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Antimicrobial, antioxidant and anti-inflammatory properties of Valeriana jatamansi and Nardostachys jatamansi

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ABSTRACT

For a long period of time, plants have been a valuable source of natural products for maintaining human health. In the last decade, there has been a more intensive study for natural therapies. The use of plant compounds for pharmaceutical purposes is gradually increasing in the world. The Himalaya is credited all over the world as a treasure of medicinal and aromatic plants, which in turn prove as a treasure of bioactive agents. Among these, a good fraction of species is critically endangered. Valerian originated from the Latin word "Valere" meaning 'to be in good health'. Valerian and Nardostachys (a substitute) belong to the Valerianaceae (now Caprifoliaceae) family. Due to similar species name and some common components, these plants are used as the substitute of each other. The root extracts of Valeriana jatamansi and Nardostachys jatamansi in various solvents are investigated for their antimicrobial property using the agar diffusion method. The antioxidant properties of both plant root extracts are analyzed using DPPH assay. The root extracts are further subjected to Lipoxygenase enzyme inhibition assay (a kit based method) to determine the anti-inflammatory properties. Methanolic extracts of Valerian show better antimicrobial effect compared to Nardostachys. It also shows the better anti-oxidant property. In contrast, antifungal and the anti-inflammatory property are shown by Nardostachys is better as compared to Valerian. According to the results obtained, it can be concluded that the plants cannot be really used a substitute; as they show different properties.

Keywords— *Valerian, Jatamansi, Antimicrobial, Anti-Inflammatory, Anti-oxidant*

1. INTRODUCTION

Worldwide herbal medicines have been used traditionally for the prevention and treatment of various diseases; as these are a rich source of bioactive phytochemicals and bionutrients. Many of these products complement conventional pharmaceuticals in the treatment and prevention of diseases.[1] The herb *Valerian* is found in the Himalayan region of India, Nepal, Bhutan, Burma, Pakistan, and Afghanistan.[2] *Valerian* and *Nardostachys* both have similar common names and the nomenclature is always confusing. *Valerian* is used for its calming and antispasmodic properties and is taken in the form of tea, tincture, capsules or tablets to treat nervousness and insomnia, muscle spasms and pain.[3] *Nardostachys* is used in perfumery, hair and massage oils and is also beneficial to treat disorders of digestive and respiratory systems.[4] Due to similar species name and some common components, these plants are used as a substitute of each other. [5]

However, the Antimicrobial activity needs to be evaluated and confirmed for these plants. The present study evaluates the Antibacterial and Antifungal potential of root extracts of both the plants. Anti-oxidant property of the extracts is analyzed using DPPH radical scavenging assay. The anti-inflammatory properties are estimated using Lipoxygenase inhibition test (a kit based method). The comparative study is carried out to determine the efficiency of both the plant root extracts. The results show a difference in properties of both these plants which suggests that these plants cannot be used as a substitute to each other.

2. MATERIALS AND METHODS

- **Plant material**: The authenticated plant material is collected from KUBG, Dept. of Botany, University of Jammu and Kashmir, Srinagar Campus. The roots are cleaned and disinfected with 15% H_2O_2 followed by washing with double distilled water. Shade dried roots are powdered with the help of an electronic blender and the powder is stored in air tight bottles, at room temperature prior to use.
- **Chemical requirements:** HPLC grade Water, Ethanol and Methanol are used as solvents for extraction.
- **The extraction procedure:** The extracts are prepared in a Soxhlet apparatus using continuous hot percolation method at 55^0C for at least 8Hrs. The prepared crude extracts are concentrated using rotary evaporator under vacuum at room temperature. All these concentrated extracts are stored at 4^0C in air tight bottles and used within one week for the experiments.

2.1 Screening of Antimicrobial activity

2.1.1 Microorganisms used: The bacterial cultures used in the study are Gram-positive (*S. aureus, S. epidermidis, Brevibacterium epidermidis*) and Gram negative (*E. coli, K .pneumoniae, S. abony, P. aeruginosa*). Cultures are preserved on sterile Nutrient Agar slants by serial sub-culturing after one week and were stored at 4^0C. A loopful of freshly grown overnight culture is evenly suspended in the sterile saline sample in a suspension tube and the Optical Density is adjusted to 0.1 to get uniform cell density for each culture prior to the study.

