IJPSR (2015), Vol. 6, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 October, 2014; received in revised form, 10 December, 2014; accepted, 04 February, 2015; published 01 June, 2015

SCREENING OF SOLVENT EXTRACTS OF *VALERIANA JATAMANSI* FOR ISOLATION OF ANTIMICROBIAL COMPOUND

Pradeep Babu[1*], Satish K. Verma [2] and Abhishek Mathur [3]

Uttarakhand Technical University (UTU) [1], Institute of Transgene Life Sciences [3], Dehradun (U.K), India
Govt. K.L. (PG) College [2], Allahabad (U.P), India

Keywords:

Antimicrobial activity, *Valeriana jatamansi*, pathogenic and drug resistant strains, valerenic acid

Correspondence to Author:
Pradeep Babu

Uttarakhand Technical University, Dehradun (U.K), India

E-mail: biotech.pradeep@yahoo.com

ABSTRACT: In the present study, the hydro-alcoholic and hexane extracts of the plant, *Valeriana jatamansi* (Family: Valerianaceae) were screened for antimicrobial activity against pathogenic and drug resistant strains. The non polar extracts did not showed any activity against any of the pathogenic strains. Amongst these extracts the hydro-alcoholic extracts of the plant, *Valeriana jatamansi*, showed potent antimicrobial activity against almost all the pathogens studied. These extracts also showed potent antibacterial activity against multidrug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the ranges from 0.3 mg/ml to 0.7 mg/ml. The potent hydro-alcoholic extract was further subjected for isolation and characterization of potent antimicrobial compound via chromatographic and spectroscopic techniques. The pure compound isolated, Valerenic acid was obtained as colorless liquid with a penetrating aroma; slightly soluble in water, soluble in alcohol, and ether. It melts at -34 ^{0}C and boils at 186 ^{0}C. The compound showed R_f value 0.37 similar to that of the standard compound, Valerenic acid as detected by TLC. The retention time of the pure compound purified was found to be 4.300 minutes as compared to the Standard reference compound, Valerenic acid which showed its retention time 4.393 minutes while eluting out through the column as determined by HPLC. The active compound was further subjected to FT-IR analysis which confirmed the TLC and HPLC data.

INTRODUCTION: Natural products and their derivatives represent more than 50% of the drugs in clinical use in the world. One of the paramount reasons for pursuing natural products chemistry resides in the actual or potential pharmacological activity to be found in alkaloids, terpenoids, coumarins, flavanoids, lignans, glycosides etc. Since the advent of antibiotics in the 1950's, the use of plant derivatives as a source of antimicrobials has been virtually non-existent [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(6).2641-48

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(6).2641-48

Antimicrobial plant extracts have been recognized as a future source of new antimicrobials in the event of the current downturn in the pace at which these are being derived from micro-organisms [2]. These antimicrobial compounds isolated from plants are found to be very effective in comparison to traditional medicines and produces no side effects.

The biological activity of many plants has been known through scientific research and any literature search via the internet or elsewhere, would reveal that numerous new publications are added to the scientific literature every day. In the present investigation, *Valeriana jatamansi* extracts (hydro-alcoholic and hexane) were screened for antimicrobial activity against pathogenic and drug

resistant microbial strains in lieu of isolation and identification of antimicrobial compound.

MATERIALS AND METHODS:

Plant Materials Collection:

The parts of the plant, *Valeriana jatamansi* were collected during September, 2010 from local gardens of North West Himalayan Garhwal regions of Uttarakhand and were further identified by Dr. Ajay Swami, Reader & HOD, Dept. of Botany, Chinmaya Degree College, Hardwar (U.K), India.

Preparation of Plant extracts:

The plant parts were separated, washed with distilled water, dried under shade and pulverized. The method [3] for preparation of extracts was slightly modified. Briefly 20 g portions of the powdered plant material was soaked in different solvents i.e. hydro-alcohol (50%v/v) and hexane for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatmann filter paper No. 1 (Whatmann, England) The filtrates obtained were concentrated in vacuo using water bath at 30^0C. The potent extracts of the plants were subjected for isolation and characterization of active principles.

Determination of Antimicrobial activity:

Culture Media:

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India while Soyabean Casein Digest agar/broth procured from Hi Media Pvt. Bombay, India was used for antibacterial assay.

