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Evaluation of antimicrobial activities and phytochemical constituents of extracts of *Valeriana wallichii*

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ABSTRACT

Valeriana wallichii DC, known as 'Tagar, or Ganthoda ' is a wild herb common in the temperate Himalaya, has been used since long in Ayurvedic and Unani systems of medicine. The ancient Indian Charak Samhita has described tagar as cure for obesity, skin disease, gastrointestinal conditions, and snake poisoning. It has been discussed among the bitter tasting and fragrant group of plants. The rhizome and root extracts of Valeriana wallichii DC in various solvents were investigated for its antimicrobial effect. The crude extracts were tested against gram positive Staypholococcus aureus, Staypholococcus epidermidis and gram negative Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis bacteria and fungi Aspergillus niger, Candida albicans, M. furfur to find out their antimicrobial activity using agar diffusion method. Ethanol and Methanol solvent extracts showed significant antimicrobial activity ranging from 13-18mm diameter of zone of inhibition especially against fungal pathogens as compared to bacterial pathogens. In continuation with these evaluations, the most promising extract in polar solvent was investigated by GC/MS. Components identified in the solvent extract constituting Cyclopentaneacetaldehyde; Quinoline; Aristolene; Cyclohexane, 2,4-diisopropenyl-l-methyl-l-vinyl, Cyclopentane,1-(3-methylbutyl); Tricyclo[5.1.0.0(2,4)oct-5-ene-5-propanoic acid; Ascorbic acid2,6-dihexadecanoate; 9,12-Octadecadienoic acid; Pentadeconic Acid,4 hexadecyl ester; Valeric acid as the major constituents.

Key words: *Valeriana wallichii*, Antimicrobial, GC/MS.

INTRODUCTION

Plants have been an important source of medicine for thousands of years. As per World Health Organization estimates, up to 80 percent of people still depend on traditional remedies such as herbs for their medicines. Today, Ayurvedic, Hoemoeo and Unani physicians utilize numerous species of medicinal plants that found their way a long time ago into the Hindu Material Media [1]. The twentieth century set a trend for introducing a new generation of botanical therapeutics that includes plant derived pharmaceuticals, multicomponent botanical drugs; dietary supplements, functional foods and plant produced recombinant proteins. Many of these products will soon complement conventional pharmaceuticals in the treatment, and prevention of diseases.

Valerian (Syn: *V. jatanamansi*) is a member of the *Valerianaceae* family that includes upto 250 species, commonly called as Indian valerian [2]. It is indigenous to the temperate Himalayas and found in India, Bhutan, Burma, Pakistan and Afghanistan. The parts of the plants used for therapeutic purposes are mainly the roots and the rhizomes. It is used in anxiety, insomnia, epilepsy, failing reflexes, hysteria, neurosis and sciatica [2, 3]. It is also

considered useful as potent tranquilizer, [4] and for hypertension [5]. However, its antibacterial and antifungal potential is needed to be explored. The present investigation aimed to evaluate antibacterial and antifungal potential of *V. wallichii* root and rhizome extracts in various solvents polar.

MATERIALS AND METHODS

Chemicals

Methanol and Ethanol used for extractions were of HPLC grade, and distilled water , were purchased from M/S Merck ltd. Mumbai.

Plant Material

The plant material was collected from Lord's International Medicinal Plant Nursery, Aurangabad. The dried roots and rhizome were cleaned and disinfected with 15%H_2O_2 followed by wash with a distilled water, crushed into powder sample using an electronic blender. The powdered sample was stored in bottle at room temperature prior to analysis.

Extraction Procedure

To prepare stock solution, 25g powder was added to 250ml of solvents (w/v, 25 g 250mL^{-1}) the solvents used for extraction were distilled water, ethanol and methanol. Each extract was shaken for at least 6 hours and after that each extract was passed through sterile Whatman filter paper No. 1. The final filtrates as 25% crude extracts was then thus concentrated aseptically on rotary evaporator under vacuum at 20 ^{0}C and they were filter sterilized. All extracts were stored at -20°C in air tight bottles and used within one week for the experiments.

Microorganisms used

The bacterial organisms included in the study were Gram positive (*S.aureus, S.epidermidis*) and Gram Negative *(E.coil, K. pneumoniae P.mirabilis, P.vulgaris, P. aeruginosa)*The cultures were grown in Erlenmeyer flasks (250 ml) containing 100 ml Nutrient broth and were maintained in nutrient agar medium and preserved on the same medium at 4^o C. A loop full of bacterial cultures were inoculated individually in the medium and incubated under agitation (150 rpm) at specified temperatures prior to the assay.

The fungal organisms used in the present study were *Aspergillus niger, Candida albicans and Malassezia. furfur*
The fungal cultures were maintained on potato dextrose agar (PDA) (*Aspergillus niger, Candida albicans*) and Pityrosporum Agar (*Malassezia. furfur*) and preserved on the same medium at 4^{o}C. The cultures were subcultured periodically (5-7 days) under stationary condition on the same medium at 28 ±2^{o}C.

