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Antimicrobial effectiveness of Indian Valerian (*Valeriana Jatamansi* Jones ex Roxb.)

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Valeriana jatamansi Jones belongs to the family Caprifoliaceae, commonly known as Indian valerian distributed in Pakistan, Afghanistan, Himalayas and China. It is a perennial herbaceous plant having multiple therapeutic uses. The study was aimed to screen out antifungal and antibacterial potential of *Valeriana jatamansi*, an important medicinal plant of high mountainous regions. Different fractions were prepared and tested against the selected bacterial and fungal species causing diseases in human beings. In bacterial activity, four species *S. mutans*, *S. marcescence*, *S. aureus,* and *Methicillin resistance staphylococcus aureus* (MRSA) were selected. Five fractions of methanolic extracts, n-hexane, chloroform, ethyl acetate, and aqueous were prepared are tested against the above bacterial species. The methanolic extracts showed a high zone of inhibition (46-52%) against all the selected bacterial species followed by the n-hexane fraction. The lowest antibacterial activity was exhibited by aqueous fractions. Antifungal activity was also tested against four fungal species i.e. *Polyspondylium pallidum, Aspergillus flavus, Fusarium oxysporum and Alternaria alternate.* The highest antifungal activity was shown in methanolic extracts (34-48%) against all the selected fungi followed by ethyl acetate fraction (34-37%) against *P. pallidum* and *F. oxysporum* and the lowest antifungal activity was shown by an aqueous fraction. It was concluded that *Valeriana jatamansi* are good source of antifungal and bacterial drugs having multiple therapeutic uses.

Keywords: Antibacterial; Antifungal; inhibition; Fractions; *Valeriana jatamansi*

INTRODUCTION

Medicinal plants use everywhere on this planet but in Asia, their usage is about 80% and Latin America and Africa are 50-70%. c. According to World Health Organization (WHO), 80-90% of drugs were prepared from medicinal plants (Guerra-Rodríguez et al. 2020). Plants consist of two types of metabolites primary and secondary metabolites. Primary metabolites help in plant growth and development and the metabolic pathways). The medicinal properties of plants are due to the secondary metabolites (Rastegari et al. 2019). Modern techniques also play a very important role and have given amazing importance to medicinal plants by identifying, isolating, and purifying active metabolites (Anand et al. 2019). About 25% of medications and 60% of the anti-inflammatory and anticancer drugs are provided to the markets. Antibiotics also play a very important role against different diseases caused by microbes although less effective in some stages (Anand et al. 2019). It is recorded that about 50% of the deaths globally and the third major cause of death are these microbial diseases. Fungi have dual threatening effects; they not only affect our crops but also cause toxicities and allergies (Deshmukh et al. 2019). In this regards the current research work was designed to evaluate the antifungal and antibacterial activity *of Valeriana jatamansi* (Dhiman et al. 2020).

Morphological characteristics

Valeriana jatamansi is a herbaceous perennial plant (Jugran et al. 2019). It grows up to 50cm in height and possesses a thick root system of about 6-10cm. It has an herbaceous stem that is several in number and 15 45cm in length. The leaves are of two types i.e. cauline and radicle. The cauline leaves may be small, lobulated, or entire while the radicle leaves are toothed, sinuate, ovate, or cordate (Kirk 1899). It also possesses a stalk and

2.58cm in length. The flower is plant is dioecious. i.e male and female plants arise in different plants with corymbose inflorescence. The calyx and corolla are five in number. Corolla is lobed and funnel-shaped. The fruits are covered with persistent sepals. Both flowers and fruits appear in March and April. After that seed ripening is started (Ashton et al. 1988).

Therapeutic properties

The therapeutic properties of *V. jatamansis* are used were known after obtaining its fraction (Jugran et al. 2019). These fractions showed their effect against different diseases caused due to bacterial and fungal diseases in the study we find that the fraction which was obtained from the selected plants caused inhibition of four bacterial and four fungal species which were selected from different hospitals of Khyber Pakhtunkhwa Pakistan (Pretorius et al. 2003). Some of the other therapeutic properties of *V. jatamansi* as epilepsy, and skin diseases.

