Pharmacogn J. 2020; 12(3): 551-556
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Pharmacognostic Studies on Methanolic Extract of Leaves of *Vitex negundo* Linn

Kiran Sharma*, Manish Yadav, Kavita Attri

ABSTRACT

Vitex negundo Linn (verbenaceae), known as Nirgundi is important medicinal plant with variety of phytoconstituents having significant pharmacological activities. It has anti-inflammatory, analgesic, anti-histaminic, anti-oxidant, anti-bacterial, hepatoprotective, anti-implantation, laxative, larvicidal, anti-arthritic, anticonvulsant and effective against snake venom activity. Two compounds namely vitexin and negundoside are reported to have anti-cancer and hepatoprotective activity respectively. For safe and effective use of herbal drugs in a formulation, proper standardization of herbal drugs is necessary. So, in this research paper basic pharmacognostic studies on *Vitex negundo* leaf extract like physicochemical parameters-ash values and extractive values, Fluorescence analysis, phytochemical screening, TLC profile with different solvent systems, behavior with different reagents and metal analysis was done. The findings throw light on preliminary standardization of this important medicinal plant.

Key words: Vitex negundo (VN), Ash values, Extractive values, Fluorescence analysis, TLC.

Kiran Sharma*, Manish Yadav, Kavita Attri

SGT College of Pharmacy, Gurugram, Haryana- 122505, INDIA.

Correspondence

Kiran Sharma

SGT College of Pharmacy, Gurugram, Haryana- 122505, INDIA.

E-mail: kiransharmapharma@gmail.com

History

- Submission Date: 29-12-2019;
- Review completed: 05-02-2020;
- Accepted Date: 20-02-2020.

DOI : 10.5530/pj.2020.12.83

Article Available online

http://www.phcogj.com/v12/i3

INTRODUCTION

Vitex negundo Linn., belonging to family Verbenaceae is commonly known as Nirgundi. The white flowered variety is known as Sinduvaara, whereas the blue flowered variety is known as Nirgundi or Sephaali. The Leaves of *Vitex negundo* (VN) contain irridoid glycosides like negundoside and flavonoids such as vitexin, which is a flavonol glycoside besides casticin and the glycosides, luteolin-7-glucoside and a-D-glucoside of tetrahydroxy monomethoxy flavones. Also, two pentacyclic triterpenoids, betulinic acid and ursolic acid, along with an aliphatic alcohol, β-sitosterol and p-hydroxybenzoic acid have been isolated from leaves. Dried powder of roots contain hentriacontane and stigmasterol. The seeds contain p-hydroxybenzoic acid, glucose and the triterpene vitextriterpene.[1-3] The water extract of the leaves, when administered to rats, exhibited anti-inflammatory, analgesic and antihistaminic activity.[4-6] The *Vitex negundo* leaf extract is known to exhibit antioxidant activity.[7] The methanol, ethanol, petroleum ether and chloroform extract of leaves and bark of VN exhibited significant antibacterial activity.[8] The active constituent negundoside isolated from leaves of VN showed hepatoprotective activity against carbon tetrachloride using human liver cells.[9-11] Research has also proved the anti-implantation effect of VN.[12] The crude aqueous extract of VN are known to produce significant laxative activity in a dose dependant manner.[13] *Vitex negundo* is also known to attenuate calpain activation and catractogenesis in selenite induced cataract.[14] The petroleum ether extract of VN served as a potent larvicidal agent and also acted as a promising repellant against various adult vector mosquitoes.[15] It has been also found that VN extract produce reduction of oxidative stress.[16] The methanol extract of VN showed significant anti-arthritic activity in Complete Freund's adjuvant induced paw edema in rats.[17] The methanol extract of VN showed anticonvulsant activity.[18] The VN root extract significantly acted against snake venom.[19]

MATERIALS AND METHODS

Collection and authentification of plant

The plant material of VN was collected from plantation field been situated in a small village called as Shiroor, 60 Kms from Pune and the voucher specimen (08-123) was kept at departmental herbarium of ARI. The plant material was cleaned and dried in the shade and powdered to 40 mesh and stored in an airtight container at 25 °C.

