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Estimation of phytochemicals, antioxidant, antimicrobial, and brine shrimp lethality activities of *Valeriana jatamansi* Jones

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Abstract

Herbal medicine has been widely utilized in traditional medicine for treating various ailments. This study aimed to quantify the phenolic, flavonoid, and tannin content in different plant extracts, assess their antioxidant capacity, and evaluate their antimicrobial efficacy. Among the extracts tested, methanol exhibited the highest total phenolic content (TPC) at 166.5 ± 2.0 mg GAE/g and total flavonoid content (TFC) at 103.87 ± 5.47 mg QE/g, whereas hexane displayed the lowest TPC (16.03 ± 2.22 mg GAE/g) and TFC (8.27 ± 3.88 mg QE/g). The methanolic extract demonstrated the strongest antioxidant activity with an IC_{50} of 103.3 ± 1.53 g/mL, compared to the hexane extract's IC_{50} of 524.7 ± 0.89 µg/mL. Antimicrobial testing revealed that aqueous, ethanolic, and hexane extracts effectively inhibited Klebsiella pneumoniae with a zone of inhibition of 17 mm, comparable to the positive control neomycin (24 mm). The ethanolic extract showed substantial minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against K. pneumoniae and Staphylococcus aureus (3.12 mg/mL and 6.25 mg/mL, respectively), relative to the control (0.0078 mg/mL and 0.015 mg/mL). Additionally, cytotoxicity assays showed LC_{50} values of 1905.46 µg/mL for the ethanolic and 2137.96 µg/mL for the methanolic extract. These findings suggest that the studied plant extracts, particularly methanolic and ethanolic, have significant bioactive potential, positioning them as promising sources for future drug development against infectious diseases.

Keywords

Valeriana jatamansi, TPC, TFC, DPPH, antimicrobial, MIC, MBC, toxicity

Article information

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1 Introduction

Medicinal plants have long played a critical role in human health, providing therapeutic compounds that have been used since ancient times to manage and treat diseases. These plants, which contain bioactive compounds in various organs, serve either as direct therapeutic agents or as essential precursors for drug synthesis. A distinction exists between medicinal plants that have been scientifically validated for their therapeutic effects and those consid-

ered medicinal but lack thorough scientific study, underscoring the need for continued research into these natural resources [1, 2].

Natural materials, including plant parts, animal products, and microorganisms, have a historical foundation in medicine, with fossil evidence indicating that humans have utilized plants for medicinal purposes for at least 60,000 years. The chemical diversity found in natural compounds has developed over millions of years, providing an array of biologically active substances with potential pharmacological effects [3]. Phytochemicals, the bioactive constituents produced by plants, are essential metabolites that contribute to various functions such as plant growth, pollination, and defense against pathogens. These compounds are not only crucial for plant survival but also play an important role in protecting plants from environmental stressors, including disease and UV radiation [4, 5].

Phytochemicals can be classified into primary and secondary metabolites. Primary metabolites, like proteins, carbohydrates, and nucleic acids, are indispensable for basic plant functions, while secondary metabolites, including phenolics, flavonoids, and terpenoids, arise from more specialized metabolic pathways. These secondary compounds are often associated with defensive functions, including antibacterial, antifungal, and antiviral properties, making them of great interest for therapeutic use [6, 7]. Antioxidants, flavonoids, and phenolic compounds found in medicinal plants help combat oxidative stress, which is linked to degenerative diseases such as cancer, atherosclerosis, and gastric ulcers [8, 9].

One notable medicinal plant is *Valeriana jatamansi* Jones, a perennial herb from the Valerianaceae family, native to the Himalayan region and traditionally valued for its broad spectrum of medicinal properties [10, 11]. Distributed from Afghanistan to China, *V. jatamansi* Jones has been used to treat various conditions, including neurological disorders, insomnia, and bacterial infections. Its bioactive profile includes valepotriates, lignans, sesquiterpenoids, flavones, and other phytochemicals, supporting its application in ailments such as epilepsy, skin diseases, and obesity [12–14]. In addition to its historical significance in traditional medicine, studies have highlighted the plant's anti-inflammatory, sedative, antioxidant, and neuroprotective effects [15].

Despite its longstanding use, comprehensive studies examining the phytochemical composition and biological properties of the aerial parts of *V. jatamansi* Jones remain limited. This study aims to fill this research gap by analyzing the phytochemical constituents and evaluating the biological activities of the aerial parts of *V. jatamansi* Jones, contributing to a deeper understanding of its therapeutic potential.

2 Materials and Methods

2.1 Chemicals

For this study, high-purity analytical-grade solvents-methanol, ethanol, ethyl acetate, dichloromethane, and hexane- were sourced from Merck and Fischer Scientific to ensure optimal extraction conditions. EDTA disodium salt dihydrate was supplied by SRL, while Merck provided additional reagents including sodium chloride, boric acid, and fused calcium chloride. Key materials such as the Folin-Ciocalteu (FC) reagent, Resazurin, and microbial culture media including Mueller Hinton Broth, Nutrient Agar, and Mueller Hinton Agar were obtained from HieMedia and LOBA CHEMI Pvt. Ltd. The use of high-quality reagents and culture media was essential for achieving precision in both the phytochemical analyses and microbiological assays.

