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Original Research Article

Antioxidant Capacity and Antimicrobial Effects of Nardostachys Grandiflora Leaves Extracts

S. Saklani[1], V. Gupta[2], R. Sharma[3], G. Kaur[2], M. Kawra[1], P. Sakshi[1], M. Maithani[4]

[1]Department of Pharmaceutical Chemistry, Hemvati Nandan Bahuguna Garhwal University, Srinagar, Pauri Garhwal, India

[2]Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot, India

[3]Faculty of Pharmaceutical Sciences, ICFAI University, Baddi, Himachal Pradesh, India

[4]Department of Pharmaceutical Sciences, Hemvati Nandan Bahuguna Garhwal University, Srinagar, Pauri Garhwal, India

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Corresponding Author: M. Maithani

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Abstract:

Nardostachys grandiflora, a high-altitude medicinal herb traditionally used in Ayurvedic and Tibetan medicine has been recognized for its diverse pharmacological properties. The present study aimed to evaluate the antioxidant capacity and antimicrobial potential of leaf extracts obtained using different solvents. Antioxidant activity was assessed through standard assays including DPPH radical scavenging, revealing a concentration-dependent free radical scavenging effect. Antimicrobial activity was tested minimum inhibitory concentration (MIC) methods. Results demonstrated that methanolic extract exhibited the highest antioxidant activity (IC_{50} = 332.65 µg/ml) and significant antibacterial effects, with inhibition zones ranging from 16.2 to 19.0 mm against B. subtilis, S. aureus, P. vulgaris, and K. pneumoniae. These findings suggest that N. grandiflora leaves are a promising source of natural antioxidants and antimicrobial agents, supporting their potential application in nutraceutical and pharmaceutical formulations. These in-vitro findings required to be validated in-vivo models and toxicity assessments before therapeutic use.

Keywords: Nardostachys Grandiflora; Antioxidant Capacity; Antimicrobial Effects; Leaves Extracts.

Introduction

Nardostachys grandiflora DC is a plant belongs to the family Valerianaceae and is commonly known as Indian spikenard or musk root [1]. The herb N. grandiflora is known to be a popular medicinal and aromatic plant. It is a reputed Ayurvedic herb and used in various multiple formulations. It has also been mentioned in the Holy Bible and Quran [2]. It is a small, perennial, rhizomatous, herb which grows in steep, moist, rocky, undisturbed grassy slopes of India, Nepal, China, Tibet and Bhutan from 2200 m to 5000 m above sea level [3]. Rhizomes of 2.5 to 7.5 cm occurs in short pieces of reddish-brown colour with elongated cylindrical shape, has dark grey colour and typical smell. Leaves are sessile and ovate. The leaves are rosy, slightly pink or blue in dense cymose. Flowers are slightly pink in colour. It has an agreeable odour with bitter aromatic taste and is used as substitute for valerian. The plant grows to about 1 m in height and has pink, bell-shaped flowers. Spikenard rhizomes (underground stems) occur in short pieces, has dark grey colour and typical smell. Its rhizomes are used in traditional medicines in different medicinal system. Jatamansi has been widely used for medicine and in perfumery for centuries in India [4]. It is valuable in Ayurveda, N. grandiflora is used for nervous headache, excitement, menopausal symptoms, flatulence, epilepsy and intestinal colic. In combination with cold water, the oil is considered to be effective against nausea, stomach ache, flatulence, liver problems, jaundice and kidney complaints, insomnia and headache [5]. Externally, the oil is added to a steaming bath to treat inflammation of the uterus. The oil has also been reported in ophthalmic formulations and as an antidote. Oil is reported to be useful in the treatment of atrial flutter. The roots and rhizomes of N. grandiflora have been used to treat epilepsy, hysteria and mental weakness. It also exhibits cardio protective activity and used in the treatment of neural diseases. The essential oil obtained from the roots of jatamansi showed various pharmacological activity including antimicrobial, antifungal, hypotensive, antiarrhythmic and anticonvulsant activity [6]. Their extracts also possess antispasmodic and stimulant properties which are useful in the treatment of fits and heart palpitations and it can also be used to regulate

constipation, urination, menstruation and digestion [7].

