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Antibacterial activity of *Valeriana jatamansi* against extended-spectrum β-lactamase producing Gram-negative bacteria causing urinary tract infections

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ABSTRACT

Objective: To find out the antibacterial activity of *Valeriana jatamansi* (*V. jatamansi*) rhizomes against the extended-spectrum β-lactamases (ESBLs) producing isolates of Enterobacteriaceae family.

Methods: Confirmation of ESBLs producing *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Hafnia alvei* isolated from urinary tract infections was performed by double disc diffusion assay. Antimicrobial susceptibility of all ESBLs producing isolates was determined by disc diffusion method following guidelines of Clinical and Laboratory Standards Institute. Successive extraction of rhizomes of *V. jatamansi* was performed with hexane, chloroform and methanol using Soxhelt apparatus. These extracts were tested against the ESBLs producing isolates using well diffusion method.

Results: Hexane extract showed significant results as compared to chloroform and methanol extracts with the maximum zone of inhibition (21 mm) while ciprofloxacin and amikacin were used as standard drugs.

Conclusions: Findings of the study suggested that hexane extract of *V. jatamansi* can be used in combination with other antibiotics as alternative treatment for urinary tract infections caused by ESBLs producing strains of Enterobacteriaceae.

1. Introduction

Urinary tract infections (UTIs) are the most recurrent infections after the respiratory tract infections encountered in community which results in high morbidity rate and worse effect on economy in terms of treatment[1]. Complicated infections of urinary tract can lead to abnormalities of urinary tract which in turn cause urinary retention. Nevertheless, occurrence of UTIs varies with sex and age, and females are more affected than the males[2]. Children aging from 1 to 10 years may consider more susceptible to UTIs[3]. UTIs are very common nosocomial as well as community acquired infection caused by bacteria, virus and fungi. Out of these pathogens, Gram-negative bacteria particularly, Enterobacteriaceae including *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* are the most drastic uropathogens[4,5]. Emergence of multidrug resistance among Enterobacteriaceae has become the most alarming factor in case of UTIs which has overcome most of the therapeutic options[6]. Extended-spectrum β-lactamases (ESBLs) enzymes are one of the major elements responsible for resistance among Enterobacteriaceae with ability to inactivate the 3rd generation cephalosporin and monobactam by hydrolysing β-lactam ring[7].

An investigation of the intensive care units of various hospitals was accompanied in the United States where the prevalence of ESBLs producing isolates of *K. pneumoniae* was recorded from range of 3.6% to 14.4%[8]. Rapid emergence of ESBLs producing *E. coli* has been reported in a recent study from Pakistan[9]. Acquisition of multidrug resistance to commonly prescribed drugs leads scientists to introduce alternatives of these drugs that could be more potent for treatment of such infections and pathogens.Plants produce many secondary metabolites that have been familiar for biological activities[10]. Among these, few plants have been reported for their antimicrobial activities against multidrug resistant bacteria including the ESBLs producing *E. coli* and *K. pneumoniae*[11,12].

Valeriana jatamansi (*V. jatamansi*) is a perennial herb also known as *Valeriana wallichii* which belongs to family Valerianaceae[13]. Major phytochemicals of *V. jatamansi* rhizomes are valepotriates, hesperidins, isovalerate, 6-methyl apigenin and sesquiterpenoids whereas essential oils such as valerenic acid, maaliol, valeranone, viridiflorol and terpineol were the most prominent constituents[14].

Rhizomes of *V. jatamansi* exhibit antioxidant, nematicidal, antiepileptic, antispasmodic and anxiolytic and sedative effect[15-20]. No data were available for antibacterial activity of *V. jatamansi* against

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ESBLs producing Enterobacteriaceae. The current study was aimed to evaluate the antibacterial activity of native medicinal plant prevalent in hilly areas of Pakistan, *V. jatamansi* against the extended-spectrum β-lactamases producing Gram-negative bacteria isolated form UTIs.

2. Materials and methods

2.1. Plant material and extraction

Rhizomes of *V. jatamansi* plant were collected from the forest of Azad Jammu Kashmir, Pakistanin in October 2015 and were authenticated by Professor Dr. Zaheer Ahmed (Department of Botany, Government College University, Lahore). The plant was completely dried in shadow and pulverized to make a fine powder by passing through a sieve and stored in amber glass bottle. Dried powder of rhizomes of *V. jatamansi* were successively extracted in Soxhelt apparatus by using the solvents of hexane, chloroform and methanol and solvents were evaporated in the rotary vacuum evaporator[21].