Inoculum:

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6h and the suspensions were checked to provide approximately 10^5 CFU/ml. The bacterial strains were inoculated separately in Soyabean Casein Digest broth for 6h and the suspensions were checked to provide approximately 10^5 CFU/ml.

Fungal strains used for the study:

The clinical fungal test organisms used for study were *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Bacterial strains used for the study:

The multidrug resistant (MDR) bacterial strains (Super bugs) isolated from clinical samples of infected patients viz. *Staphylococcus aureus* and *Pseudomonas aeruginosa* procured from Dept. of Microbiology, Shoolini University, Solan (H.P) were used for the study. Besides MDRs other different pathogens viz. *Micrococcus luteus* ATCC 9341, *E.coli* ATCC 8739, *E.coli* mutans ATCC 11105, *Salmonella abony* ATCC 6017, *Lactobacillus plantarum* ATCC 8014 and *Staphylococcus epidermidis* ATCC 12228 were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antifungal activity:

The agar well diffusion method [4] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1mg/ml) was used as positive control and fungal plates were incubated at 37^oC for 72h. The diameters of zone of inhibition observed were measured.

Determination of antibacterial activity:

The same procedure was adopted for antibacterial activity. Soyabean Casein Digest Agar (SCDA) was used for bacterial cultures. The culture medium was inoculated with the bacterial strains separately suspended in Soyabean Casein Digest broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks. Standard antibiotic (Fucanazole, Chloramphenicol concentration 1mg/ml) was used as positive control and culture plates were incubated at 37^oC for 72 h (for antifungal assay) and for 24 h (for antibacterial assay). The diameters of zone of inhibition observed were measured.

Determination of MIC and MBC:

The antimicrobial plant extracts were then after evaluated to determine MIC and MLC values. The serial dilution method [5, 6] was adopted by using N-saline for diluting the plant extract and was incubated for 48h. The minimum dilution of the

plant extract that kills the microbial growth was taken as MLC (Minimum lethal count) while the minimum dilution of plant extract that inhibits the growth of the organism was taken as MIC.

Phytochemical screening of the extract:

The portion of the dry extract was subjected to the phytochemical screening using the method adopted [7, 8]. Phytochemical screening was performed to test for alkaloids, saponin, tannins, flavanoids, steroids, sugars and cardiac glycosides [9].

- **Test for alkaloids:**
  The 0.5g of the plant extract was dissolved in 5ml of 1% HCl and was kept in water bath for about 2 minutes. 1ml of the filtrate was treated with Dragendroff's reagent Turbidity or precipitation was taken as indicator for the presence of alkaloids.

- **Test for Tannins:**
  About 0.5g of the sample was dissolved in 10ml of boiling water and was filtered. Few ml of 6% $FeCl_3$ was added to the filtrate. Deep green colour appeared confirmed the presence of Tannins.

- **Test for Flavanoids:**
  About 0.2gm of the extract was dissolved in methanol and heated for some time. A chip of mg metal was introduced followed by the addition of few drops of conc. HCl. Appearance of red or orange color was Indicator of the flavanoids.

- **Test for Saponin:**
  About 0.5g of the plant extract was stirred with water in the test tube. Frothing persists on warming was taken as an evidence for the presence of saponin.

- **Test for Steroids:**
  Salkowaski method was adopted for the detection of steroids. About 0.5g of extract was dissolved in 3ml of chloroform and filtered. To the filtrate, conc. H_2SO_4 was added to form a lower layer. Reddish brown color was taken as positive for the presence of steroids ring[10].
- **Test for Cardiac glycoside:**
  About 0.5 g of the extract was dissolved in 2ml of glacial acetic acid containing 1 drop of 1% $Fecl_3$. This was under laid with conc. H_2SO_4. A brown ring obtained at the interphase indicates the presence of deoxy sugar. A violet ring appeared below the ring while in the acetic acid layer a greenish ring appeared just above ring and gradually spread throughout this layer.

- **Test for reducing Sugars:**
  1ml each of Fehling's solutions, I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2-5 minutes. The production of a brick red precipitate indicated the presence of reducing sugars.