The genus Valeriana (Valerianaceae), containing about 200 species, is distributed throughout the world. In the history, the early uses of valerian were in the most part due to its bitter and aromatic qualities. N. grandiflora is a plant indigenous to India has been prescribed in this country since 800 B.C. for a diversified group of ailments like sleeping disorders, nervous disorders, for its stimulating effect, as a bitter tonic, antipyretic, antispasmodic, and antiseptic [8-9]. In the Unani system of Medicine, N. jatamansi DC is used as hepatoprotective, cardio tonic, diuretic and analgesic. In China, N. chinensis (N. jatamansi) is used for stomachic and sedative effects [10]. Traditionally in Nepal N. jatamansi has been used in treatment of epilepsy, hysteria, convolutions, heart palpitations, intestinal colic, and antiarrhythmic activities and is an important component in Ayurvedic formulations [11]. Pharmacologically, N. jatamansi has shown antioxidant activity, hepatoprotective activity, cardiotonic, antihyperlipidemic, respiratory, hair growth and anti-hyperglycemic effects [12]. The chemical composition of N. jatamansi DC is highly complex containing volatile essential oil and other biological active compounds. Although all parts including roots and rhizomes have significant and different medicinal properties. N. jatamansi has been shown to be a neuroeffective drug; it helps in improving learning disorders, alleviating aggressiveness, stubbornness, restlessness, and insomnia, and it has fewer side effects and is more efficient than commercially available drugs like amphetamine and chlorpromazine [13]. Methanolic and aqueous extracts have shown pronounced activity against amnesia and dementia in mice [14]. N. jatamansi has shown insecticidal activity against Sitophyluszeamais and Tribolium castaneum [15]. Preliminary clinical studies with jatamansone reported reduced incidence of aggressiveness, restlessness, stubbornness and insomnia. In a study conducted on hyperkinetic children, jatamasnone, D-amphetamine and chlorpromazine were compared for efficacy. Jatamasnone and amphetamine significantly improved behaviour in reducing aggressiveness and restlessness [16]. All parts of the plant including roots and rhizomes have significant and different medicinal properties. Due to high medicinal value of various parts of Nardostachys grandiflora, the authors planned the research study for antioxidant and antimicrobial activity of leaves of Nardostachys grandiflora plant.

Material and Methods

Plant Material: Leaves of Nardostachys grandiflora were collected from Joshimath region (Chamoli District) of Uttarkhand and were identified by Department of Botany, H.N.B.G.U Srinagar, Uttarakhand. (Herbarium no. GUH1362). The fresh leaves were hydro-distilled for 8hrs to get the essential oil. Dried leaves were successive extracted for extracts in different solvent (petroleum ether, chloroform, and methanol). The extracts as well as oil were stored for further analysis.

Determination of chemical composition by GC–MS analysis:

Phytochemical analysis: Preliminary phytochemical screening of Nardostachys grandiflora extracts were performed for the detection of phyto-constituents like alkaloids, carbohydrates, flavonoids, triterpenoids, amino acids, and saponins using standard methods as per standard guidelines [17, 18].

DPPH radical scavenging assay:

Antioxidant activity: The antioxidant activity of each extract of Nardostachys grandiflora was done by 1, 1-Diphenyl-2-Picrylhydrazyl radical scavenging [19, 20]. The free radical scavenging activity of different extracts of N. grandiflora and standard gallic acid was measured as radical scavenging capability or hydrogen donating using the DPPH. In this test, about 0.1 mM solution of DPPH was prepared in methanol and then 1.0 ml of this solution was added to 3.0 ml of the respective extracts with different concentrations. Absorbance was measured at 515 nm after the incubation of 30 minutes. The decrease in absorbance of the reaction mixture indicates increase infree radical scavenging activity. The antioxidant activities were expressed as IC_{50} (the concentration of extracts in μg/ml that inhibits the formation of DPPH radicals by 50%).

Screening of antimicrobial activity

Preparation of Mc farland standard: 0.5Mc equivalent turbidity was prepared by using standard method (Chessbrough M, 2000).

Antibacterial activity assay: For antibacterial activity, petri plates were prepared by pouring about 25ml of Brain Heart Infusion Agar (BHI), Cetrimide Agar, Luria Bertanii Agar and Muller Hinton Agar in each plate for Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia and Proteus vulgaris respectively. One plate for standard antibiotic tetracycline was prepared. Then, 100μl of standardized inoculum suspension spreaded uniformly on respective agar plates and dried for 5 minutes. The impregnated discs as well as antibiotic discs were then placed on the agar surface with the help of forceps. The plates were then kept undisturbed for 10-15 minutes followed by incubation at 37℃ for 24hrs. The Zone of Inhibition (ZOI) obtained is directly proportional to the sensitivity of test organisms to the extract [21].

