

Preliminary Estimation of Antioxidant Activity and Phytochemicals of Methanol Solvent of *Vitex negundo* Linn Leaves

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Abstract : 'Nirgudi' plant *Vitex negundo* Linn. from family Verbenaceae is important medicinal plant in Indian Pharmacopoeia with variety of bioactive phytochemicals having importance in pharmacology and tribal medicines. In the present study the phytochemical compounds present in the methanol extract of vitex negundo linn leaves extract analyzed and the free radical scavenging capacity of the extract. The experimental data showed that the methanol extract of vitex negundo linn contain alkaloids, carbohydrate, glycosides, amino acids, proteins, tannins and phenolic compounds. The free radical scavenging capacity of the sample was measured by DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. The IC₅₀ value of the ascorbic acid which is used as the standard is 14.97 while for sample is 46.84. the methanol extract of vitex negundo leaves shows good antioxidant property.

Keywords : Phytochemical; antioxidant; Methanol extract; *Vitex negundo* Linn; Leaves.

Introduction :

Natural products are provided with numerous therapeutics effects and used as traditional medicines, since ancient times. Conventional plants are rich in various bioactive compounds that stimulate the immune system and protect against many diseases. Many of these compounds have passed clinical trials, and several others still remain unexplored [1]. Natural products may be of plant or animal origin, although herbal bioactive products are derived from the plant origin, thus increasing the demand for systematic evaluation of novel bioactive compounds [2].

Plants are the rich sources of bioactive metabolites where every compound has antimicrobial properties. One such plant is *Vitex negundo* L. (Verbenaceae) commonly known as 'Nirgudi' in India. The genus *Vitex* has around 270 known species, ranging from shrubs to trees in the tropical, sub-tropical regions and temperate zones [3]. The *Vitex* is used as a folk medicine in India, Bangladesh, China, Indonesia, Nepal, Pakistan, Philippines, and Sri Lanka [4,5]. *Vitex negundo* is one such plant which is highly potential in the treatment of toothache, inflammation, eye disorders, leukoderma, spleen enlargement, skin-ulcers, auto immune disorders and sexually transmitted diseases. The extracts of this plant are used as tonics, vermifuge, lactagogue, antipyretic and anti-histaminic agents. The anti-oxidant potential is present in the leaves extracts with anthelmintic, dysmenorrhoeal, anti-hyperglycaemic, antifilarial and antibacterial activity [6]. Leaves are mostly used for the treatment of eye diseases, inflammation, leukoderma, toothache, spleen enlargement, skin-ulcers, gonorrhoea, rheumatoid arthritis, and bronchitis. Along with these, leaves are used as a vermifuge, lactagogue, tonics, antibacterial, antipyretic and antihistaminic agents [7].

Previous phytochemical studies on *V. negundo* have afforded several types of compounds, such as volatile oils, lignans, flavonoids, iridoids, terpenes (triterpenes, diterpenes, sesquiterpenes), and steroids [8]. Even though, it constitutes a rare medicinal shrub with high biological value, very little survey research work has been done on this species in our country. Taking into account of these ethnobotanical importance, the chemical constituents of the leaves of *V. negundo* have been investigated. The present study analyses the preliminary phytochemical analysis and evaluation of antibacterial activity of the *Vitex negundo* Linn.

