International Journal of Pharmacognosy and Life Science

E-ISSN: 2707-2835
P-ISSN: 2707-2827
www.pharmacognosyjournal.com
IJPLS 2023; 4(2): 01-11
Received: 01-04-2023
Accepted: 03-05-2023

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Phytochemical analysis, antimicrobial activity and antioxidant activity of *Valeriana jatamansi*

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DOI: https://doi.org/10.33545/27072827.2023.v4.i2a.85

Abstract
Valeriana jatamansi, formerly known as *Valeriana wallichii*, is a rhizome herb of the genus Valeriana and the family Valerianaceae also called Indian Valerian or Tagar-Ganthoda, The genus Valeriana, with about 200 species, belongs to the family Valerianaceae and has a distribution throughout the world. The Indian Valerian has long been used in Ayurveda (Charak, Samhita, and Susruta) and Unani systems of medicine, which describe its use in obesity, skin disease, insanity, epilepsy and snake poisoning. The crude drugs from roots/ rhizomes and Valerian-derived phytomedicines are used as mild sedatives in the pharmaceutical industry. The activity is largely attributed to the presence of valepotriates. Plant extracts from medicinal plants had been used for many centuries, to treat several health disorders. The active constituent present in the plant plays a remarkable role in curing diseases. In this aspect, *Valeriana jatamansi* is widely used in the indigenous system of medicine. Different parts of the plant root, rhizome, seed and flower have medicinal values. The root of the plant is specifically rich in aroma. The active constituents present in the plant are alkaloids, flavonoids, saponins, tannins and essential oil.

Keywords: *Valeriana wallichii*, Indian Valerian, Ayurveda, Unani systems of medicine, phytomedicines, medicinal values, etc.

Introduction
Valeriana jatamansi is a perennial herb with a cylindrical rhizome, covered with brown to deep grayish fibers, rootstock woody, long and stout, covered with fibers from the petioles of withered leaves. Stem 10-60 cm, more or less pubescent upwards, often glaborate below, sub scapose. Adventitious roots thin, branched and red to brown in color. Flower rosy, pale pink or blue in dense cymes. Flower-heads usually 1, bracts 3 or 5, 6 mm long, usually pubescent corolla-tube 6 mm long somewhat hairy within, as are the filaments below. Fruit 4 mm long, covered with ascending white hairs, crowned by the ovate, acute, often dentate, calyx-teeth. Odor slight and aromatic and taste is acrid, slightly bitter and aromatic (Quan, *et al.*, 2020; Bhatt, *et al.*, 2012) [3, 1].

The different bio-chemical substances main active constituents are sesquiterpenes, coumarins, iridoids, organoids, and sesquiterpenes include valerenic acid, and other derivatives including valeranone and valerenal. Moreover, organoids include pinoresinol-4-o-d-glucoside and lignans 8'-hydro-xypinoresinol. Iridoids include valepotriates (Valtrate, didrovaltrate, acevaltrate, and isovaleryl hydroxyvalerate). Alkaloids include chatinine, nordel porphine, morpholine, thaliperphine, nantenine, phenanthrene, phoebine, dehydroaphine, valerine, valeriane, and oxoaporphine. Flavonoids in Valeriana mainly contain acacetin, hesperidin and methylapigenin, diosmetin, luteolin, quercetin, kaempferol, linarin, and luteolin. The other constituents are volatile oil, essential oil, sugar, bitter extractive matter, starch, gum, resin, and ketones (Tan, *et al.*, 2016) [2].

The genus Valeriana belonging to the family Valerianaceae contains around 250 types of species which is distributed in all temperate regions of world. Among them in India, 16 species were found and out of them two subspecies of genus and five species in habitat at higher altitude range of Central Himalayas. The most popular name for this genus is "Valeriana." *Valeriana jatamansi* is commonly known as India Valerian, Muskbala, Sugandhbala (Hindi), and Tagar (Sanskrit) (Bhattacharya, *et al.*, 2000, 2009) [4]. Indian Valeriana is a small herbaceous species distributed in tropical and temperate Himalaya up to

