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Original Article

PHYTOCHEMICAL SCREENING, ELEMENTAL AND FUNCTIONAL GROUP ANALYSIS OF *VITEX NEGUNDO* L. LEAVES

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ABSTRACT

Objective: To analyse elemental profile, evaluate functional groups and find out phytochemical constituents of *Vitex negundo* L. leaves.

Methods: Determination of primary and secondary metabolites for various extracts by qualitative methods. Elemental analysis was carried out by using inductively coupled plasma mass spectrometry (ICP-MS) technique and the functional groups have been determined by using Fourier Transform Infrared Spectroscopy (FTIR) technique.

Results: Soluble extractive percentage of material has been found the maximum in the aqueous extract (6.75%) followed by methanolic extract (4.35%) and acetone extract (1.8%). Phytochemical screening of material revealed the presence of carbohydrates, proteins, amino acids, steroids, cardiac and anthraquinone glycosides, saponins, flavonoids, tannins and phenolic compounds. The elemental analysis revealed Na, Mg, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, Se, Mo, Li, B, Al, P, Cd, As, Ba, and Hg. FTIR technique was used to identify various functional groups present in three different extracts of the material.

Conclusion: The phytochemical screening of three different extracts of the material showed the presence of most of the primary and secondary metabolites in aqueous and methanolic extract than acetone extract. The soluble extractive value was found the maximum in aqueous extract hence aqueous extract is most effective for studying the pharmacological activity of this plant. Elemental analysis showed the presence of trace elements in sufficient concentrations and traces of heavy and toxic metals. The FTIR study revealed the presence of essential functional groups in three different extracts of the material. The present investigation is most essential to discover innovative, dynamic and novel drugs for curing various newly emerged dangerous health problems.

Keywords: Phytochemical screening, Elemental analysis, FTIR, ICP-MS, Secondary metabolites

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INTRODUCTION

Herbal medicine is as old as a human race and is being used for the primary health care of human being since ancient period. Traditional medicinal plants contain therapeutic active substances, which is a precursor for the synthesis of herbal drugs [1]. Important drugs have been derived from plant resources directly or indirectly. Phytochemicals such as alkaloids, flavonoids, phenolic compounds, glycosides etc. play a significant role in pharmacological properties of plants. Screening of phytochemicals gives an idea about medicinal properties and values of plants. *Vitex negundo* L. belongs to the family Verbenaceae. It is woody, aromatic shrub, commonly known as nirgudi in Marathi and five-leaved chaste tree in English. In Ayurveda, it is used as antihelmentic and in folklore system, it is used in the treatment of asthma, cancer, jaundice, liver disorders, wounds, rheumatism, joint pains, antiallergic agent etc [2]. Extract of nirgudi has been shown various biological activities such as antibacterial [3], antifeedant [4], antifungal [5], antilarval [6], insecticidal [7], pesticidal activity [8], analgesic activity [9], anti-inflammatory activity [10, 11], anticonvulsant activity [12], antioxidant activity [13, 14] etc. Almost all parts of nirgudi like leaves, roots, bark, fruits, flowers and seeds are used for medicinal purpose in the form of powder decoction, juice, oil, dry extract etc [15]. *Vitex negundo* used as folk medicine in most of the states of India.

Phytochemical constituents of medicinal plants have been considered to be a basic requirement in the discovery of potent medicines and remedies on various diseases in ayurvedic and nutraceutical research. Medicinal properties of any plant are depending on the presence of phytoconstituents and nutritive elements as well as minerals. Medicinal plants are effective against various health problems due to their pharmacological efficacy which is depends on their elemental concentrations [16]. Likewise, phytochemical screening, elemental and functional group analysis of plant extract is also essential to evaluate medicinal properties of *Vitex negundo.* Hence present research was designed to evaluate soluble extractive percentage, phytochemical screening, elemental and functional group analysis of *Vitex negundo* leaves.

MATERIALS AND METHODS

Chemicals and reagents

Millions reagent, ninhydrin reagent, benedicts reagent, glacial acetic acid, acetone, tannic acid (Loba), picric acid (Himedia), methanol (Himedia), multi-element standards (Fluka make, USA), sodium nitropruside, ferric chloride (Sigma-Aldrich).