Extraction of plant

VN leaves were washed and dried at 55 °C in an air dryer for 48 h. Dried plant material was powdered separately with a Wiley mill (model-4276 M, Thomas, Scientific, USA) to pass a 20 mesh sieve and stored and stored in a sealed plastic bag. About 500 mg of various powders were taken in a 5 ml volumetric flask, mixed with 5ml of methanol and vortexed for two minutes followed by sonication (33 MHz, Roop telesonic, India) at room temperature for min. The process was repeated thrice for complete extraction. After sonication methanolic extracts were combined and evaporated to dryness in vacuo. Dried extract was obtained as 13.1 g.

Physico-chemical analysis

The Physicochemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed in Indian Pharmacopeia, 1996 and the WHO guidelines on quality control methods for medicinal plant

Cite this article: Sharma K, Yadav M, Attri K. Pharmacognostic Studies on Methanolic Extract of Leaves of *Vitex negundo* Linn. Pharmacog J. 2020;12(3):551-6.

materials.[20,21] Fluorescence analysis was carried out according to the method of Chase and Pratt (1949) and Kokoski, *et al.* (1958).[22,23] Preliminary phytochemical screening was carried out using standard procedures described by Kokate (1986) and Harborne (1998).[24,25] For performing the TLC, leaves were extracted with various solvents in the order of increasing polarities such as carbon tetrachloride, benzene, petroleum ether, ethyl acetate, chloroform, acetone, methanol, ethanol and water so as to obtain all the non-polar, semi-polar and polar constituents that are soluble in these solvents. So, all these extracts of VN leaves were used for estimation by TLC method, so that we can have an idea about the polarity and number of constituents present in these extracts.

RESULTS AND DISCUSSIONS

Physicochemical studies

VN was analyzed for moisture content, total ash, water insoluble ash, acid insoluble ash which gives an idea about amount of organic and inorganic constituents present in the samples. Also foaming index, swelling index, loss on drying, pH and electrical conductivity studies were also performed. These studies give an overview of amount of saponins, moisture content, nature of salt and ions present in the sample Table 1.

Extractive values

VN leaf extract gives different range of yield in different solvents. The highest yield was obtained in water extract and least yield was obtained in petroleum ether extract. The results are shown in Table 1.

Phytochemical screening

Phytochemical screening proved that VN leaf extracts has alkaloids, glycosides, flavonoids, fixed oils, carbohydrates, amino acids, terpenoids and steroids. Hence, the phytochemical screening gave an idea about the various classes of chemical compounds present in different solvents Table 2.

TLC behavior of VN leaves

The purpose behind performing the TLC of each extract was to have an idea about the no. of components in each solvent's extract and R_f values were determined. The R_f value of 0.2 confirmed the presence of marker compound as shown in Table 3 as reported by *Sharma et al.*[26]

Fluoroscence behavior of VN leaf extracts

The fluorescence of VN leaf extract with different solvents were determined under visible, short and long UV light as mentioned in

Table 1: Physicochemical parameters of *V. negundo* leaves.

S.No.	Parameters	Leaf
1.	Total Ash	0.934
2.	Acid insoluble ash	0.857
3.	Water soluble ash	0.077
4.	Loss on drying at 110˚C	5.60
5.	Foaming index	1.4
6.	Swelling index	0.1
7.	pH (2% w/v)	5.12
8.	Electrical conductivity	2.60
	Extractive values	
9.	Cyclohexane	0.068
10.	Carbon tetrachloride	0.062
11.	Petroleum ether	0.039
12.	Chloroform	0.098
13.	Acetone	0.143
14.	Ethanol	0.376
15.	Methanol	0.437
16.	Ethyl acetate	0.093
17.	Water	0.420
18.	Benzene	0.032

Table 2: Phytochemical screening of VN leaves extract.

Chemical test	Pet ether extract	Alcohol extract	Aqueous extract
Alkaloids			
Dragendorff's	-	+	-
Mayer's	-	+	-
Hager's	-	+	-
Glycosides			
Fehling's	-	-	+
Legal	-	-	+
Keller Killiani	-	-	-
Tannins			
Phenazone	-	-	-
Lead acetate	-	+	+
Carbohydrates			