an altitude of 3000 m between 1500 and 1800 m in Khasi and Jaintia hills; this has been used as an ingredient for herbal medicine in Indian system of medicine and substitutes for European Valeriana officinalis in India. Geographically, this species is available in different temperate regions and has diverse genetic and morphological features, which will affect the level of active constituents present (Jugran, *et al.*, 2013) [6]. *V. jatamansi* is a hairy dwarf and rhizomatous perennial herb growing up to 0.5 m with thick root stacks, covered with horizontal descending fibers and pubescent stem and radical leaves 1–3 cm in diameter. Flowers are white or tinged in pink color, fruits are crowned by persistent pappus calyx. This species is gynodioecious, the fruiting and flowering time occurs during the period of March–June and mode of proliferation is both sexual through seeds and abiogenetic through rhizome. *V. jatamansi* used in Ayurvedic and Unani system of medicine. The roots and rhizomes are used to treat insomnia, blood and circulatory disorders, asthma, dry cough, jaundice, seminal weakness, cardiac debility, and skin diseases. Its herbal oil is widely in use for making perfumery preparation and in insect-repellent formulation. The *V. jatamansi* root contains 0.8% of essential oil and used in pharmaceutical industries and in hair preparations (Rajkumar, *et al.*, 2013; Sundaresan, *et al.*, 2012) [7, 8].

Plant extracts from medicinal plants had been used for many centuries, to treat several health disorders. The active constituent present in the plant plays a remarkable role in curing diseases. In this aspect, *Valeriana wallichii* is widely used in the indigenous system of medicine. It is also known as *Valeriana jatamansi*. Different parts of the plant root, rhizome, seed and flower have medicinal values. The root of the plant is specifically rich in aroma. The active constituents present in the plant are alkaloids, flavonoids, saponins, tannins and essential oil. The therapeutic action of the plant is due to presence of major chemical constituent flavonoids. The herb is beneficial in treating insomnia, nervous problems, snakebite, hysteria and also as analgesics. Many pharmacological activities *viz*., anti-inflammatory, antispasmodic, antipsychotic, antimicrobial, anthelmintic, antioxidant, cytoprotective and had been reported in different plant extracts. Based on many scientific researches this article is reviewed to reveal the therapeutic aspects of the herb for the benefit for further research (Raina and Negi, 2015) [9].

Medicinal uses of Jatamansi

Natural brain nervine tonic and a memory enhancer, which has calming, peacefulness and relaxation features. it is an endangered ayurvedic medical herb had been used since the ancient times for many medicinal purposes.in the market, it is available in the form of root, oil, and powder. *Valerian Jatamansi* is a known calming herb in ayurveda and unani because of its medicinal values a number of studies have been done for its efficacy in respect of nervous system. in ayurveda, it is prescribed against stress, spasm, epilepsy, convulsion and hysteria. in fact, it is one of the excellent herbs to treat epilepsy (Sahu, *et al.*, 2016; Pant, *et al.*, 2021, 2022) [10, 17].

1. The essential oil, known as spike nard oil, possesses antiarrhythmic activity with possible therapeutic useful to treat auricular flutter.
2. The oil exerts a hypotensive effect and in moderate doses it has a distinct depressant action on the central nervous system.
3. The volatile oil from the rhizomes is very effective in leprusy.
4. The rhizome in combination with other drugs, is prescribed in snake bite and scorpion-sting
5. Jatamansi have been traditionally used in treatment of wide range of disorders, which include digestive system, circulatory system, nervous system, respiratory system, urinary system, reproductive system and skin diseases.
6. It is used to impart black colour to hair and prevents greying of hair. its medicated oil with almond is highly useful for smooth and silky hair.
7. It is beneficial for hyperactive children and helpful to reduce hyperactivity, restlessness and aggressiveness.
8. It is therapeutically very important, alleviates pain and swelling and shows the properties like carminative and aromatic.
9. It has hepatoprotective characteristics thus useful in hepatitis, prevent enlargement of liver and jaundice.
10. The oil helps to relax and calm the body and mind thereby ensures to relieve from headache and migraine.
11. Its underground stem is used in preparation of powerful aromatic essential oil.
12. The root of the plant is used in making of oil to treat insomnia and birth-related problems.
13. It is used as analgesic and diuretic in unani.
14. Used as making of perfumes and dies.
15. The root of the powder is used to treat intestinal worms.