2.2. Collection and identification of bacteria

Bacterial isolates subjected for identification of ESBLs production were isolated from urine samples collected from urology department of tertiary care hospital in Lahore, Pakistan. Gram-negative bacteria were identified by colony morphology on MacConkeyagar (Merck, Darmstadt, Germany) and confirmed by biochemical tests using commercially available kits, API 20E Identification System (BioMérieux, France) and RapIDTM ONE System (Remel, USA).

2.3. Detection of ESBL producing isolates

All bacterial isolates were tested for production of ESBLs following the guidelines of Clinical and Laboratory Standards Institute (CLSI) by using double disc diffusion method[22]. Two plates were streaked for each isolate. Ceftazidime (30 μg) and ceftazidime (30 μg) + clavulanic acid (10 μg) were applied on one plate and cefotaxime (30 μg) and cefotaxime (30 μg) + clavulanic acid (10 μg) on other. Minimum distance between discs and edges was kept at 15 mm. Muller-Hinton agar (Merck, Darmstadt, Germany) plates with sensitivity discs were incubated at 37 ˚C for 16–18 h. Zone of inhibition around each disc was measured and recorded. Bacterial isolates were considered phenotypically confirmed for ESBL production if the zone measured by combination disc was enhanced by $\geqslant$ 5 mm in comparison to zone measured by single disc as per CLSI guidelines.

2.4. Antimicrobial susceptibility testing

Further exploration of antibacterial resistance of all ESBLs producing isolates against different antibacterial agents were done by using disc diffusion method[9]. Antibiotics used for disc diffusion method were amikacin (30 μg), colistin sulphate (10 μg), fosfomycin (50 μg), ciprofloxacin (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), gentamicin (10 μg), imipenem (10 μg), nalidixic acid (30 μg), piperacillin-tazobactam (100 μg + 10 μg), trimethoprim-sulfamethoxazole (1.25 μg + 23.75 μg) and ticarcillin-clavulanic acid (75 μg + 10 μg). Zone of inhibition around each disc was measured and results were interpreted using guidelines of CLSI[22].

2.5. Antibacterial assay of plant extracts

The antibacterial activity of plant extracts against ESBLs producing bacteria was carried out by agar well diffusion method. Muller-Hinton agar plates were prepared and wells were generated on solidified plate with the help of borer having diameter of 7 mm. Well-isolated colonies of test specimen were suspended in 1.5 mL demineralized water and turbidity was attuned analogous to 0.5 McFarland of turbidity standards. An amount of 50 μL of each dilution (1 mg/mL, 5 mg/mL and 10 mg/mL) of plant extracts and 50 μL of two standard drugs *i.e.* ciprofloxacin and amikacin (1 mg/mL) were injected into wells and incubated at 37 ˚C overnight. Three plates were used for each bacterial isolate and zones of inhibition were measured. Assay was repeated for three times to observe reproducible result[23].

3. Results

3.1. Bacterial identification and ESBL detection

A total of 50 isolates of Gram-negative bacteria were collected from clinical laboratory of a tertiary care facility at Lahore, Pakistan. Out of 50 isolates, 19 isolates were ESBLs producing bacteria as confirmed by double disc diffusion assay. On the basis of biochemical characterization, out of 19 isolates, eight isolates were *E. coli*, five isolates were *Enterobacter aerogenes* (*E. aerogenes*), four isolates were *K. pneumoniae* and other two isolates were Hafnia alvei (*H. alvei*).

3.2. Antimicrobial susceptibility testing

All ESBLs producing isolates were further subjected for antibacterial susceptibility using 12 commonly used antimicrobials belonging to nine different groups by disc diffusion method. All isolates expressed high levels of resistance to cefotaxime (84%), ceftazidime (78%), whereas 68% isolates were resistant to nalidixic acid, gentamicin, ticarcillin-clavulanic acid and trimethoprim-sulfamethoxazole. Nearly 63%, 42%, 36% and 15% isolates were resistant to ciprofloxacin, colistin sulphate, amikacin and fosfomycin respectively. On the basis of susceptibility patterns, all isolates were defined as multidrug resistant. Imipenem was the only compound to which all of the isolates were susceptible as shown in Table 1.

Table 1
Antimicrobial resistance among ESBLs producing isolates [*n* (%)].