The fungal cultures used are *Candida albicans* and *Malassezia furfur*. The fungal cultures are maintained on sterile Sabauroud's Agar slant and sterile Pityrosporum Agar slant, respectively. The cultures are subcultured periodically and stored at 4^0C.[2] Saline suspension of freshly grown culture with adjusted O.D. is taken for the microbial study.

2.1.2 Diffusion assay for antimicrobial activity testing: The screening of antimicrobial activity of Aqueous, Ethanolic and Methanolic root extracts of both the plants is done by Agar cup method (a standard diffusion assay) using Muller Hinton Agar (Hi-Media, India). Wells of 5mm diameter is punched out aseptically, in the pour-plated agar medium containing the test organism (around10^6 CFU/ml). 50µl of the extract is pipetted out into the wells whereas 50µl of Ethanol, Methanol, and Sterile Distilled Water served as a Control. After incubation, the diameter of inhibition is measured. The diameters of zone of inhibition due to extracts are compared with those produced by the commercial control antibiotics, such as Ampicillin (10µg/ml). In the antifungal assay, the lawn is made on PDA by mixing fungal inoculums. Wells are made by sterile cork borer and 50µl of the extract is added to respective wells. Intraconazole (10µg) is used as a positive control whereas Sterile Distilled Water and 60% Ethanol and Methanol are used as controls. The plates are kept at 4^0C for half an Hr for pre-diffusion of the extracts and then transferred to the incubator at 37^0C. After incubation, the diameter of the zone of inhibition is measured. Both, antibacterial and antifungal tests are performed in triplicates and observed values of the zone of inhibition are expressed as mean values of the triplicate readings.

2.1.3 Determination of Minimum Inhibitory Concentrations (MICs): The extracts with promising antimicrobial activity (with a zone of inhibition more than 10mm) are subjected for the Minimum Inhibitory Concentration (MIC) assay to find out the lowest concentration of extracts that inhibit the growth of the test organisms. MIC assay is performed using the crude extracts with a concentration range of 100 to 1000µl/ml. After incubation at 37^0C for 24 to 96 Hrs, the highest dilution showing microbial growth inhibition is recorded as Minimum Inhibitory Concentration (MIC) respectively, for each pathogen.[2]

2.2 Screening of Antioxidant properties by DPPH Assay

Plant extracts are mixed with DPPH (0.1mM) in Ethanol solution. After 20 min incubation in dark, at room temperature, absorbance readings are taken at 517nm on a spectrophotometer. Various concentrations of plant extracts (0.1-1mg/ml range) are prepared in alcohol (Ethanol/Methanol) used as a solvent. Ascorbic acid of different concentrations is used as a standard. The activity of various extracts (Ethanolic and Methanolic) of both the plants (*Valeriana jatamansi* and *Nardostachys jatamansi*) is measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical.[9]
The inhibitory percentage of DPPH is calculated according to the following equation:

$$\%\text{Inhibition} = \frac{100\%\ \text{Initial Activity (IA)} - \text{Inhibitor}}{100\%\ \text{Initial Activity (IA)}} \times 100\%$$

The % inhibition is plotted as a function of the inhibitor concentration to determine the IC50 value.

2.3 Anti-inflammatory assay

Inflammation is a process in which the body's immune system protects us from harmful stimuli such as pathogens to prevent infections and to restore the body cells to a normal state. Lipoxygenases are members of a class of non-heme iron-containing dioxygenases that catalyze the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-1, 4-pentadiene system to give an unsaturated fatty acid hydroperoxide. In mammals, lipoxygenases carry out the first step in the arachidonic acid cascade. 5- and 15-Los lead to the biologically active lipoxins, whereas 5-LO leads to 5, 6-epoxy-leukotrienes which are involved in a variety of inflammatory responses, including neutrophil chemotaxis, vascular permeability, and smooth muscle contraction. In contrast, it is suggested that animal 15-LO products act as anti-inflammatory agents. This implies that the 5- and 15-LO pathways may play a role in regulating inflammation.[6-7]The assay kit (purchased from Genetix Biotech Asia Private Limited) provides an accurate and convenient method for screening lipoxygenase inhibitors. This assay measures the hydroperoxides generated from the incubation of a lipoxygenase (5-, 12-, or 15-LO) with either arachidonic or linoleic acid. The root extracts are screened for the anti-inflammatory property using this method. The inhibitory percentage of Anti-inflammatory property is calculated as below:

$$\%\text{Inhibition} = \frac{100\%\ \text{Initial Activity (IA)} - \text{Inhibitor}}{100\%\ \text{Initial Activity (IA)}} \times 100\%$$

The percent inhibition is plotted as a function of the inhibitor concentration to determine the IC50 value.

3. RESULTS

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. The use of plant extracts and phyto-chemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. These plants are used as a substitute to each other, due to the similarity in species name (*jatamansi*); as well as due to the presence of some common components like Valeric acid. But the overall activity

of plants is due to a combination of various components and not only due to one marker compound. With the results obtained in this study, we can confirm that the antimicrobial, anti-inflammatory and antioxidant activity of both plants are not similar and one cannot use *Nardostachys* as a substitute for *Valerian*.

3.1 Results of antimicrobial assay

The results are shown in the following tables.

Table 1: Antimicrobial activity of the various extracts of *Valeriana jatamansi*

Microorganisms	The diameter of Inhibition zone*(mm)		
	Water	Ethanol	Methanol
S.aureus	-	11	18
S.epidermidis	-	13	17
B.epidermidis	-	12	19
C.diptheriae	-	10	14
E.coli	-	12	18
K.pneumoniae	-	11	17
S.abony	-	11	16
P.aeruginosa	6	16	23
C.albicans	-	-	11
M.furfur	-	-	9

**All the values are average of three determinations*

Table 2: Antimicrobial activity of the various extracts of *Nardostachys jatamansi*

Microorganisms	The diameter of Inhibition zone*(mm)		
	Water	Ethanol	Methanol
S.aureus	-	9	12
S.epidermidis	-	8	13
B.epidermidis	-	9	12
C.diptheriae	-	-	-
E.coli	-	9	11
K.pneumoniae	-	-	9
S.abony	-	-	10
P.aeruginosa	-	10	12
C.albicans	4	11	16
M.furfur	-	9	11

All the values are average of three determinations

Diffusion assay showing the antimicrobial activity of root extracts of both plants

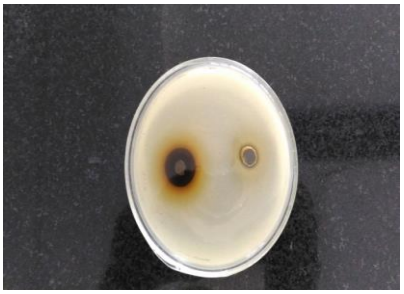

Fig. 1: Zone of inhibition using *V. jatamansi* extract

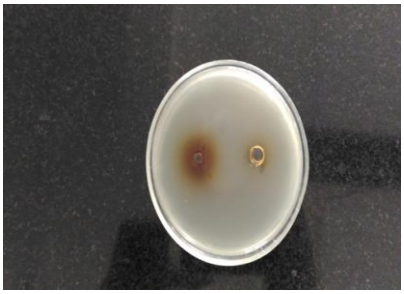

Fig. 2: Zone of inhibition using *N.jatamansi* extract

Table 3: MIC of the Methanol extract of *V.jatamansi*

Microorganisms	MIC µl/ml	Microorganisms	MIC µl/ml
S.aureus	400	*K.pneumoniae*	500
S.epidermidis	400	*S.abony*	800
B.epidermidis	300	*P.aeruginosa*	200
C.diptheriae	800	*C.albicans*	600
E.coli	400	*M.furfur*	900

3.2 Results of Antioxidant and Anti-inflammatory assay

The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical scavenging activity. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, it then leads to a loss of this violet colour. Lesser the IC50 value better is the Antioxidant activity. Free radical scavenging activities of the various extracts are presented in the following table.