MATERIALS AND METHODS

Antibacterial Activity

Plant specimen collection and processing. The plant *V. jatamansi* was collected from several locations in Khyber Pakhtunkhwa (Sher et al. 2015). The plants were washed and dried in the shade before being dried and cooked in an oven. Plants were dried in a newspaper for a week and then powdered with the help of grinder and subjected for extraction. (Balasubramanian et al. 2016).

Preparation of Extraction and Fractionation

The active metabolites were extracted using the cold maceration techniques (Rasheed et al. 2018). One-half of the powdered plant material was dipped in ethanol and incubated at 40°C for five days. The substance was filtered three times, yielding a clear filtrate. At 40°C, the filtrate was evaporated using a rotatory evaporator (Organization 1988). After drying the extract, it was diluted in 100mL distilled water. . Using a separating funnel, the solution was fractionated using several organic solvents such as ethanol, n-hexane, chloroform, methanol, and ethyl acetate (Wijanarko and Rifa'i, 2020). By using a rotatory evaporator, all of the fractions were concentrated and designated for that solvent fraction.

Media preparation:

The antibacterial activity was determined using the agar well diffusion method. To liquefy 25g of Luria Broth, one liter of distilled water was used, and the PH of Miller powder was set to 7.0 (Khushnood et al. 2019). The media was placed in a 250mL autoclave flask and autoclaved. The flask was filled with the selected four bacterial stains and maintained overnight at 150RPM at 37°C (Menacho-Melgar et al. 2021). After that, the agar was solidified, and five holes were tunneled using a sterilized borer. In the tunnel, the inoculum was placed. The bacterial and fungal species were chosen because they were commonly found in local hospitals in Khyber Pakhtunkhwa (KP) and showed resistance to a variety of treatments (Hamayun et al. 2021).

Test for bacterial strains:

Serratia marcescens, MRSA *(Methicillin resistance staphylococcus aurous), Streptococcus mutans*, and *staphylococcus aurous* were the four bacterial strains chosen, three of which were gram-positive and one of which was gram-negative. *Serratia marcescens* is a gram-negative bacterium (Pesewu et al. 2008).

Test for Fungal Strains:

Four fungal strains were chosen for antifungal activity: *Fusarium oxysporum*, *Aspergillus flavus, Polyspondylium pallidum,* and *Alternaria alternate* (Hamayun et al. 2021).

Measurement of Zones of inhibition:

The extracts were diluted in dimethyl sulfoxide (20 mg/mL) as a negative control. As a positive control, Cefotaxime (standard antibiotics) was utilized (Basile et al. 2010). About 75L of plant fractions were poured into the medium wells, and the petri dishes were placed in an incubator for 24 hours at 37°C. The diameter of each transparent zone was measured when the incubation period was completed. To calculate the standard data, the experiment was repeated several times.

RESULTS AND DISCUSSION

The current study was carried out to find the effects of various fractions of *Valeriana jatamansi* against bacterial and fungal species (Jugran et al. 2021).

Antibacterial Activity of the Crude Extracts of *Valeriana jatamansi:*

The antibacterial ability of all fractions produced from medicinal plants was tested against four bacteria: *Staphylococcus aureus, Streptococcus mutans, Serratia marcescens,* and MRSA (Methycillin resistance *Staphylococcus aureous*) (Al-Snafi, 2015). These organisms were chosen as experimental organisms because of their harmful character, which was mostly observed during screening in different hospitals in Khyber Pakhtunkhwa. These species were primarily responsible for human ailments, thus we chose them to test (Gelagle 2018).The effectiveness of our plant extract against the selected pathogens. The bacterial pathogen was treated with 6 mg/mL of the extract and each fraction, which after findings showed the effectiveness of the plant extract and fractions by Owais et al. 2005 as shown in Table 1. The inhibition zones by Cr. Met. Ext were 12.0±0.34 (46.15%), for *S. aureus*, 13.0±0.45 (46.43%), for *S. mutans*, 11.0±0.97 (46.30%), for *S. marcescens* and13.0±0.68 (52.38%) MRSA. The inhibition zones by *n*- hexane fraction were 10.0±0.33 (38.46%), for *S. aureus*, 12.0±0.55 (42.86%), for *S. mutans* 06.0±0.54 (28.57%),

