WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 1373-1383. Research Article ISSN 2277– 7105

# COMPARATIVE PHYTOCHEMICAL STUDY OF ROOTS VERSUS SMALL BRANCHES OF *VITEX NEGUNDO* L. USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC ULTRA-VIOLET DETECTION METHOD

**S. C. Verma*, E. Vashishth, S. Subhani, R. Singh, P. Pant, M. M. Padhi, A. Kumar**

Central Council for Research in Ayurvedic Sciences, 61-65, Institutional Area, Opp.-D-Block, Janakpuri, New Delhi-110058, India.

Article Received on
30 Nov 2014,

Revised on 24 Dec 2014,
Accepted on 19 Jan 2015

***Correspondence for Author**
**Dr. S. C. Verma**
Central Council for Research in Ayurvedic Sciences, 61-65, Institutional Area, Opp.-D-Block, Janakpuri, New Delhi-110058, India.

## ABSTRACT

*Vitex Negundo* L., family Verbenaceae is an important medicinal plant and immensely used in the Indian System of Medicine to cure human diseases. Roots of *V. negundo* have been reported for various medicinal properties such as relieving intermittent fever, thirst, body pain, to cure rheumatism, dyspepsia, piles and as an anthelmintic. *V. negundo* is commonly known as Nirgundi in India. Chemo-profiling screening of two parts of *V. negundo* plants revealed variations in phytochemicals within roots and small branches. The unique patterns of the chromatographic fingerprint were validated by analyzing roots and small branches of *V. negundo*. Our results revealed that the chromatographic fingerprint combined with similarity measurement could efficiently identify and distinguish *V. negundo* roots and small branches. The phytochemical fingerprint profiling of small branches and roots of *V. negundo* were found similar as an official part of *V. negundo* plant i.e. root, therefore small branches may be used in place of roots and vice-versa after comparison and confirmation of same pharmacological activities. The method can also be used for identification of different *V. Negundo* species and adulterants.

**KEYWORDS:** *V. negundo* L., HPTLC–UV detection, phytochemical fingerprint profiling analysis.

**Abbreviations: HPTLC–UV**, high performance thin layer chromatography-ultra violet detection; $R_f$, retention factor; **min**., minutes; **Sm. Br.**, small branches; **Rt.** Root.

## INTRODUCTION

*Vitex Negundo* L. (Fig. 1) belongs to family Verbenaceae commonly known as Nirgundi.[1] The family includes 80 genera and about 800 species. In Sanskrit word Nirgundi can be used for plant or any substance which protects the body from the diseases and it is an herb, which is mentioned in Ayurveda with a number of uses. It is a large, woody, aromatic, deciduous shrub, growing to 3 m at a medium rate with seven rings per 2.5cm of radius giving a mean annul girth-increment of 2.3 cm having typical five foliate leave pattern.[2-4] The bark is thin and grey in colour.[5] It shows its flowering time from September to October. The scented flowers are hermaphrodite (have both male and female organs) and are pollinated by insects.[1] The shrub can be reproduced readily from cuttings and it produces the root-suckers which are useful for planting against soil-erosion.[5] *V. negundo* has the common name "Chaste tree" since Athenian women used the leaves in their beds to keep themselves chaste during the feasts of Ceres. The name “Chinese Chaste tree” is derived from one of its therapeutic activity which depresses the sexual desire. *V. negundo* seeds itself into landscaped beds and can become somewhat weedy.[4, 6] It commonly bears digitate, tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes.[7] It is widely distributed in the tropic and warm region, covers throughout the greater part of India at warmer zones and ascending to an altitude of 1500 m in outer, Western Himalayas.[1,3,7] In India it is found in Assam, Bihar, Delhi, Himachal Pradesh, Hubei, Hunan, Jammu and Kashmir, Jiangsu, Jiangxi, Karnataka, and Kerala. The plant shows its better growth in light (sandy) and medium (loamy) soils requires well-drained soil and can also able to grow in nutritionally poor soil. The plant prefers acid, neutral and basic (alkaline) soils. It cannot grow in the shade because it requires dry or moist soil.[1] It thrives in humid places or along water courses in wastelands and mixed open forests.[7] *V. negundo* showed the presence of compounds such as carbohydrates, proteins, calcium oxalate, volatile oils, starch, saponin, phenols, saponins, xathoproteins, triterpenoids, tannins and flavonoids.[8] In the literature major compounds identified through GC-MS were 1H-indene, cyclododecanol, patchoulane, 1,2-dihexylcyclopropene-3-carboxylic acid, 2-heptenoic acid, (+) aromadendrene, trans-caryophyllene, 7-oxabicyclo [4.1.0] heptane, cyclohexane, farnesol, pentadecane and 1-octanol.[9, 10] The other constituents previously isolated from the plant include eight lignans ( negundin A, negundin B, 6-hydroxy4-(4-

