Vitex negundo L. (VCE)

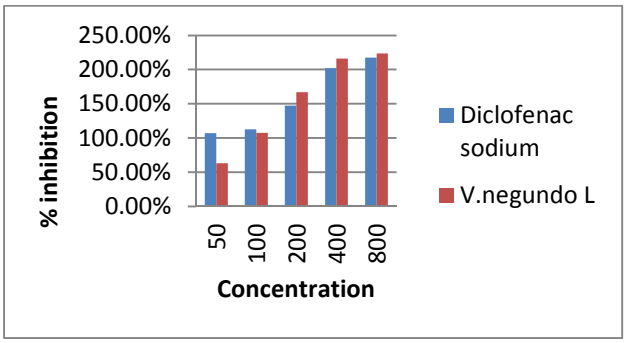

Fig. 2: In-vitro anti-arthritic activity by inhibition of Bovine serum albumin protein Denaturation of Vitex negundo L.

Guntur.

Extraction of the plants material: Leaves of *Vitex negundo L.* were dried at room temperature and crushed to fine powder in a mechanical grinder. 15 grams of powdered material was macerated with 150 ml of chloroform (polar solvents) and stored in an airtight conical flask for 48 hours. Plant extract was filtered through double layered muslin cloth and sterile Whatman No1filter paper.

Extraction was carried at 40-60ºC in soxhlet apparatus and then concentrated under reduced pressure by using rotary evaporator. Finally the extract was stored at 4 ºC until use.

Phytochemical screening: A portion of crude extract was subjected to preliminary qualitative screening of phytochemical such as alkaloids, saponins, Phytosterols, phenolics, tannins, flavonoids, steroids, terpinoids and cardiac glycoside [17].

In-vitro anti-arthritic activity: Anti-arthritic activity of the plant extract was studied by using in-vitro inhibition of albumin denaturation [18]. 5ml of reaction mixture consists of 0.2ml of egg albumin (from fresh hen's egg), 2.8ml phosphate buffered saline (p^H-6.4) and 2ml of extract. Different concentrations of extracts (50,100,200,400,800 µg /ml) were used for the determination of effective dose. 5ml of double distilled water served as control. The samples were initially incubated at 37^0C for 15min and at 70^0C for 5min. After cooling the sample to room temperature, absorption was measured at 660nm using UV visible Spectrophotometer (model). Different concentrations of Diclofenac Sodium (50,100,200,400,800 µg/ml) was used as reference drug .The percentage inhibition of protein denaturation can be calculated as

%f inhibition = 100 × [V t /Vc-1]

Where V t =absorbance of test sample, V c=absorbance of control.

DPPH free radical scavenging assay: DPPH (1, 1-diphenyl-2-picrydrazyl) is widely used for free radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH in methanol solution in the presence of hydrogen-donating antioxidant due to the formation of the non-radical form DPPH. DPPH (3.9432 mg) was dissolved in methanol and make up to 100 ml to obtain a final concentration of 100 µM stock solution. Different concentrations of test solution were prepared in DMSO (3 µg to 100µg/ml). 3 ml of the reaction mixture consists of 2.80 ml of DPPH and0 .2 ml of extract of varied concentrations. After 50 minutes of incubation in dark, absorbance was measured at 517 nm by using X-mark micro plate spectrophotometer (Bio-RAD). Ascorbic acid (Vit- C) was used as standard. % inhibition of DPPH radical scavenging activity was calculated by the formula,

% inhibition = {(A-B) /A} ×100.

Where; A= Difference in absorbance of control with and without DPPH,

B= Difference in absorbance of test sample with and without DPPH.

