International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 3, Issue 2, 2011

Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACTS OF *VITEX LEUCOXYLON* L.F.

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Received: 09 Nov 2010, Revised and Accepted: 08 Dec 2010

ABSTRACT

Leaves of *Vitex leucoxylon* were shade dried, powered and was extracted using solvents Petroleum Ether, Dichloromethane, Methanol, Ethanol, and Aqueous. Preliminary phytochemical screening of the crude extracts revealed the presence of alkaloids, flavonoids, steroids, terpenoids, phenols and carbohydrates. The presence of these bioactive constituents is associated with the antimicrobial activity of the plant. Disc diffusion method revealed the high activity against microorganisms, *Vibrio cholera, Salmonella paratyphi, Enterobacter aerogenes and Escherichia coli* a group of gram negative bacteria. The gram positive bacteria were not as susceptible as gram negative bacteria. The results confirm that *Vitex leucoxylon* can be used as source of drugs to fight infections caused by susceptible bacteria.

Key words: antibacterial activity, ethanolic extract, phytochemical, *Vitex leucoxylon*

INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medical use over thousands of years constitute the most obvious choice for examining the current search for therapeutically effective new drugs such as anticancer drugs, (Dewick, 1996), antimicrobial drugs (Phillipson and Wright, 1996) and anti hepatotoxic compounds (Evans, 1996). About 80% of the world's population depends on herbal medicines and the governments of third world countries unable to sustain a complete coverage with western type drugs have encouraged the rational development of traditional treatments. The chemical constituents of plant medicines are a part of the physiological activities of living plants and hence they are believed to have a better compatibility with the human body. The World Health Organization (WHO) has reported that around 21,000 plants have been used for medicinal purpose in the world. About 500 higher species have been thoroughly investigated as potential source of new drugs. Nearly 119 pure chemicals were extracted from 90 plant species (Sara old field, 1992). There is a growing tendency all over the world to shift from synthetic to natural hared products including medicinal plants. Thus medicinal plants constitute a group of industrially important crops which bring appreciable income to the country by way of export (Bhattacharjee, 1998). The use of medicinal plants has increased in recent years in spite of the advances made in the field of chemotherapy. The reasons proposed (Magherini, 1998) are the use of medicinal plants as materials for the extraction of active pharmacological agents or as precursors for chemico-pharmaceutical hemi synthesis. Also there is the increased use of medicinal plants in industrialized countries for galenic preparations and herbal medicines.

In developing countries, a large proportion of the rural population depends on biodiversity for livelihood, nutrition, and health. Changing forestland to agriculture may, in the short term, slightly enhance the nutritional status of the population, but also leads to a loss of important medicinal plants and can expose people and livestock to diseases resulting from ecosystem imbalance (Gerard bodeker, 2006). Many higher plants are major sources of natural products and are used as pharmaceuticals, agrochemicals, flavors and fragrance, ingredients, food additives and pesticides (Balandrin and Klocke, 1988). Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as a tool in discovering new biologically active molecules has been productive in the area of antibiotics (Gerhartz, et al., 1985; Kroschwitz and Howe-Grant 1992). Medicinal plants are valuable antimicrobial agents and are a source of many potent and powerful drugs (Srivastava, 1996). The different parts used include root, stem, flower, fruit, twigs, exudates and modified plant organs. Expressing grave concern that many medicinally important plants were under threat the Chiang Mai Declaration highlighted "The urgent need for international cooperation and coordination to establish programs for conservation of medicinal plants to ensure that adequate quantities are available for future generations". (Mulabagaal and Tsay, 2004).

Vitex leucoxylon L.f. is an endemic tree found in peninsular India and Sri lanka. It reaches to a height of 12 metres. Leaves are 3- 7 foliolate with an elongate petiole of about 10 cm. Flowers are in corymbose panicles,cream and purplish within. Fruits are seen throughout the year. Trees are mostly seen along river banks and hills. *Vitex leucoxylon* is one of the useful medicinal tree in India. The tree yields a nice grayish brown wood, which like that of *V. altissima,* also contains flavones, readily soluble in water and imparting it a yellowish green tinge; heartwood not distinct. The wood is lustrous, with a smooth feel, straight-grained and fine and even-textured, tough. The pharmaceutically active extracts of *V. leucoxylon* have shown hypoglycemic and anti-inflammatory properties. Stem decoction to cure whooping cough, dried leaf powder smoke as remedy for asthma, leaf paste to wash scars of pox wounds, root bark to reduce fever, fresh twigs as insecticides and dry leaf powder to preserve food grains. Leaf extract is also suggested by native healers for inhibiting the increase of blood sugar. It is used as a traditional medicine for relieving headache and catarrh. The pharmacological studies revealed anti-psychotic, anti-depressant, analgesic, antiinflammatory, anti-parkinsonian and anti-microbial activities of aqueous and ethanolic extracts of leaves of *V. leucoxylon* have studied the anti-inflammatory and wound healing properties of the crude alcoholic extract of the leaves in acute inflammation model (Makwana HG et al) The roots and bark are astringent and the roots are reported to be used as a febrifuge. (Alluri et al 2009) The wood is mostly used for constructional and general purposes, and for cart-wheels,

