April 2009 Volume 3 Issue 2

Trade Science Inc.

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 3(2), 2009 [60-62]

Preliminary phytochemical evaluation and antibacterial studies on vitex *Altissima* L.

H.S.Ravikumar Patil[1]*, H.A.Sayeswara[2], H.Gurumurthy[1], H.K.Makari[1], M.Abhilash[3], K.L.Naik[2]

[1]Department of Biotechnology, GM Institute of Technology, Davanagere-577006, Karnataka, (INDIA)

[2]Department of Zoology Sahyadri Science College(Autonomous) Kuvempu University, Shimoga, Karnataka, (INDIA)

[3]Department of Biotechnology, Oxford College of Engineering, Banglore, Karnataka, (INDIA)

E-mail : patil_varuni @rediffmail.com

Received: 7th January, 2009 ; Accepted: 12th January, 2009

ABSTRACT

Preliminary phytochemical and antibacterial activity of petroleum ether, chloroform and methanol leaf extracts of *Vitex altissima* was investigated. The extracts showed the presence of alkaloids saponins, sterols flavonoids, phenols, tritepenoids, tannins etc., at different concentrations. Antibacterial activity was tested against both gram +ve and gram -ve organisms. Among the extracts tested methanol leaf extract exhibited broad spectrum antibacterial activity against the tested organisms. Maximum activity is exhibited against Enterobacter aerogenes which was followed by *Pseudomonas aeruginosa Bacillus cereus, Proteus vulgaris, Staphylococcus aureus, Alcaligenes faecalis,* Escherichia coli and Bacillus subtilis. Petroleum ether and chloroform leaf extract exhibited less activity.

KEYWORDS

Vitex altissima;
Preliminary phytochemistry;
Antibacterial studies.

INTRODUCTION

Phytomedicines represent a vast untapped source of drugs. They arc effective in treating infectious diseases simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[8]. Over last 20 years, a large number of plant species have been evaluated for their antimicrobial activity[3]. It is estimated that, today the plant materials provide the models for 50% of Western drugs (Robbers et al.[15], which can be attributed to the various phytochemicals present in it. Further these diverse chemical groups exhibits wide range of physiological and pharmacological activity. By realizing the importance of the plant based active constituents several workers explored many plant genetic resources for their efficacy in controlling the pathogenic bacteria viz *Indigofera* species[13]. Piper aduncum[12], *Solanum torvum*[1], *Enantia polycarpo*[2]. *Vitex altissima* L., (small sized tree) belongs to the family Verbenaceac. found distributed in dry deciduous forests of Karnataka state, India[9]. *V.altissima* is commonly known as peacock chaste tree (English) and naviladi (Kannada). The tribal groups residing in the Davanagere district Karnataka state use leaves as antiseptic in the treatment of wounds, jaundice and fever[9]. Several active constituents like flavonoids, verbascoside[16], vitexin[14] have been reported from the leaves of this plant. This indicated that this plant genetic resource remained unexplored for their antibacterial activity. Hence efforts have been made to evaluate the antibacterial potency of *V.altissima* on both gram +ve and gram -ve bacteria.

MATERIAL AND METHODS

Plant material

Leaves of *V.a1tissima* L., were collected from the Joldal range of Davanagere. The plants were identified by the first author and the taxonomic authenticity was confirmed by referring to herbarium specimens at Madras herbarium, Botanical Survey of India, Southern Circle, Coimbatore. The voucher specimen is deposited in the Kuvempu University herbaria for future reference.

Phytochemical extraction

Leaves were shade dried and powdered mechanically. Powdered materials were subjected to successive soxhlation and exhaustively extracted with petroleum ether, chloroform and methanol for 48 hrs. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi). Solvents were removed completely on water bath and dried in the dessicator. The dried extracts were subjected to phytochemical tests[5] to detect the various chemical groups present in it.