2.2 Plant Collection and Identification

The aerial parts of *Valeriana jatamansi* Jones were collected from the Far Western region of Nepal (Sayal Gaupalika-02, Doti). Details such as the scientific name, local name, plant parts used, and ethnomedicinal applications are summarized in **Table 1**. The specimen was authenticated by research officers at the Herbarium Center, where it was assigned the voucher code 01KATH162457. A visual representation of the *V. jatamansi* Jones medicinal plant is provided in **Figure 1**.

Figure 1: Aerial parts of *V. jatamansi* Jones

Table 1: Description of *V. jatamansi* Jones

Scientific name	*V. jatamansi* Jones
Local name	Sugandhawal
Family	Valerianaceae
Parts used	Aerial parts
Traditional uses	Curing blood diseases, burning sensation, cholera, skin disease, throat troubles, and ulcers.
Reference	[16]

2.3 Preparation of Extract

After collection, the plant material was thoroughly cleaned, followed by shade drying to preserve its phytoconstituents. It was then finely powdered in the grinding mill, ensuring a uniform consistency for further use. Approximately ten grams of *V. jatamansi* Jones powder was dissolved in 200 mL of six different solvents, ranging in polarity from more polar to less polar: water, methanol, ethanol, ethyl acetate, dichloromethane (DCM), and hexane. The content kept in maceration was shaken every 24 hours for three days. The contents were then filtered, and the filtrate was dried using a rotatory evaporator, maintaining a temperature of 40-45 °C.

2.4 Qualitative Phytochemical Analysis

Qualitative phytochemical screening was conducted using standard protocols [17–19] to identify various metabolites present in *V. jatamansi* Jones. The screening targeted a range of phytochemicals, including glycosides, flavonoids, alkaloids, phenolic compounds, terpenoids, steroids, carbohydrates, saponins, tannins, fixed oils, and lipids. This analysis provided an overview of the bioactive constituents within the plant extract.

2.5 Estimation of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the plant extracts was determined using the Folin-Ciocalteu colorimetric method, as described by Lu et al. [20]. In triplicate, 96-well plates were prepared with 20 L of plant extract, 100 L of 10% Folin-Ciocalteu reagent (diluted 1:10), and 80 L of 1M Na_2CO_3. The reaction mixture was incubated for 30 minutes at room temperature until a blue color developed. Absorbance was then measured at 765 nm using a spectrophotometer. TPC was quantified as milligrams of gallic acid equivalent per gram of extract dry weight (mg GAE/g), based on a gallic acid standard curve ranging from 7.5 to 100 g/mL.

2.6 Estimation of Total Flavonoid content (TFC)

The total flavonoid content of the plant extracts was assessed using the aluminum chloride method as described by Ahmed et al. [21]. In triplicate, 20 L of plant extract, 100 L of distilled water, and 60 L of ethanol were added to 96-well plates. This was followed by the addition of 10 L of a 10% aluminum chloride ($AlCl_3$) solution and 10 L of 1M potassium acetate (CHCOOK) solution. The reaction mixture was kept to incubate at room temperature for 30 minutes. Absorbance was then measured at 415 nm using a spectrophotometer. The total flavonoid content was written as milligram of quercetin equivalent per gram of extract dry weight (mg QE/g), with quantification based on a standard calibration curve for standard quercetin having the value from 7.5 to 100 g/mL.

2.7 Evaluation of Antioxidant Activity

The positive control, quercetin, was serially diluted from the original concentration of 20 g/mL down to 0.625 g/mL, while the crude plant extract was diluted from 640 g/mL to 5 g/mL. In triplicate, 100 L of each diluted plant extract and the positive control were added to a 96-well plate. The initial absorbance was recorded at 517 nm. Following this, each well received 100 L of DPPH reagent, and the mixture was incubated for 30 minutes. The final absorbance was measured again at 517 nm. Methanol and 50% DMSO served as negative controls. This formula quantifies the ability of the extracts to scavenge free radicals, providing insight into their antioxidant potential [22, 23].

Radical scavenging capacity $= \frac{A\ control - A\ sample}{A\ control} \times 100\ \%$

Where, $A_{control}$ = absorbance of control, A_{sample} = absorbance of sample

Inhibitory concentration (IC_{50}) was calculated using GraphPad Prism (version 8.0.2.263).

2.8 Evaluation of Antimicrobial Activity

Antibacterial activity was assessed using the agar well diffusion method on Mueller Hinton Agar (MHA) plates [24, 25]. The test microorganisms, including *Shigella sonnei* (ATCC 25931), *Staphylococcus aureus* (ATCC 43300), *Klebsiella pneumoniae* (ATCC 700603), and *Escherichia coli* (ATCC 25312), were cultivated in Mueller Hinton Broth (MHB) and incubated at 37 ^{o}C for 24 hours. The turbidity of the broth was adjusted to a 0.5 McFarland standard to ensure uniform bacterial density. Using a cork borer, wells were created in the agar plates, which were then filled with 50 L of the plant extract. Negative control wells received 50% DMSO, while positive control wells were filled with

50% neomycin. After allowing for 15 minutes of diffusion, the Petri dishes were incubated for 18 to 24 hours at 37 °C. Following incubation, the zones of clearance were observed and measured to evaluate the antibacterial efficacy of the plant extract.