hydroxy-3methoxy)-3-hydroxyl methyl-7 methoxy -3,4 dihydro-2-napathaldehyde, vitrofolal, (+) – iynoiresinol, (+)–iynoiresinol-3α-0-β-D-glucoside, (+)(-)(-) pinorecinol, (+) – diasyringaresinol, irridoid glycoside, (2-p-hydroxy benzyl mussaenosidic acid), flavonones (5,3',dihydroxyl-7,8,4'trimethoxyflavonone and (5,3'dihydroxy-6,7,4'trimethoxy flavonone), flavone (vitexicarpin), β-sitosterols essential oils (α–pinene, linalool, terpinyl acetate, beta caryophyllene), non diterpene (vitedoin B), pentacyclic triterpenoids, (beutinilic acid, ursolic acid), flavonoid glycoside, luteolin, agnuside, negundoside, isoorientin.[3,10] The root, leaves, twigs and seeds contain a large number of compounds with varying structure, diterpenoid, camphene, casicin, glucononitol, α-pinene, citral, β-caryophyllene, orientin, isorientin, corymbosin, flavonoid glycosides, triterpenoids, long chain unsaturated fatty acids, iridoid glycosides and lignin.[3] The root of *V. negundo* contains vitexoside, flavonoid glycoside, agnuside and R-dalbergiphenol[11] lignans (agnucastoside A, B, C and aucubin, agnuside, mussaenosidic).[4] Bark contains vanillic acid, p-hydroxybenzoic acid, luteolin and two leucoanthocyanidins i.e 6, 8 di-*O*-methylleucocyanidin-7 and orhamnoglucoside. The leaves contain an alkaloid nishidine, flavonoids like flavones, luteolin-7-glucoside, casticin, iridoid glycoside, an essential oil and other constituents like artemetin, benzoic acid, β-sitosterol, C-glycoside , carotene, friedelin, vitamin-C.[11] 5-hydroxy-3, 6, 7 trimethoxy (3,4dimtoxypheny) 4H chrome-4-on, 5, 7-dihydroxy-2-(3, 4 dihydroxyphenyl)- 4H chromen-4-one, Agnuside.[4] 5-hydroxy 3,6,7,3', 4'-pentamethoxyflavone.[12] 12,6'-p-hydroxybenzoyl mussaenosidic acid; 2'-p-hydroxybenzoyl mussaenosidic acid, 5, 3'-dihydroxy-7,8,4' trimethoxyflavanone; 5, 3'-dihydroxy-6, 7, 4'-trimethoxyflavanone, angusid; casticin; nishindine; gluco-nonitol; p-hydroxybenzoic acid; sitosterol.[13] α-elemene, δ- elemene, β-elemene, β-eudesmol, camphor, camphene, careen, 1,8-cineol, 1-oceten-3-ol, γ-terpinine, α-phellendrene, β-phellendrene, α-guaiene, abieta-7,13-diene, neral, geranial, bornyl acetate, nerolidol, β-bisabolol, cedrol and vitexicarpin.[11] Essential oil of fresh leaves, flowers and dried fruits contains δ-guaiene; guaia-3,7-dienecaryophyllene epoxide; ethyl-hexadecenoate; α-selinene; germacren-4-ol; caryophyllene epoxide; (E)-nerolidol; β-selinene; α-cedrene; germacrene D; hexadecanoic acid; p-cymene and valencene.[14] The leaves and twig of *V. negundo*, having a stilbene derivative, characterised as 4,4'- dimethoxy-trans-stilbene, along with five flavones, 5,6,7,8,3'4'5- heptamethoxy, 5-hydroxy 6,7,8,3'4'- pentamethoxy (5-odesmethylnobiletin), 5 hydroxy-6,7,8,3',4',5-hexamethoxy (gardenin A), 5 hydroxy-6,7,8,4'-tetramethoxy (gardenin B) and 5 hydroxy-7,3',4',5'-tetramethoxyflavone (corymbosin). Terpinen-4-ol, α-terpineol, sabenine, globulol, spathulenol, β-farnesene, farnesol, bis (1,1dimethyl) methylphenol, α-pinene, β-pinene, linalool, terpinyl acetate, caryophyllene epoxide, caryophyllenol along with