Phytochemical constituents

V. jatamansi main active constituents are sesquiterpenes, coumarins, iridoids, organoids, and sesquiterpenes include valerenic acid, andother derivatives include valeranone and valerenal. Moreover, organoids include pinoresinol-4-o-d-glucoside and lignans 8'-hydro- xypinoresinol. Iridoids include valepotriates (valtrate, didrovaltrate, acevaltrate, and isovaleryl hydroxyvalerate). Alkaloids include chatinine, nordelporphine, morpholine, thaliperphine, nantenine, phenanthrene, phoebine, dehydroaphine, valerine, valeriane, and oxoaporphine. Flavonoids in Valeriana mainly contain acacetin, hesperidin and methylapigenin, diosmetin, luteolin, quercetin, kaempferol, linarin, and luteolin. The other constituents are volatile oil, essential oil, sugar, bitter extractive matter, starch, gum, resin, and ketones (Yang, *et al.*, 2011; Patan, *et al.*, 2018) [11, 12].

Antioxidant Activity

Antioxidant activity Methanol, and distilled water extracts of the dried roots of *V. jatamansi* were extracted and assessed for their polyphenol and flavonoid content. The antioxidant activity of the two solvent extracts of *V. jatamansi* roots (100 µg/ml) and was assessed by 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH). In another study, the antioxidant activity and anti-inflammatory activity were studied by hydroalcoholic extracts of *V. jatamansi* using DPPH free radical scavenging and the percentage (%) inhibition of proteins denaturation, respectively, using diclofenac sodium as the reference standard. A substantial decrease of various mediators of inflammation was seen due to the presence of bioactive compounds such as flavonoids, tannins, and polyphenols.

The percentage antioxidant activity (AA%) of the root extract was obtained using the DPPH (2, 2-diphenyl-1-

picryl-hydrazyl) radical absorbance assay, according to the procedure described by (Choi *et al*.) with some changes. The reaction mixture contained sample and DPPH in ethanol at different concentrations (25, 50, 75, 100, µg/mL). When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The positive control was ascorbic acid at the same sample concentration. The changes in colour (from deep violet to light yellow) were read (Absorbance (Abs)) at 518 nm after 30 min of reaction using a UV-VIS spectrophotometer. The reaction occurred in 30 minutes, and soon after that the absorbance was read in the spectrophotometer at 518 nm (Thusoo, *et al.*, 2014) [13].

Antibacterial activity

The antibacterial activity was explored using methanol and distilled water extracts from rhizomes of *V. jatamansi*. Extended spectrum B-lactamase produced by Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae. The inhibition was significant with methanol and distilled water extract and can be given along with other antibiotic. The methanol, and distilled water extracts of *V. jatamansi* were evaluated for their antimicrobial effect against strains Pseudomonas aeruginosa, *E. coli*. Among them, the hydroalcoholic extracts with doses of 0.3–0.7 mg/ml shown better antibacterial activity against S. aureus, P. aeruginosa by cup plate method using Soyabean Casein Digest Agar medium, and chloramphenicol was standard antibiotic used at 1 mg/ml (Habeeb, *et al.*, 2016) [14].

Phytochemical Analysis

The root extracts of *Valeriana jatamansi* in various solvents are investigated for their phytochemical composition, using various qualitative tests.

The plant extract was assessed for the existence of cyanogenic glycosides, phenols, tannins, anthocyanins, proanthocyanidins, flavonoids, catechins, steroids, triterpenoids, saponins, resins, alkaloids, and quaternary bases by the phytochemical analysis (screening) using typical standard methods (Wang, *et al.*, 2017; Saini, *et al.*, 2022) [15, 18].

Material and Methods

Preparation of Plant Extract

1. Add 5gm of rhizomes powder in 100ml Ethanol or Distilled water.
2. These mixtures were shaking in rotatory shaker for 18 hours.
3. Filter the solvent with the help of Whattman Filter paper.
4. Collect it in beaker
5. The filter obtains kept on water bath/ Hot air oven at 40-45 degree Celsius for evaporation when solvent will evaporate at last when extract will remain in beaker.
6. Collect in air tight vials and store at 4 degree Celsius.