Molisch's	-	-	+
Fehling's	-	-	+
Phenol			
Ferric chloride	-	+	-
Terpenoids			
Liberman Burchard	+	+	-
Salkowski	+	+	-
Steroids			
Liberman	+	+	-
Amino acids			
Ninhydrin	-	+	-
Millon's	-	+	-
Biuret	-	+	-
Gums and mucilages			
Iodine	-	-	+
Water	-	-	+
Alcohol	-	-	+
Fixed oil			
Spot	+	+	-
Essential oil			
Sudan III	-	-	-
Tincture alkana	-	-	-
Odour	-	-	-
Saponin			
Foam	-	-	+
Resins			
Sulphuric acid	-	-	-
50% nitric acid	-	-	-
Water	-	-	-
HCl + water	-	-	-
Bromine vapour	-	-	-
Litmus	-	-	-

+ Present, - Absent

Table 3: TLC behavior of different extracts from leaves of VN.

Extracts	Solvent system	No. of spots	R_f values
Cyclohexane	Toluene: Ethyl acetate: Formic acid (8:2:0.2)	3	0.97,0.96, 0.66
Chloroform		9	0.13, 0.18, 0.21, 0.28, 0.36, 0.41, 0.53, 0.69, 0.93
Petroleum Ether		4	0.15, 0.38, 0.57, 0.94
Chloroform		4	0.18, 0.52, 0.8, 0.91
Acetone		8	0.06, 0.14, 0.22, 0.35, 0.46, 0.52, 0.86, 0.93
Ethanol		5	0.21, 0.34, 0.48, 0.60, 0.76
Methanol		10	.09, 0.15, 0.21, 0.26, 0.33, 0.39, 0.44, 0.74, 0.57, 0.94
Ethyl acetate		4	0.09, 0.18, 0.55, 0.88
Benzene		8	0.06, 0.15, 0.25, 0.35, 0.45, 0.51, 0.58, 0.87

The R_f value 0.21 confirms the presence of marker compound.

Table 4. This study was performed to assess the greatest UV absorbance activity of different extracts, the extract showing the greatest intensity was thought to posses the more UV radiation absorbing capacity and which can further be used as a sunscreen protective agent in cosmetics.

Behavior of VN leaves with different reagents

Behavior of VN leaves powder with different reagents was evaluated to study its reaxtion and stability with that reagent. Stability studies e.g. solubility, hydrolysis and degradation were performed to interpret the amount of degradation by noticing some visual characters such as brown color showed complete degradation of the components as shown in Table 5.

Metal analysis

The acid insoluble ash was taken and by means of the atomic absorption spectroscopic method the metal analysis of VN leaf powder was done. The metal analysis results obtained revealed that Iron (Fe) was present in the highest amount (456) and calcium was present in the least amount (0.03). All the concentrations were expressed in ppm as shown in Table 6.

Table 4: Fluorescence behavior of different extracts from the leaves of VN.

S. No.	Extract	Visible light	Short UV light	Long UV light
1.	Cyclohexane	Greenish yellow	Yellowish green	Amber color
2.	Carbon tetrachloride	Dark yellowish Green	Dark purple	Purple
3.	Petroleum ether	Greenish yellow	Yellowish green	Amber
4.	Chloroform	Dark herbage green	Purple	Purple
5.	Acetone	Dark herbage green	Dark purple	Red
6.	Ethanol	Dark herbage	Dark herbage green	Purple
7.	Methanol	Dark herbage green	Dark purple	Purple
8.	Ethyl acetate	Herbage green	Dark purple	Red
9.	Water	Brown	Blood color	Blood color
10.	Benzene	Dark herbage green	Purple	Purple

Table 5: Behaviour of VN powder with different reagents.

S.No.	Chemical reagent	Observations
1.	Conc. sulphuric acid	Particles float on surface, brown in color
2.	Conc. Hydrochloric acid	Particles float on surface, on shaking particles remain suspended, brown in color
3.	Conc. Nitric acid	Particles float on surface, on shaking particles remain suspended, rust in color, thick consistency
4.	Acetic acid	Particles float on surface, slowly settle, on shaking particles remains suspended, brown in color
5.	Sodium hydroxide	Particles float on surface, slowly settle, on shaking particles remains suspended, amber in color
6.	Potassium hydroxide	Particles float on surface, slowly settle, on shaking particles remain suspended, amber in colour.
8.	Ferric Chloride	Particles float on surface, amber in colour, on shaking particles remain suspended.
9.	Iodine	Particles float on surface, slowly settle, on shaking particles remain suspended, yellowish brown in colour.
10.	Organoleptic characters	Colour: Dark green, Odour: odourless, Taste: Tasteless, Touch: soft.

