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Preliminary Phytochemical Screening and Antibacterial Activity of *Vitex Leucoxylon. (L.)* (Leaf) Against Various Bacterial Species

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ABSTRACT

The aim of the present study was to evaluate the qualitative analysis of phytochemicals and antibacterial activity of various solvent (Petroleum ether, Benzene, Chloroform, Methanol and Water) extracts of *Vitex leucoxylon (L.)* leaves. Antimicrobial activity of different solvent extracts of Vitex *leucoxylon* leaves were tested against Gram positive and Gram negative bacterial strains by observing the zone of inhibition. The bacteria used in the study were *Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli Bacillus subtilis, Lactobacillus delburkii.* It was observed that aqueous showed maximum inhibitory effect on all tested bacteria where as Petroleum ether, Benzene, Chloroform, Methanol extracts showed maximum inhibitory effect on all tested bacteria except *Pseudomonas putida* compared to other solvent extracts.

Keywords: Antimicrobial activity, Gram positive, Gram negative, phytochemicals, *Vitex leucoxylon (L.),* zone of inhibition

INTRODUCTION

Plants and their active constituents play an important role in the prevention of a variety of ailments. Most of the species of the genus *Vitex* are used therapeutically in ancient Indian systems of medicine especially, Ayurveda and Siddha. The genus *Vitex* contains about 270 species distributed around the world. These species contain a variety of potentially bioactive molecules, such as iridoids, flavonoids, diterpenoids, derivatives, and phytosteroids. Most of these species possess analgesic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, antihistamine, and antiasthmatic properties. (Ajay Kumar Meena *et al.* 2011).

Vitex leucoxylon L. (Tella vavilli-Telugu) is a large deciduous tree with spreading head and trunk and belongs to the family Verbenaceae. It is widely distributed in the forests of Deccan Peninsula and Sri Lanka. Herbal extracts, specifically the extract obtained from *V. leucoxylon (L.)* is found to cure diabetics, inflammatory diseases, liver disorders and free radical mediated diseases. Roots and the bark of *V. leucoxylon (L.)* are astringent and febrifuge. Leaves are used in traditional medicine for relieving headache and fever (Chanda, 1982). Sarma *et al.* (1990) studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of *V. leucoxylon (L.)* in acute inflammation model. General pharmacological studies deals with antipsychotic, ati-depressant, analgesic, anti-inflammatory, anti-Parkinson's and anti-microbial activities of aqueous and ethanolic extracts (Makwana *et al.*, 1994). Rao *et al.* (1997) isolated many pharmaceutically important bio active compounds like β-sitosterol, dimethyl terphthalate, vitexin, isovitexin, agnuside and aucubin from the leaves.

MATERIAL AND METHODS

Plant material:
Leaves of the *Vitex leucoxylon (L).* Were collected from the Mulikavanam Dravidian University, Srinivasavanam, Kuppam, Chittoor District, Andhra Pradesh. The plant specimen was botanically identified and authenticated by comparing the herbarium specimen (SVUBH/2012/L-11673) available at Sri Venkateshwara University Thirupathi, chittoor District, Andhra Pradesh.

***Fig-1. Different parts of selected plant Vitex leucoxylon (*L.)**

Tested Microorganisms:
Antibacterial activity of the leaves extract was investigated against six bacterial isolates which were ob tained from Microbial Type Culture Collection (MTCC) and American Type Culture Collection (ATCC) from Institute of Microbial Technology, Chandigarh. These include gram positive and gram negative bacteria *Staphylococcus aureus* (ATCC 6538) *Pseudomonas auriginosa* (ATCC 9027) *Pseudomonas putida, Escherichia coli* (ATCC 9837) *Bacillus subtilis* (ATCC 9856) *Lactobacillus delburkii*. The tested microorganisms were cultured on Nutrient agar (HiMedia, Mumbai) (for bacteria at 35 ± 2^{o}C for 24 hrs) .The reference strains of bacteria were maintained on Nutrient agar (Hi Media, Mumbai). The cultures were sub cultured regularly (every 30 days) and stored at 4^{o}C.

Preparation of the Extracts:
The collected leaves were washed with deionized water and dried for one hour at room temperature. The dried leaves of *Vitex leucoxylon (L).* (100 gm for each solvent) were grinded in motor and pestle using solvents (petroleum ether, benzene, chloroform, methanol and water – each solvent 500 ml). The obtained extracts were filtered by using Whatmann No. 1 filter paper. The residual extracts were stored in refrigerator at 4^{o}C in small and sterile plastic bottles.