2.9 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined following the standard procedure described by Sarker et al. [26]. The bacterial inoculum was prepared to a final concentration of 106 CFU/mL by diluting the 0.5 McFarland turbidity culture in Mueller Hinton Broth (MHB) at a ratio of 1:100. A 5 L inoculum was then introduced into each well of a 96-well plate. Neomycin served as the positive control in this assay. After sealing the plate with a sterile lid, it was incubated at 37 °C for 20 to 24 hours. Following incubation, 0.003% resazurin was added to each well, and the plates were incubated for an additional 3 to 4 hours at 37 °C. Wells that remained blue indicated no bacterial growth, while those that turned pink signified the presence of bacterial growth. The lowest concentration of the extract at which bacterial growth was inhibited was recorded as the MIC. To ascertain the MBC, the contents of the wells were streaked onto nutrient agar plates, which were subsequently incubated at 37 °C for over 18 hours. The absence of bacterial colonies on the agar plates indicated the minimum bactericidal concentration of the crude plant extracts.

2.10 Brine Shrimp Lethality Assay (BSLA)

The toxicity of the plant extracts was assessed using a standard procedure [27]. The pH of the artificial seawater was adjusted to a range of 8 to 8.5 by adding 1M NaOH. Various concentrations of the plant extract were prepared, including 1000, 800, 500, 250, 100, and 10 μg/mL. Each test tube was filled with 4 mL of the prepared seawater solution. In duplicate, 10 nauplii were added to each test tube along with 500 V. μL of the respective plant extract. Potassium dichromate solution served as the positive control, while artificial seawater acted as the negative control. After 24 hours of incubation, the number of dead nauplii in each test tube was counted. The percentage of nauplii mortality was calculated using the following formula:

$$\% \text{ mortality} = \text{Number of dead nauplii/Total number of nauplii} \times 100$$

Y= mx + c is the linear equation that may be found using the Probit value table, where Y is the Probit value at 50% mortality, m is variable, and c is the intercept. Calculating the lethal concentration (LC_{50}). This method provided insight into the potential toxicity of the plant extracts on aquatic organisms.

2.11 Statistical Analysis

Data collection and analysis from the Gen5 Microplate Reader were conducted using Microsoft Excel. The results for total phenolic content (TPC) and total flavonoid content (TFC) were reported as mean ± standard deviation. For antioxidant activity, the findings were expressed as mean ± standard error of the mean (SEM). The inhibitory concentration (IC) values were computed using GraphPad Prism software (version 8.0.2.263). This analytical approach ensured a comprehensive evaluation of the data obtained from the experiments.

3 Results

3.1 Qualitative Phytochemical Analysis

The results of qualitative phytochemical screening of the extract of *V. jatamansi* Jones(in different solvents) are shown in **Table 2**.

Table 2: Qualitative phytochemical screening of plant extracts

Phytochemicals	**Test**	**Plant extracts**
Alkaloids	Dragendorff's test	-
Carbohydrates	Molish's test	+
Reducing sugars	Fehling test	-
Glycosides	Borntrager's test	+
Amino acids	Xanthoproteic test	+
Flavonoids	Alkaline reagent test	+
Phenols	$FeCl_3$ test	+
Tannins	Braymer's test	+
Terpenoids	Salkowski's test	+
Anthraquinones	Borntrager's test	+
Phytosterols	Salkowski's test	+

Note: (+) = present, (-) = absent

3.2 Total Phenolic Content (TPC)

The phenolic content of the various solvent extracts of *Valeriana jatamansi* is summarized in **Table 3**.

The methanolic extract exhibited the highest total phenolic content (TPC) value of 166.5 ± 2.0 mg GAE/g, while the hexane extract displayed the lowest TPC value of 16.03 ± 2.22 mg GAE/g, as indicated in the table below. These results highlight the significant variation in phenolic compound concentrations among the different solvent extracts.

Table 3: TPC values for various solvent extracts

Plant Extracts	TPC (mg GAE/g)
Aqueous	93.9 ± 3.89
Methanol	166.5 ± 2.0
Ethanol	105.66 ± 5.42
Ethyl acetate	74.55 ± 3.62
Dichloromethane	34.18 ± 2.74
Hexane	16.03 ± 2.22

3.3 Total Flavonoid content (TFC)

The flavonoid content of the various plant extracts is presented in **Table 4** below. The methanolic extract demonstrated the highest total flavonoid content (TFC) value of 103.87 ± 5.47 mg QE/g, while the hexane extract exhibited the lowest TFC value of 8.27 ± 3.88 mg QE/g. These findings indicate a significant variation in flavonoid concentrations across the different solvent extracts.

