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Original Research Article

Evaluation of Phytochemical Compounds in Leaf Extract of *Vitex Negundo* L. Using TLC, UV-VIS and FTIR Analysis

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ABSTRACT

Objective: The present study was carried out to investigate the medicinally active substances present in the methanolic leaf extract of *Vitex negundo* by using the analysis of TLC, UV-VIS and FTIR.
Methods: In the present investigation, chromatographic techniques such as Thin Layer Chromatography (TLC) analysis was used to separate and isolate flavonoid compound from the crude leaf extract of *Vitex negundo* L. The solvent system of TLC was n-Butanol, Acetic acid and water in the ratio of 4:1:5 was used and its R_f value was detected. For UV-VIS Spectrophotometric analysis, the leaf extract of *Vitex negundo was* scanned in the wavelength ranging from 250-900 nm by using Perkin Elmer Spectrophotometer and the characteristic peaks and their absorption values were detected. For FTIR Analysis, the leaf exract of *Vitex negundo* was focused in the transmittance ranging between 400-4000cm^1 on a Perkin Elmer Spectrophotometer system and the characteristic peak values and their functional groups were detected.
Results: From TLC analysis result, a spot was identified with R_f value was 0.84.This R_f value was compared with literature data showed that the presence of flavonoid compound as quercetin. The UV-VIS profile showed the peaks at 206,243,249 and 295 nm with the absorption values 1.1489, 4.1168, 3.8773 and 2.0162 respectively. The result of UV-VIS spectroscopic analysis confirms the presence of phenols and Flavonoids in the vitex negundo extract. The results of the present FTIR study confirms the presence of Phenol, Alkane, Alkene, Carboxylic acid, Aromatic compound, Nitro compound, Alcohol, Benzene and Bromo alkanes compounds.
Conclusion: The results of the present study were revealed that the presence of phenols, flavonoids and functional groups of the *Vitex negundo* which indicates the medicinal importance of this plant and also used to identify the plant in the pharmaceutical industry.

Key words: *Vitex negundo* L., TLC, UV-VIS, FTIR, Phytochemicals, Medicinal importance.

INTRODUCTION

Medicinal plant research includes much more than the discovery of new drugs. This field has been expanding to also include diverse subjects as negotiation of power based on medicinal plant knowledge. [1] Plants generally contain both primary metabolites as well as secondary metabolites. The different phytoconstituents present in plants include anthraglycosides,

arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents would help in determining various biological activities of plants. Natural products, either as pure compounds or as standardized plant extracts, provideunlimited opportunities for new drug. [2]

A variety of techniques can be used to determine and estimate the presence of such phytoconstituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. Thin layer chromatography (TLC) is among various chromatographic methods, a comparatively simple, rapid and convenient method frequently used for identifying many pharmaceutical substances. [3] Thin layer Chromatography (TLC) is a method of analysis in which the stationary phase, a finely divided solid is spread as a thin layer on a rigid supporting plate and the mobile phase, a liquid is allowed to migrate across the surface of the plate. Although separation efficiencies equivalent to those obtained with gas or high-pressure liquid chromatography cannot be obtained by this method, it has the advantages of speed, versatility and simplicity. [4]

UV-Visible Spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis and involves measuring the amount of ultraviolet absorbed by a substance in solution. Instrument which measure the ratio or function of ratio of the intensity of two beams of light in the UV-Visible region are called Ultraviolet-Visible spectrophoto-meters. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. [5]

FTIR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures and it has been used as a requisite method to identify medicines in Pharmacopoeia of many. [6] FTIR can be employed to determine the structure of unknown composition and the intensity of the absorption spectra associated with molecular composition or content of the chemical group. [7,8] The FTIR method measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By attaining IR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites. [9] At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites. [10-12]

