

Evaluation of Phytochemical and Thin Layer Chromatographic Study of terpenoids from Leaves Extract of *Vitex negundu* Linn.

Asmita Singh¹, Manisha Mathur², Ajay Kumar², Gajendra Pal Singh⁴

Ph.D Student, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

Lecturer, Department of Botany, R.R College, Alwar, University of Rajasthan, Jaipur, Rajasthan, India

Scientist, Biochemistry Division, Indian Veterinary Research Institute, Bareilly, U.P, India

Assistant Professor, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

ABSTRACT: Folk medicines of almost all civilizations of the world abound in herbal remedies. Majority of the traditional medicines used in healthcare are obtained from plants *Vitex negundu* Linn. is one of the important medicinal plants having many therapeutic uses. The present study deals with preliminary phytochemical screening and TLC investigation of *Vitex negundu* Linn. In qualitative analysis, the phytochemical compounds such as steroids, triterpenoids, sugars, alkaloids, phenolic compounds, flavonoids were screened in leaf methanolic extract by using standard methods. Phytochemical screening of plant revealed the presence of alkaloids, flavonoids steroids reducing sugar, glycosides and Terpenoids. The present study revealed that *Vitex negundu* Linn important source of many therapeutically and pharmacologically active constituents.

KEYWORDS: *Vitex negundu*, Terpenoids, Phytochemical analysis, Flavonoids, Triterpenoids, Therapeutically

I. INTRODUCTION

Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. In the present era of drug development and discovery of newer drug molecules, many plant products are evaluated on the basis of their bioactive constituents. The curative properties of medicinal plants are mainly due to presence of various complex chemical substances of different compositions which occur as secondary metabolites [1]. Typically, bioactive compounds of plants are produced as secondary metabolites [2]. Every living body, from one cell bacterium to million cell plants, processes diverse chemical compounds for their survival and subsistence. All compounds of biological system can be divided into two broad arenas. One is primary metabolites, which are the chemical substances aimed at growth and development, such as carbohydrates, amino acids, proteins and lipids. Another is secondary metabolites, which are a group of compounds other than primary metabolites believed to help plant to increase their overall ability to survive and overcome local challenges by allowing them to interact with their surroundings. These natural bioactives compounds provided an opportunity to obtain natural extracts that could be potentially used [3,4].

Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub growing to a small tree and bears tri- or penta-foliolate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. Although, all parts of *V. negundo* are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarrhal fever, rheumatoid arthritis, gonorrhoea, sinuses, scrofulous sores, bronchitis and as tonics, vermifuge, lactagogue, antibacterial, antipyretic, antihistaminic, analgesic,

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insecticidal,ovicidal, feeding deterrence, growth inhibition and morphogenetic agents. The present paper is aimed to study the phytochemical constituents and identification of two pharmaceutically valuable terpenoids from methanolic extract of leaves of vitex negundo.

II.REVIEW OF LITRATURE

Plant derived material or preparation with therapeutic or other human health benefits, which contains either raw or processed ingredients from one or more plants. The World Health Organization have currently encouraged, recommended and promoted herbal remedies in healthcare programmed because of much lower cost and are for comparatively safer. Various medicinal plants have been identified and thoroughly studied using modern scientific approaches.

Higher plants are warehouses of assorted bioactive constituents or phytochemicals which find ample use in the pharmaceutical industry. Namdeo [5] states that about a quarter of all prescribed pharmaceuticals in advanced countries contain compounds that are directly or indirectly, derived from plants. Phytochemicals or secondary metabolites usually occur in complex mixtures that differ among plant organs and stages of development [6,7]. Knowledge of the phytochemical constituents is very essential to enable investigation of the actual effectiveness of the plant in medicine. Principle indigenous systems are homeopathy, herbalmedicines (medical herbalism) and aromatherapy. Many of these indigenous plants have shown very significant therapeutic activities like hepatoprotective,antiinflammatory, antihelmintic etc. [8].

Vitex negundo (Verbenaceae) commonly known as Nirgundi. The Sanskrit word nirgundi literally means that which protects our body from all diseases[9].It is one of the most usable herbs mentioned in all Samhitas of Ayurveda and has various synonyms like sinduka,sitabhiru, vanaka, bhutakesi, anilamanjiri etc[10]. The plant parts contain various chemicals such as alkaloids, glycosides, tannins, flavonoids, carbohydrates. The fresh leaves extract exhibited anti-inflammatory, analgesic and antihistaminic properties[11].It has also been reported that the extract has mosquito repellent effects[12], antiparasitic[13], antimicrobial effects[14,15]. The leaves are astringent, febrifuge, sedative, tonic and vermifuge[16]. Anti-inflammatory and pain suppressing activities of fresh leaves of *Vitex negundo* Linn are attributed to prostaglandin synthesis inhibition [17]. The extracts also possess the ability to combat oxidative stress by reducing lipid peroxidation owing to the presence of flavones, vitamin C and carotene[18]. Rooban et al[19]evaluated the antioxidant and therapeutic potential of *Vitex negundo* Linn flavonoids in modulating solenoid-induced cataract and found it to be effective. Yunos et al.[20] and Jana et al.[21] established anti-inflammatory properties of *Vitex negundo* Linn extracts in acute and subacute inflammation. Anti-inflammatory and pain suppressing activities of fresh leaves of *Vitex negundo* Linn are attributed to prostaglandin synthesis inhibition[22], antihistamine, membrane stabilising and antioxidant activities [23].Leaf extracts of *Vitex negundo* Linn were determined to possess anti-oxidant potential by [24].