Table 4: TFC of different solvent extracts of *V. jatamansi* Jones

Plant Extracts	TFC (mg QE/g)
Aqueous	94.63 ± 6.11
Methanol	103.87 ± 5.47
Ethanol	93.87 ± 3.41
Ethyl acetate	54.93 ± 5.61
Dichloromethane	30.69 ± 4.41
Hexane	8.27 ± 3.88

3.4 Antioxidant Activity

The antioxidant activity of the plant extracts was assessed using the DPPH assay, with the IC values for the different solvent extracts of *Valeriana jatamansi* summarized in **Table 5**. The methanolic extract exhibited the lowest IC value of 103.3 ± 1.53 V μg/mL, indicating a strong antioxidant activity. In contrast, the hexane extract showed a significantly higher IC_{50} value of 524.7 ± 0.89 V μg/mL, reflecting lower antioxidant capacity compared to the other extracts. These results highlight the varying effectiveness of different solvents in extracting antioxidant compounds from the plant. The inhibition of radical scavenging activity is displayed in **Figure 2**.

Table 5: Antioxidant potential (IC_{50}) values of different solvent extract

Plant Extract	IC_{50} (g/mL)
Aqueous	104.3 ± 0.41
Methanol	103.3 ± 1.53
Ethanol	155.7 ± 0.34
Ethyl acetate	161.3 ± 1.14
Dichloromethane	323.8 ± 0.14
Hexane	524.7 ± 0.89
Quercetin (Standard)	3.49 ± 2.29

Note: Quercetin = Standard antioxidant

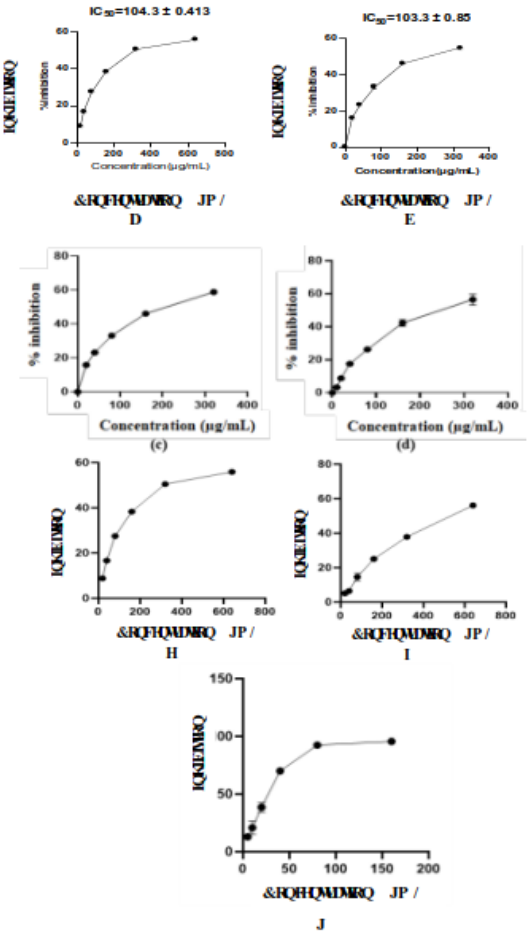

Figure 2: A plot showing % inhibition against the concentration of (a) aqueous (b) methanol, (c) ethanol, (d) ethyl acetate, (e) dichloromethane, (f) hexane extract, and (g) standard quercetin

3.5 Antibacterial Activity

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method, targeting both Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Shigella sonnei*, *Klebsiella pneumoniae*, and *Escherichia coli*). The results of the antibacterial tests are presented in **Ta-**

ble 6 and illustrated in **Figure 3**. Among the extracts, the aqueous, ethanolic, and hexane extracts exhibited the highest zone of inhibition against *Klebsiella pneumoniae*, measuring 17 mm. Conversely, the aqueous extract demonstrated limited antimicrobial activity against *Escherichia coli*, with a zone of inhibition of only 9 mm. These findings suggest varying levels of antibacterial efficacy among the different extracts against the tested microorganisms. For ease of interpretation, the antibacterial activities are displayed in the bar diagram in **Figure 4**.

Table 6: Zone of Inhibition (ZOI) of plant extracts against different bacteria

Plant Extracts	**Bacteria**	**ZOI of Sample (mm)**	**ZOI of Positive Control (Neomycin) (mm)**
Aqueous	*Klebsiella pneumoniae*	17	24
	Escherichia coli	9	22
	Shigella sonnei	15	24
	Staphylococcus aureus	15	23
Methanol	*Klebsiella pneumoniae*	15	24
	Escherichia coli	12	22
	Shigella sonnei	13	24
	Staphylococcus aureus	12	23
Ethanol	*Klebsiella pneumoniae*	17	24
	Escherichia coli	13	22
	Shigella sonnei	12	24
	Staphylococcus aureus	11	23
Ethyl acetate	*Klebsiella pneumoniae*	15	24
	Escherichia coli	12	22
	Shigella sonnei	15	24
	Staphylococcus aureus	13	23
Dichloromethane	*Klebsiella pneumoniae*	14	24
	Escherichia coli	15	22
	Shigella sonnei	11	24
	Staphylococcus aureus	10	23
Hexane	*Klebsiella pneumoniae*	17	24
	Escherichia coli	14	22
	Shigella sonnei	11	24
	Staphylococcus aureus	11	23