especially in Tamilnadu. Owing to its attractive colour, small and regular pores, and the broad and conspicuous medullary rays showing very small flecks of silvery grain, the wood is recommended for decorative cabinet or other work (The Wealth of India, Raw material). This paper presents a preliminary phytochemical investigation of *Vitex leucoxylon,* which are responsible for the antibacterial activity of the extracts of the leaves on selected bacterial species.

MATERIALS AND METHODS

Collection of plant materials

The specimens were collected in Palni hills, of the Western Ghats South India. They were duly processed and mounted in standard Herbarium sheets. A voucher specimen is deposited in the Rapinat Herbarium (RHT) of ST. Joesph's College, Tiruchirapalli, South India and the specimen number is RHT 56156. The leaves were collected in the month of May 2007.

Processing of plant material

The leaves were washed and dried over a period of two weeks. The dried samples were milled into fine powder by pounding manually with a clean and sterile mortar, stored in sterile cellopohane bags in a cool dry place till further use.

Extraction of plant material

Dried ground leaves of 50 grams were extracted in soxlet sequentially in 300ml of Petroleum Ether, Dichloromethane, Methanol, Ethanol, and Aqueous. The process was run for 48 hrs after which the sample was concentrated using rotatory evaporator and freeze dried to powdered form. The dried extracts were weighed and kept in labelled sterile specimen bottles.

Preliminary phytochemical investigatons

The major secondary metabolites classes such as terpenoids, steroids, flavonoids, alkaloids, saponins and glycosides were screened according to the common phytochemical methods described by Harborne (1998).

Microbial strains

The microbial strains used are *Enterobacter aerogenes; Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC 15380, *Salmonella paratyphi; Vibrio cholerae; Staphylococcus aureus ATCC 25923 and Streptococcus faecalis*. The bacterial isolates were cultured on nutrient agar and incubated at 37^{o} C for 24 h and the microorganisms were repeatedly sub-cultured in order to obtain pure isolation. Morphological and biochemical reactions were carried to ascertain proper identification. They were inoculated into nutrient agar slants and stored at 4^{o}C. Overnight broth culture of the respective bacteria strains were adjusted to turbidity equivalent to 0.5 McFarland standards. (0.2 ml culture of the organisms was dispensed into 20 ml sterile nutrient broth and incubated for 24 h and standardized at 1.5 x 10^{6} CFU/ml by adjusting the optical density to 0.1 at 600nm PERKIN-ELMER UV-spectrophotometry) (Tereschuk et al., 1997).

Disc diffusion method

The disc diffusion method (Rosoahaivio and Ratsimanga-urverg, 1993) was used for antimicrobial activity. The dried extract was reconstituted with Dimethyl sulfoxide (DMSO) to obtain a stock solution of 200 mg/ml^{-1}, 100 mg/ml^{-1}, 50 mg/ml^{-1} and 25 mg/ml^{-1} were prepared. Overnight broth culture of the respective bacteria stains were adjusted to turbidity equivalent to 0.5 McFarland standards. Nutrient agar (NA) (Hi Media Laboratories Pvt.Ltd. Mumbai) plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacterial strains. What man filter paper (no.1) discs of 6mm diameter were impregnated with 10µl of the solution of crude extracts (at 200mg/ml) prepared using DMSO at solvent. The discs were evaporated at 37ºc for 24hr. Discs prepared with only the corresponding volume of DMSO was used as negative control. The plants were incubated at 37ºc for 24hr. Antimicrobial activity was evaluated by measuring diameter of the inhibition zone (IZ) around the discs. The assay was repeated twice.

The control antibiotic Gentamycin (10 µg) for gram positive bacteria and Kanamycin for gram negative bacteria were used. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract.