for *S. marcescens* and 11.0±0.56 (44%) MRSA. The inhibition zones by Cr. Met. fraction were 09.0±0.58 (34.62%), for *S. aureus*,14.0±0.98 (50%), for *S. mutans*, 0.0±0.00 (0%), for *S. marcescens* and 08.0±0.33 (32%) MRSA. The inhibition zones by Chloroform fraction were 10.0±0.46 (38.46%), for *S. aureus*,14.0±0.66 (50%), for *S. mutans*,12.0±0.87 (57.14%), for *S. marcescens* and 12.0±0.98 (48%) MRSA. The inhibition zones by Ethyl acetate fraction were 06.0±0.44 (23.08%), for *S. aureus*, 07.0±0.56 (25%), for *S. mutans*, 0.0±0.00 (0%), for *S. marcescens* and 08.0±0.44 (32%) MRSA. It is obvious to say that the plant extract is very effective against selected bacterial pathogens with significant results **(**Table 1 &3, Figure 1&3)

Table 1: Antibacterial activity of the crude extracts of *Valeriana jatamansi.*

Bacterial Species	Standard	Cr. Methanolic. Ext.		*n*- hexane		$CHCl_3$		EtOAc		Aqueous	
		ZOI	%	ZOI	%	ZOI	%	ZOI	%	ZOI	%
S. aureus	26.0±0.44	12.0±0.34	46.15	10.0±0.33	38.46	09.0±0.58	34.62	10.0±0.46	38.46	06.0±0.44	23.08
S. mutans	28.0±0.66	13.0±0.45	46.43	12.0±0.55	42.86	14.0±0.98	50	14.0±0.66	50	07.0±0.56	25
S. marcescens	21.0±0.34	11.0±0.97	52.38	06.0±0.54	28.57	0.0±0.00	0	12.0±0.87	57.14	0.0±0.00	0
MRSA	25.0±0.76	13.0±0.68	52	11.0±0.56	44	08.0±0.33	32	12.0±0.98	48	08.0±0.44	32

Table 2: Antifungal activity of crude extracts of *Valeriana jatamansi.*

Fungal Species	Standard	Cr. Met. Ext		*n*- hexane		$CHCl_3$		EtOAc		Aqueous	
		ZOI	%	ZOI	%	ZOI	%	ZOI	%	ZOI	%
A. flavus	100.0±0.00	45.0±0.55	45	35.0±0.23	35	00.0±0.00	0	00.0±0.00	0	00.0±0.00	0
A. alternate	100.0±0.00	34.0±0.69	34	00.0±0.00	0	00.0±0.00	0	00.0±0.00	0	00.0±0.00	0
F. oxysporum	100.0±0.00	45.0±0.52	45	00.0±0.00	0	00.0±0.00	0	37.0±0.10	37	00.0±0.00	0
P. pallidum	100.0±0.00	48.0±0.56	48	00.0±0.00	0	00.0±0.00	0	34.0±0.34	34	00.0±0.00	0

Table 3: Anova: Two-Factor for antibacterial significance

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Rows	103.6	3	34.53333	6.148368	0.008942	3.490295
Columns	139.8	4	34.95	6.222552	0.005981	3.259167
Error	67.4	12	5.616667			
Total	310.8	19				

Table 4: Anova: Two-Factor for antifungal significance

ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	127.6667	3	42.55556	9.206731	0.001068	3.287382
Columns	951	5	190.2	41.14904	3.09E-08	2.901295
Error	69.33333	15	4.622222			
Total	1148	23				