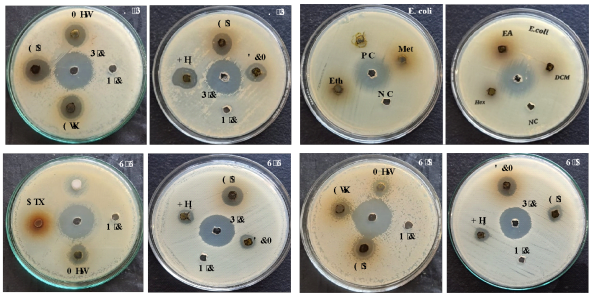

Figure 3: Antibacterial test slides against different bacterial strains, KP = *Klebsiella pneumoniae*, Met = Methanol, SS = *Shigella sonnei,* Eth = Ethanol, SA = *Staphylococcus aureus,* EA = Ethyl acetate, E. coli = *Escherichia coli,* DCM = Dichloromethane, Hex = Hexane, Aqu = Aqueous

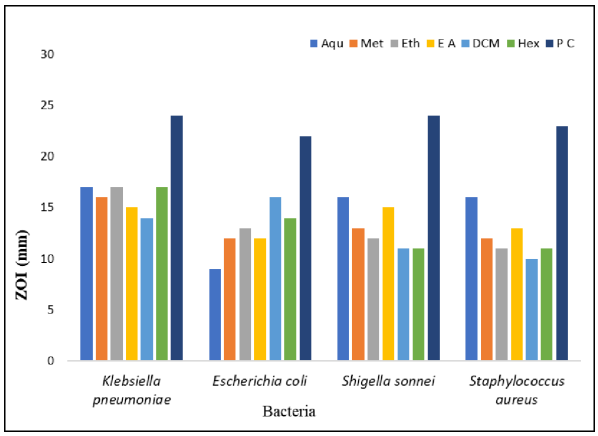

Figure 4: Bar diagram showing antibacterial activity (ZOI in mm) by different solvent extracts against *K. pneumoniae*, *E. coli*, *S. sonnei*, and *S. aureus*.

3.6 MIC and MBC

The study revealed that the ethanolic plant extract exhibited stronger antimicrobial effects than the methanolic extract, demonstrated by lower minimum bactericidal concentration (MBC) values for both *K. pneumoniae* (Gram-negative) and *S. aureus* (Gram-positive). Specifically, the minimum inhibitory concentration (MIC) and MBC values for both plant extracts against *K. pneumoniae* and *S. aureus* were 3.12 mg/mL and 12.5 mg/mL for the methanolic extract, and 3.12 mg/mL and 6.25 mg/mL for the ethanolic extract as shown in **Table 7**. This suggests that the ethanolic extract was more effective at a lower concentration. In contrast, the positive control, neomycin, showed far lower MIC and MBC values of 0.0039 mg/mL and 0.0078 mg/mL. This indicates that while the plant extracts do exhibit antimicrobial activity, they are much less potent compared to neomycin. However, the ethanolic extract still presents potential for antimicrobial applications due to its relatively lower MBC in comparison to the methanolic extract. The photographs of the experimental results are shown in **Figure 5** and **Figure 6**.

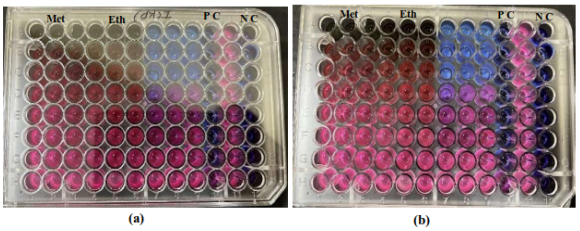

Figure 5: Well-Plates showing MIC values against (a) *K. pneumoniae* and (b) *S. aureus*, Met = Methanol, PC = Positive control, Eth = Ethanol, and NC = Negative

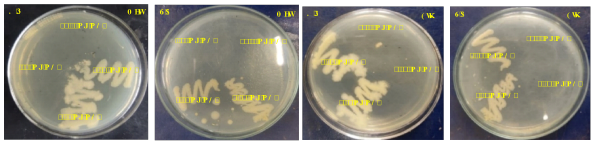

Figure 6: Petri plates showing MBC of plant extract against *K. pneumoniae* and *S. aureus*, KP = *Klebsiella pneumoniae,* Met = Methanol extract, SA = *Staphylococcus aureus,* Eth = Ethanol extract

3.7 Toxicity Analysis

The lethality of the plant extracts, measured by the LC_{50} values, showed that the ethanolic extract had the lowest LC_{50} at 1077.78 μg/mL, indicating greater toxicity to the brine shrimp nauplii than the methanolic extract, which had the highest LC_{50} at 1905.46 μg/mL **Table 8 and 9**. In this bioassay, 50% DMSO served as a negative control, in which all ten-brine shrimp nauplii survived, demonstrating that it had no toxic effects. Conversely, potassium dichromate, used as a positive control, proved highly toxic, as all ten nauplii exposed to it were dead. These findings suggest that the ethanolic extract is more toxic to brine shrimp compared to the methanolic extract, with the positive and negative controls confirming the validity of the assay.