Screening of Antimicrobial Activity

The antibacterial activity of the Ethanol, Methanol and aqueous extracts were determined by the agar well-diffusion method on Nutrient agar (HiMedia, India) medium. Using a cork borer, wells (5mm in diameter) were punched out in the agar medium and inocula containing 10^{6}CFU/ml of the test bacteria were spread plated onto the surface of the medium with a sterile spreader. The 50µl of the extract was pipetted into the wells, whilst 50µl of ethanol, methanol and sterile distilled water served as a control. The agar plates were incubated at 37ºC for 24 h and the diameter of the zone of inhibition in mm surrounding the wells was measured. The diameters of zone of inhibition due to extracts were compared with those produced by the commercial control antibiotics, Ampicillin (10µg/ml).

Antifungal activity of each plant extract against different strains was done by well diffusion assay. A lawn was made on PDA by mixing fungal inoculum. After solidification wells were made with sterile borer and 50µl of the extract was transferred into wells. Itraconazole (10µg) was placed in one of the agar plate and used as positive control and distilled water, ethanol and methanol were also used as control. The plates were kept at 4^{0}C for 2 h for pre-diffusion of the extract [6]. Plates were incubated at 28 ± 2^{o}C for 5-7 days to observe the zone of inhibition around the well. Both antibacterial and antifungal tests were performed in triplicates and observed values of zone of Inhibition were expressed as mean values of the triplicate readings.

Determination of Minimum Inhibitory concentrations (MICs)

The extracts with promising antimicrobial activity (with zone of inhibition > 10 mm) was subjected for the minimum inhibitory concentration assay to find out the lowest concentration of extract that inhibits the growth of the test organisms .MIC assay was performed using the crude extracts with concentration of 100 to 1000 µl/ml.

After incubation at 37^0C for 24 to 96 hrs, the highest dilution showing microbial growth inhibition was recorded as Minimum Inhibitory Concentration (MIC) respectively for each pathogen [7].

GC MS Analysis

The extract with promising activity was subjected for the GC /MS analysis. The 2µl of the sample was injected to the GC-MS with the oven temperature programmed as 60°-210°C at 3°C/min using Helium as the carrier gas at 1.0 mL/min. The injection size was 0.1 µL, and detector temperature of 270^0C. The eluted peaks were identified using NIST library by comparing with the mass spectral data and retention indices in the literature.

RESULTS AND DISCUSSION

Antimicrobial activity of Extract

The result of the antimicrobial activity of the tested plant extracts in water, ethanol and methanol are summarized in Table I. Out of three of the solvent extracts, methanol extract was found active against all the tested organisms. The highest zone of Inhibition (zoi =13 mm) was observed against *M. furfur,* followed by *C. albicans* (zoi= 12mm) and *A. niger*(zoi=12mm) , *S. aureus and P.mirabilis* (zoi=12mm) , respectively. Significant inhibition was also observed against *E.coli and K.pneumoniae* (zoi= 11 mm) *followed by P. aeruginosa* and *S. epidermidis* with least inhibition (zoi= 9mm). Gram positive, Gram negative microorganisms and fungal pathogens selected were susceptible to the methanol extracts of the root and rhizome of *V. wallichii*.

Ethanol extracts showed a variable activity against all the test strains. Out of ten microorganism tested *A. niger* showed the highest zone of inhibition (zoi=11mm). Gram positive strains were also significantly inhibited by the ethanol extract of *V. wallichii* rhizome and root as *S. epidermidis* and *S. aureus both* showed (zoi=10mm) inhibition, followed by *C.albicans* and *M.furfur* (zoi=9mm) .Least inhibition of ethanol extract was recorded against *P.aeruginosa* and *P. mirabilis* (zoi=8mm) while no activity was observed against *P. vulgaris* and *E. coli* (zoi=0 and 1mm) respectively for ethanol extract.

Table I: Zones of Inhibition (mm) of Various Extracts of *V. wallichii*

Microorganisms	Diameter of Inhibition Zone* (mm) with Extracts		
	W	E	M
E. coli	0	0	11
K.pneumoniae	9	10	11
P.mirabilis	0	8	12
P.vulgaris	0	1	10
P.aeruginosa	0	8	9
S.aureus	0	10	12
S. epiderdimis	0	10	9
A.niger	0	11	12
C.albicans	4	9	12
M.furfur	0	9	13

*W-Water; E-Ethanol; M-Methanol; *All the values are average of three determinations*

The aqueous extract was showed the lowest antimicrobial activity particularly against *K.pneumoniae* with zone of Inhibition (zoi=9mm) and *C.albicans* with zone of Inhibition (zoi=4mm). The results of present investigation are in agreement with the previous findings [8], in which the antimicrobial activity of oil from *V. wallichii* was found effective against animal pathogenic bacteria. The present findings also provide new lead as there are no previous records of the antimicrobial activity of *V. wallichii* against skin pathogens, more precisely against the fungal strain *M. furfur*. Earlier studies also reported that some of plant extract and oils exert a greater inhibitory activity against Gram positive bacteria [9] which was consistent with the present findings.