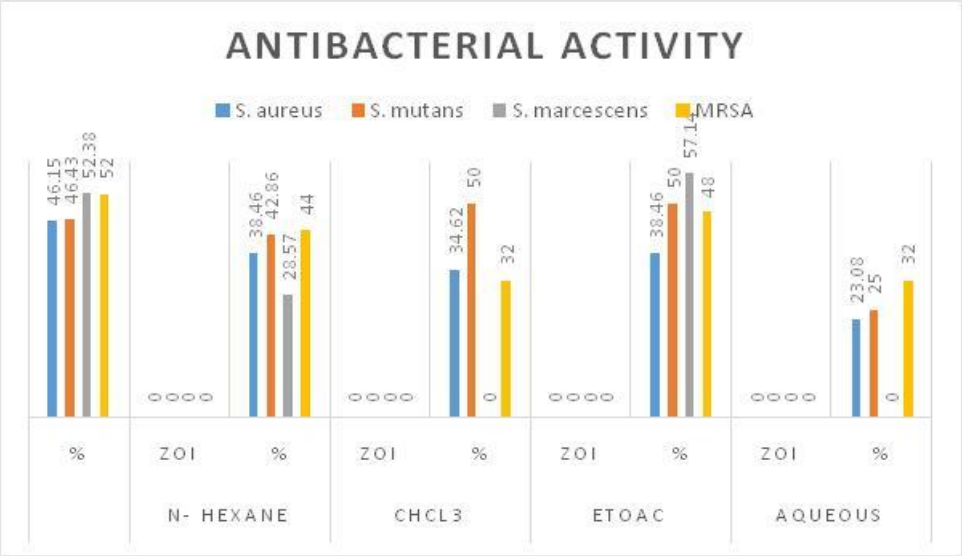

Figure 1: Trend of the Antibacterial Activity of the Crude Extracts of *Valeriana jatamansi*

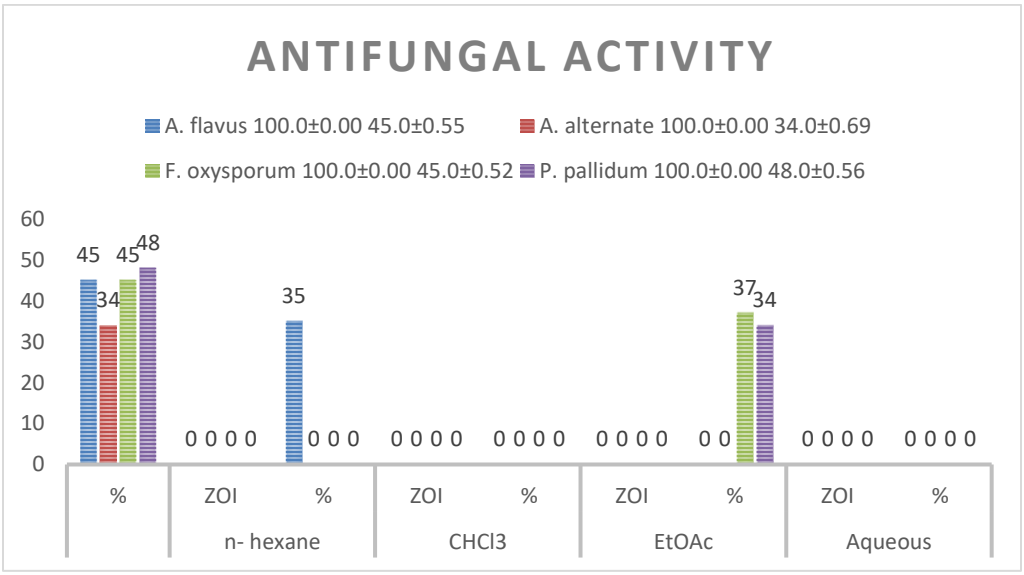

Figure 2: Trend of the Antifungal Activity of the Crude Extracts of *Valeriana jatamansi*