Table 7: MIC and MBC values for plant extract

Plant Extracts	*Klebsiella pneumoniae*		*Staphylococcus aureus*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Methanol	3.12	12.5	3.12	12.5
Ethanol	3.12	6.25	3.12	6.25
Positive control	0.0078	0.015	0.0039	0.0078

Table 8: The number of survived nauplii after treatment with methanolic and ethanolic extracts and their percentage mortality

Plant Extract	Concentration (g/mL)	Total no. of survived nauplii	% Mortality
Methanolic extract	10	25	16.67
	100	23	23.33
	250	21	30
	500	20	33.33
	800	16	46.67
	1000	15	50
Ethanolic extract	10	28	6.67
	100	23	23.33
	250	21	30
	500	20	33.33
	800	18	40
	1000	17	43.33

Table 9: The toxicity of plant extracts for both methanolic and ethanolic extracts

Plant Extracts	Linear Regression Equation	LC50 (g/mL)
Methanolic	5.0 = 0.4554x + 3.4823	2137.96
Ethanolic	5.0 = 0.6306x + 2.9269	1905.46

This table highlights the dose-dependent effect of both methanolic and ethanolic extracts on brine shrimp mortality, with the methanolic extract demonstrating slightly higher toxicity than the ethanolic extract at higher concentrations. However, the LC_{50} values demonstrate the ethanol extract was found toxic than the methanolic extract.

4 Discussion

The study confirms that the methanolic extract of *Valeriana jatamansi* Jones is particularly rich in phenolic (166.5 ± 2.0 mg GAE/g) and flavonoid (103.87 ± 5.47 mg QE/g) compounds, which were significantly higher than those in other extracts (aqueous, ethanol, ethyl acetate, dichloromethane, and hexane). Phenolic compounds, known for their redox properties, contribute to antioxidant activity by acting as reducing agents, hydrogen donors, and singlet oxygen quenchers [28]. In previous research, the methanolic extract of *V. jatamansi* Jones roots exhibited a TPC of 187.13 ± 6.8 mg GAE/g and an IC_{50} value of 78 ± 2.9 g/mL. However, in this study, the IC_{50} for the methanolic extract was slightly higher at 103.3 ± 1.53 μg/mL, and the aqueous extract had an IC_{50} of 104.3 ± 0.41 μg/mL, showing improvement from its previous IC_{50} of 154 ± 4.6 μg/mL [29]. These variations from climatic and environmental factors affect antioxidant capacity, total phenolic content (TPC), and total flavonoid content (TFC) [29–31]. The collection site has a cold temperature, organic-rich soil, moderate water supply, and minimal light exposure. These conditions influence the biosynthesis of secondary metabolites, as supported by various studies. Environmental changes can affect the production of certain medicinal plant species because the concentration of secondary metabolites (SMs) in these plants is influenced by factors such as climate and ecological conditions [32]. Plant metabolism and life depend on light because of photosynthesis. Plants must therefore be able to detect the many light spectra found in solar radiation to survive [33]. Additionally, a wide variety of secondary metabolites in the intricate biochemical interaction are affected by light at varying intensities [34]. Temperature changes sig-

nificantly influence plant growth and the metabolic pathways involved in signaling, physiological control, and defense responses. When photosynthesis is disrupted due to extreme conditions, the temperature of the primary meteorological factors can greatly affect the composition of secondary metabolites [35]. The Pearson correlation coefficients provide valuable insights into the relationships between Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Antioxidant Activity (AA), allowing us to better understand how these factors interact with one another. The strong positive correlation between TPC and TFC ($r = 0.923$, $p = 0.009$) suggests that phenolics and flavonoids might share similar biosynthetic pathways. This relationship is statistically significant at the 0.01 level. However, the negative correlations between TPC-AA ($r = -0.835$, $p = 0.038$) and TFC-AA ($r = -0.922$, $p = 0.009$) indicate that other factors may influence antioxidant activity beyond just phenolic and flavonoid contents. TPC and AA relationship is statistically significant at the 0.05 level, whereas, TFC and AA relationship is statistically significant at the 0.01 level.

Regarding antimicrobial activity, previous work reported that the methanolic extract of *V. jatamansi* Jones achieved a zone of inhibition (ZOI) of 19 mm against *E. coli* and 21 mm against *Pseudomonas* [36]. In this study, the ethanolic and hexane extracts demonstrated the highest ZOI of 17 mm, nearing the efficacy of neomycin, the positive control. Ethyl acetate extract also showed moderate inhibition, with a 15 mm ZOI against *K. pneumoniae* and *S. sonnei*. Besides phenolics and flavonoids, other compounds like vitamins, carotenoids, saponins, enzymes, and minerals may also contribute to antimicrobial activity [37].

The study further investigated the antibacterial potency of methanolic and ethanolic extracts against *K. pneumoniae* and *S. aureus*, with MIC and MBC values of 3.12 mg/mL and 6.25 mg/mL, respectively, for the ethanolic extract, and 3.12 mg/mL and 12.5 mg/mL for the methanolic extract. These results are consistent with findings from earlier research, demonstrating that *V. jatamansi* Jones extracts possess robust antibacterial properties against these pathogens [38].

