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Research Article **Botany**

Qualitative and Thinlayer chromatographic identification of phytochemical and anti microbial activity of *Vitex negundo* Linn.

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ABSTRACT

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. The phytochemical screening *Vitex negundo* leaf showed that the presence of flavonoids, steroids, tannin, saponins, glycosides, terpenoids phlobatannins. alkaloids and anthroquinones, Protein, Carbohydrate, Triterpenoids, Polyphenol. Significant amount of Flavonoids, polyphenols are present in the plant. The *in vitro* antimicrobial activities of the Vitex negundo leaf extract against bacteria were investigated. Over all, *Vitex negundo* leaves contain rich source of phytochemicals and the antibacterial activity of *Vitex negundo* leaves extract is particularly important considering the test human pathogenic bacteria.

Keywords: *Vitex negundo,* Phytochemicals, Antibacterial activity, Chromatagraphy

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INTRODUCTION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg,1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999) and About 150 phytochemicals have been studied in detail.Plant and plant products play a wide range of biological properties. Keeping in view, the present study to investigate the phytochemical and antimicrobial properties of *Vitex negundo* L.

MATERIALS AND METHODS

Plant materials:

The fully mature *Vitex negundo* leaves were collected in December 2015 from Kananthagudi East, Thanjavur, Tamil Nadu, and India.

Preparation of alcoholic extract:

The leaf of *Vitex negundo* was first washed well and dust was removed from the leaves. Leaf was washed several times with distilled water to remove the traces of impurities from the leaf. The leaves were dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical screening

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973, 1984).

Quantitative determination of the chemical constituency

Total phenols estimated by the method of Edeoga et al., (2005). Alkaloid determine by the method of Harborne (1973). Saponin determine by the method of of Obadoni and Ochuko (2001). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994) Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Thin Layer Chromatography

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. Separation of compounds by TLC followed by the method of Harborne, (1984, 1973) and Stahl, (1969).

Determination of Antimicrobial Activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing Escherichia coli, Staphylococcus aureus spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

RESULTS AND DISCUSSION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary metabolism is important for growth and development of plants include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll’s etc. Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Attractions of pollinators, natural defense system against predators and diseases, etc., are examples of the roles of secondary metabolites (Sofowara, 1993).

In the present study was carried out on the plant sample revealed the presence of medicinally active constituents. The phytochemical characters of the Vitex negundo leaf investigated and summarized in Table-1. The phytochemical screening Vitex negundo leaf showed that the presence of flavonoids, steroids, tannin, saponins, glycosides, terpenoids phlobatannins. alkaloids and anthroquinones, Protein, Carbohydrate, Triterpenoids, Polyphenol.Quantitative analysis of phytochemicals represented in table 2. Significant amount of polyphenols are present in the plant.

Table 1: Phytochemical screening of *Vitex negundo* leaf

S.No	Phytochemical analysis	Observation	Results
1	Tannin	Blue black	+
2	Phlobatannins	Red precipitated	+
3	Saponin	Emulsion	+
4	Flavonoids	yellow	+
5	Steroids	Blue	+
6	Terpenoids	Radish brown	+
7	Triterpenoids	Violet	+
8	Alkaloids	White precipitated	+
9	Carbohydrate	Red precipitated	+
10	Protein	Pink	+
11	Anthroquinone	Pink	+
12	Polyphenol	Blue green	+
13	Glycoside	Brown ring	+

(+) Presence (-) Absence

Table 2: Qualitative analysis of *Vitex negundo* leaf

S.No	Test	Result (mg/gm)
1.	Polyphenol	121.02mg
2.	Flavonoids	70 mg
3.	Alkaloids	40 mg
4.	Saponin	25 mg
5.	Terpenoids	20 mg

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action. Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom. Some isoflavones widely used in insecticides. They might also play a role in disease resistance. Some flavonoids such as quercetin and rutin, are known to support humam health by serving antiinflamatory, antihistaminic and antiviral agents (Okwu, 2004).

