

Proximate, Phytochemical and Spectroscopic Analysis of Various Extracts of *Vitex negundo* Linn. Leaf

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ABSTRACT

Vitex negundo Linn belongs to family Verbenaceae and grows as small tree in terrestrial land. The plant is used by the traditional healers and folklore practitioners for treatment of various illnesses. Even though the plant is expected to have various pharmacological activities, there are very limited studies on its chemical profiling. The present study focussed the scientific documentation of chemical characteristics of *V. negundo*. The ethanolic extract, methanolic extract and aqueous extracts of shade dried leaves of *V. negundo* were prepared. The studies on proximate, physico chemical characteristics, phytochemical characteristics and spectroscopical analysis were carried out. The presence of various phytoconstituents including tannins, alkaloids, flavonoids were scientifically documented in triplicate analysis.

Keywords: *Vitex negundo*, Proximate Analysis, Spectroscopic Studies, Nirgundi.

INTRODUCTION

Historically, mankind has used various plants and their products for the treatment and prevention of many ailments. In recent years a significant revival on importance of natural products has been observed throughout the world [1-3].

Vitex negundo, commonly known as 'Nirgundi' is one of the medicinal plants of India that is widely used in indigenous medicine systems and by traditional healers. *Vitex negundo* belongs to Verbenaceae family and it is a woody, aromatic deciduous shrub growing to a small tree. The plant is distributed throughout India up to an altitude of 1500 m in the outer Himalayas.

The plant is an erect, 2-5m height, slender tree with quadrangular branchlets; leaves have five leaflets palmately arranged, glabrous, hairy beneath and pointed at

both ends. Flowers are bluish purple borne on axillary or terminal panicle up to 30 cm long [4-6].

V. negundo has been reported for various pharmacological properties including antimicrobial, anti-inflammatory, analgesic, antigestaltic etc (6-9). Its use has also been extended as repellent, insecticide and larvicidal (10). In Malaysia, this plant is used in traditional herbal medicine for the treatment of women health especially on menstrual cycle related issues [11]. The Food and Drug Administration of the Philippines has recently approved clinical trials for *V. negundo*, as a supplemental treatment for COVID-19 patients [12-13]. The present study scientifically documents the proximate composition, physico-chemical characteristics, phytochemical analysis and spectroscopic characterization of various extracts of *V. negundo*.

Taxonomy

Kingdom- Plantae

Clade - Tracheophytes

Clade - Angiosperms

Clade - Eudicots

Clade - Asterids

Order - Lamiales

Family - Lamiaceae

Genus - Vitex

Species - *Vitex negundo*

Synonyms - *Vitex cannabifolia*,
Vitex incisa Lam

Vernacular Names

Nirgundi - Hindi

Nochi/Nirgundi - Malayalam

Nirnochi - Tamil

MATERIALS AND METHODS

Plant Collection and Authentication

The fresh plant materials were collected from the Western ghat regions of Palakkad District, Kerala, India. The collected plant materials were authenticated by the taxonomist, from Kerala Forest Research Institute, (Research Institute under Government of Kerala), Thrissur. The plant material was processed and herbarium was prepared as per the standard guidelines. Voucher specimen is maintained in the Department of Biochemistry and Pathology, National Ayurveda Research Institute for Panchakarma (NARIP), Cheruthuruthy for ready reference.

Extraction [14]

The air-dried, powdered plant leaf material was extracted by various methods using different solvents. The hot solvent extraction for 8 hours was done by Soxhlet method using ethanol. Methanolic extract was prepared by cold extraction method. The plant material was soaked in methanol for overnight 10-12 hours and filtered through Whatman filter paper No. 1. The aqueous extract was prepared as per the protocol prescribed in Ayurvedic pharmacopeia of India. The solvent was

removed under reduced pressure at suitable temperature using rotovac equipment. The solvent free extract was aliquoted and stored in refrigerated condition for ready to use form.

Physicochemical Characteristics [15-16]

Physico-chemical analysis such as moisture content, total ash, water soluble ash, acid insoluble ash, water soluble extractive and alcohol soluble extractive were carried out as per the standard protocols developed and standardized at NARIP was used.

Phytochemical Analysis [17-19]

The phytochemical analysis was carried out as per the standard protocols that has been standardised in NARIP. It comprised of various tests including Froth's test, Libermann Burchard test, Fehling's test, Salkowski test, Dragendorff's test, Keller Killani test and Ellagic acid test.

Spectrophotometric Analysis [20-21]

Spectrophotometric characteristics were analysed for understanding the basic chemical profiling of study plant and to compare the proposition of major compounds among different solvent extracts. The ethanolic extract, methanolic extract and aqueous extract of *V. negundo* leaf at different concentrations were used for spectroscopic analysis using the concerned solvent as blank. The spectrum characteristics were measured after standardizing the procedures and analysis was confirmed by carrying out triplicate analysis at 200-800 nm.

Chemicals and Reagents

The chemicals and reagents of AR grade purchased from make of Spectrum, Nice and HiMedia were used for the present study.