Micro dilution broth method: Micro Dilution Broth Method was used to determine the Minimum Inhibitory Concentration (MIC) values of methanol extract in 96 well micro titer plates. The methanol extract was diluted to get different concentrations i.e. 250µl, 500µl, 750µl and 1000µl and then inoculated with respective bacterial strain. Standard antibiotic tetracycline was taken as positive control [22]. Then the absorbance was taken at 630nm using microquant microplate spectrophotometer.

Result and Discussion

GC-MS of Essential Oil: The GC-MS analysis of light green colored leaf oil of N. grandiflora resulted in the identification of large numbers of sesquiterpenes, aliphatic components, monoterpenes and diterpenes. Both the major as well as minor constituents were identified by their retention indices. The main compounds having the highest peak were sabinene, 1,8-cineole, borneol, bornyl acetate, α-pinene, β-pinene, terpinine-4-ol and chamazulene. Sabinene emerged as the major component of the A millefolium essential oil under tropical conditions. Results are shown in Figure 1 and Table 1.

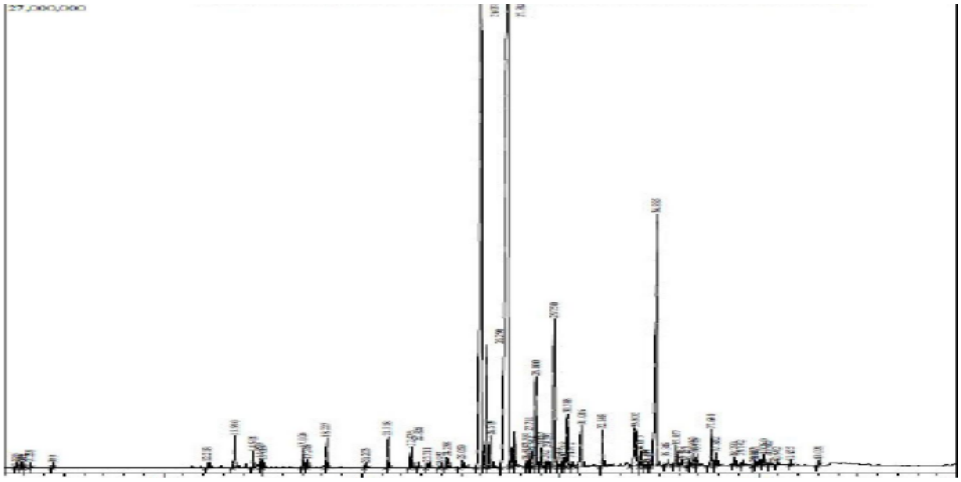

Figure 1: GC-MS Spectra of composition of Essential oil

Table 1: Essential Oil Composition of Nardostachys grandiflora

S.No	Compound	Molecular Formula	Molecular Weight	Rf
	α-pinene	$C_{10}H_{16}$	136	931
	β-Patchoulene	$C_{15}H_{24}$	204	1378
	β-Gurjunene	$C_{15}H_{24}$	204	1413
	γ-Elemene	$C_{15}H_{24}$	204	1433
	α-Humulene	$C_{15}H_{24}$	204	1436
	Aromadendrene	$C_{15}H_{24}$	204	1445
	Alloaromadendrene	$C_{15}H_{24}$	204	1465
	α-Selinene	$C_{15}H_{24}$	204	1473
	α-Panasinsen	$C_{15}H_{24}$	204	1518
	Nerolidol	$C_{15}H_{26}O$	222	1561
	Ledol	$C_{15}H_{26}O$	222	1565
	Spathulenol	$C_{15}H_{24}O$	220	1575
	Globulol	$C_{15}H_{26}O$	222	1585
	Cubenol	$C_{15}H_{26}O$	222	1614
	β-Eudesmol	$C_{15}H_{26}O$	222	1630
	Cadinol	$C_{15}H_{26}O$	222	1641
	Muurolol	$C_{15}H_{26}O$	222	1655
	Bulnesol	$C_{15}H_{26}O$	222	1664
	Jatamansone	$C_{15}H_{26}O$	222	1667
	n-tetradecanol	$C_{14}H_{30}O$	214	1679
	α-Bisabolol	$C_{15}H_{26}O$	222	1685
	5-neo-Cadranol	$C_{21}H_{31}O$	302	1699
	cis-Farnesal	$C_{15}H_{26}O$	222	1705
	Hexadecanal	$C_{16}H_{32}O$	240	1712