Organoleptic properties determination
Organoleptic properties (color, consistency/texture and odour) of the plant extract *Vitex leucoxylon* (L). In different solvents were characterized and noted in the (table 1).

Phytochemical Analysis of Plant Extracts:
All the extracts were dissolved in di methyl sulphoxide (DMSO) except water that was dissolved in Distilled water prior to use (Kubmarawa et al, 2004). The plant extract of all the solvents were used for the phytochemical analysis (Khyade and Vaikos, 2009) for the identification of various classes chemical compounds using the standard protocol (Harborne, 1984; Kumar *et al*, 2009; Govaerts, 2011)

Experimental Design:
The prepared leaf extracts of *Vitex leucoxylon* (L). Using different solvents like Petroleum ether, Benzene, Chloroform, Methanol and aqueous (Water) were brought to the room temperature before use. The Laminor air Flow hood was sprayed with 70% ethanol and the U V light is switched on for half an hour for sterilization and the autoclaved Nutrient Agar Medium was poured in to the sterile petriplates inside the chamber and left for 30 minutes undisturbed for solidification.

After solidification the bottom of the petriplate was divided in to five equal parts using the marker for each of the leaf extract to be poured on the discs were prepared using Whatmann No 1 filter. The overnight bacterial inoculum of *Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli, Bacillus subtilis, Lactobacillus delburkii* 100µl of each inoculum were spreaded in separate petriplates with Nutrient Agar Medium using the sterile L- rod. Using the sterile forceps the whatmann filter paper dics were placed in the divided region in the petriplate and marked for the specific leaf extract.10µg of the leaf

extract were poured using the micro pippet after all the leaf extracts poured on the discs the petriplate lid was closed and sealed using the parafilm and kept inverted inside the incubator at 37^{o}C for 10-12 hours and observed.

RESULTS

Orgnaoleptic Properties:
The color, texture and odor of the plant extracts in different solvents (*Vitex leucoxylon* (L.) in wet conditions were characterized (Table 1). The Petroleum ether extracts were better than the corresponding aqueous and other organic extracts in retaining the natural fragrance of the plants. This may be due to the preservative ability of methanol (i.e. reducing breakdown of organic compounds by microorganisms), its enhanced extraction capability (i.e. more fragrant components extracted) or a combination of both. The aqueous extract was found foamy and pleasant smell among all the extracts. The petroleum ether extract was darker where as methanolic and water extracts was found light green in color.

Phytochemical Analysis of the extract:
Preliminary phytochemical analysis of the (*Vitex leucoxylon (L.)* extract (Petroleum ether, Benzene, Chloroform, Methanol and Aqueous) showed the presence of Alkaloids Anthraquinones, Carbohydrates Proteins, Amino acids, Phenolic compounds and Tanins by the series of tests conducted as shown in the (Table 2).

Antibacterial activities
Among all the extract tested aqueous extract were found to be the most active than corresponding organic extracts (table 3). Aqueous extract was found to be active against

Table1: Organoleptic properties of leaves extract of (*Vitex leucoxylon (L.)*

Plant Extract Dissolved in the solvent	Texture	Color	Odor
Petroleum ether	Semisolid	Dark greenish	Pleasant smell
Benzene	Semisolid sticky	Black green	Alcoholic leafy smell
Chloroform	Semisolid	Green brown	Pungent smell
Methanol	Liquid	Light green	Characteristic odor
Water	Semisolid, Foamy	Light reddish green	Agreeable/ pleasant smell

Table 2: Preliminary phytochemical analysis of (*Vitex leucoxylon (L.)* Leaf extract

Phytochemical tested	Test used	Leaves extracts				
		P. Ether	Benzene	Chloroform	Methanol	Aqueous
Alkaloids	Mayer's test	-	+	-	-	-
	Wagner's test	-	+	-	-	-
Anthraquinones and Carbohydrates	Molish's test	-	-	+	-	-
	Fehling's test	-	-	+	-	-
	Barfoed's test	-	-	-	-	-
	Benedict's test	+	+	++	+	+
Proteins and Amino acids	Biuret's test	+	-	-	-	-
	Ninhydine test	+	-	-	-	-
Phenols and Tanins	Ferric chloride test	++	++	-	++	-
	Alkaline reagent test	-	++	-	-	-
Flavanoids	Alkaline reagent test	-	++	-	-	-
	Lead acetate test	-	++	-	-	-