Drugs	*E. coli* (*n* = 8)	*K. pneumoniae* (*n* = 4)	*E. aerogenes* (*n* = 5)	*H. alvei* (*n* = 2)
Amikacin	3 (38)	2 (50)	1 (20)	1 (50)
Cefotaxime	7 (88)	4(100)	3 (60)	2 (100)
Ceftazidime	7 (88)	2 (50)	4 (80)	2 (100)
Ciprofloxacin	6 (75)	3 (75)	2 (40)	1 (50)
Colistin sulphate	3 (38)	2 (50)	3 (60)	0
Fosfomycin	1 (13)	1 (25)	1 (20)	0
Gentamicin	6 (60)	3 (75)	2 (40)	2 (100)
Imipenem	0	0	0	0
Nalidixic acid	7 (88)	3 (75)	3 (60)	0
Piperacillin/ tazobactam	2 (25)	2 (50)	1 (20)	1 (50)
Ticarcillin-clavulanicacid	7 (88)	2 (50)	2 (40)	2 (100)
Trimethoprim-sulfamethoxazole	6 (75)	2 (50)	3 (60)	2 (100)

3.3. Antibacterial assay of plant extracts

Antibacterial activity of hexane extract of *V. jatamansi* rhizomes was established against different ESBLs producing isolates of *E. coli*, *K. pneumoniae*, *E. aerogenes* and *H. alvei* at concentrations of 1 mg/mL, 5 mg/mL and 10 mg/mL as shown in Table 2. Hexane extract showed the enormous antibacterial activity against all isolates of *K. pneumoniae* and the maximum zone of inhibition (21 mm) was produced by the *K. pneumoniae*

6bn at concentration of 10 mg/mL. Remarkable results were recorded against eight ESBLs producing isolates of *E. coli* where the maximum zone of inhibition (18 mm) was produced by *E. coli* 241lyt with hexane extract at concentration of 10 mg/mL. Moderate antibacterial activity of hexane extract of *V. jatamansi* was observed among isolates of *E. aerogenes* and *H. alvei* ranged from 12 mm to 18 mm in diameter.

Table 2

Antibacterial activity of hexane extract of *V. jatamansi* (mm).

Bacterial name	Ciprofloxacin	Amikacin	Hexane extract of rhizomes		
	1 mg/mL	1 mg/mL	1 mg/mL	5 mg/mL	10 mg/mL
E. coli 1051xv	10.23 ± 0.54	11.45 ± 0.23	-	-	-
E. coli 125lyx	-	-	13.09 ± 0.87	14.94 ± 0.52	15.80 ± 0.28
E. coli 135lut	-	-	-	13.74 ± 0.23	14.09 ± 0.78
E. coli 163lyt	-	-	-	-	-
E. coli 147lyt	-	-	12.45 ± 0.15	13.04 ± 0.28	14.74 ± 0.45
E. coli 247ltr	10.29 ± 0.56	11.24 ± 0.56	-	-	-
E. coli 231ltr	11.09 ± 0.87	11.89 ± 0.14	12.55 ± 0.78	13.25 ± 0.89	14.05 ± 0.82
E. coli 241lyt	10.41 ± 0.25	10.09 ± 0.87	15.24 ± 0.85	16.65 ± 0.23	18.47 ± 0.73
E. aerogenes 2eg	11.99 ± 0.25	10.24 ± 1.33	10.45 ± 0.37	11.24 ± 0.75	12.12 ± 0.14
E. aerogenes 3mp	10.05 ± 0.25	11.25 ± 0.63	16.14 ± 0.45	17.78 ± 25	18.01 ± 0.21
E. aerogenes 2bn	-	-	-	-	-
E. aerogenes 3mb	-	-	-	-	-
E. aerogenes 4mn	-	-	-	-	-
K. pneumoniae 9bn	10.25 ± 0.52	11.05 ± 0.85	12.25 ± 0.74	13.25 ± 0.23	13.75 ± 0.57
K. pneumoniae 2bn	11.04 ± 0.25	10.41 ± 0.52	13.14 ± 0.85	14.14 ± 0.96	15.12 ± 0.45
K. pneumoniae 6bn	11.34 ± 0.37	11.01 ± 0.25	19.91 ± 0.87	20.45 ± 0.47	20.92 ± 0.45
K. pneumoniae 4nt	12.09 ± 085	11.14 ± 0.74	10.12 ± 0.45	11.04 ± 0.56	12.56 ± 0.85
H. alvei 2wey	-	-	11.05 ± 0.23	12.01 ± 0.87	12.52 ± 0.25
H. alvei 5wey	-	-	-	11.45 ± 0.47	12.45 ± 0.47

All values were expressed as mean ± SD (n = 19).

