Vitex negundo medicinal plant anylisis by morden HPTLC technique

DB Dupare

Department of Botany, Shri Dr. R.G. Rathod Arts and Science College, Murtizapur, Akola (MS), India

Abstract
Plants have been used traditionally in local people for treatment of bone fracture. The plant parts (extract) identified e.g. (root) serve as major source of active ingredient and products of secondary metabolites e.g alkaloid, terpenoids etc. used in curing diseases. The phytochemical analysis of *Vitex negundo* L was evaluated to ascertain some of the secondary metabolites that exhibit medicinal properties. The results of phytochemical screening of ethanol crude root extract of *Vitex negundo* Lrevealed the presence of alkaloids, tannins, saponins and flavonoids. These metabolites observed by various techniques like solvent extraction by ultra-sonicator, rota-vapour, thin layer chromatography column separation and HPTLC technique.

Keywords: Phytochemical, Medicinal plant, traditional, bone fracture

1. Introduction

Vitex negundo L, commonly known as "Nirgudi." in almost all hot places of India specially Amravati region. It is fairly common in field side which grown in hotter part of the India. Plant *Vitex negundo* Lis extensively utilized for the treatment of pharmaceutical disorders antioxidative, antitumor, antidiabetic, antifungal, antibacterial, properties [1]. It has been reputed in Siddha system of medicine as a remedy to treat bone fracture; plants are an essential and integral component in the world of prescription medicine and have the ability to make various chemical constituents like flavonoids, alkaloids, and steroids.

The present study is to review the overall information on the taxonomical classification, morphology, distribution, traditional uses, phytochemical constituents and recent scientific investigations of *Vitex negundo* L.

2. Materials and Methods

Vitex negundo Lwere collected during the month of February2015-Octomber2015, from melghathill area near to Amravati region Maharashtra, India. The fresh root were separated and kept for shade drying. Dried root material was powdered using mechanical grinder and heat in microwave oven to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

2.1 Preparation of extracts

Dried *Vitex negundo* L. root powder mixed with ethanol and keep in ultra-sonicator for half an hour to mix all chemical constituents in ethanol solvent was subjected to successive extraction in a soxhlet extractor using ethanol and water. The extracts were filtered and the filtrates were concentrated under Rota-vapour at room temperature to obtain the extracts as solid residues.

2.2 Primary Phyto-chemical screening

Phyto-chemical screening was performed using standard procedures is given below.

2.2.1. Test for Terpenoids (Salkowski test): The 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

2.2.2. Test for Flavonoids: Three methods were used to test for flavonoids. First, dilute ammonia (5ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. Third, a portion of the extract was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. In all the cases, a yellow coloration indicating the presence of flavonoids was observed.

2.2.3 Test for Saponins: The 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and the mixture is observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

2.2.4 Test for Tannins: The 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

2.2.5 Test for steroids (Liebermann-Burchard's test): One ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [2].

2.3 Thin Layer Chromatography (TLC): TLC analysis was carried out for the plant extracts dissolved in ethanol and water solvent. For the analysis the silica gel sheet was used, fresh root extracts were analyzed using TLC. The sheets are kept in TLC Chamber for one hour, depending on the polarity of the eluted fractions to be analyzed. The sheets were treated with 1% ninhydrin diluted to acetone.

2.4 HPTLC Technique: HPTLC analysis was carried out for the plant extracts dissolved in ethanol. The HPTLC characterization perform at Sophisticated Instrumentation Centre for Applied Research & Testing (SICART)**,** Sardar Patel Centre for Science & Technology, Charutar Vidya Mandal Vallabh, Vidyanagar.

3. Results and Discussion

3.1 Phytochemical Investigation: The Phytochemical screening of *Vitex negundo* L. showed positive results as the tests like Terpenoids, Flavonoids, Saponins, Tannins and steroids. This data clear that there is presence of various phytochemical in *Vitex negundo* L. plant extract

Table 3.1: Phytochemical screening of extracts of medicinal plants

Sr no	Test perform	ethanolic extract	aqueous extract
1	Terpenoids	+	+
2	Flavonoids	+	+
3	Saponins	+	+
4	Tannins	+	-
5	Steroids	+	+

3.2. Quantitative spectrophotometric analysis for phenolic content and flavonoids:

The total phenolic and flavonoids content of plant aqueous extract were determined spectrophotometrically using the tannic acid and quercetin standard calibration curves, respectively [3]. Both standard curves showed linearity with R_2 value 0.962 and 0.956. The total phenolic and flavonoids content was found as per given table 3.2. as antioxidant used in medicinal application to cure bone fracture.

Table 3.2: Total phenolic and flavonoids contain in *Vitex negundo* L plant

Sr. no	Plant name	Phenolic (ug/ml)	Flavonoids (ug/ml)	Alkaloids (ug/ml)	Steroids (ug/ml)
1	***Vitex negundo* L.**	9.568	25.672	2.265	34.233

3.3 TLC purification of the extracts

The TLC of ethanolic extract of *Vitex negundo* L. plant is shown in (Figure 3.c) with their RF values. From the figures it is evident that there are many components that are responsible for the antioxidant activity. Hence, further investigations are required to isolate, purify and characterize those compounds which are responsible for the antioxidant activity used in medicinal application to cure bone fracture.