Phytochemical Analysis

In plants the naturally occurring chemical compounds are phytochemicals. They give organoleptic properties and colour to the plant. In many places, as a dietary accessory they are comfortably approachable but dormant health advantages of phytochemicals are only reachable from the utilization of whole plant. Phytochemicals are beneficial to boost up immunolatory responses and also provide immunity against many diseases. Some phytochemicals are known to reveal medicinal and physiological activities which are phenols, tannins, flavonoids, saponins, carbohydrates, alkaloids, phytosterols etc. Therapeutic or curing activities of plants were conventionally proclaimed to have medicinal properties by small researchers. In worldwide medicinal plants the presence of phytochemicals checked in recent researches. Anti-inflammatory and anti-nociceptic activities of Variance, are the significant properties of conventional medicinal plants against many pathogens. So because of the presence of bioactive constituents medicinal plants show these medicinal properties

Phytochemical screening

The test were done to find the presence of the active chemical constituents such as alkolids, glycosides, terpanoids, and steroids, flavonoids, reducing sugar and tannin by following procedure:

Proteins: Biruet test to 2ml of the test solution added 5 drops of 1% copper sulphate solution and 2ml of 10% NaoH. Mix thoroughly. Formation of purple or violet colour confirmed proteins.

Tannins: Small amount of extract was mixed with water and heat on water bath. The mixture was Filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

Anthraquinones: 1ml of extract was boiled with 10% HCL for few minutes in water bath. It was filtered and allowed to cool, Equal volume of CHCL3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heat. Formation of rose-pink colour indicate the presence of anthraquinones.

Glycosides: Glycolysis are compound which upon hydrolysis give rise to one or more sugar (glycogen) and a compound which is not a sugar (a glycine). The extract was hydrolysed with HCL solution and neutralised with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides.

Reducing Sugar: The extract was shaken with distilled water and filtered. The filtrate was boiled with few drops of Fehling's solution A and B for 5 minutes. An orange red precipitates forms.

Flavonoids: 1ml of extract was dissolved in diluted NaOH and HCL was added. A yellow solution that turns colourless indicates the presence of flavonoids.

Phlobatannins: 1 ml of extract was dissolved in distilled water and filtrated. The filtrate was boiled with 2% HCL solution. Red precipitate shows the presence of phlobatannins.

Amino Acids: 1ml of extract was treated with few drops of ninhydrin reagents. Appearance of purple colour shows the presence of amino acids.

Terpenoids (Salkowski) Test: 1ml of extract was mixed with 2ml of chloroform (CHCL3) and concentrated H2So4 was carefully added to form a layer. A reddish brown

colouration of the interface was formed to indicate positive results for the presence of terpenoids.

Screening of Antimicrobial Activity

Chemical used for analysis of antimicrobial activity: Nutrient agar media, nutrient broth, disc

Test organisms: *Pseudomonas* and *E.coli*

Preparation of (Medium Nutrient Broth)

Composition	Amount for ml	Amount for 100ml
Beef Extract	3.00gm	0.30gm
Peptone	5.00gm	0.50gm
NaCl	5.00gm	0.50gm
Distilled Water	1000ml	100ml
pH	7.0	7.0

After mixing the chemicals shown in the table above, the nutrient agar media was autoclaved at 15lbs, and 121degree Celsius for 15minutes.

Preparation of Bacterial Inoculum: Prepared nutrient broth is transferred into sterile test tubes, each test tube was containing 10ml of broth. All work is done in Laminar air flow for maintaining sterilized conditions. Different bacterial cultures were taken from the already cultured plates by using sterile inoculating loop and inoculate into the separate test tubes. Inoculated test tubes were incubated at 37 degree celcius in BOD incubator for 24 hours.

Test for Antimicrobial Properties: For antimicrobial test against bacteria two methods used:

Preparation of different concentrations of extracted sample: Two samples of dried extract were taken weighing 0.1gm, 0.2 gm, 0.3 gm, 0.4 gm. Each sample was dissolved in 10 ml of distilled water.

A. Procedure for performing the disc diffusion test

1. First made the nutrient agar plates.
2. Approx. 100ul of inoculum was poured on the plates by micropipette.
3. Culture was spread by a glass spreader.
4. Whatman disc were prepared.
5. A disc of control and four discs of different concentrations of sample were loaded on each plate.
6. Plates were then incubated 37 degree Celsius for overnight.
7. The zones of inhibition was observed and recorded against control.

B. Procedure for performing the Agar well diffusion method

1. First made the nutrient agar plates.
2. Approx. 100ul of inoculum was poured on the plates by micropipette.
3. Culture was spread by a glass spreader.
4. 5 wells in each plate were prepared by using the tip of micropipette.
5. A control and four different concentration of samples were loaded in the wells.
6. Plates were then incubated 37 degree Celsius for overnight.
7. The zone of inhibition was observed and recorded against control.

