ORIGINAL ARTICLE

Phytochemical analysis and antibacterial activity of *Vitex agnus-castus*

S. Arokiyaraj, K. Perinbam, P. Agastian[1], R. Mohan Kumar[2]
Department of PG and Research, Sathyabama University, Chennai - 119, [1]Department of Plant Biology and Biotechnology, Loyola College, Chennai - 34, [2]Astagiri Herbal Research Foundations, Chennai, India

The leaves of *Vitex agnus-castus* was sequentially extracted in hexane, ethyl acetate, methanol and aqueous medium and studied for *in vitro* antibacterial property. The ethyl acetate extract was found to be most active against all the bacterial species tested except *K. pneumoniae.* The best MIC value (0.312 mg/ml) was seen against MRSA. Active ethyl acetate extract was further studied for HPTLC fingerprint and phytochemical analysis. HPTLC analysis confirmed segregation of eight individual compounds with individual R_f values and peak area percentage. The results of phytochemical screening of extract revealed the presence of terpenoids, steroids, flavonoids and carbohydrates. This analysis revealed the high antibacterial activity in active ethyl acetate from *Vitex agnus- castus.*

Key words: Antibacterial activity, drug resistant, minimum inhibitory concentration, phytochemical analysis, *vitex agnus-castus*

INTRODUCTION

Vitex agnus-castus (Verbenaceae), commonly called "chasteberry", a small deciduous tree that grows in Asia, Europe (especially in Mediterranean region) and North America. It bears slender spikes of violet blue, 8-10 cm flowers. It is popularly used in folk medicine to treat ovarian insufficiency, uterine bleeding, premenstrual syndrome, fibroid cysts, infertility and acne in teenagers.[1-3] It has also been traditionally used as a digestive aid, sedative and anti-infective.[4] There have been several reports on its chemical constituents. It includes iridoid glycosides (agnuside, aucubin); flavonoids (vitexin, kaempferol, casticin, quercetagetin); progestins (progesterone, hydroxy progesterone, androstenedione); alkaloids (viticin); volatile oil (1,8-cineol, limes, α-pinenes, β-pinenes) and essential fatty acids (palmitic acid, oleic acid, stearic acid).[5-6] Several other *Vitex* species are also reported to possess biological activities Viz. *Vitex rotundifolia* has repelling activity against *Aedes aegypti* mosquitoes, *Vitex negundo* L act as a larvicidal agent of mosquito's and antioxidant. *V. pinramidata*, *V. pubescens, V. gaumeri* are folk remedies to treat diarrhea, gastro intestinal affections, malaria, colds and cough spells.[7]

Infectious disease is the number one cause of death accounting for approximately one-half of all deaths in tropical countries. Death from infectious diseases, ranked 5th in 1981, has become the 3rd leading cause of death in 1992, with an increase of 58%.[8]

More than hundreds of plants world wide are used in traditional medicine as treatments for bacterial infection.[9] Although many have been treated by conventional pharmaceutical approaches, there is a growing interest in the use of natural products by the general public. In addition to the pharmaceutical industry continues to examine their potential as sources of novel growth factor, immunomodulatory and antimicrobial activity.[10]

Literature study showed antibacterial activity of this plant, but activity against clinical isolates and drug resistant has not been reported. Considering the medicinal value of this plant we evaluated the antibacterial potential, HPTLC fingerprint and phytochemical analysis of *Vitex agnus-castus* (Leaves).

MATERIALS AND METHODS

Plant Collection and Extraction

Leaves of *V. agnus* were collected from botanical garden University of Madras, Maduravoil (Chennai, Tamil Nadu, India). A specimen (61/July/2007) was deposited at the department herbarium, Loyola College, Chennai. Collected materials were washed thoroughly, shade dried in open air and grounded into powder. The powder was sequentially extracted by maceration in hexane (10.6 g) during 72 hr. Residues were further extracted with ethyl acetate (42.5 g) and methanol (40.4 g) following the same procedure. Final extraction was performed with distilled water (33.6 g). The plant extracts were concentrated using rotary flash evaporator and preserved at 4°C in airtight bottle until assay.

