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ARTICLE

Preliminary Phytochemical Screening Of *Vitex negundo*, Linn.

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Abstract

Vitex negundo Linn. commonly called 'nochhi' is having great demand on Ayurveda, due to its medicinal value. A study is designed to explore the preliminary phytochemical analysis of *Vitex negundo* which is responsible for its pharmacological properties. Qualitative phytochemical screening of *Vitex negundo* Linn. was studied. Three solvents viz; water, acetone, chloroform and petroleum ether were used to obtain extracts from powdered leaves, stem and roots. The extracts were subjected to qualitative phytochemical screening using standard procedures. Results show that twelve of fourteen phytochemicals screened for were present. They are steroids, triterpenoids, glycosides, saponins, alkaloids, flavonoids, tannins, terpenoids, quinones, proteins, free amino acids and carbohydrates. In all, more phytochemicals were found present in extract prepared with chloroform. Remarkably, Phlobatannins and Oxalates were not present in all extracts from different parts. The diversity of phytochemicals found present suggests that *Vitex negundo* Linn. may serve as a source of useful drugs.

Keywords: *Vitex Negundo*, Phytochemical

Introduction

Green plants always fascinate us with their remarkable source of biochemical components. Ancient literature tells as their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa with minimal side effects (Bibitha *et al*. 2002 and Maghrani *et al*. 2005). Presence of these compounds makes them commercially significant. Now a days, many pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective medicines that are affordable to the common man.

Vitex negundo Linn., commonly called nochhi a member of the family Lamiaceae (formerly under Verbanaceae), is an evergreen medicinally important shrub distributed throughout India. The plant is bitter, acrid, thermogenic, expectorant, carminative, digestive, stomachic, anodyne, anti-inflammatory, antiseptic, cephalic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic. The roots used in vitiated conditions of vata, kapha, jajvara, cephalalgia, sprains, orchitis, gout, splenohepatomegaly, otorrhoea, inflammations ulcers, cephalagia, otalgia, arthritis, inflammation, dyspepsia, colic, verminosis, flatulence, dysentery, uropathy, wounds, bronchitis, cough, malarial fever, haemorrhoids, ysmenorrhoea, leprosy, dermatopathy, ophthalmopathy and general debility. The bark is used in vitiated conditions of vata, odontalgia, verminosis and ophthalmopathy. The flowers used in diarrhoea, cholera, fever, haemorrhages, hepatopathy and cardic disorders (Johnson and Christine, 1986, Johnson *et al*. 2008 and Warrier *et al*, 1995). The whole plant is having great demand on the market due to its medicinal value. The present study is designed to explore the preliminary phytochemical analysis of *Vitex negundo* which is responsible for its pharmacological properties.

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Materials and Methods

Collection of plant material

The leaves, bark and roots of *Vitex negundo* were collected from three different locations of Thiruvananthapuram. Collected leaves, bark and roots of *Vitex negundo* shade dried and the dried plant materials was taken and ground using motor and pestle to obtain a fine powder. The powder was further passed through a 2mm sieve to obtain finer particles. The powdered samples were stored in a clean glassware container until needed for analysis.

Preparation of plant extracts

5 gram of each sample was taken and extracted in soxhlet apparatus successively with acetone, petroleum ether, chloroform, and water. After extraction, the extracts were filtered through Whatman No.1 filter paper and stored for further phytochemical investigations

Preliminary phytochemical investigations

The major secondary metabolites like, alkaloids, flavonoids, saponins, phenols, terpenoids, nthraquinones, proteins and aminoacids, carbohydrates and glycosides were assessed according to the standard procedure described by Harborne (1998). Following standard procedures were used.

Test for Steroids and Triterpenoids (Liebermann Burchard test)

Crude extract was mixed with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was then added from the sides of the test tube and observed for the formation of a brown ring at the junction of two layers. Green coloration of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids and triterpenoids respectively.

Test for Glycosides (Keller Killiani Test)

Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Saponins (Foam Test)

Test solution was mixed with water and shaken and observed for the formation of froth, which should be stable for 15 minutes. This indicates the presence of Saponins.

Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100mL of water] and observed for the formation of reddish brown precipitate (or colouration) which indicates the presence of alkaloids.