In vitro 5- Lipoxygenase inhibition assay: Lipoxygenase inhibition assay was studied using Linoleic acid as substrate and Lipoxidase as enzyme. 5µl of enzyme was added with 175 µL of 50mM tris HCL buffer (pre incubated with 20µl test sample at 25ºc 5min). The reaction was initiated by the addition of 50µl Linoleic acid (final cons 140µM) in 50mM tris HCL buffer followed by incubation at 25c in dark for 20 minutes. The above total volume contained 185µl of reactionmiture. The assay was terminated by the addition of 65µl freshly prepared FOX reagent; after that termination the absorbance was taking at 595 nm up to 30 minutes at25ºc. % of inhibition of 5-Lipoxygenase was calculated by using the formula,

% of inhibition of 5-Lipoxygenase = (Absorbance of sample – Absorbance of control) – (Absorbance of sample – Absorbance of back ground) / (Absorbance of control – Absorbance background)

α- Amylase Inhibition Assay: The plant extract were pre-incubated with 50 µl of α-Amylase (α- Amylase from ex-porcine pancreas was dissolved in water to make a stock of 1mg/ml) at room temperature for 20 minutes and 50µl of starch solution was added. The mixture was further incubated at 37ºc for 10 minutes. The reaction was stopped by the addition of 100 of DNA reagent (10 mg of DNA was dissolved in 2ml of 2N NaoH and make up to 5ml distilled water; 3 gm of sodium potassium tartrate was added to the above and mad up to 100 ml using distilled

Vitamin-C (Standard-drug) *V. negundo L.*

Fig. 3: Anti- oxidant activities of chloroform extracts of V. negundo L.

Curcumin (Standard drug) *V. negundo **L***

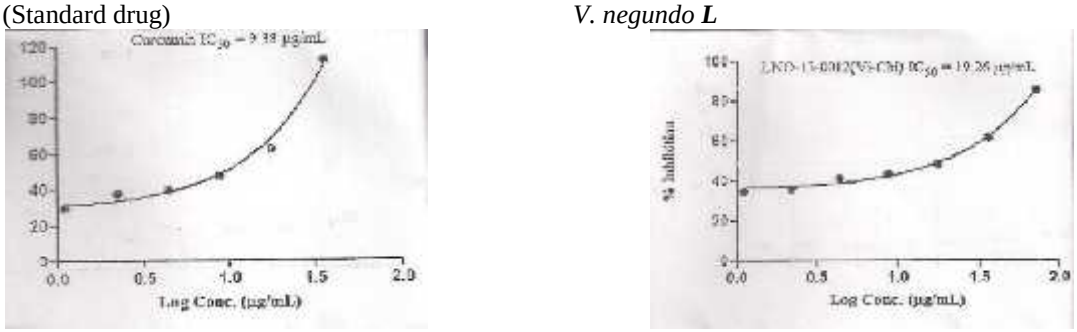

Fig. 4: Anti- Lipoxygenase activities of chloroform extracts of V. negundo L. (VCE)

Acarbose(Standard drug) V .negundo L.

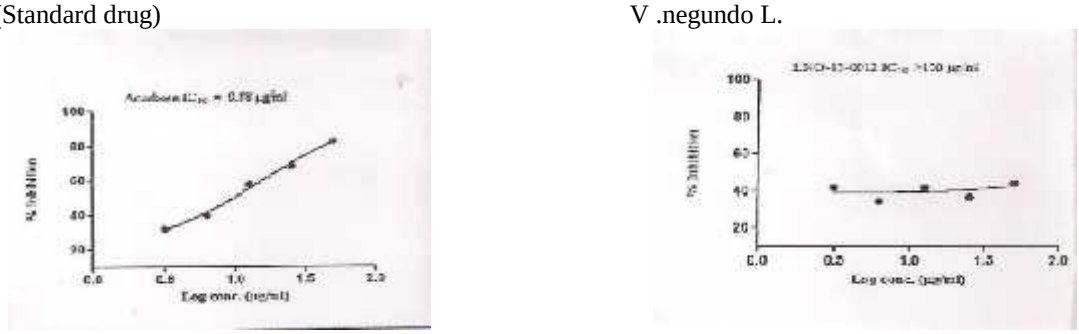

Fig. 5: Anti- obesity activities of chloroform extracts of V. negundo L. (VCE)

water) by color developed by placing the tubes in boiling water for 10 minutes (80-90º c). Finally 2.5ml distilled water was added of which 300 is transferred to 96-well plate and absorbance was read X- mark micro plate spectrophotometer (Bio-RAD) at 470nm. Assays will be carried in duplicated. Percentages of inhibition was calculated as follows,

Percentages of inhibition of Amylase = {(A-B)/A} x 100.