Instruments

UV Spectrophotometer of Agilent- Cary 60 was used for spectroscopic analysis.



Fig. 1. Herbarium of *Vitex negundo* Linn and its Authentication

RESULTS AND DISCUSSION

The authentication of the plant *Vitex negundo* Linn has been presented in Figure 1. The proximate composition analysis comprising of total extractive yield, total ash content, water soluble and acid soluble

ash concentration, solubility characteristics of extract has been presented in Table 1. The shade dried leaf of *V. negundo* had yielded nearly 28.76% extract and the total ash content was found to be 5.95 %. The characteristic of extract is found have more solubility in water than alcohol.

The phytochemical experiments had been carried out for ethanolic extract, methanolic extract and aqueous extract of shade dried leaf. The study evidenced the presence of glycosides, tannins, alkaloids and flavonoids in the extracts at moderate to high level (Table 2).

Spectrophotometric analysis was conducted extensively for all the three types of extracts at different concentrations level. The study documented the number of standard peaks at the wavelength of 200-800nm confirming the presence of various group of compounds including flavonoids, tannins, alkaloids. The experiments were confirmed by triplicate analysis (Figure 2-4 and Table 3-5).

Table1. Physico Chemical Characteristics of *Vitex negundo* L. Shade Dried Leaf

S No	Proximate Compounds	<i>Vitex negundo</i> L. (Shade Dried Leaf)
1	Extractive Yield (%)	28.76
2	Moisture content (%)	9.60
3	Total Ash (%)	5.95
4	Water Soluble Ash (%)	2.25
5	Acid Insoluble Ash (%)	1.19
6	Water Soluble Extractive (%)	19.10
7	Alcohol Soluble Extractive (%)	13.74

Table2: Phytochemical Characteristics of *Vitex negundo* L. (Shade Dried Leaf)

S. No	Phytochemical Compounds	Method	<i>Vitex negundo</i> L- Shade dried Leaf		
			Ethanolic Extract	Methanolic Extract	Aqueous Extract
1	Tannins	Ferric Chloride Test	+	++	++
2	Saponins	Froth's Test	-	-	-
3	Terpenoids	Salkowsky's Test	+	+	+++

4	Cardiac Glycosides	Keller-Killani Test	+++	++	++
5	Flavonoids	Shinoda Test	+	+	++
		Lead acetate Test	+	+	++
		Alkaline Reagent Test	+	+	+
6	Carbohydrates	Fehling 's Test	++	+++	+++
		Molisch Test	++	+	++
		Benedict's Test	+	+	+++
7	Phytosterols	Liebermann Burchad Test	++	++	+++
8	Proteins	Millon's Test	+	++	+++
9	Alkaloids	Dragendorff's Test	++	-	++
		Mayer's Test	++	-	+
		Hager's Test	++	-	+
		Wagner's Test	++	-	+
10	Anthraquinones	Modified Brontrager's Test	-	+	+

+++ Highly present ++ Medium + Low - Nil

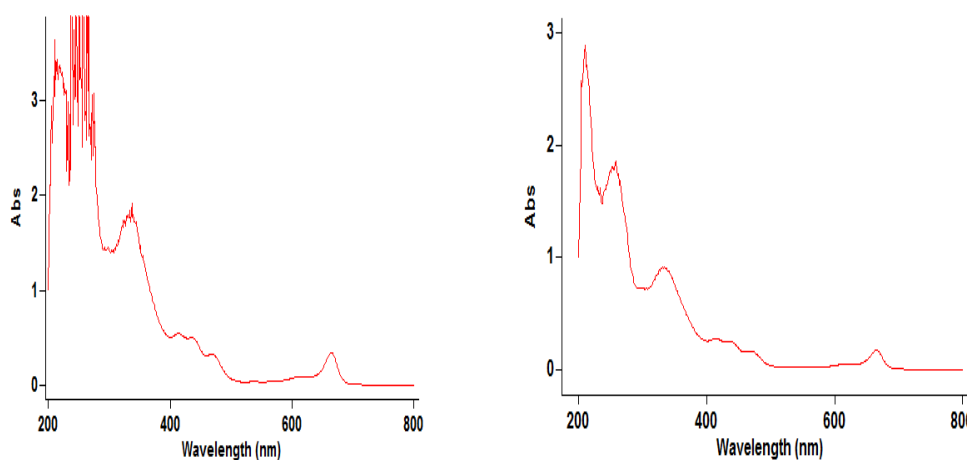


Fig. 2. UV Spectroscopic Analysis of Methanolic extract of *V. Negundo L. Shade Dried Leaf* at Different Concentration

Table 3. Comparative Data of Spectroscopic Analysis of Methanolic Extract of *V. Negundo L. Shade Dried Leaf* at Different Concentrations

<i>V. negundo L. Leaf - Methanol Concentration 0.125%</i> 200-800 nm		<i>V. negundo L. Leaf Methanol Concentration 0.0625%</i> 200-800 nm	
Wavelength	Absorbance	Wavelength	Absorbance
665.0	0.340	665.0	0.171
345.0	1.728	255.0	1.805
268.0	2.745	331.0	0.909
223.0	3.302	230.0	1.634