The leaves are discutient and are useful in dispersing swellings of joints from acute rheumatism and of the testes from suppressed gonorrhoea[25]. Nishindaside a novel iridoid glycoside[26]. and volatile constituents have been reported from the leaves of this plant.[27]. The chloroform extract of defatted seeds of *Vitex negundo* linn yielded four triterpenoids and exhibited anti-inflammatory activity[28] Traditionally the leaves of this plant are used in various diseases, to give scientific background an attempt is made to assess the efficacy of *Vitex negundo* linn leaves for its antipyretic activity in the present study.

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III. MATERIAL AND METHODS

Collection and identification of plant material

The specimen was collected from University Botanical Garden, Department of Botany, University of Rajasthan, Jaipur. The leaves of the plant were washed thoroughly 2-3 times with running tap water and then with sterile distilled water. Air dried at room temperature. After complete drying roots were powdered well using a mixer. Powdered samples were extracted through Soxhlet extraction with methanol. The crude extract was collected in amber coloured sample bottles and stored. All chemical and reagents used including the solvents were of analytical grade.

Phytochemical Analysis

Phytochemical analysis was carried out in the methanolic extract of the leaves of *Vitex negundo* Linn using standard procedures to identify constituents as described by Harbone (1984), Trease and Evans (1979) and Sofowara (1993). [29,30,31]

Test for alkaloids

Methanolic extract was warmed with 2% H₂SO₄ for two minutes. It is filtered and few drops of reagents were added and indicated the presence of alkaloids.

a) Dragendorff's test

To 5 ml of extract few drops of Dragendorff's reagent was added for the formation of orange coloured precipitate. An orange precipitation indicates the positive.

b) Mayer's test

To 5 ml of extract few drops of Mayer's reagent was added for the formation of creamy-white coloured precipitate. This precipitation indicates positive.

c) Wagner's test

To 5 ml of extract few drops of Wagner's reagent was added for the formation of reddish brown coloured precipitate. This reddish brown coloured precipitation indicates positive.

d) Hager's test

To 3 ml of extract few drops of Hager's reagent (Picric Acid (1%)- was added for the formation of prominent yellow coloured precipitate. This yellow coloured precipitation is positive.

Test for Flavonoids

A small quantity of extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the following test

a) Ammonium Test

The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration was observed at ammonium layer. This indicates the presence of flavonoids.

b) Aluminium Chloride Test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoids and diluted NaOH and HCl was added. A yellow solution that turns colorless indicates positive.

Test for Terpenoids

a) Salkowski Test

The extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of presence of terpenoids.

Test for Tannins

A small quantity of extract is boiled with 5 ml of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. Filtrates were used to the following test.

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a)Lead Sub Acetate Test

1ml of the different filtrate was added with three drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

b)Ferric Chloride Test

1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

Test for sterols

a)Liebermann-Burchard test

To a small amount of the extract few drops of chloroform, acetic anhydride and H₂SO₄ was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for proteins

a)Ninhydrin test

To the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicate the presence of protein in sample

b)Biuret test

To 3 mL of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple color. On addition of alkali, it becomes dark violet.

c) Xanthoprotein test

To 3 mL of the extract few drops of HNO₃ reagent was added for the formation of intensely yellow colour.

Test for carbohydrates

a)Molisch's test

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H₂SO₄ along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

b)Fehling's test

The extract was treated with 5 ml of Fehling's solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar

Test for Glycosides

5ml of diluted sulphuric acid was added in extracts in a test tube and boiled for fifteen minutes in a water bath. It was then cooled and neutralized with 20% potassium hydroxides solution. A mixture of 10ml of equal parts of Fehling's solution A and B were added and boiled for five minutes. A more dense red precipitate indicates the presence of glycosides

a)Baljet's Test

To 5 mL of the extract few drops of sodium picrate was added to observe yellow to orange colour.

b)Keller-Killiani test

To 5 mL of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric

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chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides

Test for saponins

a)Foam test

To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

TLC identification of two terpenoids from methanolic root extract:

Phytochemical identification of terpenoids from extract by thin layer chromatography was performed as per the method .Briefly ,the extract were drawn into capillary tubes and applied as spots on a stationary phase (silica gel coated plate)about 1 cm from the base. The plates was then dipped into a suitable solvent system (mobile phase) and placed in a well covered tank. Chromatographic tank was saturated with mobile phase at room temperature for 5 min prior to development. After that the plates were removed dried and processed for the identification of separated compounds(as colored spots) and the Rf values were calculated using the formula .