Where; A=Difference in absorbance of control with and without α-Amylase.

B=Difference in absorbance of test sample with and without α- Amylase.

Brine Shrimp Lethality test: The plant extract was subjected to the test for Cytotoxic activity against Brine Shrimp Larvae (*Artemia Salina*)).100mg of *Artemia salina* eggs was added to 200ml of sterile brine solution and aerated for 38 hours for the eggs to hatch into shrimp larvae. 5ml of sterile brine solution was pipetted into100µl of different dilutions of the extract (2.5µg/ml to 100µg/ml) in triplicates. 10 shrimps were added to each test tube and number of surviving larvae of test sample and control were observed after 24 hours the percentage lethality was determined by comparing the mean surviving larvae of controls and standard. ED50 values were obtained using fenny probed analysis software at 95% confidence limits from observed data the Brine Shrimp Lethality Test (BSLT) results are expressed in LC50 µg/ml.

RESULTS AND DISCUSSION

Plants used in traditional medicine have the potential to promote pharmacologically active natural products which can be used to treat various ailments. Several factors are known to influence the active principle present in the plants. It is necessary to point out that chemical compounds of plants greatly depend to geographical region age, local climatic seasonal and experimental condition, and genetic difference. Change in such factors cause abiotic stress which in turn responsible for change in nature of chemical compounds and their biological activities (1, 2). In present study an attempt has been made to evaluate anti arthritic potential of *V.negundoL.* In view of finding pharmacologically active lead compound from chloroform extract of this plant, phytochemical screening and immunostimulatory activities have also been carried out.

Phytochemical screening: Chloroform extracts of Vitex *negundo* L. (VCE) yield a green compound weighing 1.1g (7.33%w/w). Distribution of various secondary metabolites

Table 2: Brine Shrimp Lethality Test (ED_{50} values) of chloroform extracts of *Vitex negundo L.*

Name of the plant extract	ED50(µg/ml)	Degree(S) of freedom	UCL	LCL
Vitex negundo L.	76.77	0.103	113.24	26.08
Podophyllotoxin (Std. drug)	2.50	0.013	3.11	1.64

UCL=Upper Confidence Limit, LCL=Lower Confidence Limit

present in chloroform extract of *V. negundo L.* (VCE) was studied by phytochemical screening (Table. I). Phytochemical analysis of VCE revealed the presence of important secondary metabolites. Viz. Phenols, saponins, flavonoids, Phytosterols in the leaf extract of plant .Flavonoids and phenols were found to be active constituents in VCE (Table1).

Preliminary Phytochemical screening: Previous studies have been reported leaves of *V.negundoL*. Contain nishidine an alkaloid and, flavonoids like flavones, luteoline-7-glucoside, casticin, iriodoid glycoside an essential oil and other constituents like vitamin-c carotenes, benzoic acid, and stilbene derivatives [19]. In present study chloroform extract of leaves of *V.negundoL*. Showed significantly high amount of flavonoids and phenols in support of earlier findings (Table1).