Thin layer chromatographic separation of compounds in Vitex negundo leaf extract

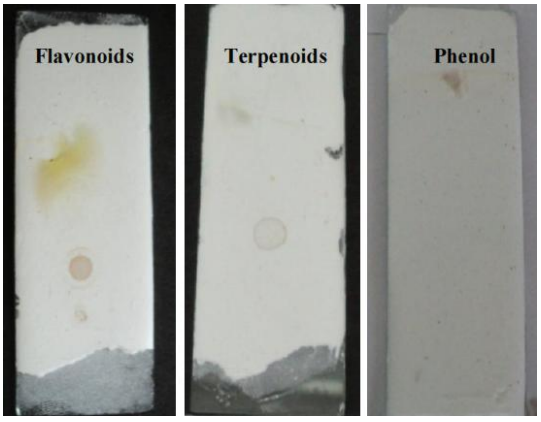
TLC is a simple, quick, and inexpensive procedure that gives the researcher a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the Rf of a compound is compared with the Rf of a known compound. Additional tests involve the spraying of phytochemical screening reagents, which cause color changes according to the phytochemicals existing in a plants extract. This has also been used for confirmation of purity and identity of isolated compounds.

Presence of phytoconstituents in particular Vitex negundo extract was confirmed by spraying TLC plates with different spraying reagents. Presence of phenol was detected visually by spraying the freshly prepared vanillin reagent (green colour), flavonoids positive reaction was formation of yellow colour spot by exposure of ammonia where as terpenoids was detected by Vanillin reagent (Figure 1). TLC profile studies in authentification for quality control.

Table 3: Thin layer chromatographic separation of compounds in Vitex negundo leaf extract

S. No	Tests	Rf-Values
1	Flavonoids	0.75
2	Terpenoids	0.60
3	Phenol	0.25

Figure 1: Thin layer chromatographic separation of compounds in *Vitex negundo* leaf extract



Antimicrobial activity

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are

resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosuppressant and are major burning global issues in treating infectious diseases (Karaman et al., 2003). This situation forced scientists to search for new antimicrobial substances with plant origin. *Staphylococcus aureus* is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections,such as cellulitis, impetigo, and folliculitis (Todar, 2007) . *Escherichia coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In some cases, virulent strains are also responsible for haemolyticuremic syndrome, peritonitis, mastitis, septicaemia and pneumonia (McCaig, 2006).

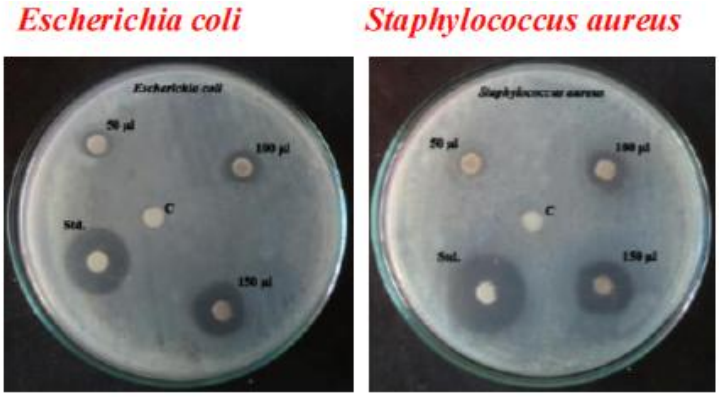
Table 4: Antimicrobial activity of Vitex negundo leaf extract

Microorganism	50µl	100 µl	150 µl	Standard	Control (Solvent)
Escherichia coli (mm)	1.98±0.13	2.74±0.19	4.91±0.34	10.46±0.73	0
Staphylococcus aureus (mm)	1.52±0.10	2.60±0.18	4.49±0.31	10.37±0.72	0

Values were expressed as Mean ± SD.
Bacterial standard – Chloromphenical

Ethanolic extract of Vitex negundo was screened against Escherichia coli and Staphylococcus aureus were evaluated using the standard agar disc diffusion method. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Nutrient agar plates were swapped with the test organism and the samples were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The *in vitro* antimicrobial activity of the *Vitex negundo* leaf extract against these bacteria was qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 2. The inhibitory activities in culture media of the Vitex negundo reported in Table 4 were comparable with standard antimicrobiotic viz. chloromphenical

Figure 2: Antimicrobial activity of *Vitex negundo* leaf extract



Plant derived phytochemical therapy may be helpful for various free radical mediated diseases. The plants and its derivatives may considered as good sources of natural phytochemicals for medicinal uses such as against cancer, diabetic mellitus, cardiovascular diseases, aging and other diseases related to radical mechanisms. Determination of the natural phytochemicals and antimicrobial compounds will help to develop new drug candidates for antimicrobial therapy.

Over all, *Vitex negundo* leaves contain rich source of phytochemicals and the antibacterial activity of *Vitex negundo* leaves extract is particularly important considering the test human pathogenic bacteria.

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