Isolation of compounds from plant extracts by Gravity Gradient Column Chromatography:

Sintered glass column fitted with stopper (i.d., 1.5 and length 100cm) was filled with silica gel G (for column chromatography) with procedure described below. The elutions of the compounds were carried out at the room temperature and pressure.

Loading of fraction:

The potent extract of the plant having the highest antimicrobial activity were subjected to liquid-liquid fractionation. The highest antimicrobial fractions were chromatographed on 2x30 cm silica gel open column using a stepwise gradient of n-hexane and increasing amount of ethyl acetate (20 % at each step); ethyl acetate with increasing amount of methanol (10 % at each step); and finally at 40 % methanol. Collected fractions were evaporated under vacuum and examined by TLC. Homogeneous fractions were pooled to give large number of different fractions. These fractions were examined using Silica gel coated TLC plates to confirm the pure compound by changing the ratios of the solvent system components.

Elution of compounds:

Once the column was filled with eluent, the column was allowed to run. A quick flow rate helps to give good separation. By adjusting the air pressure to give a swift flow rate, different coloured bands were appeared in the column. After collecting these fractions the column's progress were monitored by TLC.

Conventional preparative TLC:

Silica gel G used for thin layer chromatography (TLC) was activated in hot air oven at 110°C for one hour.

Preparation of thin layer plates and loading of sample:

The plates were developed in the solvent, Hexane: ethyl acetate: glacial acetic acid (65:35:0.5) to separate the compound. Chromatograms were detected with: a) UV 254 nm; b) sprayed the plate with the HCl-acetic acid reagent, dry in stream of cold air, heat to 110 °C for 5 minutes. Inspected the plate in visible light and under UV 366 nm; c) Sprayed the plate with the HCl-acetic acid reagent, dry in stream of cold air, place on plate heater (or in oven) at 120 °C for 2 minutes (or until color of standard has developed). The active fractions/pure compounds was scraped from the Silica gel plate and eluted from the silica gel with ethanol. The active compounds were filtered through Millipore filters (0.45µm and 0.22µm) to remove the silica gel and this yielded more of compound(s) fraction.

Structure elucidation of isolated compounds by combination of different techniques:

Identification of compound was done by using a combination of different techniques including HPLC and FT-IR Besides these characterization techniques, R_f values and melting point of the active compounds were also determined.

High-performance liquid chromatography (HPLC):

HPLC analysis was performed in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a Shimadzo LC- 2010 HPLC system (Kyoto, Japan), equipped with a Shimadzo LC 2010 UV-VIS detector with a thermostated flow cell and a selectable two wavelengths of 190 - 370 nm or 371–600nm. The detector signal was recorded on a Shimadzo LC2010 integrator.

The column used was a C-18 block heating-type Shim-pack VP-ODS (4.6mm interior diameter × 150mm long) with a particle size of 5µm. Mobile phase was designed as per the nature of the compound, containing 50% acetonitrile along with 50 % Phosphate buffer was used at a flow rate of 3.0 ml/min, column temperature 25°C. Injection volume was 40µl and detection was carried out at specific wavelength having maximum absorbance as calculated by UV absorption spectra at maximum wavelength.

Fourier Transform Infrared (FTIR) studies:

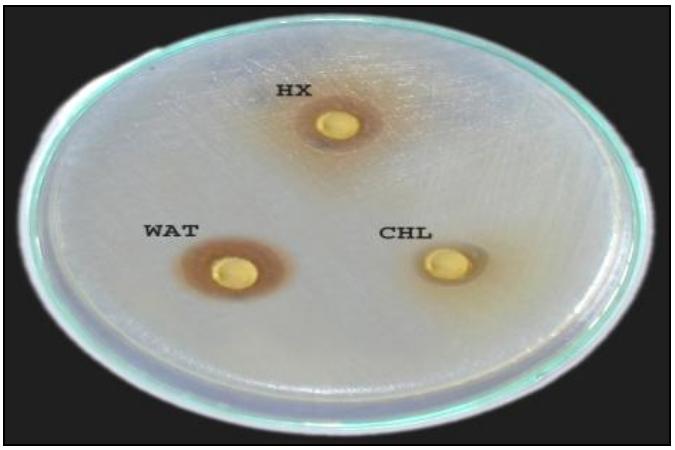
The IR spectrum of isolated compound was recorded in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a computerized FTIR spectrometer (Perkin Co., Germany) in the range of 4000-400 cm^{-1} by the KBr pellet technique.