Antibacterial screening

The bacterial strains obtained from National chemical laboratory, Pune (listed in TABLE 2) were employed for screening purpose by disc diffusion method. 25 mg, 50 mg and 100 mg of leaf extracts were dissolved in 1ml of petroleum ether, chloroform and methanol. Filter paper discs (Whatman No.1) of 5 mm-diameters were loaded with 100 of crude extract. Discs were completely dried and sterilized. 100µl of 24 hrs broth cultures were spread on sterilized nutrient agar media; impregnated discs were placed on it and incubated for 24 hrs at 370C. Streptomycin discs (10µg/disc) were used as a standard drug. The diameter of zone of inhibition in mm was recorded. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

TABLE 1: Preliminary phytochemical screening of extracts of *Vitex altissima*

Tested group	Petroleum ether extract	Chloroform extract	Methanol extract
Alkaloids	-	-	-
Saponins	-	-	+
Glycosides	-	-	+
Flavonoids	-	-	+
Triterpinoids	-	+	+
Sterols	-	-	+
Tannins	-	-	-

Note- "-" not detectable "+" detectable

RESULT

The phytochemical screening and antibacterial activity of leaf extracts of petroleum ether, chloroform and methanol were depicted in TABLES 1 and 2 respectively. The phytochemical results reveal the presence of alkaloids, saponins, phenols, triterpenoids, sterols, flavonoids and tannins.

The potency of the tested drugs against tested organisms is depicted in TABLE 2. The methanol leaf extract of *V.altissima* exhibited maximum activity against *Enterobacter aerogenes* (15.4mm), followed by *Pseudomonas aeruginosa* (12.2mm), *Bacillus cereus* (11.8mm), *Proteus vulgaris* (11.3mm), *Staphylococcus aureus (*11.2mm), *Alcaligenes faecalis* (10.5mm), *Escherichia coil* (10.2mm), *Bacillus subtilis* (10.0mm). Petroleum ether leaf extract and chloroform extracts were found to be inactive for most of the organisms tested. These data reveals that the methanol leaf extract of *V.altissima* exhibits significant antibacterial activity.

In all the cases inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity.

TABLE 2: Antibacterial effect of leaf extract of *Vitex altissima*

Tested organisms	Petroleum ether			Chloroform extract			Methanol extract			Streptomycin
	2.5mg	5mg	10mg	2.5mg	5mg	10mg	2.5mg	5mg	10mg	10µg
Bacillus subtilis NCIM-2063	0.00	0.00	0.00	0.00	0.00	2.40	8.7	9.5	10.0	15.4
Escherichia coli-NCIM-2065	0.00	0.00	0.00	0.00	1.80	3.00	8.1	9.1	10.2	16.8
Enterobacter aerogenes-NCIM-2340	0.00	0.00	0.00	0.00	3.40	4.20	12.6	14.5	15.4	18.5
Alcaligenes faecalis-NCIM-2262	0.00	0.00	2.40	0.00	0.00	1.80	8.0	9.4	10.5	18.0
Staphylococcus aureus NCIM-2079	0.00	0.00	3.10	0.00	0.00	0.00	9.2	10.0	11.2	19.0
Pseudomonas aeruginosa NCIM-2036	0.00	0.00	0.00	0.00	0.00	2.00	10.3	11.0	12.2	20.4
Proteus vulgaris NCIM-2027	0.00	0.00	0.00	0.00	0.00	1.80	9.7	10.0	11.3	17.8
Bacillus cereus NCIM-2155	0.00	0.00	0.00	0.00	0.00	0.00	9.5	10.0	11.8	19.0

FULL PAPER

DISCUSSION

The secondary metabolites of various chemical types present in the plant species are known to possess antimicrobial activities Flavonoids are found to be effective antimicrobial substance against a wide range of microorganisms. This activity is probably due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall. More lipophilic flavonoids may also disrupt microbial membrane[17]. Phenolics and polyphenols present in the plants are known to be toxic to microorganisms. The mechanism thought to be responsible for phenolic toxicity to microorganisms includes, enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl group or through more nonspecific interaction with the protein[11].