Test for Flavonoids (Alkaline reagent test)

2mL of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for Tannins (Braymer's test)

2mL of extract was treated with 10% alcoholic ferric chloride solution. Formation of blue or greenish colour solution shows the presence of Tannins.

Test for Terpenoids (Salkowki's test)

1mL of chloroform was added to 2mL of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

Test for Phlobatannins (Precipitate test)

Deposition of a red precipitate when 2mL of extract was boiled with 1mL of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for Proteins (Biuret Test)

Test solution was treated with 10% sodium hydroxide solution and two drops of 1% copper sulphate solution and observed for the formation of violet/pink color.

Test for Free Amino Acids (Ninhydrin Test)

Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Test for Carbohydrate (Benedict's test)

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Oxalate

To 3mL portion of extracts were added a few drops of ethanoic acid glacial. A greenish black colouration indicates presence of oxalates.

Results and Discussion

Results obtained for qualitative screening of phytochemicals in different parts of *Vitex negundo* Linn. is presented in Table 1. Of the fourteen phytochemicals screened for, twelve were found present in various solvent extracts. They are steroids, triterpenoids, glycosides, saponins, alkaloids, flavonoids, tannins, terpenoids, quinones, proteins, free amino acids and carbohydrates. In all, more phytochemicals were found present in extract prepared with chloroform. Remarkably, Phlobatannins and Oxalates were not present in all extracts from different parts. According to Tiwari *et al*., the factors affecting the choice of solvent are; quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractant. The logic in using different solvents when screening for phytochemicals in plant materials was clearly validated in present study. For instance, the results shows that Terpenoids were exceptionally present in petroleum ether and chloroform extracts but absent in other two extracts. Quinones showed their presence in acetone and chloroform extrats. This corroborates the reports of Misra *et al*. Proteins, free amino acids and carbohydrates showed their presence in all extracts irrespective to the solvents and plant parts.

Phytochemical screening of the extracts of *vitex negun-*

Table 2. Result of phytochemical screening of *Vitex negundo*, Linn.

Phytochemicals	Extract											
	Acetone			Petroleum ether			Chloroform			Distilled Water		
	Leaf	Bark	Root	Leaf	Bark	Root	Leaf	Bark	Root	Leaf	Bark	Root
Steroids	+	–	+	+	–	+	+	–	+	–	–	–
Triterpenoids	–	–	–	+	–	–	+	–	–	–	–	–
Glycosides	+	–	+	–	–	+	+	–	+	+	–	+
Saponins	+	–	–	+	–	–	+	–	–	–	–	–
Alkaloids	+	+	+	–	+	+	–	+	+	+	+	+
Flavonoids	–	+	–	+	+	–	–	+	–	–	–	–
Tannins	–	–	–	–	–	–	+	+	+	+	+	+
Terpenoids	–	–	–	+	+	+	+	+	+	–	–	–
Quinones	+	+	+	–	–	–	+	+	+	–	–	–
Phlobatannins	–	–	–	–	–	–	–	–	–	–	–	–
Proteins	+	+	+	+	+	+	+	+	+	+	+	+
Free amino acids	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
Oxalate	–	–	–	–	–	–	–	–	–	–	–	–

+ indicates presence and - indicates absence.

do revealed the presence of alkaloids, steroids, flavonoids, aminoacids, phenols, quiones and starch (table1). These compounds have significant application against human pathogens, including those that cause enteric infections (El-Mahmood *et al.*). The result indicates that *Vitex negundo* Linn. hold promises as source of pharmaceutically important phytochemicals. Alkaloids generally present in all extracts which play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine especially the steroidal alkaloids. Tannins are known to inhibit pathogenic fungi, is present in hexane extract. The flavonoids and phenolic compounds in plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc.

Conclusion

It is very necessary to introduce new and biologically safe and active drugs for an eco-friendly life style. Phytochemicals found present in the *Vitex negundo* Linn. indicates their potential as a source of principles that may supply novel medicines. Further studies are therefore suggested to ascertain their antimicrobial, antiplasmodic and antihelminthic activities. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies. So we can reiterates a popular local quote of the Bhangalis in the western Himalayan region of India which translates as- "A man cannot die of disease in an area where *Vitex negundo* is found" (Uniyal *et al.*).

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