$R_f \text{ value} = (\text{Distance moved by the compound}) / (\text{Distance moved by the solvent front})$

IV. RESULTS

Extraction of Plant Material

The leaves of the plant(*Vitex negundo* Linn)were washed thoroughly 2-3 times with running tap water and then with sterile distilled water .air dried at room temperature .Figs.1 (a) shows the Leaves (b) is After complete drying leaves were powdered well using a mixer. Powdered samples were extracted through soxhlet extraction with methanol. The crude extract were collected in amber coloured sample bottles and stored .All chemical and reagents used including the solvents were of analytical grade.



Figs.1 (a) shows the Leaves (b) is After complete drying roots were powdered well using a mixer.

Phytochemical Analysis









Powdered leaves of *Vitex negundo* Linn were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendroff's test, Mayer's test, Hager's test, Wagner's test), saponins, glycosides (Baljet's test, Kellar-Killianitest), carbohydrates (Molisch's test, Fehling's test), proteins (Biuret test, Xanthoprotein test), tests for tannins, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents and results are tabulated in Table 1.

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Table 1 showing Powdered leaves of *Vitex negundo* Linn were subjected to various qualitative tests for the identification of phytochemical constituents. preliminary phytochemical screening of methanolic extract of leaves of *Vitex negundo* linn.

Sl. No	Test for	Reagents	Reaction	Inference
1	Alkaloids:	Dragendroff's Reagent Mayer's reagent Wagner's reagent Hager's reagent (Picric Acid (1%)-	Orange precipitation Creamy-white precipitation Reddish brown precipitation yellow coloured precipitate	+++ 
2	Flavanoids	Dilute ammonia solution (1%).	Yellow coloration was observed at ammonium layer	++ 
3	Carbohydrates:	Molisch Reagent Copper sulphate+ potassium sodium tartarate +NaOH	Formation of red or dull violet colour at the inter phase Formation of yellow or red color precipitate	+ 
4	Terpenoids	2ml of chloroform and concentrate H2SO4 (3ml) (Lieberman Test)	Reddish brown coloration of the interface	+++ 
5	Steroids	Few drops of chloroform, acetic anhydride and H2SO4(salkowski Test)	Dark red or pink colour	+ 
7	Proteins and amino acids	0.2% ninhydrin solution Few drops of HNO3 reagent	violet color Formation of intensely yellow colour	- 
8	Saponin	distilled water	persistent foam	+ 
9	Tannins	drops of ferric chloride lead sub acetate solution	Transient greenish to black color A cream gelatinous precipitation	- 

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Figure 3: Shows TLC identification of terpenoids from methanolic leaves extract of *Vitex negundo* Linn after derivatization.

Methanolic extract was subjected to TLC in order to separate and identify the two pharmacologically valuable terpenoids present in the root extract methanolic extract of *Vitex negundo linn*. In the present study, the preliminary phytochemical screening of the root methanolic extracts revealed the presence of major bioactive compounds and TLC analysis was conducted for the detection of two valuable terpenoids which may retain a wide range of action.

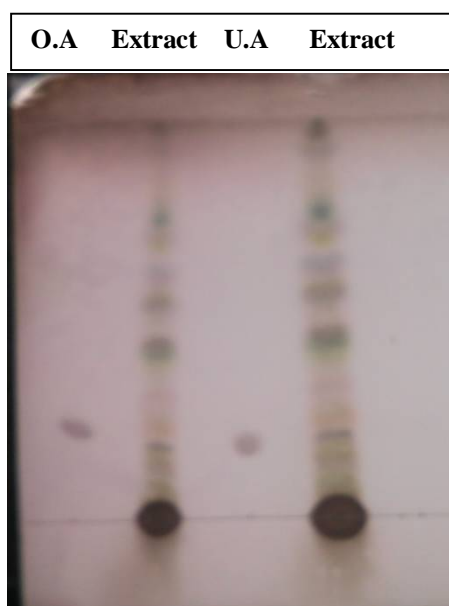


Figure 3: Shows TLC identification of terpenoids from methanolic leaves extract of *Vitex negundo* Linn after derivatization

Table 2: Results of TLC of Methanolic Extract of *Vitex negundo linn*:-

Sample	Solvent system(7.8:2.2:0.75 ml)	No. of spot	Rf Values
Root extract	Toluene:Methanol:Formic acid	7	0.19,0.20,0.45,0.5,0.58,0.66,0.83
Standard1(Oleanolic acid)	Toluene:Methanol:Formic acid	1	0.19
Standard2(Ursolic acid)	Toluene:Methanol:Formic acid	1	0.20

Stationary Phase: Silica gel. 60-120 mesh size (Merk).

IV. CONCLUSION

The selected plant is possess a multitude of phytochemical secondary metabolites which impart an unprecedented variety of medicinal uses to the plant. source of secondary metabolits. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Clinical and pharmaceutical investigations have in fact elevated the status of medicinal plants by identifying the role of active principles present in them and elaborating on their mode of action Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. These preliminary results open the way for deep studies of methanolic extract and established a significant scope to develop a

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broad spectrum use of leaves of *Vitex negundo linn* in herbal medicine and as a base for the development of novel potent drugs and phytomedicine.

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