Vitex negundo L. (Lamiaceae), a large, aromatic shrub or sometimes a small slender tree, upto 4.5 m in height found throughout the greater part of India. *Vitex* comprises of about 250 species having tri or pentafoliate leaves borne on quadrangular branches. It bears bluish- purple coloured flowers in pendent branched tormentose cymes. [13,14] Phytochemical analysis of plant showed that its leaves contains alkaloid (nishundine), flavonoids like flavones, luteolin-7- glucoside, casticin, iridoid glycosides, an essential oil and other constituent like vitamin C, carotene, benzoic acid, β-sitosterol and C-glycoside. [15] The whole plant is used in anticancer, inflammations, antiseptic, antipyretic,

diuretic, antihistamine, antioxidant, antibacterial, CNS depressant, antifungal, snake venom neutralization, mosquito repellant activity, insecticidal, larvicidal efficacy, antinociceptive, antiandrogenic, hepatoprotective, antifertility, skin aging inhibitor and anti dopaminergic effects [16] Roots play vital role in rheumatism, dyspepsia, piles etc.[17,18] Although all plant parts are used, but the leaves and root extract constitute more significant medicinal activity. [19] Therefore, the present research work was designed to investigate the phytochemical and functional constituents of *Vitex negundo* by using of TLC, UV-VIS and FTIR analysis.

MATERIALS AND METHODS

Plant Material: The leaves of *Vitex negundo* L. were collected from the region of Manapparai, Trichy district, Tamilnadu, India in the month of January. The botanical identity of the plant material was authenticated by Botanical Survey of India, Coimbatore, Tamilnadu. A voucher specimen of the plant material was deposited in our department under the number BSI/SRC/5/23/2014-2015/TECH/540 for further reference.

Chemicals: All chemicals and reagents used in the study were obtained commercially and were of analytical grade.

Preparation of Extract: *Vitex negundo* leaves were dried under shade and then powdered with a mechanical grinder to obtain a coarse powder. The powder was then subjected to continuous hot extraction process using Soxlet apparatus at 60°C with methanol (90%) for 72hrs. After extraction, the solvent was removed by rotary evaporator at 200ºC. The extract was concentrated and stored in a desiccator.

TLC analysis: Flavonoid compound of methanolic leaf extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel G were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm. In chamber with solvent system (n-Butanol: Acetic acid: Water (4:1:5) was used as mobile phase. After presaturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared ammonia reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its R_f value was calculated for sample. [20]

UV –VIS Spectrophotometric Analysis: The methanolic leaf extract was examined under UV Visible spectral analysis. The extract was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 250-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. [21]

FTIR Analysis: FTIR spectrophotometer was performed to assess the functional groups of the leaf extract. The hydraulic pellet press method was followed. Samples were taken in 1:100 ratios with potassium bromide and mixed uniformly in a porcelain dish to prepare the pellets. The transmittance was recorded between 500 and 3500 cm^{-1} on FTIR spectrophotometer (Jasco FT/IR-6300). The functional groups present in the leaves were identified from the spectra. [22]

RESULTS

Thin layer chromatogram of methanolic leaf extract of *Vitex negundo* was given in fig 1 and its R_f value was given in Table.1.TLC of methanolic extract of *Vitex negundo* revealed the presence of a spot having R_f value of 0.84 when a solvent

phase of n-Butanol : Acetic acid : Water (4:1:5) solvent system was used. This R_f value 0.84 was compared with literature data and it was identified as flavonoid compound as quercetin in the methanolic extract of *Vitex negundo*. [23]

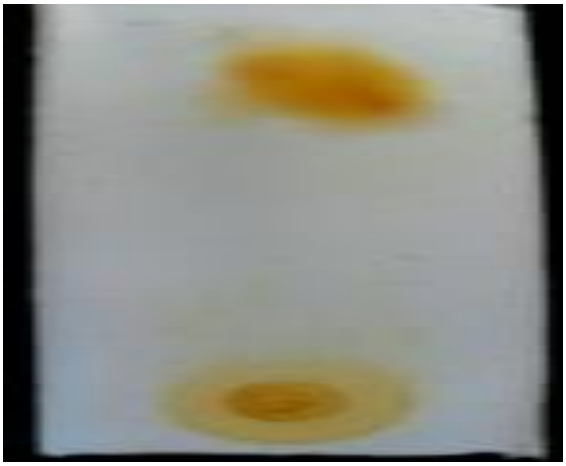

Fig 1: Analysis of flavonoid in methanolic leaf extract of *Vitex negundo* L. by TLC