viridiflorol. The leaves and twig also show the presence of volatile oil which contains ten volatile components like α-copaene, β-caryophyllene, camphene, α-thujene, α-pinene, sebinene, linalool, stearic acid and behenic acid.[11] The heartwood of *V. negundo* contains β-amyrin, epifriedelinol and oleanolic acid.[15] Seeds contain hydrocarbons, β-sitosterol, benzoic acid, phthalic acid, anti inflammatory diterpene, flavonoids, artemisin, triterprnoids, *n*-tritriacontane, *n*-hentriacontanol, n-hentricontane, n-pentatricontane, *n*-nonacosane, β-sitosterol, p-hydroxybenzoic acid and 5 oxyisophthalic acid; 3, 4-dihydroxybenzoic acid and vitedoamine A.[4,11] All parts of the plant from root to fruit possess a massive amount of phytochemical secondary metabolites which impart an unique variety of medicinal uses to the plant.[16] The plant is considered as acrid, astringent, anthelmintic, cephalic, bitter, heating, stomachic and useful in treatment of inflammations, eye diseases, spleen enlargement, asthma, bronchitis, biliousness and painful teething of children etc. It has germicidal properties. It is easily digestible and can cure morbid vata and kapha, also used in arthritis, cephalgia, otalgia, inflammatory, glandular and rheumatic swellings, intestinal worms, fever, ulcers, skin diseases, nervous disorders and leprosy.[5] It is commonly used in folk medicine as antiarthritis, anti-convulsant, anti-inflammatory, antioxidant, antifertility, antimalarial, antibacterial, antifilarial, and pesticidal. It also shows insecticidal activity, bronchial smooth muscle relaxant activity, hepato protective, laxative activity and analgesic activity.[16] Different parts of *V. negundo* have been used in traditional Indian medicine as nervine sedative and are of high value as constituents of Ayurvedic preparations such as Vishagarbha thaila, which is widely used to treat rheumatism in India. Roots are useful in rheumatism, dyspepsia, piles and as anthelmintic. The leaves possess more medicinal value and show its versatile uses in various diseases as they contain flavonoids, sterols and terpenoids.[2,3,17] The fresh aromatic leaves are useful for rheumatism and to relieve pain. It is widely used in Chinese herbal medicine. It is second most important plant used for the treatment of chronic bronchitis and cold. The leaves of plant are astringent, febrifuge, sedative, tonic and vermifuge. The leaves in the form of a paste are used for inflammatory swellings of the joints formed due to rheumatism, hydrocele and spleen enlargement. They are also used in nervous disorders and leprosy. Oil prepared from leaves is useful for growth of hair and increases the function of brain.[17,18] Decoction of the leaves of *V. negundo* is used as a bath in the puerperal state of women in India. The leaf extract has been reported to exhibit a wide range of pharmacological activities. The methanolic extract of had been found to exhibit very potent anti feedent, anti-inflammatory, analgesic and anti convulsant activities. They are used as a drug of choice for pain, inflammation and related diseases.[3] Chloroform extract of

defatted seed showed anti-inflammatory activity. It also possesses potent mosquito repelling activity against Aedes aegypti, anti-tumor and analgesic activity.[17] *V. negundo* is used in several commercial formulations and in the Ayurvedic System of Medicine.[3] The leaves are used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic and antihistaminic agents. Its extract has also shown anticancer activity against Ehrlich ascites tumour cells.[19, 20] Roots, Bark, Leaves and fruits are highly medicinal. Roots are one of the ingredients of the drug Dasmula arista; used in colitis, dysentery, diarrhoea, flatulence, fever, vomiting and colic.[11] Roots and Barks are used for relieving intermittent fever, thirst and body pain. Ripe Fruits is highly nutritious, cooling, used in treating indigestion and to improve vision.[11] Seeds used as vermicide.[2]