Plant collection and authentication

Vitex negundo L. was collected from Pune district, Maharashtra (India). The plant has been identified morphologically and authenticated by Senior Scientist D; at Botanical Survey of India (BSI), Pune, Maharashtra (India). Here a voucher specimen no. VMK 04 has been submitted.

Preparation of plant extract

Shade dried leaves of *Vitex negundo* were ground by the mechanical grinder and extracted with acetone, methanol and water by maceration process. Concentrated extracts have been used for phytochemical and FTIR spectroscopy analysis.

Determination of extractive value of *Vitex negundo* L. leaves

Three solvents i.e. methanolic, aqueous and acetone have been used to find the comparatively higher extractive value of *Vitex negundo* L. leaves [17].

Phytochemical screening

Phytochemical screening has been done for detection of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids, tannins and phenolic compounds [17, 18].

FTIR spectroscopy analysis

FTIR spectroscopy (Model-Nicoleti S 5) analysis used for determination of herbal functional groups [19].

ICP-MS analysis

Dry leaves powder of *Vitex negundo* was analysed for elemental detection which was conducted on ICP-MS technique. ICP-MS is most trusted technique for elemental analysis due to its rapid detection and capability to detect elements in extremely minute concentrations [20].

RESULTS

Maximum soluble extractive percentage of *Vitex negundo* leaves was found in the aqueous extract (6.75%) followed by methanolic extract (4.35%) and less in acetone extract (1.8%) (Table 1).

Table 1: Percentage value of soluble extractive of *Vitex negundo* L

Solvents	Weight of plant material (g)	Soluble extractive percentage (%) of *Vitex negundo* L. leaves
Aqueous	2	6.75
Methanol	2	4.35
Acetone	2	1.8

Table 2: Phytochemical screening of aqueous, methanolic and acetone extracts of *Vitex negundo* L

S. No.	Secondary metabolites	Phytochemical tests	*Vitex negundo* L. leaves		
			Methanolic extract	Acetone extract	Aqueous extract
1.	Carbohydrates	Molisch's Test	++	++	++
2.	Proteins	Millon's Reagent Test	++	-	++
3.	Amino acid	Ninhydrin Test	++	-	++
4.	Steroid	Liebermann Burchard Reaction	++	-	-
5.	Glycosides	Legal's Test	-	-	+
	a)Cardiac glycosides				
	b)Anthraquinone glycosides	Borntrager's Test	++	++	++
	c)coumarin glycosides	Fluorescence Test	-	-	-
	d)Saponin glycosides	Foam Test	-	-	++
6.	Flavonoids	Sodium hydroxide test	-	++	+
7.	Alkaloids	Mayer's Test	-	-	-
		Hager's Test	-	-	-
8.	Tannins and phenolic compounds	Dilute nitric acid test	++	++	-

"**+**= Present; -= Absent"

The phytochemical screening of three different extracts of *Vitex negundo* L. leaves showed the presence of phytochemicals like carbohydrates, proteins, amino acids, saponins, flavonoids, anthraquinones, tannins and phenolic compounds. The results of present study are helpful for the discovery of potent remedies on various diseases. Medicinal plants are main source for potent bioactive compounds. Acetone extract and aqueous extract of *Vitex negundo* leaves shows the presence of flavonoids, the present study also useful for preparing flavonoid-based drugs which have been most important in antioxidant, antiallergic, antimicrobial properties (table 2).

Table 3: Elemental analysis of *Vitex negundo* L. leaves by ICP-MS

Vitex negundo L. Leaves			
Elements	Conc (ppm)	Elements	Conc(ppm)
7 Li [1]	0.35	60 Ni [1]	3.06
9 Be [1]	0.00	63 Cu [1]	8.944
11 B [1]	73.6	66 Zn [1]	40.489
23 Na [1]	31.98	75 As [1]	0.26
24 Mg [1]	5663.8	82 Se [1]	159.8
27 Al [1]	876.3	95 Mo [1]	0.268
31 P [1]	4130.6	107 Ag [1]	0.00
39 K [1]	2332.4	111 Cd [2]	0.09
43 Ca [1]	1566.2	118 Sn [1]	0.00
52 Cr [1]	4.16	137 Ba [1]	0.00
55 Mn [1]	214.8	208 Pb [2]	0.00
56 Fe [1]	128.1	202 Hg [1]	0.12
59 Co [1]	0.71	209 Bi [1]	0.00