Similarly, chloroform extract of *V. jatamansi* rhizomes presented modest antibacterial results against all of ESBLs producing isolates included in this study at concentrations of 1 mg/mL, 5 mg/mL and 10 mg/mL as shown in Table 3. The maximum zone of inhibition (17 mm) was observed by the *E. coli* 163lyt against chloroform extract of *V. jatamansi* rhizomes at concentration of 10 mg/mL. Most of *K. pneumoniae*, *E. aerogenes* and *H. alvei* expressed moderate zones of inhibition ranged from 12 mm to 16 mm at different concentrations of chloroform extract of rhizomes of *V. jatamansi*.

Table 3

Antibacterial activity of chloroform extract of *V. jatamansi* (mm).

Bacterial name	Ciprofloxacin	Amikacin	Chloroform extract of rhizomes		
	1 mg/mL	1 mg/mL	1 mg/mL	5 mg/mL	10 mg/mL
E. coli 1051xv	10.25 ± 0.52	11.23 ± 0.45	12.58 ± 0.23	12.95 ± 0.54	13.45 ± 0.23
E. coli 125lyx	-	-	11.25 ± 0.54	11.78 ± 0.14	13.25 ± 0.25
E. coli 135lut	-	10.21 ± 0.52	10.12 ± 0.23	11.14 ± 0.14	12.78 ± 0.25
E. coli 163lyt	-	-	16.65 ± 0.52	17.01 ± 0.74	17.52 ± 0.75
E. coli 147lyt	-	-	-	-	-
E. coli 247ltr	-	-	-	-	-
E. coli 231ltr	11.09 ± 0.25	11.78 ± 0.23	12.45 ± 0.85	13.41 ± 0.52	14.08 ± 0.45
E. coli 241lyt	10.21 ± 0.45	11.12 ± 0.25	13.35 ± 0.12	14.16 ± 0.32	15.21 ± 0.25
E. aerogenes 2eg	10.23 ± 0.25	10.52 ± 0.85	10.47 ± 0.23	11.48 ± 0.23	12.12 ± 0.56
E. aerogenes 3mp	11.12 ± 0.58	10.21 ± 0.25	10.52 ± 0.23	11.24 ± 0.98	12.54 ± 0.23
E. aerogenes 2bn	10.21 ± 0.23	11.85 ± 0.21	14.45 ± 0.28	14.25 ± 0.85	16.09 ± 0.48
E. aerogenes 3mb	11.25 ± 0.25	10.25 ± 0.23	12.25 ± 0.25	13.75 ± 0.24	14.25 ± 0.52
E. aerogenes 4mn	-	-	-	-	-
K. pneumoniae 9bn	-	-	-	-	-
K. pneumoniae 2bn	11.12 ± 0.58	10.41 ± 0.52	11.12 ± 0.25	12.24 ± 0.98	13.54 ± 0.23
K. pneumoniae 6bn	11.54 ± 0.23	11.24 ± 0.98	12.54 ± 0.23	14.48 ± 0.23	16.12 ± 0.25
K. pneumoniae 4nt	12.48 ± 0.27	11.41 ± 0.52	11.52 ± 0.23	12.12 ± 0.58	13.48 ± 0.23
H. alvei 2wey	-	-	13.54 ± 0.25	13.24 ± 0.98	14.41 ± 0.52
H. alvei 5wey	-	-	-	-	-

All values were expressed as mean ± SD (n = 19).

On the contrary, methanolic extract of *V. jatamansi* rhizomes demonstrated minimum antibacterial effect against ESBLs producing isolates of *E. coli*, *K. pneumoniae*, *E. aerogenes* and *H. alvei* at concentrations of 1 mg/mL, 5 mg/mL and 10 mg/mL with the maximum zone of inhibition (16 mm) by *K. pneumoniae* 6bn at concentrations of 10 mg/mL as shown in Table 4.

Table 4

Antibacterial activity of methanolic extract of *V. jatamansi* (mm).