Anti arthritis activity: In-vitro anti arthritic activity of VCE (100 µg/ml) was evaluated and values were compared with Diclofenac Sodium, a standard drug used for arthritis pain (Fig 1 and Fig 2). VCE showed 100% inhibition of protein denaturation an indication for good protection against denaturation of proteins. VCE also showed significantly high anti- arthritic activity than standard at 200 µg/ml, 400 µg/ml and 800µg/ml concentrations of the crude extract. Denaturation of tissue proteins (albumin) is one of the well documented causes of inflammatory and arthritic diseases. Production of auto antigen in certain arthritic diseases may be due to denaturation of proteins, lyses of membrane and action of protease. Arthritis is a disease characterized by joint pain followed by bone and joint destruction of articular cartilage. Cytokines play a major role in arthritis. Deregulated expression of tumor necrosis factor-α (TNF-α) in experimental animals has been shown to cause destructive arthritis .The development of arthritis is markedly suppressed in interleukin-β (IL-1β) deficient, collagen-induced arthritis (CIA). Interleukin-6 (IL-6) gene disrupted mice are resistant to antigen that pro-inflammatory cytokines (TNF-α, IL-1β, and IL-) play a role in arthritis and are potential targets for therapy [20]. Denaturation of proteins as one of causes of rheumatoid arthritis was also well documented. Production of auto antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. The mechanism of denaturation probably involves alteration in electro statics, hydrogen hydrophobic and disulphide bonding [21]. Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many neutral serine proteinases. Leukocyte proteinase play an important role in the development of tissue damage during inflammatory reaction and significant level of protection was provided by proteinase inhibition [22].

In present study, chloroform extracts of leaves of *V. negundo L.* showed good protection against denaturation of protein. This is the first time report in plant extracts i.e., from leaves of *V. negundo L*. Against inhibition of albumin denaturation. The percentage of inhibition of protein denaturation has been increased with increased concentration of extracts in both the plants (Fig 2) we found that 100% inhibition at a concentration of100 µg/ml similar to that of diclofenac sodium. A standard drug used for arthritic joint pains. An important mechanism of anti arthritis activity is the membrane stability modulating effect. Protective effect on heat and hypotonic saline-induced erythrocyte lyses I s know to be a very good index of anti arthritis activity of any agent [23]. HRBC membrane is analogous to the liposomal membrane and its stabilization implies that the extracts may as well as stabilize liposomal membrane. Stabilization of HRBC by hypo tonicity induced of anti arthritis activity [24].

Anti oxidant activity: DPPH free radical scavenging assay revealed the ability of antioxidant in scavenging free radical. Antioxidant activity of VCE extract was expressed as the concentration that inhibits 50% DPPH free radical (IC_{50}). The tested samples showed significantly high activity with IC50 value of >100 µg/ml when compared to Vitamin – C whose IC50 value is 3.15 µg/ml (Fig 3). With increasing concentration of the extract the inhibition of DPPH free radical scavenging activity was also increased. VCE showed good DPPH free radical scavenging effect even at low concentration and emphasized significant antioxidant activity.

In living systems free radicals are generated as part of the body's normal metabolic process. Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological condition such as anemia, arthritis, inflammation, neuro degeneration and ageing process [25]. Free radical scavenging potential of the chloroform extracts of the leaves of *Vitex negundo L.* was evaluated by in vitro DPPH assay. In this method the antioxidants present in the plant extracts reacted with DPPH, which is a stable free radical and converted to 1, 1-diphenyl-1, 2-picryl,hydrazine. The previous study reported leaf extracts of *V. negundo L.* Other than chloroform were determined to possess strong anti oxidant potential. Our results were also similar and positive in support of earlier report. The strong reducing power of phenols and flavonoids present in VCE could be responsible for antioxidant activity.

Anti inflammatory activity: VCE has been tested for anti-inflammatory activity by In vitro 5- Lipoxygenase inhibition assay and results were compared with curcumin (standard drug). Different concentrations of VCE (5, 10, 15, 20 µg/ml) were tested for anti- inflammatory activity

and the extract showed potential anti- inflammatory activity in all concentrations.(fig 4). VCE showed 5-lipoxygenase inhibitory activity with IC_{50} value of 19.26 µg/ml at a concentration of 10µg/ml, compared with curcumin with IC_{50} value of 9.38µg/ml. It was also observed % of 5-Lipoxygenase inhibition was found to be increased with the increased concentration of the extract as in the case of curcumin a proven anti- inflammatory compound.