Antioxidant Properties

Valeriana jatamansi, formerly known as *Valeriana wallichii*, is a rhizome herb of the genus Valeriana and the family Valerianaceae also called Indian Valerian or Tagar-Ganthoda, not to be confused with ganthoda, the root of Indian long pepper. It is a herb useful in Ayurvedic medicine used as an analeptic, antispasmodic, carminative, sedative, stimulant, stomachic, and nervine. The present study is aim to understand the plant's role and the value as an antioxidant. Antioxidant are gaining importance due to the increase in the reactive oxygen species generated in our cells due to the modern lifestyle. Along with being an antidiabetic drug, if the extract are found to be potent antioxidant, its pharmacological value would greatly increase. All the chemicals, reagents and solvents used in this study were of analytical grade and produced locally 3.15 DPPH Radical**.**

Scavenging Assay

Antioxidant Activity: 1ml of four concentrations (25, 50, 75, 100 mg/ml) of each extract of the plant dissolved in methanol was added to 1ml DPPH in methanol with 1ml pure methanol was used as a control (Figure 11&12). The reaction mixture was mixed and incubated in the dark at room temperature for 2 hours. Their absorbance was then read at 517 nm. The DPPH scavenging ability of plant extracts was calculated using the following equation.

$$\text{Antioxidant activity (\%)} = \frac{(A_0 \quad A_1)}{A0 \text{ X } 100}$$

(Where A_0 is the absorbance of control and A1 is the absorbance of Sample)

Result and Discussions

Antimicrobial Activity of *Valeriana jatamansi* through Kirby- Bauer method

1. Bacteria: Pseudomonas

Control: Streptomycin

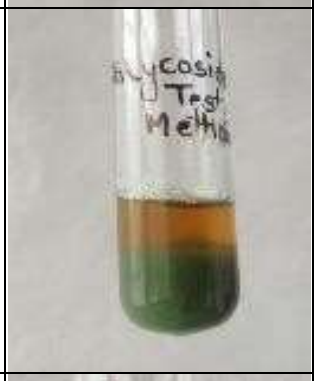
Extract	Disc (In mm)	Conc. (10 ul)	Conc. (20 ul)	Conc. (30 ul)	Conc. (40 ul)	Control
Methanol Extract	5.5 mm	20 mm	16 mm	16 mm	15 mm	30 mm
Distilled Water	5.5 mm	16 mm	18 mm	21 mm	25 mm	25 mm

2. Bacteria: *E. coli*

Control: Tetracycline

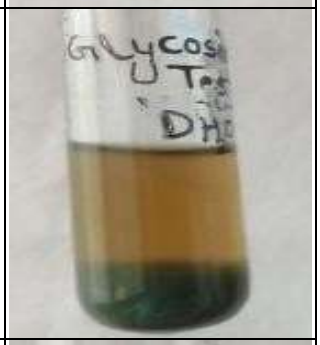
Extract	Disc (in mm)	Conc. (10 ul)	Conc. (20 ul)	Conc. (30 ul)	Conc. (40 ul)	Control
Methanol Extract	5.5 mm	13 mm	15 mm	12 mm	11 mm	19 mm
Distilled Extract	5.5 mm	13 mm	15 mm	14 mm	18 mm	20 mm

Table 1: Phytochemical Tests (Methanol extract)

S NO.	Tests	Colour	Result	Photos
1.	Proteins Test	Brown colour	Negative	
2.	Tannins Test	Dark green	Positive	
3.	Anthraquinones Test	No colour change	Negative	
4.	Glycosides Test	Yellow colour	Positive	
5.	Reducing Sugar Test	Green precipitates	Negative	

6.	Flavonoids Test	Light orange colour	Negative	
7.	Phlobatannins Test	No colour change	Negative	
8.	Amino Acids Test	No colour change	Negative	
9.	Terpenoids Test	Reddish brown colour	Positive	

Table 2: Phytochemical Tests (Distilled Water extract)

S. No.	Tests	Colour	Result	Photos
1.	Proteins Test	Brown colour	Negative	
2.	Tannins Test	Dark green	Positive	
3.	Anthraquinones Test	No colour change	Negative	
4.	Glycosides Test	Yellow colour	Positive	
5.	Reducing Sugar Test	Green precipitates	Negative	