RESULTS AND DISCUSSION:

Antimicrobial activity:

In this study, the hydro-alcoholic and hexane extracts of *Valeriana jatamansi* were screened for their antimicrobial activity against multi-drug resistant strains (isolated from Clinical samples, pus and blood of patients) and other pathogenic strains. Hydro-alcoholic extract of the plants showed significant antimicrobial activity in comparison to hexane extract against all the pathogens and against MDR *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Only hydro alcoholic extract of the plant showed potent antifungal activity against *Aspergillus niger* but none of the extract showed any anti-fungal activity against *Candida albicans*. The results are shown in **Table 1 and 2; Fig. 1 (a) and (b)** and **2**. Hydro-alcoholic extract showed potent antibacterial activity against multidrug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the ranges from 0.3mg/ml to 0.7mg/ml. *aeruginosa* in the ranges from 0.3mg/ml to 0.7mg/ml.



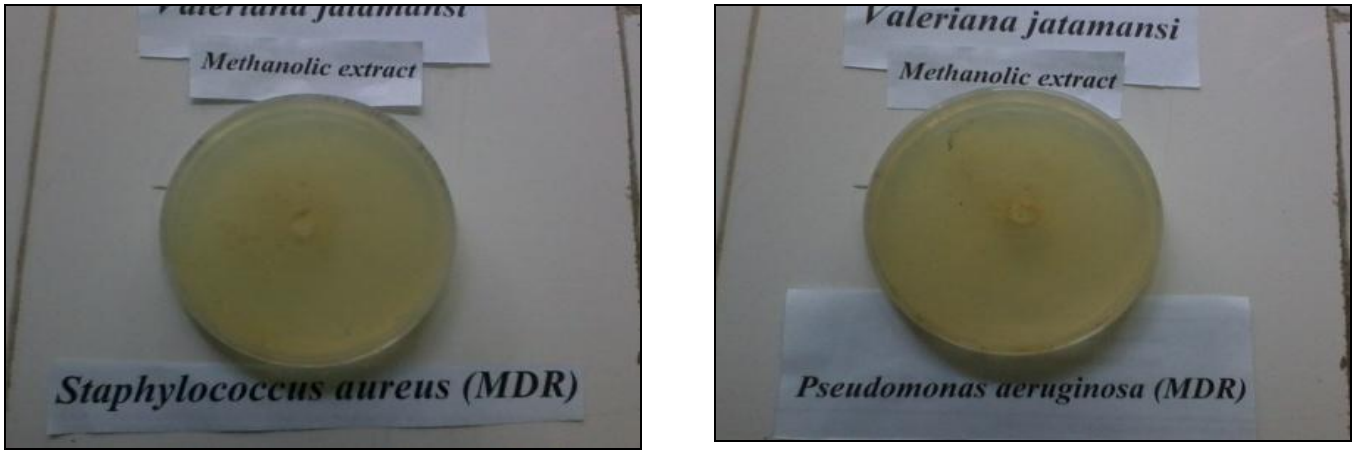


FIG. 1 (a: ANTIMICROBIAL ACTIVITY OF *VALERIANA JATAMANSI* AGAINST PATHOGENIC AND DRUG RESISTANT STRAINS.