Elemental analysis of *Vitex negundo* leaves by ICP-MS technique has been confirmed the presence of pharmaceutically active, major, minor and trace elements. The results of elemental analysis were recorded in table 3, which revealed presence of B (73.6 ppm), Na (31.98 ppm), Mg (5663.8 ppm), P (4130.6 ppm), K (2332.4 ppm), Ca (1566.2 ppm), Cr (4.16 ppm), Mn (214.8 ppm), Fe (128.1 ppm), Ni (3.06 ppm), Cu (8.944 ppm), Zn (40.489 ppm), Se (159.8 ppm) and Mo (0.268 ppm). Some heavy metals have been found in trace and higher amount such as Li (0.35 ppm), as (0.26 ppm), Cd (0.09 ppm), Hg (0.12 ppm) and Al (876.3 ppm). Toxic metals like Be, Ag, Sn, Ba, Pb and Bi were found totally absent in *Vitex negundo* leaves.

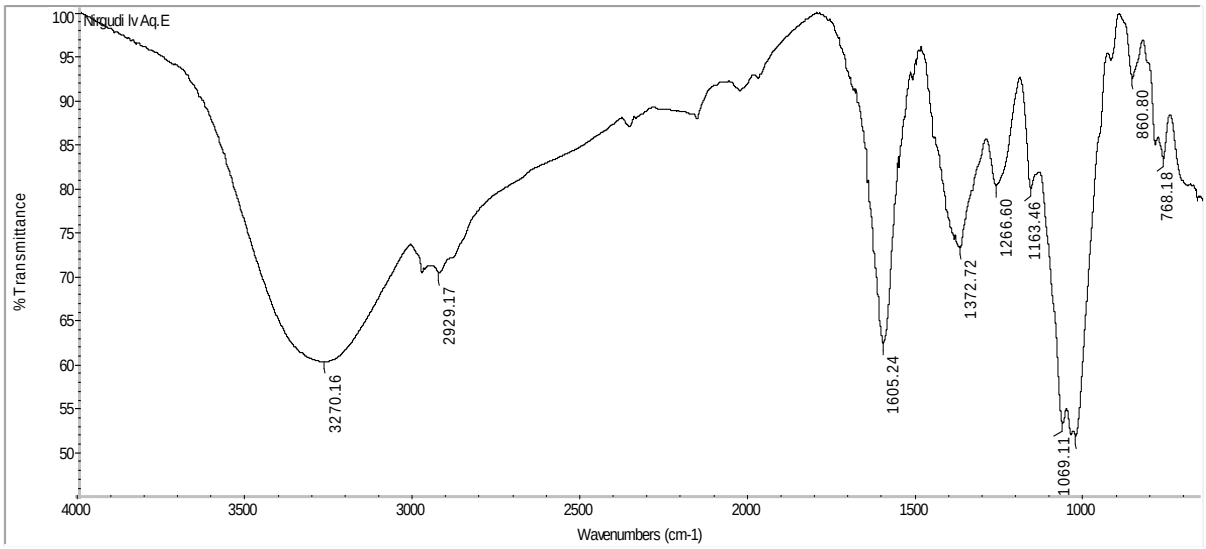

Fig. 1: FTIR spectrum aqueous extract of *Vitex negundo* leaves

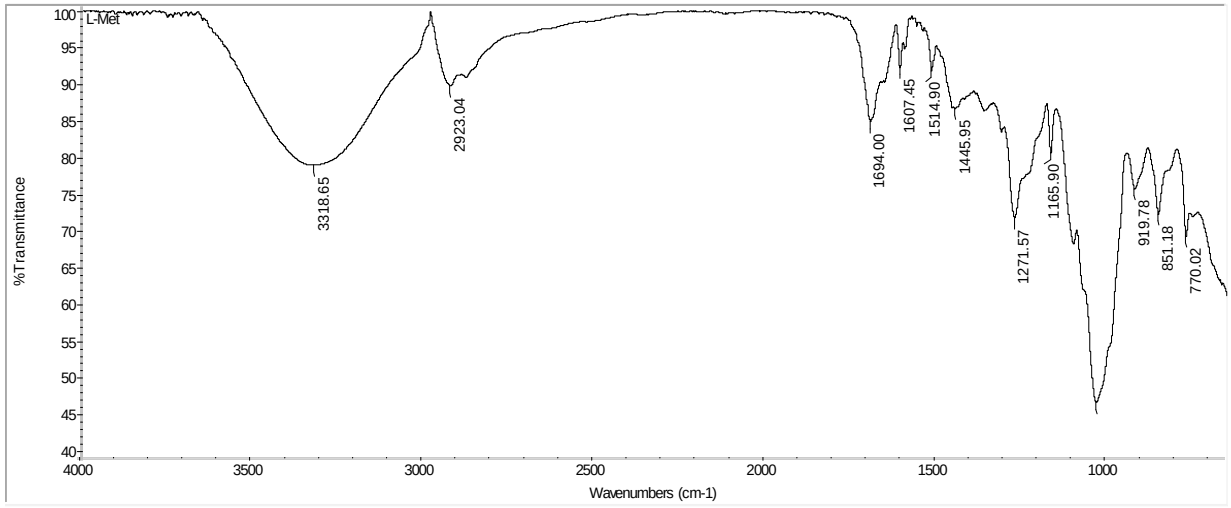

Fig. 2: FTIR spectrum methanolic extract of *Vitex negundo* leaves

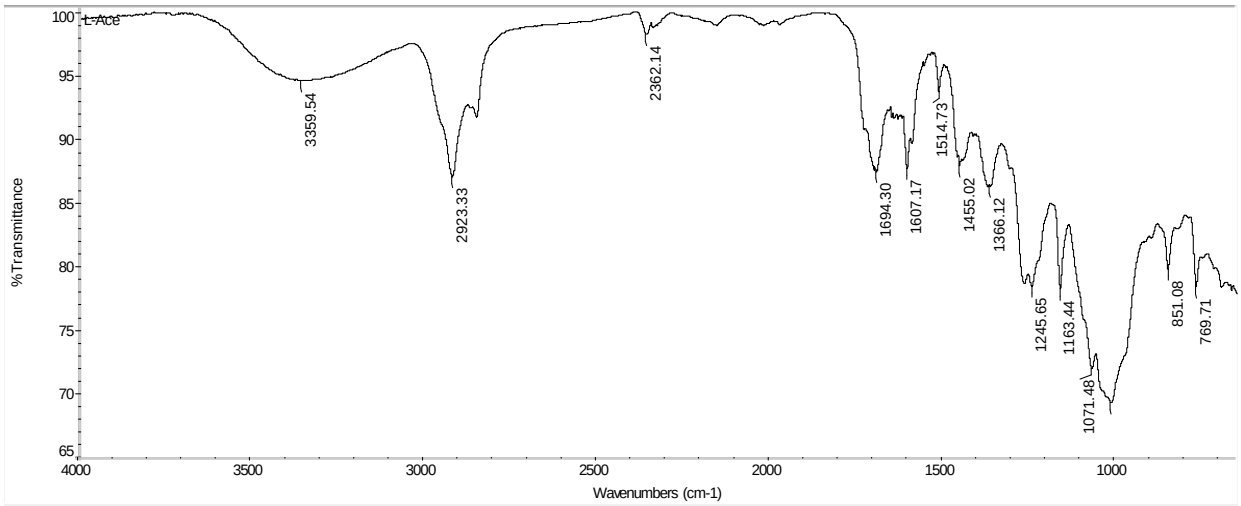

Fig. 3: FTIR spectrum acetone extract of *Vitex negundo* leaves

Table 4: Spectra analysis of *Vitex negundo* L. leaves extracts

S. No.	*Vitex negundo* leaves functional groups assignment	Component (peaks)	Absorption spectrum, frequency (cm^{-1})			
			Wave number cm^{-1} (Reference article) [21, 22]	Aqueous extract	Methanolic extract	Acetone extract
1	Hydroxyl Compound	O-H Stretch (Strong, Broad)	3200-3400	3270.16	3318.65	3359.54
2	Asymmetric stretching	C-H vibration (Medium stretch)	2915-2935 2800-3000	2929.17	2923.04	2923.33