5 Conclusion

In conclusion, *Valeriana jatamansi* demonstrates significant medicinal potential due to its diverse phytochemical composition, which includes high levels of phenolics, flavonoids, and other bioactive compounds. Among the solvent extracts, the methanolic extract exhibited the highest total phenolic content (TPC) and total flavonoid content (TFC), correlating with its strong antioxidant capacity. In contrast, the hexane extract displayed the lowest TPC and TFC, alongside the weakest antioxidant potential. Antimicrobial activity assays revealed that the aqueous, ethanolic, and hexane extracts effectively inhibited *K. pneumoniae*, with the ethanolic extract showing superior minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values, indicating a potent antimicrobial effect. Additionally, the ethanolic extract's lower lethal concentration (LC_{50}) compared to the methanolic extract suggests enhanced toxicity, further highlighting its bioactivity. The comprehensive phytochemical and biological profile of *V. jatamansi* underscores its value as a promising candidate for drug discovery. Its diverse therapeutic properties make it a viable source for the development of new antimicrobial and antioxidant agents. Future studies are essential to fully explore the pharmacological potential and underlying mechanisms of action of *V. jatamansi* Jones, paving the way for its integration into modern medicinal applications.

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References

[1] A. Sofowora. Research on medicinal plants and traditional medicine in africa. *J. Altern. Complement. Med. N. Y. N*, 2(3):365–372, 1996.

[2] W. C. Evans. *Trease, and Evans' Pharmacognosy*. Elsevier Health Sciences, 2009.

[3] H. Yuan, Q. Ma, L. Ye, and G. Piao. The traditional medicine and modern medicine from natural products. *Mol. Basel Switz.*, 21(5):559, 2016.

[4] R. J. Molyneux, S. T. Lee, D. R. Gardner, K. E. Panter, and L. F. James. Phytochemicals: The good, the bad and the ugly? *Phytochemistry*, 68(22-24):2973–2985, 2007.

[5] A. King and G. Young. Characteristics and occurrence of phenolic phytochemicals. *J. Am. Diet. Assoc.*, 99(2):213–218, 1999.

[6] K. Bone and S. Mills. *Principles and Practice of Phytotherapy: Modern Herbal Medicine*. Elsevier Health Sciences, 2013.

[7] F. Bourgaud, A. Gravot, S. Milesi, and E. Gontier. Production of plant secondary metabolites: A historical perspective. *Plant Sci.*, 161(5):839–851, 2001.

[8] B. Mwamatope, D. Tembo, I. Chikowe, E. Kampira, and C. Nyirenda. Total phenolic contents and antioxidant activity of senna singueana, melia azedarach, moringa oleifera, and lannea discolor herbal plants. *Sci. Afr.*, 9:e00481, 2020.

[9] R. Al-Tohamy, S. S. Ali, K. Saad-Allah, M. Fareed, A. Ali, A. El-Badry, N. A. El-Zawawy, J. Wu, J. Sun, G.-H. Mao, and P. F. Rupani. Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from egyptian flora. *J. Appl. Biomed.*, 16(4):289–300, 2018.

[10] N. Tlili, W. Elfalleh, H. Hannachi, Y. Yahia, A. Khaldi, A. Ferchichi, and N. Nasri. Screening of natural antioxidants from selected medicinal plants. *Int. J. Food Prop.*, 16(5):1117–1126, 2013.

[11] M. M. Iwu, A. R. Duncan, and C. O. Okunji. New antimicrobials of plant origin. *Plant Biology*, 6:405–410, 1999.

[12] W. Guo, X. Li, X. Guo, and J. Ding. Effects of rhinoncomimus latipes on the growth and reproduction of persicaria perfoliata, an invasive plant in north america. *Biocontrol Sci. Technol.*, 21(1):35–45, 2011.

[13] I. D. Bhatt, P. Dauthal, S. Rawat, K. S. Gaira, A. Jugran, R. S. Rawal, and U. Dhar. Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of valeriana jatamansi jones. *Sci. Hortic.*, 136:61–68, 2012.

[14] A. K. Jugran, S. Rawat, I. D. Bhatt, and R. S. Rawal. Valeriana jatamansi: An herbaceous plant with multiple medicinal uses. *Phytother. Res. PTR*, 33(3):482–503, 2019.

[15] A. K. Maurya and V. K. Agnihotri. Valeriana jatamansi: Bioactive compounds and their medicinal uses. *Curr. Top. Med. Chem.*, 24(9):757–796, 2024.

[16] S. Thusoo, S. Gupta, R. Sudan, J. Kour, S. Bhagat, R. Hussain, and M. Bhagat. Antioxidant activity of essential oil and extracts of valeriana jatamansi roots. *BioMed Res. Int.*, 2014:1–4, 2014.

[17] E. Iqbal, K. A. Salim, and L. B. L. Lim. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of goniothalamus velutinus (airy shaw) from brunei darussalam. *J. King Saud Univ. - Sci.*, 27(3):224–232, 2015.

[18] R. Gul, S. U. Jan, S. Faridullah, S. Sherani, and N. Jahan. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from ephedra intermedia indigenous to balochistan. *Scientific World Journal*, 2017:5873648, 2017.