Bacterial name	Ciprofloxacin	Amikacin	Methanolic extract of rhizomes		
	1 mg/mL	1 mg/mL	1 mg/mL	5 mg/mL	10 mg/mL
E. coli 1051xv	10.25 ± 0.75	11.54 ± 0.52	11.25 ± 0.12	12.85 ± 0.47	13.25 ± 0.58
E. coli 125lyx	-	-	-	-	-
E. coli 135lut	-	-	-	13.45 ± 0.23	14.87
E. coli 163lyt	-	-	-	-	-
E. coli 147lyt	-	-	-	11.25 ± 0.87	12.74 ± 0.52
E. coli 247ltr	-	-	-	-	-
E. coli 231ltr	11.42 ± 0.64	11.12 ± 0.71	11.45 ± 0.55	12.41 ± 0.21	13.47 ± 0.85
E. coli 241lyt	-	-	-	-	-
E. aerogenes 2eg	-	-	-	-	-
E. aerogenes 3mp	11.12 ± 0.52	10.01 ± 0.52	11.21 ± 0.52	12.01 ± 0.52	13.14 ± 0.52
E. aerogenes 2bn	10.25 ± 0.85	11.24 ± 0.52	15.65 ± 0.21	16.12 ± 0.25	16.64 ± 0.52
E. aerogenes 3mb	-	-	-	-	-
E. aerogenes 4mn	-	-	-	-	-
K. pneumoniae 9bn	-	-	-	-	-
K. pneumoniae 2bn	11.12 ± 0.52	10.12 ± 0.85	14.41 ± 0.85	15.14 ± 0.75	16.41 ± 0.25
K. pneumoniae 6bn	11.45 ± 0.25	11.12 ± 0.52	15.01 ± 0.24	16.14 ± 0.44	16.74 ± 0.14
K. pneumoniae 4nt	-	-	-	-	-
H. alvei 2wey	-	-	13.25 ± 0.51	13.41 ± 0.41	14.54 ± 0.32
H. alvei 5wey	-	-	-	-	-

All values were expressed as mean ± SD (n = 19).

4. Discussion

High prevalence of ESBLs producing isolates were significantly observed among patients suffering from UTIs which is similar to previously documented reports[24,25]. All ESBLs producing isolates included in this study were significantly resistant to number of antibiotics tested.

In a previous report from India, moderate antibacterial activity of different medicinal plants such as *Phyllanthus amarus*, *Prosopis spicigera*, *Zingiber officinale* and *Trachyspermum ammi* against ESBLs producing bacteria and multidrug resistant isolates was documented[11]. Whereas, remarkable antibacterial activity of *V. jatamansi* was demonstrated against ESBLs producing bacteria isolated from urine included in present investigation. Similarly, researchers from Iran determined the antibacterial activity of *Zataria multiflora* against ESBLs producing *K. pneumoniae* from urinary tract and findings were parallel to present study[26]. In the same way, Thulasi and Amsaveni reported significant antibacterial activity of methanolic extracts of *Cassia auriculata* against ESBLs producing *E. coli* which was in contrast to present study where methanolic extracts of *V. jatamansi* showed least activity against ESBLs producing *E. coli*[21]. On the contrary to previous study, hexane extract of *V. jatamansi* exhibited remarkable antibacterial effect against ESBLs producing *E. coli* isolated from urine included in present study[21]. Antibacterial action of *Coccinia grandis* against ESBLs producing *E. coli* isolated from urine was determined in previous study which revealed the fact that ethanol was better solvent for extraction[27]. However, present study showed that hexane extract of *V. jatamansi* was found to be active against most of ESBLs producing bacteria isolated from urine. Moderate antibacterial effect of methanolic extract of *V. jatamansi* was previously observed against the non-ESBLs strains of *Staphylococcus aureus*[28]. Whereas, least activity of methanolic extract of *V. jatamansi* against ESBLs producing isolates was observed in this study. To the best of our knowledge, very limited data are available on antibacterial activity of *V. jatamansi* and this is first

report on antibacterial activity of *V. jatamansi* against ESBLs producing Gram-negative bacteria isolated from UTIs. Alarming fact of emergence of multidrug resistant pathogens is a serious concern in therapeutics and search for alternative therapeutic agents such as natural plant products is exceptionally significant. Conclusively, our finding revealed that hexane extract of *V. jatamansi* showed the remarkable results which may be due to non-polar compounds present in it and could be a drug of choice in the treatment of UTIs, and in combating the hazards of drug resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

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