Botanical Description of Plant *Vitex negundo* Linn

Vitex negundo L. is a bushy shrub or small tree about 10-20 ft tall. Bark thin, grayish brown or grayish white, blaze yellow, branchlets terete or obtusely quadrangular, slightly pubescent, nodes annulate, internodes 3-9 cm long. Leaves palmately compound with 3-5 foliolate, rarely more, leaflets lanceolate-elliptic or ovate-lanceolate, middle leaflets 5-14 × 2-4 cm across, petiolules 1-3.5 cm long, lateral leaflets 2.5-4 × 0.8-1 cm across, petiolules 0.2-0.5 cm long, base cuneate or acute, margin entire or serrate, apex acuminate, chartaceous, dark green sparsely pubescent above, paler greyish pubescent beneath, lateral veins 10-18 on either side of the midrib, subparallel, margin arcuate, impressed above and prominent beneath, pubescent on midrib beneath, reticulate veinlets, petiole stout, slender, canaliculated, about 2-10 cm long. Inflorescence terminal panicles, sometimes dichotomously or trichotomously branched, about 10-30 cm long, peduncles, slender, obtusely quadrangular, pubescent, about 3-8 cm long, bracts leaf like, lanceolate, caduceus. Flowers bisexual, many, fragrant, pedicels about 1-3 mm long, Calyx campanulate, 5 toothed, teeth acute, purplish stripes inside, pubescent outside, about 3 × 2 mm across, Corolla sub infundibular, 5 lobed, 2-lipped, blue, purple or lavender, upper lip 2-lobed, light blue, lobes ovate, apex truncate, lower lip 3 lobed, midlobe obovate, light blue, concave, apex truncate, about 1 mm long, lower lobe, dark purple with white tinge, villous near the base, apex acute about 4 × 5 mm across, Corolla tube narrow, ampliate towards the apex, densely villous at throat with whitish hairs, pubescent outside. Stamens 4, didynamous, exserted, filaments slender, filiform, about 4-5 mm long, exserted, anthers oblong, light brown with white tinge, 2-celled, Ovary bicarpellary, 4 lobed, obovoid, glabrous, about 1 mm long, style slender, about 8 mm long, stigma bilobed, subequal, subulate. Fruit drupaceous, obovoid or subglobose, about 3-5 mm in diameter, green, glabrous, black when ripe, fruiting calyx cupular, pubescent [9-10].

Material and Methods:

The random surveys for collection of plant material from the Botanical Garden, College of Life Science, CHRI, Gwalior (M.P.) were conducted during June-December 2017. Fresh leaves of *Vitex negundo* were collected and washed thoroughly. The leaves were shed dried at the room temperature. The leaves without any moisture were blended until it becomes a fine powder. The solvent was evaporated and solid mass obtained was tasted for various phytochemical parameters such as alkaloids (Mayer's test, Wagner's test, Hager's test). Carbohydrates (Molish test, Fehling's test, Benedicts's test, Barfoad's test), Glycosides (Borntrager's test, Legal's test), proteins and amino acids (Millon's test, Nitric acid test, Biuret test, Ninhydrine test), Foam test, Phenolic compounds and tannin (Ferric chloride test, Gelatin test, Lead acetate test, alkaline reagent test). The procedures involved is describe in earlier reports [11-12].

Result and Discussion:

In the present study, methanol extract of leaves of *Vitex negundo* Linn. was tested for Phytochemical constituents present in the it, and antioxidant activity also measured and IC₅₀ value was calculated.

Evaluation of Phytochemical Constituents:

The medicinal properties of the plants are due to some chemical substances that produced some certain definite physiological action in the human body these non-nutritive components are called as phytochemicals [13-14]. The secondary metabolites found in plants are active principles of many drugs. Therefore, the investigation for the basic phytochemicals of the extracts for the major phytoconstituents is also vital [15-16]. Traditionally used medicinal plant produced variety of 415 compounds which are known for the therapeutic action [17-18]. Saponins one of the phytoconstituents used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation [19]. Tannins are reported for their anti-bacterial and anti-herpetic activities and provides defense [20]. alkaloids defense from chronic disease [21]. Steroids and triterpenoids show the analgesic properties.

The present study discloses that methanol extract of *Vitex negundo* Linn shows the presence alkaloids, carbohydrates, proteins, amino acid, phenolic compounds and tannins the results showed in table 1.

Table 1a: Preliminary phytochemical screening of vitex negundo methanol extract.

S. No.	Phytoconstituents	Methanol extract
1.	Carbohydrates	+ve
2.	Amino acids	+ve
3.	Protein	-ve
4.	Glycosides	-ve
5.	Alkaloids	+ve
6.	Flavonoids	+ve
7.	Tannins	+ve
8.	Phenols	+ve
(+ve)=Positive, (-ve)=Negative		

Table 1b

S. No.	Reagent	Observations
1.	Detection of carbohydrate	
A.	Molish test	+ve
B.	Fehling's test	+ve
C.	Benedic test	-ve
D.	Barfoad's test	+ve
2.	Detection of Glycosides	
A.	Borntrager's test	-ve
B.	Legal's test	-ve

As per Table 1b, out of four tests, three are positive for carbohydrate and the glycoside are found to be absent. The positive foam test indicate presence of saponin. The phenolic compounds are tannins are found to be present and hence it was decided to carry out screening of antioxidant activity.

