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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY ON *VITEX NEGUNDO*

[1]C. MERLIN ROSE, [2]L. CATHRINE

[1,2]Department of Chemistry, Holy Cross College, Tiruchirappalli, Tamil Nadu, India
Email: merlinrosemphil@gmail.com, cathrinehcc@gmail.com

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ABSTRACT

Leaves of *Vitex negundo* were shaded, dried, powdered and were extracted using three different solvents petroleum ether dichloromethane and ethanol. Preliminary phytochemical screening of the extracts revealed that the presence of simple phenols, terpenoids, flavonoids, anthraquinones and carotenoids. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. The leaf extracts of *vitex negundo* solvented by ethanol, showed the spectrum of inhibition on *salmonella paratyphi*. Most of the bacterial pathogens like *salmonella paratyphi, klebsiella pneumonia, vibrio cholera, streptococcus mutans* and *E.Coli* were found to be susceptible in leaf extracts of the *vitex negundo*. Petroleum ether leaf extract of *vitex negundo* showed good activity against *salmonella paratyphi* and *entrobactor.*

Keywords: *Vitexnegundo*; Phytochemical; antibacterial activity.

INTRODUCTION

Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in light modern scientific knowledge[1]. *Vitex negundo (verbenaceae)* commonly known as Nirkundi or Nallanocci. It is an aromatic large shrub or small tree about 3m in height with quadrangular branches and almost found throughout india, ascending to 1500m in the outer Himalaya, fairly common in waste lands, on road side, the banks or streams or in moist places near deciduous forests[2]. The essential oil of *vitex negundo* leaves showed significant antifungal activity against *trichloroderma viride, fusarium sp., collectrotrichum* and *helminthosporium* [3].An ointment made from the juice is applied as hair-tonic. It is constituent of the Ayurvedic preparations "Vishagarba thailla" [4, 5]. The extraction of the leaves showed anti-cancer activity against *Ehrlich ascites* tumer cells [6, 7]. The seed extracts of *V. negundo* interfere with male reproductive function without producing adverse toxicity in other vital organs [8]. The ash of the plant is a source of potassium carbonate and is reported to be used as alkali in dyeing[9]. Leaf extract of Azadirachta indica inhibited the growth, sporulation and germination of Alternaria alternata. Leaf extract of *Cinnamomum Camphora, Vitex negundo* were less effective [10]. Significant activity against *Escherichia coli, Klebsiella aerogenes, Proteus vulgaris* and *pseudomonas aerogenes* was shown by the extract of *vitex negundo*[11]. The pentacyclic triterpenoids betulinic and ursolic acids isolated from leaves of *vitex negundo*, showed a very mild antibacterial activity[12]. The plant extract was effective against *curvularia lunata* and *rhizopus nodules*[13]. Though v.*negundo* also finds use as a food crop and source of timber[14, 15]. The present study is designed to explore the preliminary phytochemical and antimicrobial analysis of *vitex negundo* which is responsible for its pharmacological properties.

MATERIALS AND METHODS

Plant material

The leaves of *Vitex negundo* were collected at the Rapinat Herbarium and Center for molecular systematic, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The leaves were dried, ground into powder and stored in polythene bags before use.

Extraction of plant material

Dried ground leaves of 50 grams were extracted in soxhlet sequentially in 300ml of petroleum ether dichloromethane and ethanol. The process was run for 48 hrs at 31^{0}C until complete exhaustion of the material. At the end of 48 hrs, the extracts were distilled. After distillation those extracts were stored in a refrigerator for the analysis. The compound in the leaf extracts were separated using TLC technique [16]. Then the compounds were identified using UV spectroscopy.

Preliminary phytochemical investigations

The major secondary metabolites like, alkaloids, flavonoids, saponins, phenols, terpenoids, anthraquinones, proteins and aminoacids, carbohydrates and glycosides were assessed according to the standard procedure described by Harborne [17].

Antibacterial screening test

Disc diffusion method

The paper disc diffusion method was used to determine the antibacterial activity of the extracts prepared from the *vitex negundo* leaves using standard procedure[18]. Its essential feature is the placing of filter paper discs with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculam. Routine direct application of discs to plates seeded with clinical material is not recommended because of problems with inoculam control and mixed cultures.