3	Ester/Phosphorus function	P-H-stretching (Weak stretch)	2325-2425	--	--	2362.14
4	Saturated fatty acid	(Medium stretch)	1600-1800	--	1694	1694.3
5	Amine	N-H (Medium stretch)	1550-1650	1605.24	1607.45	1607.17
6	Aromatic compounds	C=C (Weak/medium stretch)	1400-1600		1514.9	1514.73
				--	1445.95	1455.02
7	Alkanes	C-H (Medium stretch)	1350-1480	1372.72	--	1366.12
8	Aromatic compounds (Oxidized Nitrogen Function)	N-O (Medium stretch)	1080-1360	1266.6	--	--
9	Carboxylic acid and derivatives	C=O (Strong stretch)	1210-1320	--	1271.57	1245.65
10	Amine	N-H (Medium stretch)	1080-1360	1163.46	1165.9	1163.44
11	Ether	C-O (Strong stretch)	1000-1300	1029.44	1032.18	1071.48
						1015.91
12	Branched chain Alkanes	C-H (Weak bend)	919-922	--	919.78	--
13	1 ° and 2 ° Amines	N-H (Medium strech)	660-900	860.8	851.18	851.08
		C-N		768.18	770.02	769.71

The results of FTIR analysis of *Vitex negundo* leaves extracts have been recorded in table 4, fig. 1, fig. 2 and fig. 3. The present investigation of FTIR analysis revealed the presence of probable phytoconstituents as per the identified herbal functional groups from the *Vitex negundo* leaves extracts. Characteristic peaks for hydroxyl compounds O-H (Stretch) were obtained in aqueous (3270.16 cm^{-1}), methanolic (3318.65 cm^{-1}) and acetone (3359.54 cm^{-1}) extracts. C-H vibrations of asymmetric stretching have been found in aqueous, methanolic and acetone extracts of *Vitex negundo* leaves such as 2929.17 cm^{-1}, 