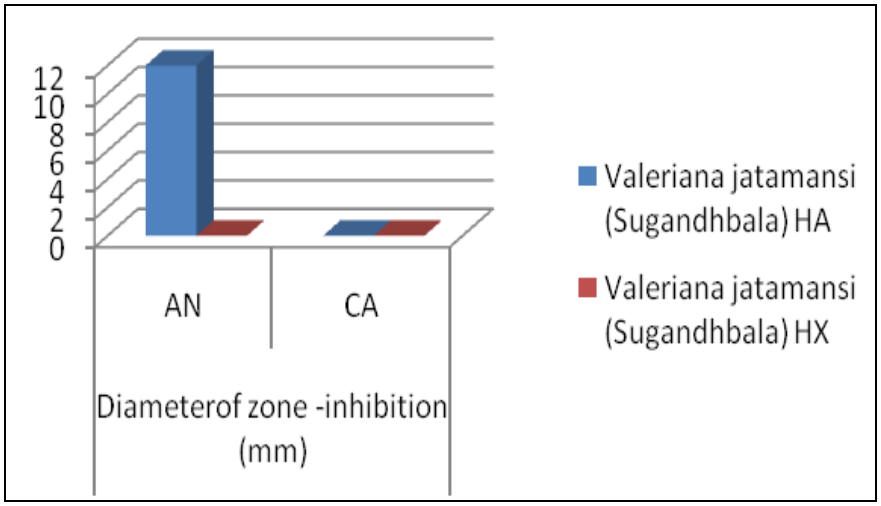


FIG.1: (b): ANTIFUNGAL ACTIVITY OF THE EXTRACTS OF *VALERIANA JATAMANSI*

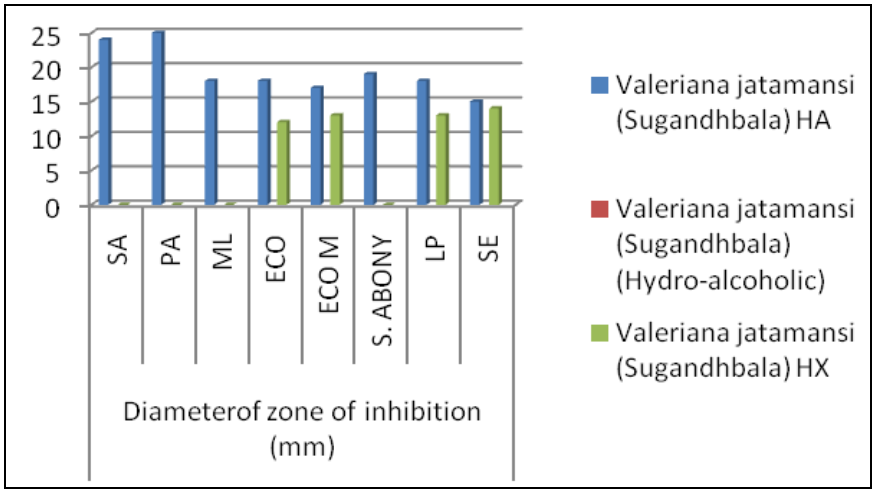


FIG.2: ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *VALERIANA JATAMANSI*

TABLE 1: ANTIFUNGAL ACTIVITY OF THE EXTRACTS OF *VALERIANA JATAMANSI*

Plants	Solvent Extract	Diameterof zone of inhibition (mm)		MIC(mg/ml)		MLC(mg/ml)	
		AN	CA	AN	CA	AN	CA
Valeriana jatamansi (Sugandhbala)	HA (Hydro-alcoholic)	12	NA	0.5	NA	0.7	NA
	HX (Hexane)	NA	NA	NA	NA	NA	NA

*AN, *Aspergillus niger*; CA, *Candida albicans;* NA, No activity

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *VALERIANA JATAMANSI*

Plants	Solvent Extract	Diameterof zone of inhibition (mm)							
		SA	PA	ML	ECO	ECO M	S. ABONY	LP	SE
Valeriana jatamansi (Sugandhbala)	HA (Hydro-alcoholic)	24	25	18	18	17	19	18	15
	HX (Hexane)	NA	NA	NA	12	13	NA	13	14

*SA, *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; ML, *Micrococcus luteus*; ECO, *E. Coli*; ECO M, *E.coli mutans*; S. ABONY, *Salmonella abony*; LP, *Lactobacillus plantarum*; SE, *Staphylococcus epidermidis;* NA, No activity

Phytochemical Screening of plant extracts:

The results showed that alkaloids and reducing sugars are present specifically in hydro-alcoholic extract of the plant while steroids and saponin are found in hexane extract of the plant. The results are shown in **Table 3**.