	n-Pentadecanol	$C_{15}H_{32}O$	228	1776
	n-Octadecane	$C_{18}H_{38}$	254	1795
	Vomifoliol	$C_{13}H_{20}O_3$	224	1837
	n-nonadecane	$C_{19}H_{40}$	268	1896
	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1923
	n-Eicosane	$C_{20}H_{42}$	282	2001
	Octadecanol	$C_{18}H_{38}O$	270	2080
	Manool	$C_{20}H_{34}O$	290	2105

Phytochemical analysis: The solvent extracts viz. petroleum ether, chloroform, methanol and water were used for phytochemical screening. The results showed the presence of phytochemicals viz. carbohydrates, alkaloids, flavonoids, phenols, saponins and tannins in the Methanolic extract of the leaves of N. grandiflora while chloroform extracts showed the presence of phenols and tannins. Water (aqueous) extracts were found to have carbohydrates, alkaloids, flavonoids, saponins, tannins and glycosides as shown in Table 2.

Table 2: Phytochemical screening of different extract of Nardostachys grandiflora

S. No.	Test	PE	CE	ME	AQE
1.	Carbohydrates- Molisch's test	-	-	+	+
	Fehling's test	-	-	+	+
	Benedict's test	-	-	+	+
2.	Alkaloids- Mayer's test	-	-	+	+
	Wagner's test	-	-	+	+
	Dragendroff's test	-	-	+	+
3.	Glycosides- Killani's test	-	-	-	+
	Legal's test	-	-	-	+
4.	Phenols- Folin – Ciocltеau's test	-	+	+	-
5.	Flavonoids- H_2SO_4/Mg test	+	+	+	+
6.	Saponin- Foam's test	+	+	+	+
7.	Tannins-Gelatin's test	-	+	-	+

Anti-oxidant activity: The results of the antioxidant activity suggest that, polar extracts of Nardostachys grandiflora had significant antioxidant potential in comparison to non-polar extracts. The result of antioxidant activity follows the order as- Methanolic extract> water (aqueous) extract> petroleum ether extract> chloroform extract as shown in Table 3**.**

Table 3: Antioxidant activity of different extracts of Nardostachys grandiflora

Extracts and Standard	DPPH activity IC50 (µg/ml)
PEE	658.66
CE	741.23
ME	332.65
AQE	515.13
Standard (Ascorbic acid)	123.21

Antimicrobial activity: The antimicrobial activities of polar and non-polar solvent extracts of the leaves of Nardostachys grandiflora were determined against different microorganisms. The maximum zone of inhibition was recorded in methanolic extract against all the bacterial strains in comparison to other extracts as shown in Table 4.

The minimum inhibitory concentrations (MIC) of methanolic extract for B. subtilis, S. aureus, P. vulgaris, K. pneumonia was found 500µl and P. aeriginosa, E. coli was 250µl.

Table 4: Antimicrobial Activity of Solvent Extracts of Leaves of Nardostachys grandiflora

Microorganism	Diameter of zone of inhibition (mm)				
	ME	AQE	CE	PE	Tetracycline
B. subtilis	18.1	No ZOI	14.7	No ZOI	21.5
S. aureus	18.5	No ZOI	No ZOI	7.5	22.8

P. aeriginosa	16.2	8.9	12.9	7.1	20.4
P. vulgaris	18.8	No ZOI	No ZOI	No ZOI	22.4
E. coli	16.8	No ZOI	No ZOI	No ZOI	21.7
K. pneumnia	19.0	7.5	12.8	No ZOI	22.1

Conclusion

The present study reports the essential oil composition of Nardostachys grandiflora, growing in Uttarakahand Himalayas. Thirty-two constituents were identified on the basis of GC-MS from the essential oil of Nardostachys grandiflora major constituents being jatamansone, cadenol, caderenol and vomifoliol. The methanolic extracts displayed moderate antioxidant activity and antimicrobial activities. These in-vitro findings required to be validated in-vivo models and toxicity assessments before therapeutic use. Due to the presence of large number of chemical compounds in Nardostachys grandiflora, it has many important applications in the treatment of various ailments. These in-vitro findings required to be validated in-vivo models and toxicity assessments before therapeutic use.

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