2923.04 cm^{-1} and 2923.33 cm^{-1}. Ester/Phosphorus function P-H (stretch) was recorded only in acetone extract at 2362.14 cm^{-1} and functional group for saturated fatty acids was recorded in the methanolic extract at 1694 cm^{-1} and acetone extract at 1694.3 cm^{-1}. The present study confirms the presence of amines N-H(stretch) and C-N (stretch) in all extracts but at different characteristic peaks, in aqueous extract at 1605.24 cm^{-1}, 1163.46 cm^{-1}, 860.8 cm^{-1}, 768.18 cm^{-1}, in methanolic extract at 1607.45 cm^{-1}, 1165.9 cm^{-1}, 851.18 cm^{-1}, 770.02 cm^{-1} and in acetone extract at 1607.17 cm^{-1}, 1163.44 cm^{-1}, 851.08 cm^{-1}, 769.71 cm^{-1}. Aromatic compounds C=C (stretch) and oxidized nitrogen function aromatic compounds N-O (stretch) have been recorded at 1514.9 cm^{-1}, 1445.95 cm^{-1} in methanolic extract, at 1514.73 cm^{-1}, 1455.02 cm^{-1} in acetone extract and at 1266.6 cm^{-1} in aqueous extract. Alkanes and branched chain alkanes were found in the aqueous extract at 1372.72 cm^{-1}, in acetone extract 1366.12 cm^{-1} and in the methanolic extract at 919.78 cm^{-1}. The characteristic peaks detected at 1271.57 cm^{-1} for methanol extract and 1245.65 $cm^{-1}$for acetone extract is assigned to Carboxylic acid and derivatives C=O (stretch). Ether C-O (stretch) has been obtained in all extracts at 1029.44 cm^{-1} in aqueous extract, at 1032.18 cm^{-1} in methanolic extracts and at 1071.48 cm^{-1}, 1015.91 cm^{-1} in acetone extract.