**Figure 1: *Vitex Negundo* L. Plant.**

**Figure 2: Small Branches**

**Figure 3: Root**

**Taxonomic / Scientific Classification**[11]

| **Kingdom** | **Plantae - Plants** |
|---|---|
| Sub Kingdom | Tracheobionta - Vascular plants |
| Super Division | Spermatophyta - Seed plant |
| Division | Magnoliophyta - Flowering Plant |
| Class | Magnoliopsida - Dicotyledons |
| Sub Class | Asteridea |
| Order | Lamilales |
| Family | Verbenaceae |
| Genus | *Vitex* L. |
| Species | *negundo* L. |

## MATERIALS AND METHODS

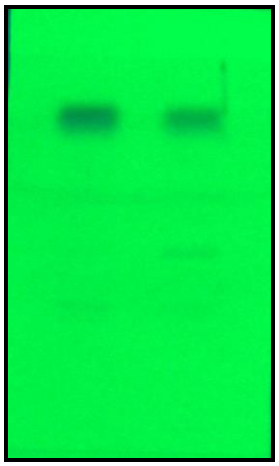
**Plant Materials and Chemicals:** Root (Fig.2) and Small branches of stem (Fig.3) of *V. negundo* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer, Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

**Sample preparation:** The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30± $2^0$C and relative humidity 50 ± 5%) and powdered in an electric grinder. Conventional extraction of root and small branches of stem of *V. negundo* were performed at room temperature (28$^o$ ± 3$^o$C) with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, ethyl acetate and ethanol. Dried and powdered parts of *V. negundo* (10 g each) were extracted three times (3 × 50 mL) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no. 1 and the solvents were removed under vacuum at 50°C, separately and concentrated up to 10 mL to get the sample solution of 100 mg $mL^{-1}$. 5 µL of each sample was applied separately to TLC plate for the development of fingerprints.

**HPTLC-UV detection Method:** High Performance Thin Layer Chromatography was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 $F_{254}$ (E. Merck). Both samples (Root and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 µL syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene: Ethyl acetate*: *Acetic acid 9:1:0.5 (v/v/v)* and as mobile phase for both *n*-hexane extract was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with vapours of mobile phase for 20 min. The plates were dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints as evident in Figures 4 – 5. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 6 using CAMAG Reprostar and WinCATs software (V 1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs were performed with same procedure in the mobile phase of *Toluene: Ethyl acetate: Acetic acid 7: 3:0.5 (v/v/v)* for both the extracts and then visualized in

λ 254 nm, λ 366 nm and white light using CAMAG Reprostar and WinCATs software as shown in Figure 7-12.