Phenol compounds are also known to inhibit plant Lipoxygenase in the process of inflammation. Phenol compounds may block the cascade process of Arachidonic acid metabolism by inhibiting Lipoxygenase activity and may serve as scavenger of reactive free radical which is produced during Arachidonic acid metabolism. In present work, VCE showed good inhibition activity against Lipoxygenase, attributed to the presence of secondary metabolites like flavonoids and alkaloids. VCE has shown potential inhibition against Lipoxygenase also revealed existence of flavonoids in significant amount in VCE(table1) may impart anti inflammatory activity .

Anti obesity activity: Porcine pancreatic α-amylase with 0.21 min^{-1} was taken as 100% enzymatic activity. Chloroform extract of the selected plant were investigated for their α-amylase activity .VCE showed lowest α-amylase inhibition activity with an IC_{50} values were >100 µg/ml, when compared with standard acarbose whose IC_{50} value was 9.88µg/ml (Fig5). VCE showed α-amylase enzyme inhibition activities were moderately significant.

Many natural resources have been investigated with respect to suppression of glucose production from carbohydrates. α- amylase activity inhibition is considered to be effective to control diabetes and obesity. Therefore effective and nontoxic inhibitors of α-amylase have been sought. Our results revealed VCE showed moderately significant α- amylase inhibition.

Cytotoxicity: Chloroform extracts *of V.negundo L.* were investigated for their toxicity against Brine Shrimp Lethality Test. The toxicity of chloroform extracts of leaves of *V.negundo L.* showed significant toxicity (Table2). The tested sample VCE showed higher toxicity and ED 50 value was 76.77 (µg/ml) after 24 hours of exposure. Results were compared with standard Podophyllotoxin whose ED_{50} value was 2.50 (µg /ml)

Presently there is an increasing interest in herbal medicines accompanied by increased laboratory investigations to study pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Brine shrimp lethality assay represents a rapid, in expensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates well with cytotoxicity and antitumor properties. In present study cytotoxicity of VCE found to be biologically active and cytotoxicity was moderately high. These results indicate chloroform extract of V. *negundo L*. is associated with pharmacological properties and best bioactivity.

CONCLUSION

The results of this study demonstrated that chloroform extracts of leaves of *V. negundo L*. showed potential anti arthritic activity and also possess immunostimulatory activities such as anti oxidant, anti inflammatory anti obesity and anti microbial activities. Results may be attributed the presence of active principles in chloroform extract and further investigation is being advocated for the identification of lead molecule with pharmacological significance.

ACKNOWLEDGEMENT

Authors are thankful to UGC-SAP(BRS) programmed of Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, A.P and UGC –MRP (F.NO:40-132-2011(SR) for financial assistance and Central Instrumentation Centre, Acharya Nagarjuna University, Guntur, A.P for Instruments.