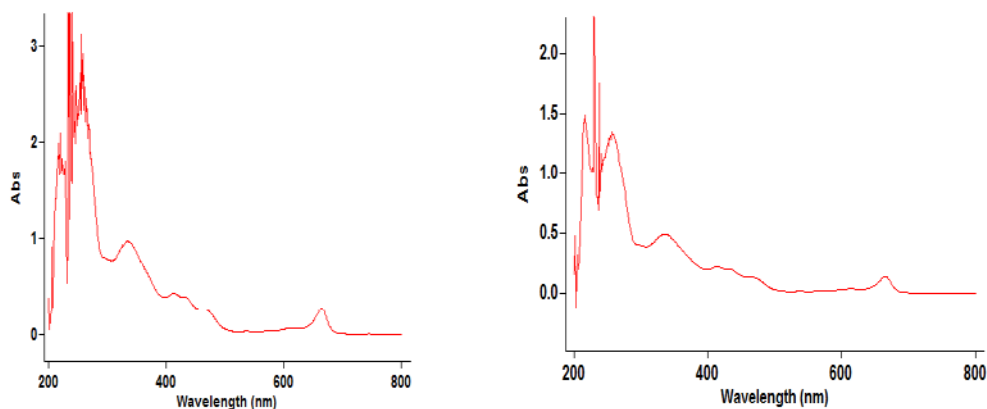


Fig. 3. UV Spectroscopic Analysis of Ethanollic Extract of *V. Negundo L. Shade Dried Leaf* at Different Concentration

Table 4. Comparative Data of Spectroscopic Analysis of Ethanollic Extract of *V. Negundo L. Shade Dried Leaf* at Different Concentrations

<i>V. negundo L. Leaf Ethanol Concentration 0.125% 200-800 nm</i>		<i>V. negundo L. Leaf Ethanol Concentration 0.0625% 200-800 nm</i>	
Wavelength	Absorbance	Wavelength	Absorbance
665.0	0.269	664.0	0.136
254.0	2.611	254.0	1.307
250.0	2.298	250.0	1.251
272.0	1.893	242.0	1.112

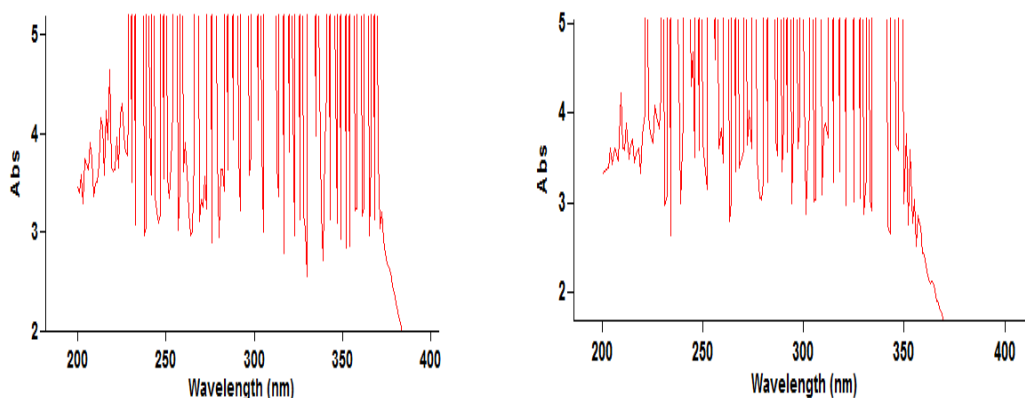


Fig 4. UV Spectroscopic Analysis of Aqueous Extract of *V. Negundo L. Shade Dried Leaf* at Different Concentration

Table 5. Comparative Data of Spectroscopic Analysis of Aqueous Extract of *V. Negundo L. Shade Dried Leaf* at Different Concentrations

<i>V. negundo L. Leaf Aqueous Concentration 1/20200-800 nm</i>		<i>V. negundo L. Leaf Aqueous Concentration 1/40200-800 nm</i>	
Wavelength	Absorbance	Wavelength	Absorbance
272.0	3.559	273.0	4.027
225.0	4.297	259.0	3.823
222.0	3.952	226.0	4.089

CONCLUSION

Majority of the population in the world depending on plants for their primary health care needs. *Vitex negundo* Linn is one such plant used by traditional healers in India for management of various health issues and has not been explored much in detail. The present study scientifically documented proximate analysis, physico-chemical characteristics, phytochemical analysis and spectroscopic characteristics of ethanolic extract, methanolic extract and aqueous extract of *V. negundo*. Presence of various phytoconstituents at different dilutions levels were confirmed by triplicate analysis. The study gives an overall understanding for its medicinal value and provides supporting basic data to the researchers for initiating further studies on biopotency of this plant.

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Conflict of Interest: Nil

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