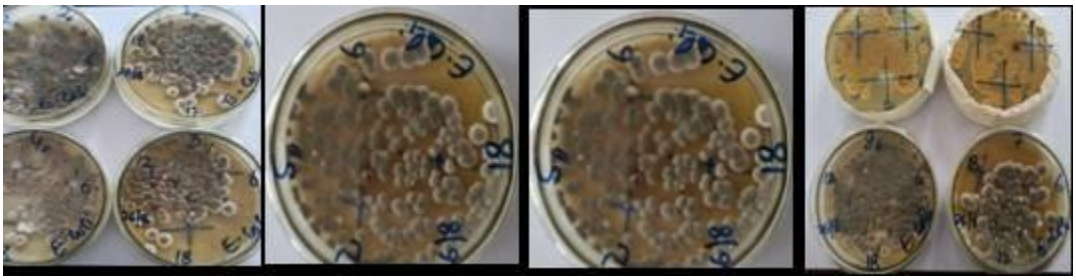

Figure 3: Antibacterial Activity of the Crude Extracts of *Valeriana jatamansi*

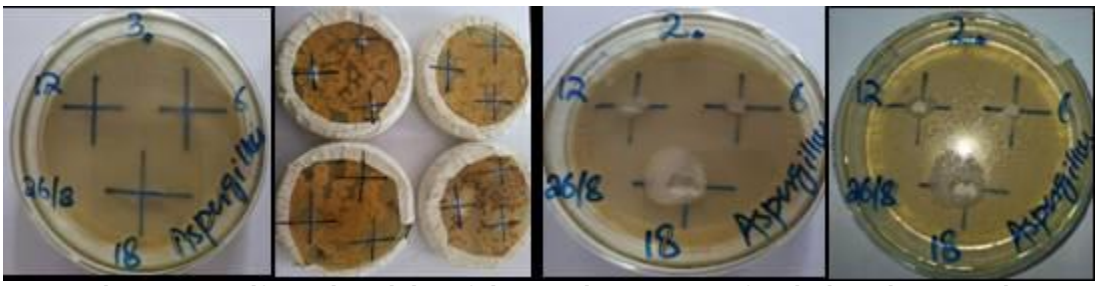

Figure 4: Antifungal Activity of the Crude Extracts of *Valeriana jatamansi*

Antifungal Activity of the Crude Extracts of *Valeriana Jatamansi:*

The same five fractions which were studied in antibacterial activity were also studied in antifungal activity.

Among the fraction, the least effective fraction was aqueous which completely failed in inhibiting the fungi with a 0.00± 0.00 zone of inhibition (Owais et al. 2005). The inhibition zones by Cr. Met. Ext were 35.0 ± 0.44, for *P. pallidum*, 45.0 ± 0.55, for *A. flavus*, 57.0 ± 0.23 for *F. oxysporum* and 65.0 ± 0.54 *A. alternate.* The inhibition zones by n-hexane were for *F. oxysporum* 35.0±0.23 and 00.0±0 for all other three. The inhibition zones by $CHCl_3$ were 00.0±0 for all selected pathogens. The inhibition zones by EtOAc were 34.0±0.34, for *P. pallidum*, 00.0±0.00, for *A. flavus*, 37.0±0.10 for *F. oxysporum*and00.0±0.00for *A. alternate.* After the overall checking of results, it is obvious to say that the plant extract is very effective against selected fungal pathogens with significant results (Table 2 & 4, Figure 2 & 4). The aqueous fraction is least effective, while $CHCl_3$ extract is moderate, and while Cr. Met. The extract is most efficient.

CONCLUSION

It is clear from the results that *Valeriana jatamansi* plays an important role in antibacterial and antifungal activities. Methanolic and n-hexane were comparatively more active in the five fractions obtained from plant detritus, demonstrating that polar solvents dissolved more properly in solvents. It was concluded from the results that *Valeriana jatamansi* are good source of antifungal and bacterial drugs.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

KUR and MNK designed and performed the experiments and also wrote the manuscript. MI, LS, QA, FZ, MA, and RUA performed antibacterial activity, antifungal activity, and data analysis. FAO and KUR designed experiments and reviewed the manuscript. All authors read and approved the final version.

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