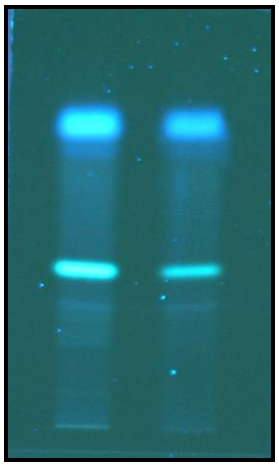
## RESULTS AND DISCUSSION



1 2

254 nm

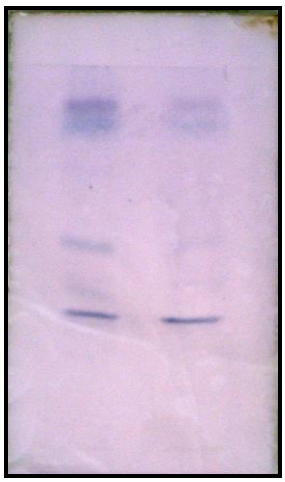
**Figure 4**



1 2

366 nm

**Figure 5**

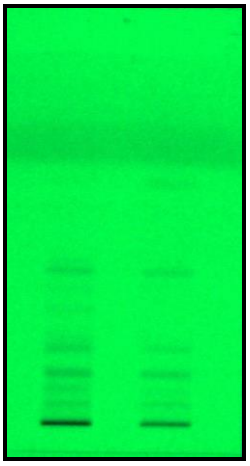


1 2

After derivatization with anisaldehyde sulphuric acid reagent

**Figure 6**

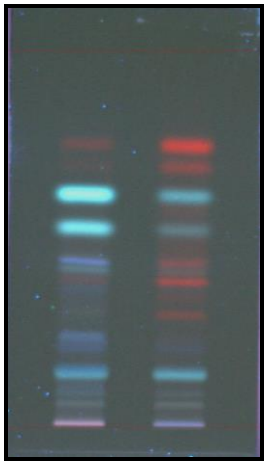
**Figure 4-6: TLC fingerprint of *n*- hexane extract of *V. negundo* (1= Rt.; 2= Sm. Br.)**



1 2

254 nm

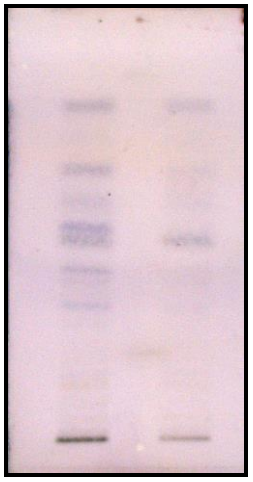
**Figure 7**



1 2

366 nm

**Figure 8**

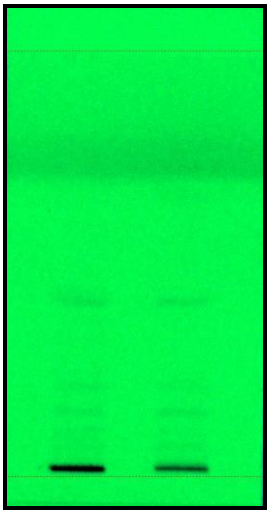


1 2

After derivatization with Anisaldehyde sulphuric acid reagent

**Figures 9**

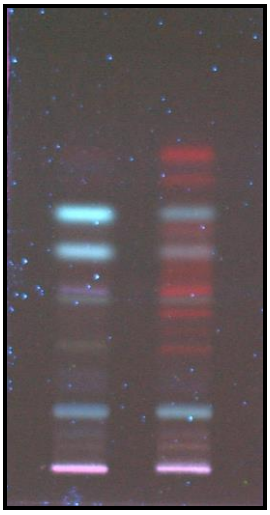
**Figure 7-9: TLC fingerprint of ethyl acetate extract of *V. negundo* (1= Rt.; 2= Sm. Br.)**



1 2

254 nm

**Figure 10**



1 2

366 nm

**Figure 11**

1 2

After derivatization with Anisaldehyde sulphuric acid reagent

**Figures 12**

**Figure 10-12: TLC fingerprint of ethanol extract of *V. negundo* (1= Rt.; 2= Sm. Br.)**

**Table 1: $R_f$ value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *V. negundo* (Rt. and Sm. Br.) at different wave-lengths.**

| Wave-length | *n*- Hexane extract | | Ethyl acetate extract | | Ethanol extract | |
|---|---|---|---|---|---|---|
| | Root | Small branches | Root | Small branches | Root | Small branches |
| **254** | 0.83 | 0.47, 0.83 | 0.07, 0.11, 0.15, 0.22, 0.32, 0.37, 0.42 | 0.07, 0.11, 0.15, 0.22, 0.42, 0.64 | 0.06, 0.14, 0.20, 0.41 | 0.06, 0.14, 0.20, 0.41 |
| **366** | 0.23, 0.34, 0.41, 0.80 | 0.34, 0.41, 0.80 | 0.10, 0.15, 0.20, 0.41, 0.44, 0.52, 0.62, 0.74 | 0.10, 0.15, 0.30, 0.37, 0.43, 0.52, 0.62, 0.69, 0.74 | 0.11, 0.15, 0.29, 0.41, 0.43, 0.53, 0.63 | 0.11, 0.15, 0.29, 0.33, 0.39, 0.41, 0.44, 0.53, 0.63, 0.69, 0.71, 0.76 |
| **Visible light after derivatization** | 0.33,0.40, 0.53, 0.82, 0.88 | 0.33, 0.82, 0.88 | 0.15, 0.35, 0.44, 0.52, 0.56, 0.70, 0.86 | 0.15, 0.44, 0.52, 0.70, 0.86 | 0.36, 0.44, 0.52, 0.56, 0.71, 0.89 | 0.52, 0.71, 0.89 |

No such study was found in literature for comparative phytochemical study of root versus small branches of *V. negundo* Linn by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of root and small

branches of *V. negundo* revealed that many similarities in phytochemical fingerprints were found and evident in Table-1 and Fig. 4-12.