TABLE 3: PHYTOCHEMICAL SCREENING OF THE ACTIVE CONSTITUENTS

Valeriana jatamansi	Phytochemical constituents						
	Alkaloids	Flavanoids	Tannins	Steroids	Saponin	Glycosides	Reducing sugars
Hydro-alcoholic extract	+	_	_	_	_	_	+
Hexane extract	_	_	_	+	+	_	_

* +, present; _, absent

Isolation and Identification of the antimicrobial compound:

Column Chromatography and Thin Layer Chromatography:

The pure compound isolated, Valerenic acid (pentanoic acid or propylacetic acid in systemic naming) is a member of short chain straight fatty acids. It was obtained as colorless liquid with a penetrating aroma; slightly soluble in water, soluble in alcohol, and ether. It melts at -34 ^{0}C and boils at 186 ^{0}C. The compound showed R_f value 0.37 similar to that of the standard compound, Valerenic acid. The pure compound, valerenic acid (as compared with that of standard) was further subjected for antimicrobial and antioxidant screening (data not shown). The pure compound fraction was further subjected to HPLC and FTIR analysis. The results are shown in **Fig. 3 to 6**.

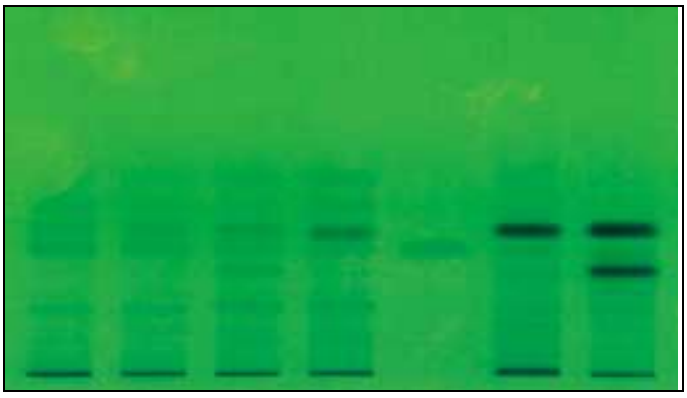


FIG.3: TLC CHROMATOGRAM OF VALERENIC ACID ISOLATED FROM *VALERIANA JATAMANSI* (VIEWED IN UV LIGHT AT 254 nm)

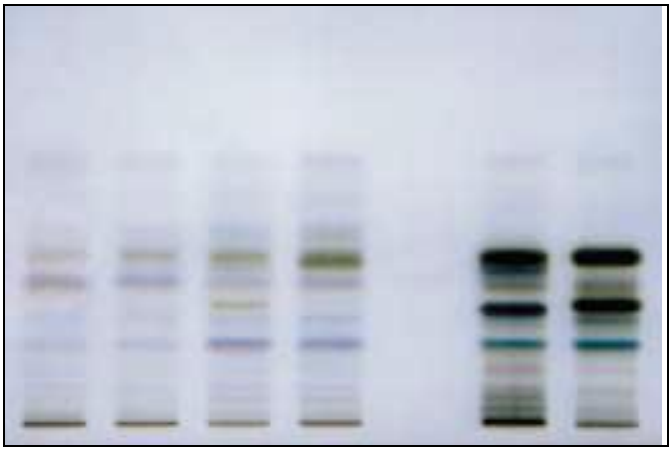


FIG. 4: TLC CHROMATOGRAM OF VALERENIC ACID ISOLATED FROM *VALERIANA JATAMANSI* (SPRAYED WITH HCL-ACETIC ACID REAGENT, VIEWED IN VISIBLE LIGHT)

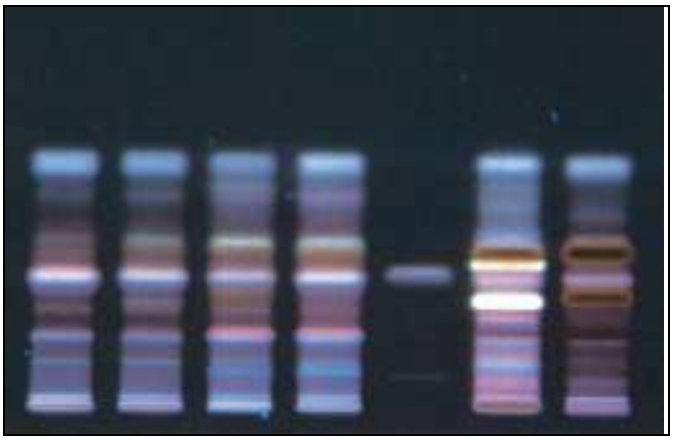


FIG. 5: TLC CHROMATOGRAM OF VALERENIC ACID ISOLATED FROM *VALERIANA JATAMANSI* (SPRAYED WITH HCL-ACETIC ACID REAGENT, VIEWED IN UV LIGHT AT 366 nm)