6.	Flavonoids Test	Light orange colour	Negative	
7.	Phlobatannins Test	No colour change	Negative	
8.	Amino Acids Test	No colour change	Negative	
9.	Terpenoids Test	Reddish brown colour	Positive	

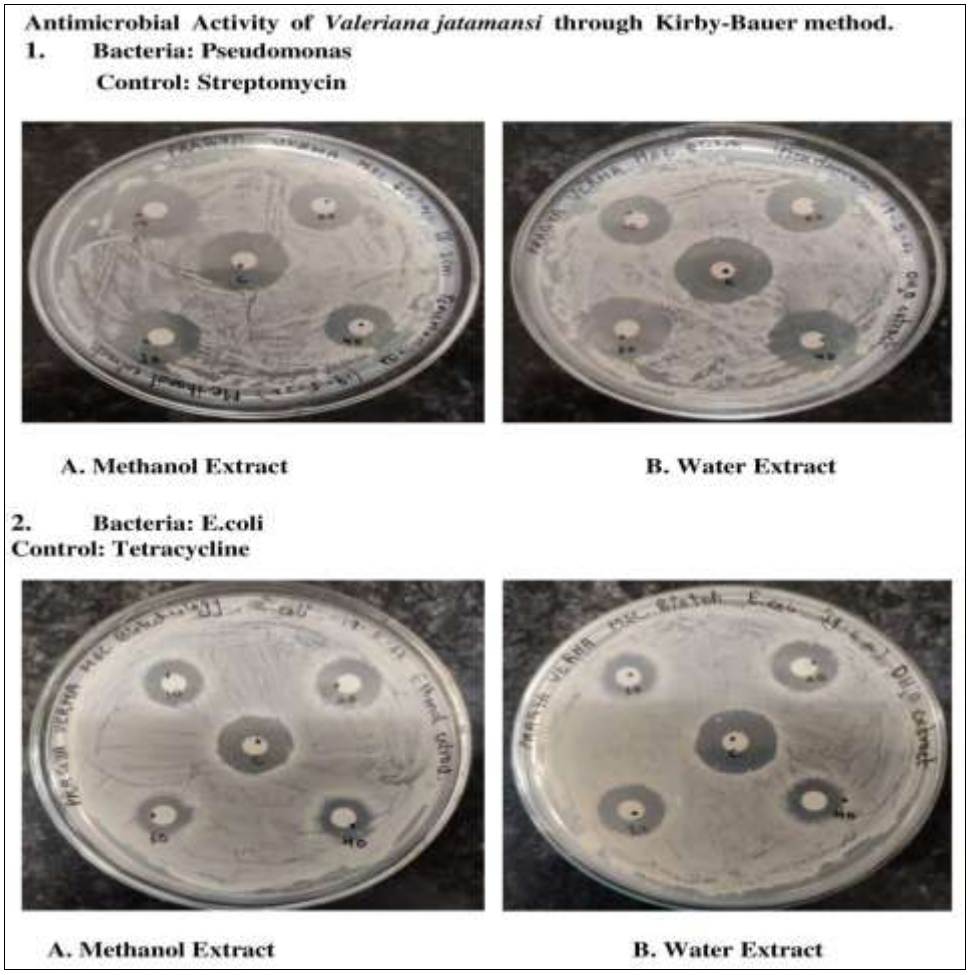


Fig 1: Antimicrobial Activity of *Valeriana jatamansi* through Kirby- Bauer method.