Phytochemical fingerprints of *n*-hexane extract of root and small branches showed one and two bands respectively, out of which, one band at $R_f$ 0.83 (black) was similar under UV detection at 254 nm. Under 366 nm UV detection, root and small branches showed four and three bands respectively, out of which three bands at $R_f$ 0.34 (light blue), 0.41 (light blue) and 0.80 (blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, root and small branches both were showed five and three bands respectively, out of which three bands at $R_f$ 0.33 (blue), 0.82 (blue), 0.88 (blue) were found similar as represented in Table 1 and Fig. 4-6.

TLC plate of ethyl acetate extract of root and small branches showed under 254 nm, seven and six bands respectively, out of which four bands were found similar at $R_f$, 0.11,0.15, 0.22 and 0.42 (all are black) while under 366 nm detection, eight and nine band were visible respectively in extract of root and small branches, out of which, six bands at $R_f$ 0.06 (yellow) ,0.10 (yellow),0.15 (blue), 0.52 (light blue),0.62 (light blue) and 0.74 (red) were found similar. After derivatization under white light detection, seven and five bands were visible in extract of root and small branches respectively, out of which five bands at $R_f$ 0.15 (yellow), 0.44 (blue), 0.52 (brown) 0.70 (brown), 0.86 (brown) were observed similar as evident in Table 1 and Fig. 7-9.

TLC plate of ethanolic extract of root and small branches visualized under 254 nm, both extracts showed four similar bands at $R_f$ 0.05, 0.14, 0.20 and 0.40 (All are greenish black) and under detection at 366 nm seven and twelve bands were observed, out of which, six bands at $R_f$ 0.11(brown), 0.15 (light blue), 0.29 (brown),0.41 (pink), 0.53 (light blue) and 0.63 ( (light blue) were found similar. After derivatization the TLC plate was visualised under white light, six and three bands were observed in of root and small branches respectively, out of which three bands at $R_f$ 0.52, 0.71, 0.89 (all are blue) were found similar as evident in Table 1 and Fig. 10-12.

## CONCLUSION

Phytochemical fingerprint profiling of various parts of *V. negundo* indicated that different types of phytoconstituents present in each part but many similarities in fingerprinting were found in root and small branches. The phytochemical fingerprint profiling of small branches

of *V. negundo* were similar with root as an official part of *V. negundo* plant, therefore small branches may be used in place of root and vice-versa after comparison and confirmation of same pharmacological activities. The $R_f$ helped in evaluation of phytochemical diversity in different parts of *V. negundo*. The phytochemical diversity was found more in root followed by small branches at one geographical region. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of root and small branches of *V. negundo* have been given an idea about the presence of various phytochemicals similarities in their reported parts. The TLC spots provided valuable clue regarding presence or absence of various phytochemicals or metabolites of the plants.

**ACKNOWLEDGMENTS**

Authors are thankful to Director General, Central Council for Research in Ayurvedic Sciences, New Delhi to provide the financial support under IMR scheme for this research work. Authors are grateful to Dr. R. K. Tiwari, NVRI&H, and Lucknow for providing the genuine plant materials and K. Basu, Arbro Pharma for scaning the TLC, respectively.

