

**VOLATILE CONSTITUENTS, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ESSENTIAL OIL FROM *NARDOSTACHYS JATAMANSI* DC. ROOTS**

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**Summary**

Essential oil of *Nardostachys jatamansi* DC. roots was extracted by hydro distillation and analyzed by GC-MS. Out of 26 components, 8 were identified from their fragmentation pattern. Identified Components were ledene oxide[II] (13.021%), patchouli alcohol(9.582%), [-]-spathulenol(2.672%), globulol(1.876%), 4-[3,3-dimethyl-but-1-ynyl]-4-hydroxy-2,6,6-trimethylcyclohex-2-enone(1.849%), magastigma-4,6[E], 8[Z]-triene(1.015%), aristolene(0.997%) and  $\beta$  - vatirenene(0.932%). These components were identified first time from essential oil of *N. jatamansi* DC. roots

The antibacterial activity of the essential oil of *N. jatamansi* was determined by paper disc diffusion method against both Gram positive and Gram negative bacteria (*Bacillus subtilis* ATCC6633, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Enterobacter aerogenes*).

Among Gram positive bacteria *N. jatamansi* essential oil exhibited maximum antibacterial activity against *B. subtilis* followed by *S. aureus*.

In case of Gram negative bacteria only *K. pneumoniae* and *E. aerogenes* were found to be sensitive to *N. jatamansi* essential oil while *S. typhimurium* and *P. aeruginosa* were resistance to *N. jatamansi* essential oil.

The results of antioxidant activity of essential oil of *N. jatamansi* showed that it was able to reduce the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to yellow-colored DPPH-H reaching 99.04% of DPPH scavenging effect comparative to butylated hydroxy toluene(BHT).

**Key words:** *N. jatamansi* DC root, Essential oil. MS, Antibacterial activity, antioxidant activity

### **Introduction**

In recent years, the antimicrobial and antioxidant actions of substances have received much attention. This is because of the increasing interest in human health and have been studied *in vitro* and *in vivo* by many researchers. The antioxidant agents may be useful in retarding oxidative damage to cellular constituents which lead to cell injury and death. This has been associated with pathogenesis of various chronic diseases, e.g. carcinomas, coronary heart disease, and many other health problems related to advance age <sup>(1,2,3)</sup>. The antimicrobial agents protect living organisms from damages resulting in the prevention of various infectious diseases <sup>(4)</sup>.

There is a growing interest in substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animals as food components or as specific pharmaceuticals <sup>(5)</sup>. Plants are the primary sources of naturally occurring antioxidants for humans. It has been well known that essential oils and plant extracts have antimicrobial and antioxidant effects <sup>(6)</sup>.

*N. jatamansi* DC, is a perennial herb, belongs to the family Valerianaceae and commonly found in Himalayas. The plant has a rich history of medicinal use and has been valued for centuries in Ayurvedic and Unani systems of medicine. It is used as a stimulant, antiseptic, insect repellent and for the treatment of epilepsy, hysteria, convulsive affections, stomachache, constipation and cholera. The essential oil of *N. jatamansi* also has medicinal properties. In combination with cold water, the oil is considered to be effective against nausea, stomachache, flatulence,

liver problems, jaundice, kidney complaints, insomnia and headache. Externally, the oil is added to a steaming bath to treat inflammation of the uterus. The oil is also used in eye compounds and as poison antidotes. Oil is reported to be useful in the treatment of atrial flutter<sup>(7,8)</sup>.

The aim of the present study was to determine the chemical composition of *N. jatamansi* essential oil as well as antioxidant and antibacterial activities to find natural antioxidant and bacteriocides that are safe to humans and environment.

### **Materials and Methods**

#### **Extraction of oil:**

The *N. jatamansi* roots were collected from the local market. They were cleaned from extraneous matter. The essential oil was extracted through hydro-distillation by reverse Dean Stark apparatus<sup>(9)</sup>. The steam distillate was removed, dried over anhydrous sodium sulphate and stored at low temperature.