Minimum Inhibitory Concentration (MIC)

The most promising extract in methanol was subjected for the Minimum Inhibitory Concentration assay against seven microbial pathogens having inhibition zone >10 mm were subjected for MIC determination .200 µl mL^{-1} was recorded as the least concentration inhibiting the against the *M. furfur* whereas, 300 µl mL^{-1} was recorded as the MIC value for methanol extract against Gram positive and fungal pathogens. As shown in table II MIC value of 500 µl $mL^{-1}$was observed for methanol extract for Gram negative bacterial pathogens. Results of the present study shares

resemblance with the other studies recorded with the minimum inhibitory concentrations of crude extract against the food pathogens ranged from 200- 400 µg/ml for several of the extracts incuding *V. wallichii* [10].

Table II: MIC of the Methanol Extract of *V. wallichii*

Microorganisms	MIC µl/ml
E. coli	500
K. pneumonia	500
P. mirabilis	500
S. aureus	300
A. niger	300
C. albicans	300
M. furfur	200

GC MS Analysis of extracts

Fraction components enlisted in Table III were identified by the GC MS analysis. Retention time, area %, molecular formula and molecular weights of the various molecules were determined and Library search was carried as NIST GC/MS spectral database and by comparing with the mass spectral data and retention indices with literature values.

Table III: Components identified by GC MS analysis

S.No.	Compound	M. F.	CAS No.	Mol. Wt
1	Cyclopentaneacetaldehyde	C10H14O2	5951-57-5	166
2	Quinoline	C10H13N	1780-19-4	147
3	Aristolene	C15H24	0-00-0	204
4	Cyclohexane,2,4-diisopropenyl-l-methyl-l-vinyl	C15H24	515-13-9	204
5	Cyclopentane,1-(3-methylbutyl)	C10H18	3789-15-9	138
6	Tricyclo[5.1.0.0(2,4)oct-5-ene-5-propanoic acid	C15H22O2	74793-63-8	234
7	Ascorbic acid2,6-dihexadecanoate	C38H68O8	28474-90-0	652
8	9,12-Octadecadienoic acid	C18H32O2	60-33-3	280
9	Pentadeconic Acid,4 hexadecyl ester	C15H30O2	0-00-0	326
10	Valeric acid	C16H26O2	0-00-0	250

(CAS No.: Chemical Abstracts Service Index Number; Mol Wt. Molecular Weight)

The identified components includes, Cyclopentaneacetaldehyde; Quinoline; Aristolene; Cyclohexane, 2,4-diisopropenyl-l-methyl-l-vinyl, Cyclopentane,1-(3-methylbutyl); Tricyclo[5.1.0.0(2,4)oct-5-ene-5-propanoic acid; Ascorbic acid2,6-dihexadecanoate; 9,12-Octadecadienoic acid; Pentadeconic Acid,4 hexadecyl ester; Valeric acid. The earlier studies, the essential oil of *V. wallichii* extracted from leaf and root were subjected to GC-MS. Twenty four components were identified including 3-Methylvaleric acid, maaliol and β-eurjunene as the major constituents of the leaf and root oil some of these components were allocated in the extract analysed under the present study [10].

CONCLUSION

Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines [11]. This plant-based, traditional medicine system continues to play an essential role in health care, they play dual role in the development of new drugs: they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; as phytomedicine to be used for the treatment of diseases [12]. The traditional healers use primarily water as the solvent but we found in this study that the plant extracts in alcohol (ethanol and methanol) provided more consistent antibacterial activity compared to those extracted by water. Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

The high altitude grown Himalayan plant *V. wallichii* has not been investigated for its defined antimicrobial potential. Though it has been exploited for its other uses all over the world but the indigenous plant part, especially rhizome needs to be tested adequately for its antimicrobial potential. This study highlights for the first time the ability of solvent extracts of the plant *V. wallichii* as antibacterial and antifungal through in vitro assays against skin pathogens.

The findings demonstrated promising antibacterial and antifungal activity of *V. wallichii* against major skin pathogens. The findings demonstrated under this investigation for the antibacterial, antifungal activity of *V. wallichii*

rhizome and root extract using well diffusion method showed that pathogenic strains were sensitive for the methanol extract as compared to the ethanol and water extract. Taken together, it may be concluded that an 'Indian Valerian', viz., *V. wallichii* DC (syn. *V. jatamansi*), a well-known traditional medicinal herb, would be a viable natural source for finding a good therapeutic agent against broad spectrum of skin pathogens.

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