HPTLC Finger Printing

Chromatography was performed on 3 × 10 cm HPTLC

Address for correspondence: Mr. S. Arokiyaraj, Department of PG and Research, Sathyabama University, Chennai - 119, India. E-mail: arokiyarajs@yahoo.in
Received: 20-08-2008; **Accepted**: 07-02-2009**; DOI:** 10.4103/0973-8258.54912

(Merck, Germany). The plate was prewashed with methanol and activated at 110°C for 5 min. The ethyl acetate extracted sample was applied as 4 mm bandwidth using a Camag (Muttenz, Switzerland) Linomat IV sample applicator equipped with 100 µl Syringe. A constant application rate of 5 µl/sec was used. Mobile phase was hexane: Ethyl acetate (4:6) and chromatogram were scanned at 254 nm.[11]

Phytochemical Analysis

Phytochemical screening of ethyl acetate extract for the presence of these secondary metabolites: Alkaloids (Draggendorff's), flavonoids (Shibat'as reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4-dinitro-phenyl hydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test) were evaluated according to the methods described by Edeoga *et al.*, 2005.[12]

Preparation of Test Organism

The bacterial strains included Gram positive Methicillin resistant *Staphylococcus aureus* (MRSA); Gram negative Carbapenem resistant *Acetobacter baumanii*, Ciprofloxacin resistant *E. coli* and clinical isolates of *Proteus vulgaris, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli, Enterococcus durans*, *Pseudomonas aeruginosa*. All microorganisms were obtained from the Microbiology lab, Christian Medical College, Vellore, Tamil Nadu, India and very carefully identified using standard microbiological method. All bacterial strains were maintained a MHA slants, stocks were stored at -20°C until use.

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) was performed according to the standard reference method NCCLS.[13] The extracts were dissolved in 2% dimethyl sulfoxide (DMSO). A stock solution of each extract was serially diluted in 96-well microtiter plate with Mueller Hinton broth to obtain a concentration ranging from 5 mg/ml to 0.039 mg/ml. A standardized inoculum for each bacterial strain was prepared so as to give an inoculum size of 10^5 CFU/ml in each well. Streptomycin was used as a standard antibiotic for comparative analysis with the effectiveness of various extracts against tested clinical isolate and drug resistant bacteria. Microtiter plate was kept at 37°C and incubated for 24 h. Following incubation, the MIC was calculated as lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

RESULTS AND DISCUSSION

In the HPTLC fingerprinting of ethyl acetate extract gave eight spots at the following Rf values: 0.09 (24.74%), 0.11 (22.18%), 0.20 (9.81%), 0.33 (9.33%), 0.44 (7.99%), 0.56 (11.85%), 0.72 (6.90%), 0.86 (7.20%). Purity of the sample extract was confirmed by comparing the absorption spectra at start, middle and end position of the band. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficient and cost effective. The corresponding HPTLC chromatograms are presented in [Figure 1].

The antibacterial activity of extracts of *V. agnus castus* against clinical isolates and drug resistant are summarized in [Table 1]. The MICs of the extracts ranged between 0.312 and 5 mg/ml. Among all the extracts, the ethyl acetate was found to be most active against all the tested bacterial species except *Klebsiella pneumonia* [Methicillin resistant *Staphylococcus aureus* (0.312 mg/ml), carbapenem resistant *Acetobacter baumannii* (0.625 mg/ml), ciprofloxacin resistant *E.coli* (0.625 mg/ml), *Proteus vulgaris* (2.5 mg/ml), *Salmonella typhi* (5 mg/ml), *Escherichia coli* (2.5 mg/ml), *Enterococcus durans* (0.625 mg/ml) and *Pseudomonas aeruginosa* (2.5 mg/ml)]. Hexane and methanol extract was less active than ethyl acetate. Compare to standard streptomycin ethyl acetate extract showed good activity against all the three tested drug resistant bacteria.

Similarly *Vitex doniana* showed potent activites against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, multidrug-resistant *Burkholderia*

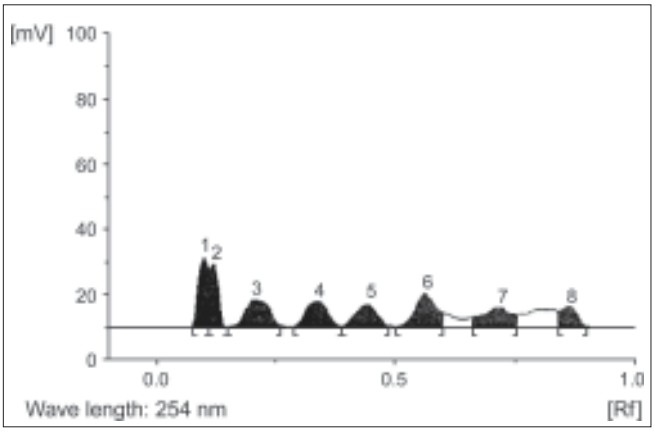

Figure 1: HPTLC fingerprint profile of ethyl acetate extract of *V. agnus castus*

Table 1: Minimum inhibitory concentration of the leaf extracts of *V. agnus-castus*

Extract	MIC in mg/ml								
	Kp	Ec	Pv	St	Pa	Ed	MRSA	CRE	CRA
Hexane	–	5	–	2.5	0.625	–	2.5	2.5	5
Ethyl acetate	–	2.5	2.5	5	2.5	0.625	0.312	0.625	0.625
Methanol	–	–	2.5	2.5	–	2.5	2.5	–	–
Aqueous	–	–	–	–	–	–	–	–	–
S (30 µg/ml)	6.25	12.5	12.5	12.5	25	25	–	–	–