Table 4: DPPH Assay showing Mean Antioxidant Activity of Methanolic extracts

Concentration (µg/ml)	*Valerian* (Ethanolic)	*Valerian* (Methanolic)	*Nardostachys* (Ethanolic)	*Nardostachys* (Methanolic)
100	17.48	29.48	13.72	22.90
200	38.68	44.70	21.95	23.70
500	64.06	78.76	38.90	44.34
800	92.47	96.81	62.55	68.60

All data are shown as the means for triplicate determination in the same extracts

Table 5: IC50 values (µg/ml) for Antioxidant and Anti-inflammatory properties

Property	*Valerian* (Ethanolic)	*Valerian* (Methanolic)	*Nardostachys* (Ethanolic)	*Nardostachys* (Methanolic)
Antioxidant	280	230	800	600
Anti-inflammatory	110	100	96	80

4. DISCUSSION

The antimicrobial activity of the extracts, in Water, Ethanol and Methanol is summarized in given tables. Out of three of the solvents, Methanol extract is found showing better activity against most of the test organisms. The highest zone of inhibition is observed against *P. aeruginosa*(zoi=23mm). Significant inhibition is also observed for Gram-positive test organisms like *B.epidermidis* and *S.aureus.* Ethanol extracts show variable activity compared to Methanolic extracts. From the results shown in the table, it is evident that *V. jatamansi* shows better antibacterial activity as compared to *N.jatamansi*. However, *N.jatamansi* extract shows more effective antifungal activity. The aqueous extracts of both plants seem to be ineffective against most of the test organisms. The results confirm the difference in activity of both these plants. The extraction of bioactive components from plant material is largely dependent on the type of solvent used in the extraction procedure. The alcoholic extracts (Ethanolic and Methanolic) show more consistent Antimicrobial activity compared to Aqueous extracts of the roots. The present findings agree with the previous studies which report the effectiveness of Valerian oil against Gram-positive organisms, including skin pathogens.[1] The results suggest the effectiveness of *Nardostachys* against fungal pathogens like *M.furfur*. The most promising Methanolic extract is subjected for the Minimum Inhibitory Concentration assay, against ten microbial pathogens having inhibition zone more than 10mm. 200µl/ml is recorded as the least concentration inhibiting *P.aeruginosa* whereas, 300µl/ml is recorded as the MIC against *B.epidermidis.* As shown in the table, the MIC values for other pathogens are in the range of 500µl/ml.

A lower IC50 value indicates higher Antioxidant activity. The VjMeth extract exhibited remarkable Antioxidant activities. In this study, DPPH radical scavenging activity of test samples increased with increase in concentration. *Valerian* Alcoholic extracts show better antioxidant activity as the IC50 value is much lesser as compared to *Nardostachys* alcoholic extracts. Whereas, Anti-inflammatory property of *Nardostachys* is comparatively better than *Valerian* extracts.

5. CONCLUSION

Valerian is a member of the Valerianaceae family that includes up to 250 species, commonly called as Indian Valerian (*Valeriana jatamansi)*.[8] *Valeriana jatamansi* has long been used in Ayurveda (Charak Samhita, Susruta Samhita) and Unani systems of medicine. The rhizomes of this plant contain volatile oil (which includes valerianic acid), volatile alkaloids (including chatinine), and iridoids (valepotriates) which have been shown to reduce anxiety and aggression and even to counteract the effects of Ethanol.[9]

In the present study, *Valeriana jatamansi* and *Nardostachys jatamansi* plant root extracts are investigated for their Antimicrobial, Anti-inflammatory, and Antioxidant potential. The study demonstrated promising Antibacterial activity of *Valeriana jatamansi* against major pathogens. It also shows remarkable Antioxidant potential with minimum IC50 value. Whereas the Antifungal and Anti-inflammatory properties are more prominently shown by *Nardostachys jatamansi* extracts. With the results obtained in this study, we can confirm that the properties of both plants are not similar and one cannot use *Nardostachys* as a substitute for *Valerian*.

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7. ETHICAL STANDARDS

This article does not contain any studies with human or animal subjects.

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