Streak plate method

The streak plate method was used[22]. In this method a sterilized loop or transfer needle is dipped into a streak plate method offers in the most practical method of obtaining discrete colonies and pure cultures. Suitable diluted suspension of organisms, which is then streaked on the surface of an already solidified agar plate with plant extract, to make a series of parallel non-overlapping streaks. The aim of this method is to check whether the organisms are growing in plant extract containing medium or not based on granite of particular microorganisms.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Phytochemical screening of the extracts of vitex negundo 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 [19]. 5-hydroxy-3, 6, 7, 3', 4'-penta methoxy flavones and 3, 5-dihydroxy-6, 7, 3', 4'-tetra methoxy flavonol were isolated from vitex negundo leaves[20].

Preliminary phytochemical screening

Chemical name	Petroleum ether	Dichloromethane	Ethanol
Alkaloids	-	+	+
Steroids	+	+	+
Triterpenoids	+	-	+
Coumarins	-	-	-
Flavonoids	+	+	+
Aminoacids	+	+	+
Carbohydrates	+	+	+
Polyoses	-	-	-
Phenols	+	+	+
Quinines	+	-	+
starch	+	-	+
Carotenoids	+	+	+
Anthraquinones	+	+	+

+ = presence; - = Absence

Antibacterial Activity

By disc diffusion method, *salmonella paratyphi* and *streptococcus mutans* express a very clear indication of inhibitory activity and they show wide spectrum of inhibition to all the solvent extracts except dichloro methane. *E.Coli* and streptococcus *mutans* were resistant to all the solvent extracts. *Klebsiela pneumoniae, Vibrio cholerae, Streptococcus mutans, Eschericia coli* have inhibitory activity next only to the *Salmonella paratyphi.* These organisms were sequentially given using their sensitivity pattern to the extract solvents by ethanol and dichloromethane. *E.Coli* and streptococcus *mutans* showed very poor susceptibility even at the higher concentrations to ethanol extract. Throughout the experiment dichloromethane solvent reveals nil inhibitory activity to the entire bacteria. The inhibition zone serially increases with the increased concentration of the extract to all bacterial culture.

Table2 shows the effect of antibacterial activity of *vitex negundo* on disc diffusion method.

In streak plate method the petroleum ether extracts, the growth of the bacterial pathogens like *salmonellaparatyphi* and *Entrobactor* were found to be maximum in both 25% and 50% concentrationand as the concentration increases the degree of susceptibility decreases and growth is absent in both 75% and 100% concentration. Table3 shows the effect of antibacterial activity of vitex negundo by streak plate method.

Table 2: Effect of antibacterial activity of *vitex negundo* on disc diffusion method-leaf

Solvents used	Pathogen used	Number of discs (D) zone of inhibition in mm			Mean±SD
		D_1	D_2	D_3	
	Klebsiela pneumoniae	7	10	12	9.66±2.51
	Vibrio cholerae	7	9	11	9.33±2
Ethanol	*Streptococcus mutans*	7	9	11	9±2
	Salmonella paratyphi	11	12	10	11±1
	Eschericia coli	12	8	9	9.66±2.08
	Klebsiela pneumoniae	6	6	7	6.33±0.57
	Vibrio cholerae	8	6	6	6.66±1.15
Dichloro methane	*Streptococcus mutans*	6	7	7	6.66±0.57
	Salmonella paratyphi	8	6	7	7.33±1
	Eschericia coli	8	7	6	7±1

Table 3: Effect of different concentrated petroleum ether leaf extract on test bacteria (Streak Plate method)

Test organism	Growth of two bacteria on different concentration leaf extract				
	Control	25%	50%	75%	100%
Salmonella paratyphi	++++	+++	++	++	+
Enterobactor	++++	+++	++	++	+

++++ = Excessive growth; +++ = Moderate growth; ++ = Less growth; + = Nil

CONCLUSION

It is very necessary to introduce new and biologically safe and active drugs eco-friendly in nature and effective as antimicrobial agents. Usually medicinal plants contain several phytochemical compounds, which are very much necessary to control the growth of the micro organisms. Uniyal et al. 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"[21]. In fine, this paper establishes that *vitex negundo* leaf extracts have good activity against *Klebsiela pneumonia, Eschericia coli, Salmonella paratyphi* and *Enterobactor.*

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