Evaluation of Antioxidant Property:

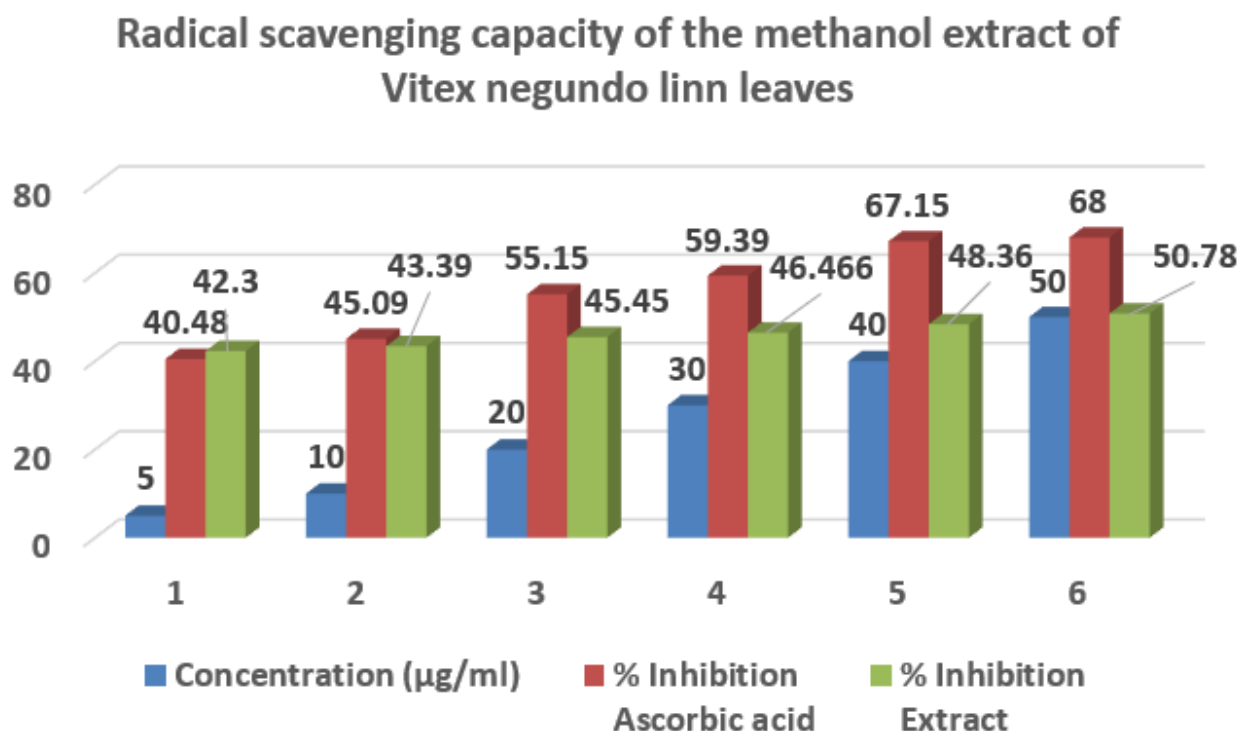
Antioxidants are the chemicals that neutralize or scavenges free radicals or reactive oxygen species (ROS) [25]. Antioxidants may be defined as radical scavenger which protects the human body against free radicals that may causes pathological conditions [22] It is also reported that ROS is implicated is a wide range of human disease like atherosclerosis and certain cancer [23]. O_2^- and OH play an important role in the maintenance of process of inflammatory and as well as pain causing tissue damage [24].

In the present study free radical scavenging capacity of the extract is measured by DPPH (2, 2- diphenyl-1-picrylhydrazyl), showed in table 2. The IC_{50} value of methanolic extract was found to be 46.84 % which is more compared to IC_{50} value of ascorbic acid. The extract shows antioxidant activity but less effective though it tests positive for phenolic compounds and tannins.