REFERENCES

1. Kiew R, Baas P. *Nytanthes* is member of Oleaceae, Pro C. Indian Acade SC 1984;3,349 -58.
2. Lutz Muller-kuhrt. Putting nature back into drug discovery. Nature Biotechnology 2003; 602.
3. Krishnaraju AV, Sundararaju D. Assessment of bioactive of Indian medicinal plant Using Brine Shrimp Lethality. Int J Appl Sci Eng 2005; 2, 125-34.
4. Nair C K N, Monahan N. Medicinal plants in India with special reference to Ayurveda. NAG Publisher, Delhi 1998; 433.
5. Bhagwandash, Kashyap L. Diagnosis and treatment of disease in Ayurveda. Concept Publishing Co, New Delhi. 1994; 254.
6. Telang R S, Chatterjee S. Studies an analgesic and anti- inflammatory activity of *Vitex* n*egundo L*. Indian J Pharmacol 1999;31,363-366.
7. Anonymous, The wealth of Indian Raw Materials. Vol.10, CSIR, New Delhi, 2003; 158-160.
8. Ayurveda pharmacopoeia of Indian. Government of Indian Publication, New Delhi, 2001; Vol.3 (1) 142-144.
9. Prabhakar A, Gupta BD, Suri KA. Hepatotective activity of 2-p-hydroybenzoyl Mussaenosidic acid, Patent no. WO 2003; 094946.
10. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. Council of Scientific and Industrial Research, New Delhi, 1986.
11. Gupta M, Mazumder UK, Bhawal SR. CNS activity of *Vitex negundo L*. In mice, Indian J Exp Biol. 1994; 37, 143-146.
12. Sharm PC, Yelne MB, and Dennis TT. Database on Medicinal plants used in Ayurveda, Vol .3. CCRAS Publication, New Delhi, Reprint Edition 2005; 450-471.
13. Nadkarni K M, Indian Materia Medical, Vol.1. Bombay popular Prakashan, 2002; 1278-1280.
14. Siddique I, Anis M, Jahan AA. Rapid multiplication of *Nytanthes abro-tritis* through In-vitro Auxillary shoot proliferation. World journal of Agricultural sciences 2006; 2,188-192.
15. Khatune NA, Islam ME, Rahman MAA, Mosaddik MA, Hague ME. In-vivo Cytotoxic Evolution of new Benzofuran derivative isolated for Nyctanthes arbor-

tristis on Ehrlich Ascite carcinoma cells in mice. Journal of Medical Sciences 2003; 3, 1659-173.

16. Diwan PV, Karwande I, Margaret I, Sattur P B. Pharmacology and biochemical Evaluations of *Tridax procumbent* on inflammation. Indian J Pharmaco 1989; 21, 1-7.
17. Harbone JB, Phytochemical methods. 3rd ed. London: Chapman and Hall, 1998.
18. Grant NH, Alburn HE, KryzanauskasC. Stabiliztion of Serum albumin by anti-Inflammatory drugs. Biochem pharmacology 1970; 19,715-722.
19. Ulusoylu M, Ondersev DV, Soyogul U, Gurkan E. The Cytotoxic and the biologic of centaurea iberica and Ferulago confuse. J Fac Pharm 2001; 18, 75-80.
20. Kambham venkateswarlu. Vitex Medicinal Values, Biological Activities, Toxicity studies and Psychopharmacological. Int J Pharmacology Res 2012; 126-133.
21. Chanpa Rani, Sunaina chawla, Manisha Mangal, AK Mangal, Subhash Kajal, Dhawan AK. Nyctanthes arbor-tristis, (Night Jasmine), A Sacred ornamental plant with immense medicinal Potentials. Vol.11. Indian Journal of Traditional Knowledge 2012; 427-435.
22. Ruthore B, Paul B, Chaudhary B P, Saxena A K, Sahu A P, Gupta Y K. Comparative Studies of different organs of *Nyctanthes arbor-tristis* in modulation of cytokines in Murine model of arthritis. Biomedicinal and Environmental science 2007; 20, 154-159.
23. Pearson CM. Development of arthritis, Periarthritis and periostitis in rats given Adjuvants. Pro Soc Exp Biol Med 1956; 91, 95-101.
24. Santosh Kumar Gupta, Amit Gupta, Gupta AK, Dhirendra pakash, Vedapal.In-vitro Anti-arthritic activity of ethanol extract of *Callicarpa macrophylla* flower. Int Res J Pharm, 2013; 4,160-162.
25. Grant NH, Alburn H E, Kryzanauskas C. Stabilization of serum albumin by anti-inflammatory drugs. Biochem Pharmacol. 1970; 19 715-722.