Table 1: Analysis of flavonoid in methanolic leaf extract of *Vitex negundo* L. by TLC

Phytoconstituent	R_f Value	Result	Literature (Gordana *et al.*, 2003)
Flavonoid	4.2/5	0.84	Quercetin

All determinations were performed in triplicates (n=3): The qualitative UV-Vis spectrum profile of methanolic leaf extract of *Vitex negundo* was selected from 250 nm to 900 nm due to sharpness of peaks and proper baseline. UV-Vis spectrum profile of methanolic leaf extract of *Vitex negundo* was given in Fig.2 and its absorption values were given in table 2.The profile showed the peaks from 250 to 900 nm and the profile showed the peaks at 206,243,249 and 295 nm with absorption values of 1.1489, 4.1168, 3.8773, and 2.0162 respectively. The UV-Vis spectrum of methanolic leaf extract of *Vitex negundo* was taken at 4.1168 and 3.8773 respectively. UV-VIS analysis result compared with literature data, The spectra for phenolic compounds (tannins) and Flavonoids typically also lie in the range of 230-290 nm. [24] The result of UV-VIS spectroscopic analysis confirms the presence of phenols and Flavonoids in the *Vitex negundo* extract.

Table 2: UV-Vis Peak Values of Methanolic Leaf Extract of Vitex negundo L.

S.No	Nanometers	Absorption values	Literature (Neha etal.,2006)
1	206	1.1489	**Phenol and Flavonoid**
2	**243**	**4.1168**	
3	**249**	**3.8773**	
4	295	2.0162	

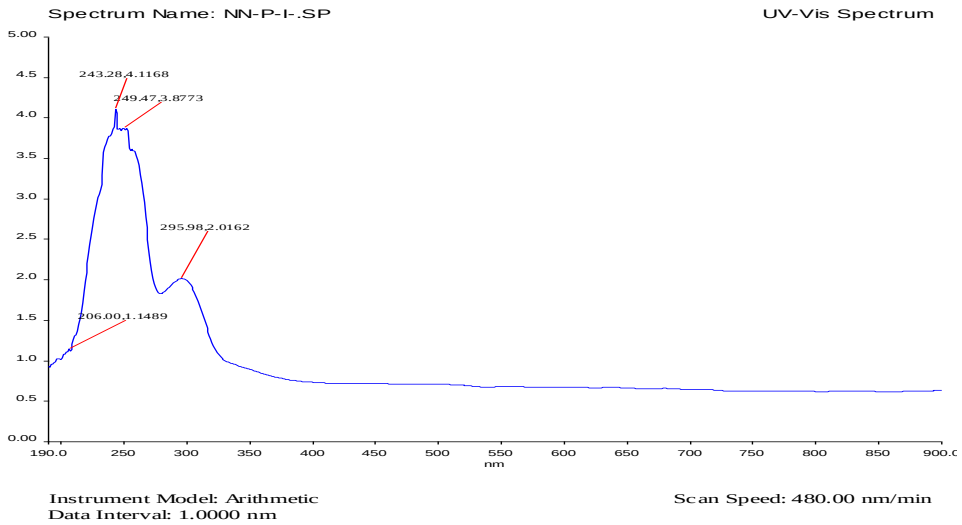

Fig 2: UV-Vis Spectrum of Methanolic Leaf Extract of *Vitex negundo* L.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR spectrum and its peak values with functional groups were represented in Fig 3 and Table 3. When the *Vitex negundo* leaf extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, and benzene and bromo alkanes compounds which shows major peaks at 3410.12, 2924.92, 2347.26, 1697.07, 1448.64, 1388.24, 1046.48, 852.44 and 600.63 respectively. [25]