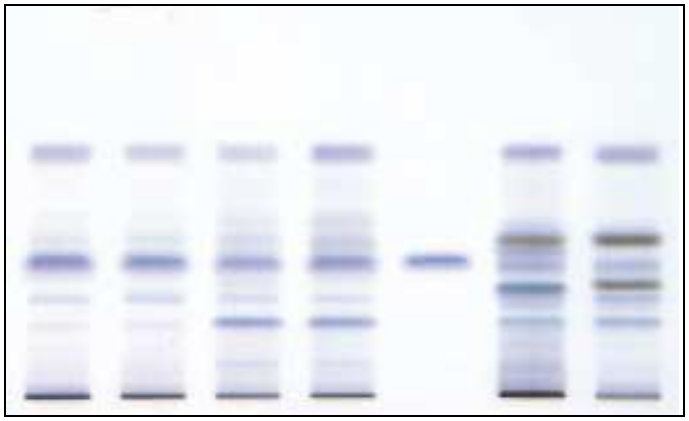


FIG. 6: TLC CHROMATOGRAM OF VALERENIC ACID ISOLATED FROM *VALERIANA JATAMANSI* (SPRAYED WITH HCL-ACETIC ACID REAGENT, PLACED IN HOT AIR OVEN AT 120^{0}C.)

HPLC analysis:

The retention time of the pure compound purified was found to be 4.300 minutes as compared to the Standard reference compound, Valerianic acid which showed its retention time 4.393 minutes while eluting out through the column. This analysis thus confirmed about the separation and identification of the purified active compound. The results are indicated in **Figure 7** and **8.**

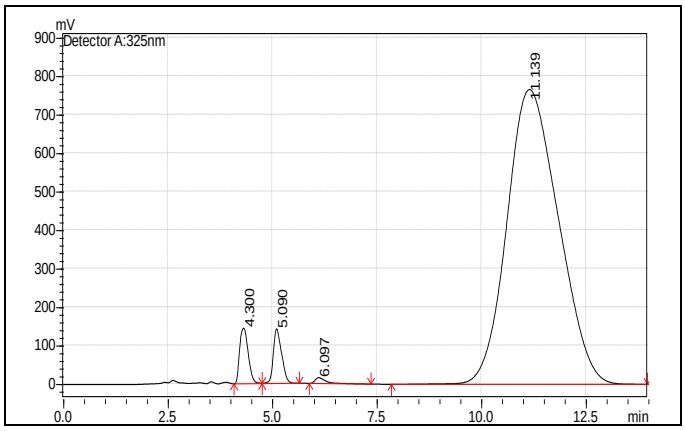


FIG.7: COMPOUND, VALERIANIC ACID EXTRACTED THROUGH HPLC

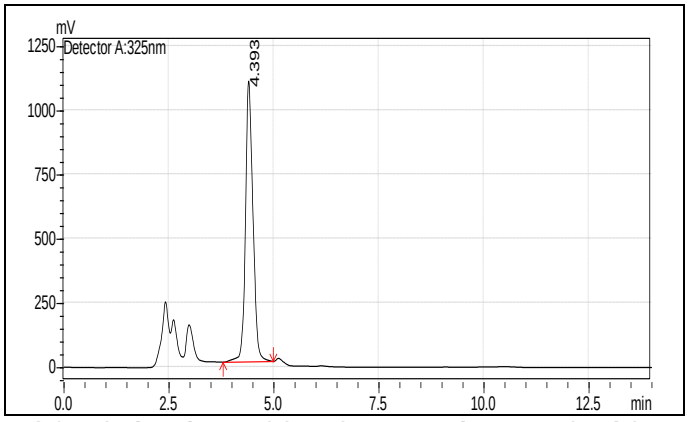


FIG.8: STANDARD COMPOUND, VALERENIC ACID CHROMATOGRAM

FT-IR spectral analysis:

The IR spectrum of isolated compound was recorded in Roorkee Research and Analytical Laboratory Pvt. Ltd., Roorkee (Uttarakhand), India using a computerized FT-IR spectrometer (Perkin Co., Germany) in the range of 4000–500 cm^{-1} by the KBr pellet technique. The active compound was isolated and identified by spectral analysis using FT-IR with literature values. The results are shown in **Fig.9.**

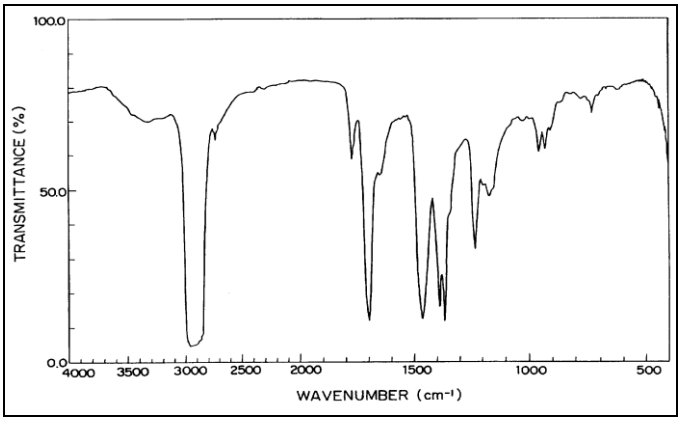


FIG.9: ISOLATED COMPOUND, VALERENIC ACID FT-IR SPECTRA

The compound was further subjected to antimicrobial activity at different doses. The results showed prominent antimicrobial activity against all the pathogens studied (results not shown).

CONCLUSION: The use of the plant in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, scientifically supported by the results obtained in this work. The present study thus suggests that compound isolated, Valerenic acid characterized is found to be most potent antimicrobial agent and can be further utilized in formulating a new antimicrobial drug. The results thus signify that the drug prepared from the compound isolated can be utilized in treatment of infections associated with such pathogens.

REFERENCES:

1. Singh SK, Pandey VD, Singh A, Singh C. Antibacterial activity of seed extracts of *Argemone mexicana* L. on some pathogenic bacterial strains. African Journal of Biotechnology 2009; 8(24): 7077-7081.
2. Mathur A, Dua VK**,** Prasad GBKS, Verma SK, Singh SK. Phytochemical Investigation and *In Vitro* Antioxidant Activity of some medicinally important plants. International Research Journal of Pharmacy 2011; 2(6):116-122.

3. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkensiana.* Journal of Ethnopharmacology 1993; 39, 171-174.
4. Perez C, Anesini C. *In vitro* antimicrobial activity of Argentine folk medicinal plants against *Salmonella typhii.* Journal of Ethnopharmacology 1993; 44: 41-46.
5. Vollekova AD, Kostalova, Sochorova R. Isoquinoline Alkaloids from *Mahonia aquifolium* stem bark is active against Malassezia sp. Folia Microbiol 2001; 46: 107-111.
6. Usman H, Abdulrahman FI and Ladan AH. Phytochemical and antimicrobial evaluation of *Tribulus terrestris* L. (Zygophylaceae). Growing in Nigeria. Res. J. Bio. Sci. Medwell Journals 2007; 2(3): 244-247.
7. Trease GE, Evans WC. 1989. Pharmacogonasy, 14th Edition, Brown Publication.
8. Harborne JB. 1993. Phytochemical method, 3rd Edition, Chapman and Hall, London. p.p. 135-203.
9. Mathur A, Verma SK, Singh SK, Prasad GBKS, Dua VK. Investigation of the antimicrobial, antioxidant and anti-inflammatory activity of the compound isolated from *Murraya koenigii*. International Journal of Applied Biology and Pharmaceutical Technology 2011; 2(1).
10. Agarwal A, Singhvi IJ, Bele D, Sharma K, Gupta SK, Karwani G, Kumawat M. Evaluation of steroids in face creams of different marketed brands. International J. Pharmacy & Technology 2011; 3(2): 2480-2486.

How to cite this article:
Babu P, Verma SK and Mathur A: Screening of Solvent Extracts of *Valeriana Jatamansi* for Isolation of Antimicrobial Compound. Int J Pharm Sci Res 2015; 6(6): 2641-48.doi: 10.13040/IJPSR.0975-8232.6(6).2641-48.