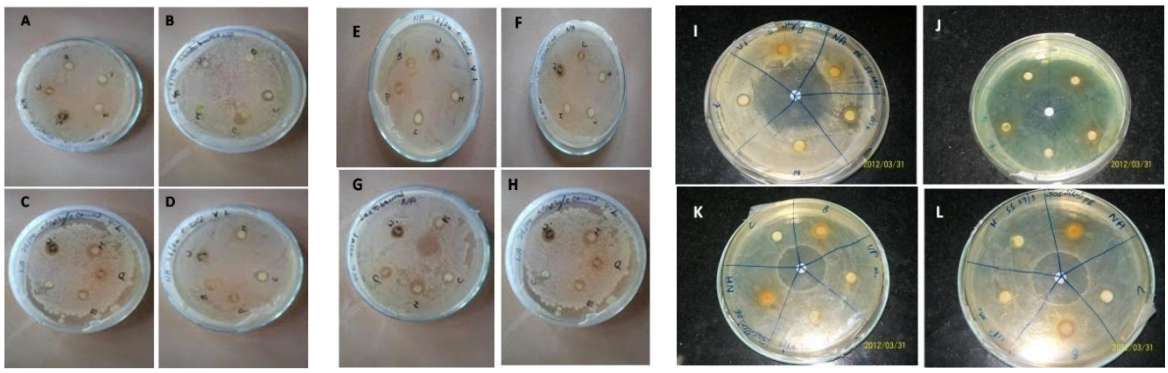
six tested bacteria (*Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli, Bacillus subtilis* and *Lactobacillus delburkii*.) were (7 mm) maximum zone of inhibition against *Staphylococcus aureus* and (3 mm) minimum zone of inhibition against *Pseudomonas auriginosa*. On the other hand methanol extract was effective against five out of six tested bacteria (*Staphylococcus aureus, Pseudomonas auriginosa, Escherichia coli, Bacillus subtilis* and *Lactobacillus delburkii*.) Petroleum ether extract was found to have maximum zone of inhibition against *Lactobacillus delburkii* (4 mm) while the minimum zone of inhibition was against *Escherichia coli* (1 mm).

Benzene extracts of leaves of *Vitex leucoxylon (L.)* were effective against four out of six bacteria tested (*Staphylococcus aureus, Pseudomonas auriginosa, Escherichia coli, Lactobacillus delburkii*) were maximium zone of inhibition showing against *Lactobacillus delburkii* (6.5 mm) while the minimum zone of inhibition against *Pseudomonas auriginosa* (3 mm). Chloroform extract was found to be produce maximum zone of inhibition against *Bacillus subtilis* (5 mm) and minimum zone of inhibition (2 mm) against *Pseudomonas auriginosa* respectively. *Tetracycline* is used as the standard drug against all the tested bacteria (*Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli, Bacillus subtilis* and *Lactobacillus delburkii*.) Were it shown the zone of inhibition in the entire tested organism uniformly (9.0, 9.5, 9.3, 10.0, 9.5, 9.6 mm respectively).

Table 3: Antibacterial activity of leaves extract of *Vitex leucoxylon (*L.)

Tested microbial strains	Plant extracts (10µg/disc)					
	Petrolem ether DIZ (mm)	Benzene DIZ(mm)	Chlorofom DIZ (mm)	Mthanol DIZ(mm)	Aqueous DIZ(mm)	Strepto-mycine DIZ(mm)
Staphylococcus aureus	0	5.0	0	7.0	7.0	9.0
P. auriginosa	2.5	3.0	2.0	4.0	3.0	9.5
P. putida	0	0	0	0	3.0	9.3
Escherichia coli	1.0	3.5	4.0	5.5	3.5	10.0
Bacillus subtilis	5.0	0	5.0	2.0	6.0	9.5
Lactobacillus delburkii	4.0	6.5	3.0	5.5	4.0	9.6

Fig-2*: Antibacterial activity of leaves extract of Vitex leucoxylon (L.)*



A: *Pseudomonas auriginosa,* B: *Lactobacillus delburkii* , C: *Staphylococcus aureus,* D: *Escherichia coli,* E: *Escherichia coli,* F: *Bacillus subtilis,* G: *Lactobacillus delburkii,* H: *Staphylococcus aureus,* I: *Staphylococcus aureus* with standard drug *Tetracycline,* J: *Pseudomonasputida* with standard drug *Tetracycline,* K: *Escherichia coli* with standard drug *Tetracycline,* L: *Bacillus subtilis* with standard drug *Tetracycline.*