Table 2: Radical scavenging capacity of the methanol extract of *Vitex negundo linn* leaves.

S. no.	Concentration (µg/ml)	% Inhibition Ascorbic acid	% Inhibition Extract
1.	5	40.48	42.30
2.	10	45.09	43.39
3.	20	55.15	45.45
4.	30	59.39	46.466
5.	40	67.15	48.36
6.	50	68.00	50.78
IC_{50} value (µg/ml)		14.97	46.84

Fig. 1



The above figure clearly depicts the percentage of **Inhibition Ascorbic acid** and **Inhibition Extract** as shown in the Table 2.

Conclusion:

The result of the present study discloses that the methanol extract of the *vitex negundo linn* leaves extract shows the positive result for the alkaloids, carbohydrate, glycosides, proteins, amino acid, tannins and phenolic compounds. The sample also shows the outstanding antioxidant activity. The IC_{50} values of the ascorbic acid which is used as the standard reference is 14.97 and for the sample is 46.84.

References:

1. Thite SV, Chavan YR, Aparadh VT and Kore BA, "Preliminary Phytochemical screening of some medicinal plants" International journal of pharmaceutical, chemical and biological sciences, 2013, 3(1), 87-90.
2. Chitra V, Shrinivas Sharma, Nandu Kayande, "Evaluation of anticancer activity of *Vitex negundo* in experimental animals: An in vitro and in vivo study" International journal of pharmtech research, 2009, 1 (4), 1485-1489.
3. Pradeep Singh, Garima Mishra, K. K. Jha, Vipin Kumar Garg, R. L. Khosa, "Chemical composition and antimicrobial activity of essential oil of leaves of *Vitex negundo* Linn. (Verbenaceae)" International journal of chemtech research, 2010, 2 (3), 1686-1690.
4. Azhar-ul-Haq, Abdul Malik, Itrat Anis, Sher Bahadar Khan, Ejaz Ahmed, Zaheer Ahmed, Sarfraz Ahmad Nawaz and Muhammad Iqbal Choudhary, "Enzymes inhibiting lignans from *vitex negundo*" Chem pharm bulletain, 2004, 52(11), 1269-1272.
5. Monali G. Desale and M.G. Bodhankar, "Antimicrobial activity of endophytic fungi isolated from *vitex negundo* Linn." International journal of current microbiology and applied sciences, 2013, 2 (12).
6. E. C.Jeyaseelan, M.K.Pathmanathan and J.P.Jeyadevan, "Inhibitory effect of different solvent extract of *Vitex negundo* L. and *Allium sativum* L. on phytopathogenic bacteria" Archive of Applied science research, 2010, 2(6), 325-331.
7. Prasanna K and Yuwvaranni S, "Preliminary phytochemical screening and antibacterial activity of *Datura metel* and *vitex negundo* against bacterial cold water disease causing organism" International journal of pharmacy and