RESULTS AND DISCUSSION

Phytochemical screening of the crude extracts of *Vitex leucoxylon* revealed the presence of alkaloids, flavonoids, terpenoids, steroids, phenolics, carbohydrates, amino acids and quinones (Table1). The presence of alkaloids is interesting, as significant quantities are used as antimalarials, analgesics and stimulants (Duke and Ayensu, 1985). The flavonoids are known to inhibit tumor growth and serve also to protect against gastrointestinal infections and are of pharmacognostic importance thus giving evidence to the use of the plant in ethnomedicine. Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows also serve to protect the plant against microbial attacks and predation by animals. The increasing reliance on the use of medicinal plants by a sizeable proportion of the people in the so-called industrial world has been traced to the extraction and development of several drugs and chemotherapeutic agents from these plants as well as from traditionally used herbal remedies (El-Mahmood et al., 2008).

Table 1: Result of the phytochemical screening of crude extracts of leaves of *Vitex leucoxylon*

S.No.	Phytochemical compounds	Leaves of crude extracts
1	Alkaloids	+
2	Flavonoids	+
3	Terpenoids	+
4	Saponins	-
5	Steroids	+
6	Cardiac Glycosides	_
7	Phenolics	+
8	Tannins	_
9	Carbohydrates & Amino acids	+

Disc diffusion method

Antibacterial activity of crude extracts of *Vitex leucoxylon* leaf were evaluated by measuring the diameters of zones of growth inhibition on some members of the *enterobacteriaceae* and the results are presented as shown in Table 2. All the test organisms were susceptible to *Vitex leucoxylon* leaf extracts though to varying degrees. karou et al. (2006) reported that the susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters varied according to strains and species, similar to the data obtained in this study. The highest zone of growth inhibition was shown by ethanol extract (2000µg) against at gram negative bacteria like *Vibrio cholera* (18 mm), *Salmonella paratyphi* (16 mm), *Enterobacter aerogenes* (15 mm) and *Escherichia coli* (14.5 mm). The growth inhibition was moderately active against gram positive bacteria *Staphylococcus aureus* (11.5 mm) and *Streptococcus faecalis* (12 mm). The ethanol methanol and pet ether extracts are more effective than dichloromethane, and aqueous extract. The large zone sizes produced by the plant extract against the test bacteria, especially the ethanol, methanol and pet ether extracts is an indication of the plant against all the test bacteria. The lowest zone of growth inhibition was dichloromethane and aqueous.

Table 2: Antibacterial activity of different solvent extracts of *Vitex leucoxylon*

S.No.	Name of the Extract	Conc. of Extract (µg.)	Zone of Inhibition (mm)						
			Gram Negative Bacteria					Gram Positive Bacteria	
			E.coli	*K.p*	*E.a*	*V.c*	*S.p*	*S.a*	*S.f*
1	Ethanol	2000	14.5	11	15	18	16	11.5	12
		1000	9	8	10	14	10	10	9
		500	-	-	-	11	8	-	-
		250	-	-	-	-	-	-	-
2	Methanol	2000	11	9	12	15	13	12	11
		1000	9	-	10	12	10	10	9
		500	-	-	-	10	9	-	-
		250	-	-	-	-	-	-	-
3	Pet ether	2000	10	12	10	11	12	11	10
		1000	9	10	-	9	10	9	-
		500	-	-	-	-	-	-	-
		250	-	-	-	-	-	-	-
4	DCM	2000	9	11	10	12	11	10	9
		1000	-	9	-	10	9	-	-
		500	-	-	-	-	-	-	-
		250	-	-	-	-	-	-	-
5	Aqueous	2000	10	12	10	13	12	11	10
		1000	-	10	-	11	10	9	-
		500	-	-	-	9	-	-	-
		250	-	-	-	-	-	-	-
6	Kanamycin		18	17	19	21	20	19	17
	Gentamycin	10	-	-	-	-	-	-	-

- No activity; E.a – Enterobacter aerogenes; E.Coli- Escherichia coli; K.p – Klebsiella pneumoniae; V.c – Vibrio cholerae; S.p – Salmonella paratyphi; S.a – Staphylococcus aureus; S.f – Streptococcus faecalis. Kanamycin and Ofloxacine – Control antibiotics. Each value represents the mean of triplicate analysis.

CONCLUSION

The result of the present study showed that the extract of *vitex leucoxylon* contains many phytochemical components. These compounds have potentially significant applications against human pathogens, including those that cause enteric infections. The present investigations in *vitex leucoxylon* for in vitro antimicrobial properties confirm that the plant has substances that exhibit measurable in vitro antimicrobial activity against some bacteria in this study. The results of the various screening tests indicate that the leaves possess some measurable inhibitory action against gram negative bacteria. *Vibrio cholera, Salmonella paratyphi, Enterobacter aerogenes* are the three important multidrug resistant bacterial strains that are found to be susceptile to the leaf extracts.

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