Assay was performed in triplicate and results are expressed in terms of the average of the three values. S (Standard) - Streptomycin, Kp - *K. pneumoniae*, Ec - *E. coli*

cepacia and *Pseudomonas aeruginosa*.[14] The petroleum ether and ethanol extracts of *V. trifolia* exhibited moderate inhibiting activity against both gram positive and gram negative bacteria.[15] The argument which help us the concept of plant extracts with traditional background helps a continuous effort to find new compounds with the potential to act against multi resistant bacteria.[16]

The phytochemical analysis of ethyl acetate extract had showed the presence of flavonoids, terpenoids, steroids, and carbohydrates. In previous findings flavonoids were found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to form a complex with extra cellular, soluble protein and bacterial cell wall: In addition more lipophilic flavonoids may also disrupt microbial membrane.[17] Secondary metabolites of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens. The most important of their bioactive compounds of plants are such as alkaloids, flavonoids, tannins and phenolic compounds. This antibacterial activity may be due to the presence of secondary metabolites.[17]

Antibacterial substances present in medicinal plants have long been regarded as the important factors in the resistance of higher plants to various bacteria. Hence researchers have always felt the need for scientifically screening of the plants, which may help the pharmacologists and phytochemists in discovering innumerable therapeutic agents.

In conclusion, the present study indicates that the plant contains potential anti-bacterial components such as flavonoids, terpenoids and steroids that may be of use for development of phytomedicine for the therapy of tested bacterial diseases. The results of this study demonstrated that, ethyl acetate extract from the leaves of *Vitex agnus-castus* showed dominant antibacterial activity against potent clinical pathogens.

ACKNOWLEDGMENT

We express our heart felt thanks to Christian Medical College, Vellore, India for providing the bacterial cultures.

REFERENCES

1. Newall CA, Anderson LA, Phillipson JD. Herbal medicines: A guide for health-care professionals. London: The Pharmaceutical Press; 1996. p. 6.
2. Blumenthal M, Burse WR, Goldberg A, Gruenwald J, Hall T, Riggins CW, Rister RS. The complete German Commission E monographs: Therapeutic guide to herbal medicines. Austin: American Botanical Council; 1998.
3. Amann W. [Improvement of acne vulgaris following therapy with agnus castus (Agnolyt)]. Ther Ggw 1967;106:124-6.
4. Christie S, Walker A. *Vitex agnus castus*, A review of its traditional and modern therapeutic use, current use from a survey of practitioners. The European Journal of Herbal Medicine 1997;3:29-45.
5. Anonymous. Chaste Tree. In: Dombek C, editor. Lawerence Review of Natural Products. St. Louis: Facts and Comparisons; 1998.
6. PDR for herbal medicines. In: Fleming T, editor. 1st ed. Montvale, NJ: Medical Economics; 1998.
7. Ahmad FB, Holdsworth DK. Traditional medicinal plants of Sabah, Malaysia Part III. Pharmaceutical Biology 1995;33:262-4.
8. Pinner R, Teutsch S, Simonsen L, Klug L, Graber JM, Clarke MJ, *et al*. Trends in infectious diseases mortality in the United States. JAMA 1996;275:189-93.
9. Martin KW, Ernst E. Herbal medicines for treatment of bacterial infections: A review of controlled clinical trials. J-Antimicrob- Chemother., 2003; 51: 241-246.
10. Ghosh S, Playford RJ. Bioactive natural compounds for the treatment of gastrointestinal disorders. Clin Sci (Lond) 2003;104:547-56.
11. Srinivas Reddy B, Kiran Kumar Reddy R, Naidu VG, Madhusudhana K, Agwane SB, Ramakrishna S, *et al*. Evaluation of antimicrobial, antioxidant and wound healing potentials of Holoptelea integrifolia. J Ethnopharmacol 2008;115:249-56.
12. Edeoga HO, OKWU DE, Mbaebie Bo. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005;4:685-8.
13. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. Wayne, PA: National Committee for Clinical Laboratory Standards; 2002.
14. Taiwo O, Xu Hx, Lee SF. Antibacterial activities of extracts from Nigerian chewing sticks. Phytother Res 1999;13:675-9.
15. Hossain MM, Paul N, Sohrab MH, Rahman E, Rashid MA. Antibacterial activity of Vitex trifolia. Fitoterapia 2001;72:695-7.
16. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. J Zhejiang Univ Sci B 2008;9:154-9.
17. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, *et al*. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistance Staphylococcus aureus. J Ethnopharmacol 1996;50:27-34.

Source of Support: Nil, **Conflict of Interest:** None declared.