#### **GC-MS analysis:**

The analysis of the essential oil was carried out on GC-MS of Agilent Technologies, Model 6890N, operating in EI mode at 70 eV equipped with a split-splitless injector. Helium used as a carrier gas at the flow rate of 1 mL/min, while HP-5MS (30 m × 0.25 mm, 0.25 μm) capillary column was used. The initial temperature was programmed at 50-140°C at the rate of 5°C/min and then 100-250°C at the rate of 3°C/min followed by a constant temperature at 260 °C for period of 20 minutes. Sample (2 μL) was injected to column programmed at 200°C and resolutions of components were attained. The components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and NIST library search from the derived fragmentation pattern of the various components of the oil.

#### **Antibacterial Assay:**

*In vitro* antibacterial studies were carried out on six bacterial strains including *B. subtilis* ATCC6633, *K. pneumoniae*, *S. typhimurium*, *P. aeruginosa*, *S. aureus* and *E.aerogenes*. Among the tested microorganisms *B. subtilis* ATCC6633 was obtained from microbiology laboratory of PCSIR labs complex Lahore and other were collected from pathological laboratory of a local hospital. All clinical isolates

were characterized to specie level according to standard microbiological techniques described by Monica Cheesbrough<sup>(10)</sup>. The cultures of bacteria were maintained in the laboratory on nutrient agar slants at 4°C throughout the study.

Paper disc diffusion method as reported by Bauer *et. al*<sup>(11)</sup> was applied with slight modification to test the antimicrobial activity of *N. jatamansi* essential oil. Normal strength nutrient agar medium (OXOID, England) was prepared and autoclaved at 121±1°C for 15 minutes. For antibacterial assay 24 hours old bacterial cultures grown at 37°C were used. Cultures were diluted 10<sup>-1</sup> in sterile ringer solution<sup>(12)</sup> to set inoculums density of approximately 10<sup>6</sup>CFU/mL which was used further for the test. Thirty micro-liters of these bacterial suspensions were inoculated to plates containing sterile nutrient agar medium using a sterile cotton swab. Each filter paper discs (6mm in diameter) impregnated with different concentrations of *N. jatamansi* essential oil separately (10µL, 15µL and 20µL) were placed on pre-inoculated culture media under aseptic conditions separately and incubated at 37°C for 24hours. The zone of inhibition was measured as the diameter (in millimeters) of the clear zone around the discs. All experiments were performed in duplicate. PenicillinG and streptomycin were used as positive controls. Control antibiotics solutions were prepared in appropriate amount (0.01g/10mL) then 25µL of each antibiotic solution was dropped on paper discs used during present study.

#### **Antioxidant activity of *N. jatamansi* oil:**

Antiradical activity was evaluated by measuring the scavenging activity of the examined *N. jatamansi* oil on the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical. The DPPH assay was performed as described by Epsin *et. al*<sup>(13)</sup>. The samples (100 µL each) were mixed with 3 ml of DPPH solution. The absorbance of the resulting solutions and the blank (with only DPPH and no sample) were recorded after an incubation time of 30 minutes at room temperature against ascorbic acid as a positive control. For each sample, 3 replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm. The percentage of radical scavenging activity was calculated using the following equation;

$$\text{DPPH scavenging effect (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A<sub>0</sub> is the absorbance of the control at 30 minutes and A<sub>1</sub> is the absorbance of the sample at 30 minutes.

### **Results and Discussion**

The essential oil was extracted by hydrodistillation from the *N. jatamansi* roots, collected from local market. The yield of oil was 0.245%. The gas chromatography coupled with mass spectrometric analysis revealed the presence of 26 compounds,

out of which 8 principal components were, identified (table 1). It was recorded that ledene oxide[II]( 13.021%), and sesquiterpine alcohol patchouli alcohol ((9.582%) were major component of the oil. [-]-spathulenol(2.672%), globulol(1.876%), 4-[3,3-dimethyl-but-1-ynyl]-4-hydroxy-2,6,6-trimethylcyclohex-2-enone(1.849%), magastigma-4,6[E], 8[Z]-triene(1.015%), aristolene(0.997%),  $\beta$  - vatirenene(0.932%), were present in considerable quantity. These components were identified first time from essential oil of *Nardostachys jatamansi* DC. roots.