[19] S. Bhandari, K. Khadayat, S. Poudel, S. Shrestha, R. Shrestha, P. Devkota, S. Khanal, and B. P. Marasini. Phytochemical analysis of medicinal plants of nepal and their antibacterial and antibiofilm activities against uropathogenic escherichia coli. *BMC Complement. Med. Ther.*, 21(1):116, 2021.

[20] X. Lu, J. Wang, H. M. Al-Qadiri, C. F. Ross, J. R. Powers, J. Tang, and B. A. Rasco. Determination of total phenolic content and antioxidant capacity of onion (allium cepa) and shallot (allium oschaninii) using infrared spectroscopy. *Food Chem.*, 129(2):637–644, 2011.

[21] I. A. Ahmed, M. A. Mikail, M. Bin Ibrahim, N. Bin Hazali, M. S. B. A. Rasad, R. A. Ghani, R. A. Wahab, S. J. Arief, and M. N. A. Yahya. Antioxidant activity and phenolic profile of various morphological parts of underutilised *Baccaurea Angulata* fruit. *Food Chemistry*, 172:778–787, 2015.

[22] T. H. A. Alabri, A. H. S. Al Musalami, M. A. Hossain, A. M. Weli, and Q. Al-Riyami. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura Metel* l. *J. King Saud Univ. - Sci.*, 26(3):237–243, 2014.

[23] L.-F. Shyur, J.-H. Tsung, J.-H. Chen, C.-Y. Chiu, and C.-P. Lo. Antioxidant properties of extracts from medicinal plants popularly used in taiwan. *Int. J. Appl. Sci. Eng.*, 3(3):195–202, 2005.

[24] M. Balouiri, M. Sadiki, and S. K. Ibnsouda. Methods for *in Vitro* evaluating antimicrobial activity: A review. *J. Pharm. Anal.*, 6(2):71–79, 2016.

[25] T. C. Abbey and E. Deak. What's new from the clsi subcommittee on antimicrobial susceptibility testing m100, 29th edition. *Clin. Microbiol. Newsl.*, 41(23):203–209, 2019.

[26] S. D. Sarker, L. Nahar, and Y. Kumarasamy. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in Vitro* antibacterial screening of phytochemicals. *Methods*, 42(4):321–324, 2007.

[27] Y. Baravalia, Y. Vaghasiya, and S. Chanda. Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of *Woodfordia Fruticosa* kurz flowers. *Iran. J. Pharm. Res. IJPR*, 11(3):851–861, 2012.

[28] L. R. Fukumoto and G. Mazza. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.*, 48(8):3597–3604, 2000.

[29] S. Thusoo, S. Gupta, R. Sudan, J. Kour, S. Bhagat, R. Hussain, and M. Bhagat. Antioxidant activity of essential oil and extracts of *Valeriana Jatamansi* roots. *BioMed Res. Int.*, 2014:1–4, 2014.

[30] S. Rawat, H. Andola, L. Giri, P. Dhyani, A. Jugran, I. D. Bhatt, and R. S. Rawal. Assessment of nutritional and antioxidant potential of selected vitality strengthening himalayan medicinal plants. *Int. J. Food Prop.*, 17(3):703–712, 2014.

[31] A. Jugran, S. Rawat, P. Dauthal, S. Mondal, I. D. Bhatt, and R. S. Rawal. Association of issr markers with some biochemical traits of *Valeriana Jatamansi* jones. *Ind. Crops Prod.*, 44:671–676, 2013.

[32] P. Pant, S. Pandey, and S. Dall'Acqua. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. *Chem. Biodivers.*, 18(11):e2100345, 2021.

[33] K. Kazan and J. M. Manners. The interplay between light and jasmonate signalling during defence and development. *J. Exp. Bot.*, 62(12):4087–4100, 2011.

[34] J. Rozema, J. Van De Staaij, L. O. Björn, and M. Caldwell. Uv-b as an environmental factor in plant life: Stress and regulation. *Trends Ecol. Evol.*, 12(1):22–28, 1997.

[35] S. Pandey, M. Carrer, D. Castagneri, and G. Petit. Xylem anatomical responses to climate variability in himalayan birch trees at one of the world's highest forest limit. *Perspect. Plant Ecol. Evol. Syst.*, 33:34–41, 2018.

[36] Z. Arshad, P. Nautiyal, S. Panwar, K. Anshika, and N. Gaurav. Phytochemical analysis, antimicrobial activity and antioxidant activity of *Valeriana Jatamansi. Int. J. Pharmacogn. Life Sci.*, 4(2):01–11, 2023.

[37] G. G. F. Nascimento, J. Locatelli, P. C. Freitas, and G. L. Silva. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz. J. Microbiol.*, 31(4), 2000.

[38] J. J. M. J. Al Zuhairi, F. Jookar Kashi, A. Rahimi-Moghaddam, and M. Yazdani. Antioxidant, cytotoxic and antibacterial activity of *Rosmarinus Officinalis* l. essential oil against bacteria isolated from urinary tract infection. *Eur. J. Integr. Med.*, 38:101192, 2020.