Table 6: Metal analysis of VN.

S.No.	Metals	Leaf
1.	N*	0.98
2.	P_2O_5 *	0.17
3.	K_2O*	0.40
4.	Ca*	0.03
5.	Mg*	0.51
6.	Zn**	78.0
7.	Cu**	37.0
8.	Fe**	456
9.	Mn**	87.0

*Values are in percentage, **Values are in ppm

CONCLUSION

The result from this study will prove to be very helpful while making any formulation of VN.

ACKNOWLEDGEMENT

I would like to Thank UGC and Dr. Ajay Sharma for his cooperation and providing the working facilities for the research work.

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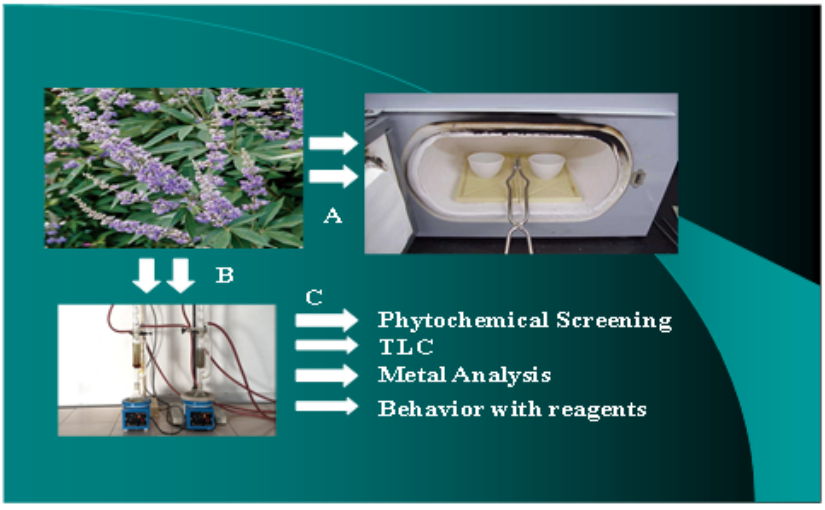
GRAPHICAL ABSTRACT

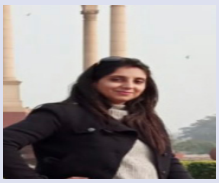


A- Determination of ash values from *Vitex negundo* plant
B- Extraction of *Vitex negundo* using solvents of different polarities
C- Pharmacognostical studies on the extracts.

ABOUT AUTHORS

Dr. Kiran Sharma has started studying phytochemistry & pharmacognosy at post-graduate level and has performed the characterization and evaluation of various active components from several medicinal plants. Her area of research is plant tissue culture and herbal drug standardization, where she has done studies on genetic transformation of *Taraxacum officinale*. She has also worked on latest techniques of plant biotechnology such as use of biotic and abiotic elicitors for increasing the yield of various phytoconstituents such as taraxerol and taraxasterol which are known to be potent anti-cancer compounds. She has to her credit around 12 publicatios in indexed journals and a book on plant biotechnology in pipeline.

Dr. Manish Yadav has started studying pharmaceutics at post-graduate level and has performed the designed, development, characterization and evaluation of various dosage forms. His area of research is floating drug delivery system and solubility studies with techniques of enhancement of solubility of poorly soluble active pharmaceutical ingredients. He worked in Ranbaxy Research Laboratory in oral control release system (OCRS) in NDDS department and have experience of pharmacovigilance in Research and analysis (R&A). He has also worked on latest techniques of Novel drug delivery systems. He has to his credit around 12 publications in indexed journals and a book on physical pharmacy in pipeline.



Ms. Kavita Attri has started studying Pharmaceutics at post-graduate level and has performed the Design, development & characterization and evaluation of various formulations. She has also worked on latest techniques of novel drug delivery systems such as Transdermal drug delivery system and process validation of Glimipride. She has to her credit around 05 publications in indexed journals and a book on pharmaceutical technology in pipeline.

Cite this article: Sharma K, Yadav M, Attri K. Pharmacognostic Studies on Methanolic Extract of Leaves of *Vitex negundo* Linn. Pharmacog J. 2020;12(3):551-6.