**Table 1:** GS/MS analysis of essential oil of *N. jatamansi*.

S. #	Components	% age	M/Z Values
1	Aristolene	0.997	M <sup>+</sup> (204,34)(189,43)(161,45)(147,43) (133,37)(105,100)(91,46)(79,14)(55,8)
2	$\beta$ -Vatirenene	0.932	M <sup>+</sup> (202,100)(187,27)(159,55)(145,63)(131,88) (117,38)(105,38)(95,26)(91,51)(77,24)(69,13)(55,9)
3	Magastigma-4,6[E], 8[Z]-triene	1.015	M <sup>+</sup> (161,100)(176,91)(147,20)(133,50)(119,82) (105,39)(91,77)(77,30)(65,15)(51,10)
4	[-]-Spathulenol	2.672	M <sup>+</sup> (220,6)(205,100)(187,33)(177,16)(159,50) (147,38) (119,58) (105,47) (79,38)(67,22)(55,17)
5	Globulol	1.876	M <sup>+</sup> (222,55)(189,62)(175,21)(161,100)(147,42) (135,42) (121,62)(109,89)(93,68)(69,57)
6	Ledene oxide[II]	13.021	M <sup>+</sup> (222,24)(202,26)(187,46)(177,39)(159,100) (151,38) (123,34)(109,60)(91,51)(81,39)(67,34)(55,24)(51,4)
7	Patchouli alcohol	9.582	M <sup>+</sup> (222,100)(207,34)(189,14)(179,17) (161,35) (138,35)(121,18)(109,28)(98,42) (67,24) (55,24)(51,2)
8	4-[3,3-dimethyl-but-1-ynyl]-4-hydroxy-2,6,6-trimethylcyclohex-2-enone	1.849	M <sup>+</sup> (234,40)(219,66)(191,48)(177,44)(163,100) (145,32)(105,40)(91,46)(77,31)(67,20)(55,23)

The data related to the *in vitro* antibacterial potential of *N. jatamansi* root essential oil against Gram positive and Gram negative bacteria along with control antibiotics are presented in table 2. The results indicated that among Gram positive bacteria *B. subtilis* exhibited maximum antimicrobial activity at 20 $\mu$ L concentration of *N. jatamansi* essential oil with an inhibition zone diameter (IZD) of 23.00 $\pm$ 0.88mm followed by *S. aureus* with an IZD of 18.75 $\pm$ 0.35mm at same concentration. These results are supportive to the findings reported by Sohail *et. al*<sup>(14)</sup> who stated that ethanolic extract of *N. jatamansi* roots was effective against both *B. subtilis* and *S. aureus*.

During present study, the biological activity of *N. jatamansi* essential oil was also evaluated against four G-ve bacteria including *S. typhimurium*, *P. aeruginosa*, *K. pneumoniae* and *E. aerogenes*. The results indicated that among tested G-ve bacteria only *K. pneumoniae* and *E. aerogenes* were sensitive to *N. jatamansi* essential oil as shown in table 2. The IZD of *E. aerogenes* was 15.00 $\pm$ 0.81mm which is greater than *K. pneumoniae* (11.00 $\pm$ 0.35mm) at 20 $\mu$ L concentration of essential oil. Sohail *et. al*<sup>(14)</sup> reported that ethanolic extract of *N. jatamansi* root showed inhibition zone diameter of 34-24mm at different concentrations (20mg/mL to 5mg/mL respectively) against *K. pneumoniae*. This is far higher than IZD of only 11.00 $\pm$ 0.35mm observed for *N. jatamansi* essential oil used against *K. pneumoniae* during current study. It may therefore be concluded that essential oil of *N. jatamansi* does not contain the active ingredient which may be present in the ethanolic extract of the same. This is in corroboration with Nimri *et. al*<sup>(15)</sup> who has reported the effect of various factors i.e. effect of solvents and method of extraction, maturity of source plant and/or sensitivity of the test strains on antimicrobial potential of the material tested. Further among test organisms both *S. typhimurium* and *P. aeruginosa* were resistance to *N. jatamansi* essential oil. Present investigation regarding *P. aeruginosa* is in accordance to Girgune *et. al*<sup>(16)</sup> who reported that *N. jatamansi* oil showed no zone of inhibition against *P. aeruginosa*. In case of positive controls (antibiotics) Streptomycin was found to be efficacious against all tested microorganisms as shown in table-2. Maximum zone of inhibition observed in case of *B. subtilis* i.e 31.00 $\pm$ 0.35mm whereas the minimum IZD was 17.00 $\pm$ 0.35mm exhibited by *K. pneumoniae*. Penicillin G was found to be effective against only *B. subtilis* and *S. aureus* as depicted in table-1.