Table 3.3 TLC purification and partition *Vitex negundo* L. plant.

Sr. No.	Plant name	N0. of Bands	Rf Value	Spraying Regents	Colour of Band appeared	Phytochemical Detected
1	*Vitex negundo* L.	6	0.11	Vanillin-sulphuric acid reagent	blue	Saponins
			0.43	5% Ferric chloride	Dark grey	Flavonoid
			0.54	5% Ferric chloride	Dark grey	Flavonoid
			0.64	FeCl3	Intense red	Phenol
			0.78	Ethanolic sulphuric acid	brown	Alkaloids
			0.83	Vanillin-phosphoric acid reagent	dark blue	Terpenoids

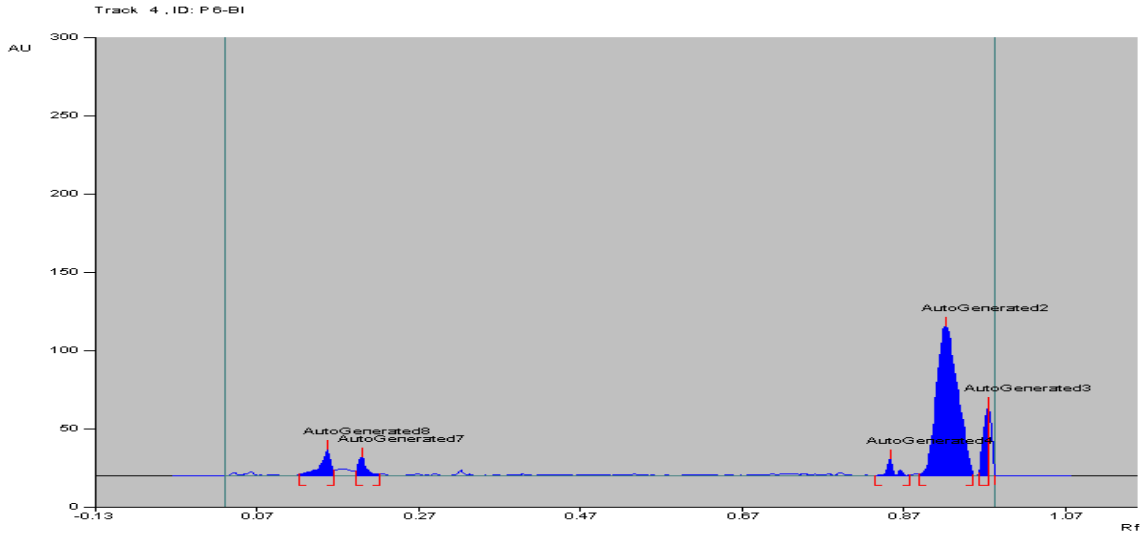
3.4 High Performance Thin Layer Chromatography (HPTLC)

The HPTLC characterization perform at Sophisticated Instrumentation Centre for Applied Research & Testing (SICART), Sardar Patel Centre for Science & Technology, Charutar Vidya Mandal Vallabh, Vidyanagar for proper identification of the seven medicinal plant. Chromatographic fingerprint profile of ethanol extracts of all plants were studied by HPTLC. For better resolution and maximum number of spots, and satisfactory resolution was obtained in the solvent Toluene: Ethyl acetate: Formic acid is given as 8:4:2. After scanning and visualizing the plates in absorbance mode at both 254nm and 366 nm range.

The results from HPTLC finger print, The Rf values ranged from 0.07 to 0.99. It is also clear from Table 3.4 and the chromatogram as shown below figure that were found to be more predominant as the percentage area is more with respectively. HPTLC plate showed different color phyto-constituents of ethanol extract. The bands revealed presence of different colour bands showing the presence of steroids, flavonoids alkaloids, terpenoids and saponins etc.

Table 3.4: HPTLC RF Value of *Vitex negundo* L

Sr. no.	Plant name	No. of Bands	Rf Value
1	*Vitex negundo* L	5	0.12,0.22,0.82,0.91,0.98.



5. Conclusion

In the present investigation, *Vitex negundo* L. Medicinal plant species used to treat bone fracture were reported. The uses of these plants to treat various illnesses by the communities. The majority of the reported species are wild and rare. These demand an urgent attention to conserve such vital resources so as to optimize their use in the primary health care system. Now a day, conservation of traditional knowledge is necessary related to modernization of the region and lack of interest in traditional patrician, in transferring it to next generation. Further advanced spectroscopic studies are required for the structural elucidation and identification of compounds.

6. Acknowledgement

Author is thankful to UGC for providing the financial assistance for minor research project for phyto-chemical study of medicinal plant. He also thankful to direct and indirect support to carried research work, field work and characterization and laboratory facilities of various department.

7. References

1. Sharma A, Sharma RA, Singh H. Phytochemical and Pharmacological Profile of Abutilon Indicum L. Sweet: A Review, Int. J. Pharm. Sci. Rev. Res., 2013; 20(1):120-127.
2. Sarkar R, Haque A, Ranjan S, Sarker M. Phyto-chemical Screening, Antioxidant and Antimirobial Effects Abutilon indicum (L.) Leaves Extracts j pharmacology Archives, 2015; 1:94-103.
3. Singh R, Mendhulkar VD. Journal of Chemical and Pharmaceutical Research, 2015; 7(6):205-211.