DISCUSSION

Aqueous and methanol extracts of *Vitex leucoxylon* (L.) were most effective against the tested microorganisms This was the first attempt to investigate the extract in different solvent on polarity basis, organoleptic properties and detailed preliminary phytochemical study of the leaf extract of this plant. Antimicrobial activity of the extracts of *Vitex leucoxylon* (L.) was first time investigated against (*Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli, Bacillus subtilis* and *Lactobacillus delburkii*.).

The Petroleum ether, benzene, Chloroform, methanol and Aqueous extract of the plants were qualitatively screened for phytochemicals using standard procedures which revealed the presence of various important bioactive chemical entities. The aqueous extracts of leaves *Vitex leucoxylon* (L.) have exhibited significant broad spectrum antimicrobial activity. However our results showed remarkable variations in the effectiveness of the leaves extract against *Staphylococcus aureus, Bacillus subtilis and Lactobacillus delburkii.*

Our study revealed that the Methanol and aqueous extract of leaves of *Vitex leucoxylon* (L.) (collected from Mullikavanam, Dravidian University, Kuppam, Chittoor district, Andhra Pradesh) was effective against *Staphylococcus aureus in* aqueous and methano leaf extracts (7 mm and 7.5 mm) at the concentration of 10µl in each disc respectively. Benzene extracts of leaves of *Vitex leucoxylon (L.) w*ere effective against four out of six bacteria tested (*Staphylococcus aureus, Pseudomonas auriginosa, Escherichia coli, Lactobacillus delburkii*) were maximium zone of inhibition showing against *Lactobacillus delburkii* (6.5 mm) while the minimum zone of inhibition against *Pseudomonas auriginosa* (3 mm). Petroleum ether extract was found to have maximum zone of inhibition against *Lactobacillus delburkii* (4 mm) while the *Escherichia coli* (1 mm).

Benzene extracts of leaves of *Vitex leucoxylon* minimum zone of inhibition was against *(L.)* were effective against four out of six bacteria tested (*Staphylococcus aureus, Pseudomonas auriginosa, Escherichia coli, Lactobacillus delburkii*) were maximium zone of inhibition showing against *Lactobacillus delburkii* (6.5 mm) while the minimum zone of inhibition against *Pseudomonas auriginosa* (3 mm). Chloroform extract was found to be produce maximum zone of inhibition against *Bacillus subtilis* (5 mm) and minimum zone of inhibition (2 mm) against *Pseudomonas auriginosa* respectively. *Tetracycline* is used as the standard drug against all the tested bacteria (*Staphylococcus aureus, Pseudomonas auriginosa, Pseudomonas putida, Escherichia coli, Bacillus subtilis* and *Lactobacillus delburkii*.) Were it shown the zone of inhibition in the entire tested organism uniformly (9.0, 9.5, 9.3, 10.0, 9.5, 9.6 mm respectively).

The chemical constituents of plants varies depending on the species, variety and part of the plant, with conditions of growth (soil, water and temperature), and with the age of the plant. The phytochemistry also varies according to the geographical regions, season and time of collection and different climatic conditions. The study of phytochemicals of *Vitex leucoxylon (*L.) reveals that the nature and amount of phytochemicals varies according to climate. For example stems, leaves and root bark of the plant from Ivory Coast, Africa contains small amount of saponins and no alkaloids, sterols, triterpenes, quinines, tannins and flavonoids. However a large amount of alkaloids was found in stem, leaves and fruits from Ethiopia. Similarly, in previous study falvonoids and steroids were absent in the leaves. However, according to our investigation, methanolic and aqueous extract were found to contain both flavonoids and steroids. However Alkaloids were absent in all tested extracts. The antagonistic results of these findings may be attributed to different geographical locations, climatic conditions for the growth of the plant.

CONCLUSION

Overall, the present study indicates the antibacterial properties of leaves extract of

vitex leucoxylon (L.) and provides some idea about phytochemical evaluation on *vitex leucoxylon (L.)* This study paves the way for further attention and research to identify the active compounds responsible for the plant biological activity. Further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect.

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