Many human physiological activities, such as stimulation of phagocytic cells, host mediated tumor activity and a wide range of anti-infective actions have been assigned to tannins[6]. Their mode of antibacterial actions may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins etc., they also complex with polysaccharides[18]. Many plant genetic resources have been analyzed for their active constituents possessing antibacterial activities for example, leaf extract of *Eupatorium odoratum* found to have broad spectrum antibacterial activity due to flavonoids, tannins and sesquiterpenes lactones[7], *Landolphia owrrience* is known to possess glycosides, flavonoids, tannins, saponins, which either individually or in combination exerts antibacterial activity[4].

In the present investigation preliminary phytochemical studies on the leaf extract of *V.altissima* revealed the presence of flavonoids, tannins, terpenes and phenols. The broad spectrum antibacterial potency of the methanol leaf extract of V.altissima may be due to the individual or combined effect of the above mentioned chemical groups. Further, the increased resistance of various bacteria towards available antibiotic drugs has initiated intensive research efforts in identifying new source of antibacterial substance. In this regard it seems essential to explore plants of ethno medicinal value which may provide a base for the development of new drugs. The findings in the present investigation offer a scientific support to the ethnomedicinal use of the plant *V.altissima* by the tribal groups of Davanagere district.

ACKNOWLEDGEMENTS

The authors are grateful to Sri G.M.Lingaraju, Secretary GMIT, Davangere Karnataka, INDIA, for their financial assistance and lab facilities. The authors wish to thank all the staff members of the Dept of Biotechnology of GMIT, Davangere Karnataka, INDIA.

REFERENCES

[1] E.O.Ajaiyeoba; Fitoter, **70**, 184-186 **(1999)**.
[2] U.Ajali; Fitoter, **71**, 315-316 **(2000)**.
[3] M.C.Castello, P.Anitha, C.Naresh, S.Madhuri; Indian J.Exp.Bio., **40**, 1378-1381 **(2002)**.
[4] G.C.Ebi, Ofoefule; Phytotherapy Research, **11**, 149-151 **(1997)**.
[5] J.B.Harborne; 'Phytochemical Methods', Chapman and Hall, London, 60 **(1998)**.
[6] E.Haslam; J.Nat.Prod., **59**, 205-215 **(1996)**.
[7] F.R.Irvine; 'Woody Plants of Ghana', Oxford University Press, London, 878 **(1961)**.
[8] M.M.Iwu, A.R.Duncan, C.O.Okunji, J. J.Janick; 'New Antimicrobials of Plant Origin', ASHS press, Alexandria, VA, 457-462 **(1999)**.
[9] B.K.Manjunatha; Flonstic composition of Davanagere district Karnataka, PhD. thesis submitted to Department of Biotechnology, Kuvempu University, Shankaraghatta, Karnataka, India, **(2002)**.
[10] B.K.Manjunatha, V.Krishna, T.Pullaiah; 'Flora of Davanagere District Karnataka', India, Regency Publications, New Delhi, India, **3**, 18-319 **(2004)**.
[11] T.L.Mason, B.P.Wasserman; Phytochemistry, **26**, 2197-2202 **(1987)**.
[12] A.L.Okunade, C.D.Hufford, A.M.CIark, L.David; Phytother.Res., **11**, 142-144 **(1997)**.
[13] B.Rajesh, M.M.Bokadia; Indian Drugs, 144-146 **(1979)**.
[14] D.S.Rao; Naturwissenschaften, **52**, 26213 **(1965)**.
[15] J.Robbers, M.Speedie, V.Tylor; Pharmacognosy and Pharmaco Biotechnology, Williams and Wilkins, Baltimore, **(1996)**.
[16] K.Taoubi, M.T.Fauvel, J.Gleye, L.Fouraste; Bull. Liaison 3roupe Polyphenols, **16**, 174-177 **(1992)**.
[17] H.Tsuchiya, M.Sato, T.Miyazaki, S.Fujiwara, S. Tanigaki, M.Ohyama, T.Tanaka, M.Linuma; J. Ethnopharmacol., **50**, 27-34 **(1996)**.
[18] C.Ya, S.F.L.Gaffney, T.H.Lilley, E.Haslam, R.W. Hemingway, J.J.Karchesy; 'Carbohydrate Polyphenol Complexation', Plenum Press, New Yark, 553 **(1998)**.