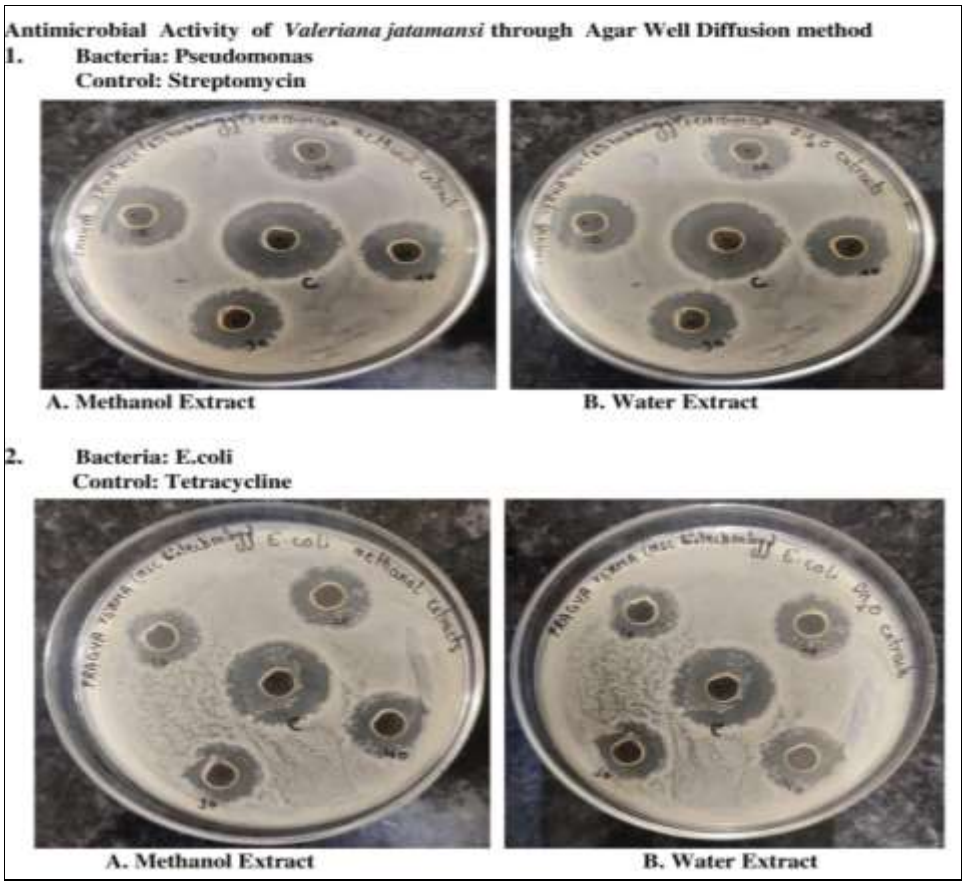


Fig 2: Antimicrobial Activity of *Valeriana jatamansi* through Agar Well Diffusion method.

Antimicrobial Activity of *Valeriana jatamansi* through Agar Well Diffusion method

1. Bacteria: Pseudomonas Control: Streptomycin

Extract	Disc (in mm)	Conc. (10 ul)	Conc. (20 ul)	Conc. (30 ul)	Conc. (40 ul)	Control
Methanol Extract	5.5 mm	21 mm	20 mm	19 mm	19 mm	27 mm
Distilled Extract	5.5 mm	19 mm	21 mm	21 mm	22 mm	26 mm

2. Bacteria: *E. coli* Control: Tetracycline

Extract	Disc (in mm)	Conc. (10 ul)	Conc. (20 ul)	Conc. (30 ul)	Conc. (40 ul)	Control
Methanol Extract	5.5 mm	16 mm	15 mm	17 mm	19 mm	25 mm
Distilled Extract	5.5 mm	19 mm	21 mm	21 mm	22 mm	26 mm

Antioxidant Activity of *Valeriana jatamansi*
Control: Ascorbic Acid OD

Conc. (mg/ml)	Methanol Extract (absorbance at 517 nm)	Distilled Water Extract (absorbance at 517 nm)
25	0.039	0.097
50	0.392	0.283
75	0.405	0.469
100	0.409	0.597

A. Antioxidant activity B. Antioxidant activity on On methanol extract Distilled water extract

C. DPPH

Fig 3: Antioxidant Activity of *Valeriana jatamansi*

Conclusion

Valeriana jatamansi is also known as *Valeriana wallichii*, is a rhizome herb of the genus Valeriana and the family Valerianaceae also called Indian Valeriana or Tagar-Ganthoda.

It is an herb used in Ayurveda (Charak, Samhita and Susruta) and Unani systems of medicine. It is use in obesity, skin disease, snake poisoning. The herb is having a life cycle lasting more than two years and grows to four feet with pinnate divided leaves and clusters of small white or pink flowers. It has a massive root system and short rhizomes. The root are hairy, spindly mass and are collected in the autumn from two years old plants. The root of *valeriana wallichii* contains alkaloids, tannins, flavonoids saponin glycosides in the methanolic extract and it was used for Pharmacognostical, Phytochemicals evaluation.

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