*Authors have no conflict of interest*

**REFERENCES**

1. Trapti R, Meenal K, Farooqui IA, Khadabadi SS. A review on ethanomedicinal uses and phyto-pharmacology of anti-inflammatory herb *Vitex Negundo*. Int J Pharm Sci Res, 2010; 1(9): 23-28.
2. Kumar NN, Pharmacognostic and Phytochemical Analysis of *Vitex Negundo* L. Int J Inov Res Sci, Eng Technol, 2014; 3(7).
3. Shah S, Dhanani T, Kumar S, Comparative evaluation of antioxidant potential of extracts of *Vitex Negundo*, Vitex trifolia, Terminalia bellerica, Terminalia chebula, Embelica officinalis and Asparagus racemosus. Inov Pharm Pharmaco ther, 2013; 1(1): 44-53.
4. Arora V, Lohar V, Singhal S, Bhandari A, *Vitex Negundo*: - A Chinese Chaste Tree. Int J Pharm Inov, 2011; 1(5): 9-20.
5. Guguloth SK, Vivekanandan L, Singaravel S, Sheik HS, Thangavel SK, Nephroprotective Activity Of *Vitex Negundo* Linn Bark Against Chemical Induced Toxicity In Experimenal Rats. Int J Adv Pharm Sci, 2011; 2(5 – 6).
6. Gilman EF, Watson DG, *Vitex Negundo* 'Heterophylla' Cut-Leaf Chaste tree, Fact Sheet ST-668, a series of the Environmental Horticulture Department, Florida Cooperative

Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Publication date: October 1994.

7. Jayasree T, Arpitha T, Kavitha R, Kishan PV, Evaluation of Anticonvulsant Activity of Ethanolic Extract of Vitex nigundo in Swiss Albino Rats. Int J Pharm Phytopharmacol Res, 2012; 1(4): 161-165.
8. Humayun S, Ibrar M, Evaluation of anti-inflammatory and analgesic activity from *Vitex Negundo* Linn. J Bio Env Sci, 2014; 4(1):164-172.
9. Sahayaraj K, Ravi C, Preliminary Phytochemistry of Ipomea Carnea Jacq. And *Vitex Negundo* Linn. Leaves. Int J Chem Sci, 2008; 6(1):1-6.
10. Tirumalasetty J, Ubedulla S, Chandrasekhar N, Kishan PV, Rasamal K, Evaluation of Antipyretic Activity of Alcoholic Extract of Vitex nigundo Leaves in PGE1 induced pyrexia model in Albino Rats. J Chem Pharm Res, 2012; 4(6):3015-3019.
11. Venkateswarlu K, *Vitex Negundo*: Medicinal Values, Biological Activities, Toxicity Studies and Phytopharmacological Actions. Int J Pharm Phytopharmacol Res, 2012; 2(2): 126-133.
12. Gautam K, Kumar P, Evaluation of Phytochemical and Antimicrobial study of Extracts of *Vitex Negundo* Linn. Int J Drug Dev Res, 2012; 4 (4): 192-199.
13. Ahirrao RA, Patel MR, Pokal DM, Pharmacognostical Studies of *Vitex Negundo* Leaves. Biol Forum Int J, 2011; 3(1): 19-20.
14. Vishwanathan AS, Basavaraju R, A Review on *Vitex Negundo* L. – A Medicinally Important Plant. Eur J Biol Sci, 2010; 3(1): 30-42.
15. Haq A, Khan SB, Flavonoid glycoside and a long chain ester from the roots of *Vitex Negundo*. Polish J chem, 2004; 78:1851-1856.
16. Kumar SK, Nagaveni P, Subahan MT, Evaluation of Anti-arthritic activity of *Vitex Negundo* Linn. Int J Res Pharma Life Sci, 2013; 1(1): 1-4.
17. Zaware BB, Nirmal SA, An overview of *Vitex Negundo* linn: Chemistry and Pharmacological profile. Res J Pharma Biol Chem Sci, 2010; 1(1): 104.
18. Mahmud S, Shareef H, Farrukh U, Kamil A, Rizwani GH, Antifungal Activities Of *Vitex Negundo* Linn. Pak J Bot, 2009; 41(4):1941-1943.
19. Amirtharaj VR, Reyaz MA, Kumar AJ, Saivishwathdindu KM, Kumar NS, Preliminary Phytochemical Studies and In vitro Cytotoxic Activies on *Vitex Negundo* (L.). Int J Res Pharma Biomed Sci, 2011; 2(4): 1800-1804.
20. Lakshmanashetty RH, Nagaraj VB, Hiremath MG, Kumar V, In vitro Antioxidant Activity of *Vitex Negundo* L. Leaf Extracts. Chiang Mai J Sci, 2010; 37(3): 489-497.