DISCUSSION

Vitex negundo has been traditionally used in ayurvedic medicine to treat several disorders such as catarrh, headache, neck gland sores, tubercular neck swellings, sinusitis, sexual debilities, nervous debility, liver complaints, inflammation of uterus, fever, diarrhoea etc [23]. The result of present research important to proves the medicinal properties of *Vitex negundo.* N. Nirmalkumar (2014) was reported aqueous soluble extractive value 22.45 % and alcoholic soluble extractive value 5.99% which indicated the maximum extractive percentage found in water extract [24] and as per the present investigation similar results have been reported, maximum extractive value has been found in aqueous extracts than methanolic and acetone extract. The phytochemical screening of *Vitex negundo* leaves extracts revealed the presence of carbohydrates, proteins, amino acids, steroids, glycosides (cardiac, anthraquinone and saponin), flavonoids, tannins and phenolic compounds. The presence of these primary and secondary metabolites suggests that the *Vitex negundo* have curative ability against several diseases. Hence present results might be important to support the ethanomedicinal and traditional medicinal properties of *Vitex negundo.* Presence of tannins and phenolic compounds proves that plants may be having antiviral, antibacterial and wound healing activity [25]. Ramesh Mani *et. al* (2013) was revealed the presence of alkaloids, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, proteins, saponin and tannins in methanolic extract of *Vitex negundo* leaves [26]. The present study also recorded same phytochemicals and also reported streroids in methanolic extract of *Vitex negundo* leaves.

The ICP-MS analysis study revealed the presence of major elements like Ca, Mg, Na, K and P have been found in maximum concentrations which are the quantity elements. Micro/trace elements such as Cr, Mn, Fe, Co, Ni, Cu, Zn, Se and Mo were quantified in sufficient concentrations. According to frieden (1985) Boron, Cobalt, Copper, Iodine, Iron, Mangenese, Molybdenum and Zinc are biologically essential elements whereas chromium, nickel, selenium are probable biologically essential elements [27].

The FTIR analysis study has confirmed the presence of vital functional groups which may be important in the synthesis of pharmaceutically active phytoconstituents. Detection of hydroxyl groups is an indication of presence of flavonoids, alcoholic and phenolic compounds [28]. *Vitex negundo* is an aromatic plant which is confirmed in present research by detection of aromatic functional groups and oxidized nitrogen functional aromatic groups. The occurrence of alkaloids in *Vitex negundo* leaves extracts confirmed by the presence of alkanes, amines, primary and secondary amines and aromatic compounds [29]. Some lipids and proteins have assymmetric streching due to C-H vibration and saturated fatty acids, which were detected in the present research. Many organic acids contain carboxylic acids and their derivatives and which are responsible for several medicinal properties.

CONCLUSION

The results of present study are helpful for the discovery of potent remedies on various diseases. Medicinal plants are main source for potent bioactive compounds. The phytochemical screening using three different material extracts showed the presence of the primary and secondary metabolites in aqueous and methanolic extract than

acetone extract. The phytochemical screening of three different extracts of *Vitex negundo* L. leaves showed the presence of phytochemicals like carbohydrates, proteins, amino acids, saponins, flavonoids, anthraquinones, tannins and phenolic compounds. Acetone extract and aqueous extract of *Vitex negundo* leaves shows the presence of flavonoids, the present study also useful for preparing flavonoid-based drugs which have been most important in antioxidant, antiallergic, antimicrobial properties. The soluble extractive value was found the maximum in aqueous extract hence aqueous extract is most effective for studying the pharmacological activity of this plant. Elemental analysis showed the presence of trace elements in sufficient concentrations and traces of heavy and toxic metals. The FTIR study revealed the presence of essential functional groups in three different extracts of the material. Present investigation is most essential to discover innovative, dynamic and novel drugs for curing various newly emerged dangerous diseases.

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AUTHOR CONTRIBUTION

These authors contributed equally to this work

CONFLICT OF INTERESTS

There is no conflict of interest between both the authors of this work

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