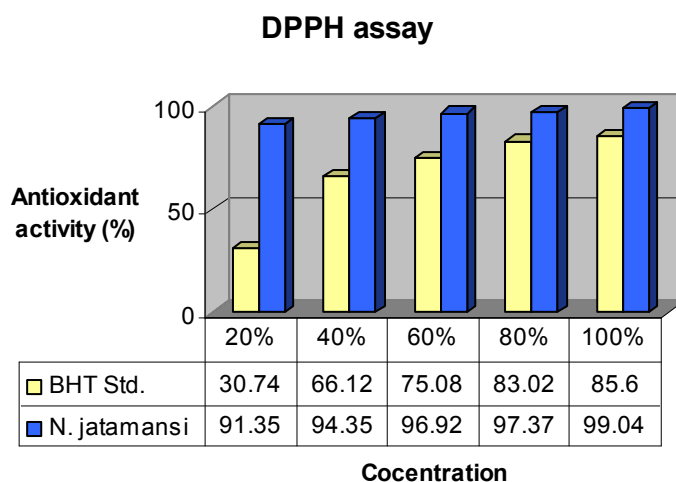
**Table 2:** Assessment of antimicrobial activity of *N. jatamansi* essential oil against six microorganisms.

Tested Micro-organisms	Gram staining/ Colony morphology	Antimicrobial activity of <i>Nardostachys jatamansi</i> essential oil and some standard Antibiotics. Zone of inhibition (mm)				
		Oil conc. 10µL/D <sup>a</sup>	Oil conc. 15µL/ D	Oil conc. 20µL/ D	Strepto-Mycin 25µg/D	Penicillin G 25µg/D
<i>Bacillus subtilis</i> ATCC 6633	G +ve rods/ White, dry surface	17.00 ±0.35	20.75 ±0.71	23.00 ±0.88	31.00 ±0.35	24.00 ±0.70
<i>Staphylococcus aureus</i> HI <sup>b</sup>	G +ve rods/ yellow, round, shiny	14.00 ±1.41	16.50 ±0.35	18.75 ±0.35	28.50 ±0.70	21.00 ±0.35
<i>Salmonella typhimurium</i> HI	G -ve rods/ Transparent white,	-	-	-	22.75 ±0.35	R <sup>c</sup>
<i>Pseudomonas aeruginosa</i> HI	G -ve rods/ green, flat-spreaded	-	-	-	21.75 ±0.35	R
<i>Klebsiella pneumoniae</i> HI	G -ve rods/ grey-white, mucoid	07.00 ±0.71	09.00 ±0.88	11.00 ±0.35	17.00 ±0.35	R
<i>Enterobacter aerogenes</i> .	G -ve rods/ large, white non-mucoid	10.00 ±0.35	13.00 ±0.71	15.00 ±0.81	25.00 ±0.36	R

<sup>a</sup>Paper disc (6mm diameter)<sup>b</sup>Hospital isolated pathogen<sup>c</sup>Resistant to antibiotic

± Standard deviation, (-) No inhibition zone

Several natural compounds are known to quench free radicals <sup>(17)</sup>. In the current study (Figure 1) essential peel oil was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 99.04% of DPPH scavenging effect. The comparison of DPPH scavenging activity of *N. jatamansi* roots oil with well known antioxidant BHT showed that root oil has stronger antioxidant potential at all concentrations.



**Figure 1:** Percentage antioxidant activity of essential oil of *N. jatamansi* DC. roots in comparison with BHT as standard reference by DPPH assay.

### Conclusions

In conclusion, this is the first study evaluating the antibacterial and antioxidant activities of essential oil from *N. jatamansi* DC roots. The results have shown that the oil has strong in vitro antibacterial activity against *B. subtilis* ATCC 6633, *K. pneumoniae*, *E. aerogenes*, *S. aureus* and antioxidant activity. So, the essential oil can be used in the treatment of diseases caused by these microbes directly or by incorporation into medicines used for the treatment of these ailments. Further studies are required to determine the mechanism of action of the essential oil for antibacterial and antioxidant activity.

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