- pharmaceutical sciences, 2014, 6 (5).
8. M.G. Dharmasiri, J.R.A.C.Jayakody, G.Galhen, S.S.P. Liyanage, W.D. Ratnasooriya, "Anti- inflammatory and analgesic activities of mature fresh leaves of *vitex negundo*" journal of Ethnopharmacology, 2003, 87, 199-206.
 9. Naseem Ahmad, Md Imran khan, Sarfraz Ahmad, Saad Bin Javad, Mohammad Faisal, Mohammad Anis, Sumbul Rehman, Syed Mohammad Umair, "Change in total phenolic content and anti-bacterial activity in regenerants of *vitex negundo* L." Acta physiol plant , Springer,2012, DOI 10.1007/s11738- 012-1120-x.
 10. Sanjay Guleria and Ashok Kumar, "Antifungal activity of some Himalayan medicinal plants using direct bioautography" Journal of cell and molecular biology, 2006, 5, 95-98.
 11. Veena Prajapati, A. K Tripathi, S. PS. Khanuja and Sushil Kumar, "Anti-insect screening of medicinal plants from kukrail forest, Lucknow, India" pharmaceutical biology (Taylor and Francis) , 2003, 41 (3), 166-170.
 12. Raghavendra H. Lakshmanashetty, Vijayananda B.Nagaraj, Madhumathi G.Hiremath and Vadlapudi Kumar, "In vitro antioxidant activity of *vitex negundo* L. leave extracts" Chiang Mai. Journal sciences, 2010, 37 (3), 489-497.
 13. M. Ashrafal Alam, M. Mostafizur Rahman, Nusrat Subhan, Muntasir Mamun Majumder, S.M Raquibul Hasan, Raushanara Akter, M. Ehsanul Haque Mazumder and Abdullah Faruque, "Antioxidant potential of the ethanol extract of the leaves of *Vitex negundo* L." Turkey journal of pharmaceutical science, 2009, 6 (1), 11-20.
 14. Kaliyaperumal Karunamoorthi, Sayeenathan Ramanujam and Rajendran Rathinasamy, "Evaluation of leaf extracts of *Vitex negundo* L. (Family: Verbenaceae) against larvae of *Culex tritaeniorhynchus* repellent activity on adult mosquitoes",Parasitol res,2008, 103 , 545-550.
 15. Chitra V. Shrinivas Sharma and Nandu Kayande, "Anti-cancer activity of *vitex negundo* in experimental animals: An in vitro and in vivo study" International journal of pharmtech research, 2009, 1 (4), 1485-1489.
 16. J. Reema Murthy, S.Venkataraman, R.Meera, Kiran S.Desmukh, N.Chidambaranathan, P.Devi, "Phytochemical investigation and antipyretic activity of leaf extract of *Vitex negundo* Linn" International journal of pharmtech research, , 2010 2 (2), 1068-1073.
 17. K.Sahayaraj and C.Ravi, "Preliminary phytochemistry of *Ipomea carnea jacq* and *vitex negundo* Linn. leaves" International journal of chemical science, 2008, 6(1), 1-6.
 18. Samreen Fatema, Mazahar Farooqui, Ghumre Parmila and Pathan Mohd Arif, "The phytochemical and antioxidant property of methanoleal extract of *Hibiscus rosasinensis* leaves" Research journal of pharmaceutical , biological and chemical sciences , 2018, 9 (3), 603-608.
 19. Seema Abbas, Hammad Saleem, M.Shoaib Ali Gill, Anam Mubashar Bajwa, Ammar Sarwar and M.Ovias Omer, "Physicochemical, phytochemical and nutritional values determination of *Suada fruticosa* (Chenopodiaceae)" Academica journal of medicinal plants, 2016, 4(8), 1-9.
 20. B. Padma Selvi, G. Prasanna and R. Anuradha, "Physicochemical and phytochemical analysis of the rhizome of *Drynaria quercifolia* L. " International journal of phytopharmacy research, 2016, 7(1), 18- 22.
 21. Nivedita and Priyanka Vijay, "Physicochemical and phytochemical analysis of *Eclipta Alba*" International journal of pharma and bio science, 2013, 4(3), 882-889.
 22. Aina DA, Jonathan SG, Olawuyi OJ, Ojelabi DO and Dutowoju BM," Antioxidant, anti-microbial and phytochemical properties of alcoholic extracts of *Cantharellus cibarius*- A Nigerian mushroom" New York science journal,2012, 5 (10).
 23. Naznin Ara and Hasan Nur, "In vitro antioxidant activity of methanolic leaves and flowers extracts of *Lippia Alba*" Research journal of medicine and medical sciences, 2009, 4(1), 107-110.
 24. Masafumi Okawa, Junei Kinjo, Toshihiro Nohara, Masateri Ono, "DPPH (1,1-diphenyl-2- picrylhydrazyl) radical scavenging activity of flavonoids obtained from medicinal plants" Biol. Pharma. Bull, 2001, 24 (10).
 25. Ahasan Dar, Shaheen Faizi, Sabira Naqvi, Talat Roome, Sadia Zikr-Ur-Rehman, Muhammad Ali, Sadiqa Firdous, Syed Tarique Moin, "Analgesic and antioxidant activity of Mangiferin and its derivatives: the structure activity relationship" Biol pharm Bull, 2005, 28(4), 596-600.