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BIOCHEMICAL AND CHROMATOGRAPHIC STUDY OF TWO TERPENOIDS IN ETHYL ACETATE EXTRACT OF LEAVES (EFV) OF VITEX NEGUNDO *LINN*

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ABSTRACT

Ethanomedicines of almost all civilizations of the world abound in herbal remedies. Most of the ethaomedicines used in healthcare are obtained from many plants Vitex negundo Linn. Vitex negundo (VN), commonly known as "chaste tree", is an ethnobotanically important plant with enormous medicinal properties. it is one of the important medicinal plants having many therapeutic uses. Different species of Vitex vary in chemical composition, thus producing different phytochemicals. The present study revealed that Vitex negundo Linn important source of many therapeutically and pharmacologically active constituents.

Keywords: *Vitex Negundu, Terpenoids, Phytochemical Analysis, Flavonoids, Triterpenoids, Therapeutically.*

Introduction

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, phytoactive 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 bioactive compounds provided an opportunity to obtain natural extracts that could be potentially used [3,4].

Nirgundi or Vitex negundo Linn. (Verbenaceae) is a woody, aromatic shrub growing to a small tree and bears tri- or penta-foliate leaves on quadrangular branches, which give rise to bluish-purple coloured flowers in branched tomentose cymes. also called a five-leaved chaste tree, is a potent ayurvedic plant, that possesses noteworthy therapeutic properties and heals several ailments including asthma, muscle spasms and anxiety.

A vast array of beneficial plant-based compounds with antioxidant and anti-inflammatory traits are present in nirgundi. These include flavonoids and phenols which display cardioprotective qualities for heart wellness, besides terpenoids and organic fatty acids that are laden with calming and analgesic properties to relieve mental stress, joint pain and muscle aches. Nirgundi is also bestowed with alkaloids such as nishindine, vitricine, which confer useful anticancer and antimicrobial effects on the body, thus combatting tumour growths and stomach infections. 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.

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Review of Literature

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. Principle indigenous systems are homeopathy, herbal medicines (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 toprostaglandin 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.

Material and Methods

Phytochemical Analysis

Phytochemical analysis was carried out in the Ethylacetate extract(EFV) 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

Ethylacetate extract of vitex leaves (EFV) was warmed with 2% H2SO4 for two minutes .it is filtered and few drops of reagents were added and indicated the presence of alkaloids.

- **Dragendroff's Test**

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

- **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 .

Test for Terpenoids

- **Salkowski Test**

The extract was mixed with 2ml of chloroform and concentrate H2SO4 (3ml) 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.

- **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.

- **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

- **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

- **Ninhydrin Test**

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

- **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.

- **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

- **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.

- **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 denser red precipitate indicates the presence of glycosides

- **Baljet's Test**

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

- **Keller-Killiani Test**

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

- **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 Ethylacetate extract of vitex leaves (EFV)

Biochemical 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.

Rf value = (Distance moved by the compound)/Distance moved by the solvent front)

Results

Preparation of Extract (EFV)

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 ethylacetate. The crude extract was collected in amber coloured sample bottles and stored. All chemical and reagents used including the solvents were of analytical grade.

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

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.

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 Ethylacetate (EFV) extract of leaves of Vitex negundo linn.

Sl. No	Test for	Reagents	Reaction	Result
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	++

Figure 3: Shows TLC identification of terpenoids from Ethylacetate (EFA) leaves extract of *Vitex negundo* Linn after derivatization.

Ethylacetate (EFA**)** extract was subjected to TLC in order to separate and identify the two pharmacologically valuable terpenoids present in the Ethylacetate (EFA) 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.

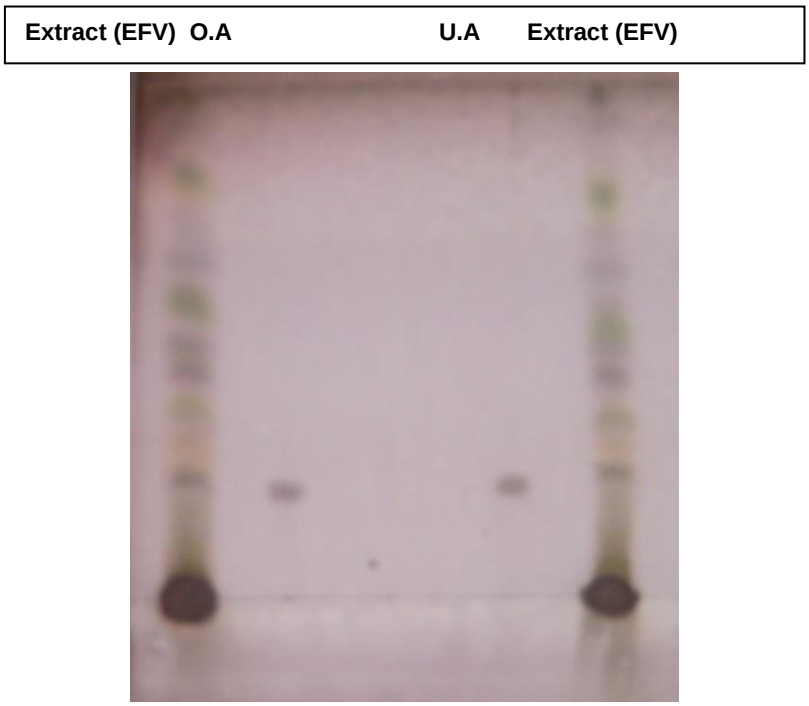

Figure 3: Shows TLC identification of terpenoids from methanolic leaves extract of *Vitex negundo* Linn after derivatization

Table 2: Results of TLC of Ethytacetate leaves Extract (EFV) of *Vitex negundo linn*:

Sample	Solvent System (7.8:2.2:0.75 ml)	No. of Spot	Rf Values
Ethytacetate leaves Extract (EFV)	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)

Conclusion

This ethanimedicinal plant bestowed with a multitude of phytochemical secondary metabolites which impart an variety of medicinal uses to the plant. VN have been validated by modern pharmacological studies, focusing on its anti-nociceptive, antiinflammatory, anti-tumor, anti-oxidant, insecticidal, antimicrobial, anti-androgenic, anti-osteoporotic, anti-cataract, hepatoprotective and anti-hyperglycemic activity, etc.and these all-curative properties of plant lie in their secondary metabolatites. 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 further investigation of Ethytacetate leaves Extract (EFV)and lead to us for more scope to develop a 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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