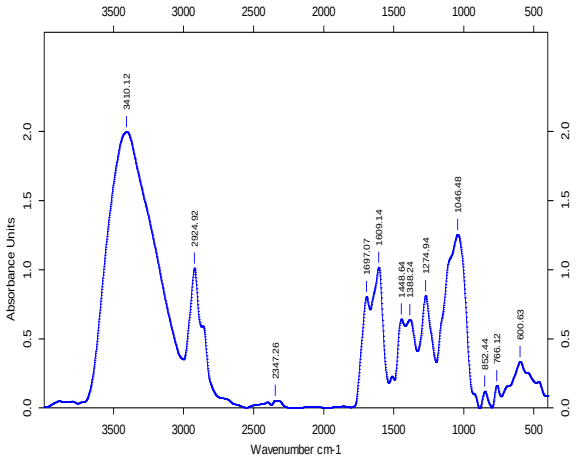

Fig 3: FTIR spectrum of methanolic leaf extract of *Vitex negundo* L.

Table 3: Functional group analysis of methanolic leaf extract of *Vitex negundo* L by using FTIR spectroscopy.

S.No	Peak Values	Bond	Functional Groups
1	3410.12	O-H	Alcohols, Phenols
2	2924.92	C-H	Alkane
3	2347.26	C=C	Alkene
4	1697.07	C=O	Carboxylic acid
5	1609.14	C=O	Carboxylic acid
6	1448.64	C=C	Aromatic compound
7	1388.24	N-O	Aliphatic Nitro compound
8	1274.94	C-O	Carboxylic acid
9	1046.48	C-O	Primary alcohol
10	852.44	C-H	Para Benzene
11	766.12	C-H	Meta benzene
12	600.63	C-X	Bromo alkanes

DISCUSSION

At present, a number of analytical tools (chromatographic and spectroscopic) have been used to analyze flavonoids in plant samples or crude drugs. Thin Layer Chromatography (TLC) is one of the most popular and widely used separation techniques because of its ease of use, cost effectiveness, high sensitivity, speed of separation as well as its capacity to analysis multiple samples simultaneously. The technique can be utilized for separation, isolation, identification and quantification of components in a mixture. It can also be utilized on a preparative scale to isolate a particular component. The present study of TLC results revealed that the presence of flavonoid as quercetin in the leaf extract of *Vitex negundo*. The results also suggest that the leaf extract of *Vitex negundo* has antioxidant and anti inflammatory properties. [26]

Spectroscopic methods have become a powerful tool for secondary metabolite profiling as well as for qualitative and quantitative analysis of the pharmaceutical and biological materials. The present study of UV-VIS spectrophotometer revealed that the presence of phenolic compound like tannin and flavonid compound which indicates the medicinal properties of this plant. Phenolic compound tannin used as antioxidant, anti inflammatory and anti cancer and flavonoid compound used as Antioxidative Activity, Hepatoprotective, Anti-Inflammatory, Anticancer and Antiviral activity of this plant extract also observed form this study. [27]

By using FT-IR spectrum, we can confirm the functional constituent's presence in the given leaf extract and even evaluate the qualities of medicinal materials. The results of the present study spectrum also revealed the functional constituents present in methanolic leaf extracts of *Vitex negundo*. The results of the present study confirms the presence of phenol, alkane,

alkene, carboxylic acid, aromatic compound, nitro compound, alcohol, benzene and bromo alkanes compounds in methanolic leaf exrtract of *Vitex negundo*. The results of the present study suggest that various medicinal properties of the *Vitex negundo*. [28] The results of the present study developed novel phytochemical marker to identify the medicinally important plant. Further advanced spectroscopic studies are required for the structural elucidation and identification of active principles present in the leaves of *Vitex negundo*.

CONCLUSION

The present study demonstrated that *Vitex negundo* has rich source of secondary metabolites. These findings suggested that *Vitex negundo* could be a potential source of natural antioxidant having great importance as a therapeutic agent and preventing oxidative stress related degenerative diseases. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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