International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685
Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 3; Issue 1; January 2018; Page No. 09-11

GC-MS analysis and Antibacterial activity of *Nardostachys jatamansi* (D.Don) DC

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Abstract
The rhizome of *Nardostachys jatamansi* (D.Don) DC. was subjected to extraction of essential oil by hydro distillation in Clevenger apparatus. The composition of essential oil so collected were determined by GC-MS system and showed the presence of 10 different compounds. The most abundant were Ledol (23.82%), Sativen (20.00%), *β*-Gurjunene (13.82%) and Valencene (7.75%). Antibacterial activity of the essential oil of *N. jatamansi* was studied. Oil exhibited moderate antibacterial activity.

Keywords: *Nardostachys jatamansi* (D.Don) DC., essential oil, GC-MS, activity

Introduction
Nardostachys jatamansi (D.Don) DC. belongs to the family Valerianaceae. It is commonly known as musk root and called as Jatamansi in Nepali. It is distributed to the northern part of alpine to sub alpine Himalayan region at an altitude of 3000-5000 m [1, 2]. It is an erect perennial herb, 10-75cm tall with dark fibers. Leaves are cauline, sessile or sub-sessile. Flowers are terminal cymes [3].

The chemical composition of *N. jatamansi* is highly complex containing volatile oil and other biological active compounds. Phytochemical constituents of *N. jatamansi* includes phenolic compounds, caffeoylquinic acid derivatives, *β*-sitosterol, lignans, neolignans, monoterpenoids, sesquiterpenoids, diterpenoids, iridoids, *β*-maaliene, calarene and nardostachnol [3, 4, 5].

N. jatamansi helps to promote physical and mental health augment resistance of the body against disease and show antioxidant, tranquillizing, hypotensive, hypolipidemic, anti-ischemic, anti-arrhythmic, hepatoprotective, anti-convulsant, neuroprotective activities, hypolipidemic and fungicidal activity [6, 7]. It is also used for the treatment of epilepsy, hysteria, convulsive affections, stomache, constipation and cholera in Ayurvedic and Unani systems of Medicine and as a stimulant, antiseptic, and insect repellent [5]. It has protective effect in Parkinsonism, epilepsy and cerebral ischemia. Jatamansi is capable of lowering norepinephrine as well as serotonin in brain [8].

Experimental
Collection of Plant Materials
The rhizome of plant was collected from Rasuwa, Nepal. The plants were identified by Department of Botany, Amrit Campus, Lainchour, Kathmandu, Nepal.

Extraction of Essential Oil
The mature rhizome of *N. jatamansi* were crushed for hydro distillation and subjected to a Clevenger apparatus for three hours. By this process about 2ml of light bluish green coloured essential oils were collected and stored in a sealed glass vials at low temperature (0-4°C) prior to analysis.

GC-MS Analysis
The essential oils sample of *N. jatamansi* was subjected to GC-MS analysis. GC-MS analysis was performed on a gas chromatography mass spectrometer GCMS-QP2010 under the following condition: injection volume 1µL with split ratio 1:50; Helium as a carrier gas with a Rtx-5MS column of dimension 30m×0.25mm×0.25µm, temperature programmed at 40, 200 and 280ºC with a hold time of 2.0, 3.0 and 4.0 min identification was accompanied by comparison of MS with those reported in NIST 05 and FFNSCI.3 libraries. It was performed in Department of Food Technology and Quality Control, Nepal Government, Babarmahal, Kathmandu, Nepal.

Antibacterial activity assays
Antimicrobial assay of essential oil of plants was performed by agar well diffusion method in Muller Hilton Agar (MHA) and the minimum bactericidal concentration of those extract was determined by micro dilution method. All the strains of bacteria was cultured in Nutrient broth (NB) and incubated at 37 ^{0} C for 18 hours. After incubation each stain were diluted with sterile distilled water. The turbidity of dilution was compared with 0.5 McFarland standards (approximately 10^8 CFU/ml). The suspensions were then diluted (1:100) in Muller Hilton Broth (MHB) to obtain 10^6 CFU/ml. Prepared inoculums were incubated for 30 minutes at 37 ^{0} C prior to use.

Plant oils (30 µl) were loaded into the respective wells with the help of micropipette. The solvent (50% DMSO) was tested for its activity as a control at the same time in the separate well. The Neomycin 20 µg/ml was used as a positive control. The plates were then left for half an hour with the lid closed so that extracts diffused to the media. The plates were incubated overnight at 37 °C. After proper incubation (18-24 hours) the

plates were observed for the zone of inhibition around well which is suggested by clean zone without growth. The ZOI were measured with the help of the ruler and mean was recorded for the estimation of potency of antibacterial substance.

Determination of Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) was determined by micro dilution method. The methanol extracts were diluted by two fold to get series of concentrations from 0.048 to 25 mg/ml in freshly prepared sterile nutrient broth. 20µl of the microorganism suspension (correspond to 10^6 CFU/ml) was added to each of the sample dilutions. These were incubated for 18 hours at 37°C and each tube content was subculture in fresh nutrient agar separately and minimum bactericidal concentration was determined that showed no growth at all.

Determination of the Minimum Inhibitory Concentration

The smallest amount of compounds required to kill or inhibit the growth of micro-organism *in vitro* can be determined by the dilution method. This amount is referred as minimum inhibitory concentration (MIC). It is a measure of potency which is expressed in terms of either µg or mg/ml. A stock solution of 25 mg/ml was prepared. This was serially diluted to obtain various ranges of concentrations between 25 mg/ml to 0.048 mg/ml.

Result and Discussion

GC-MS Analysis

GC-MS analysis of essential oils of fruits of *N. jatamansi* shows the presence of 10 different compounds. The chemical compound identified in essential oils of the fruits of the *N. jatamansi* plant are presented below:

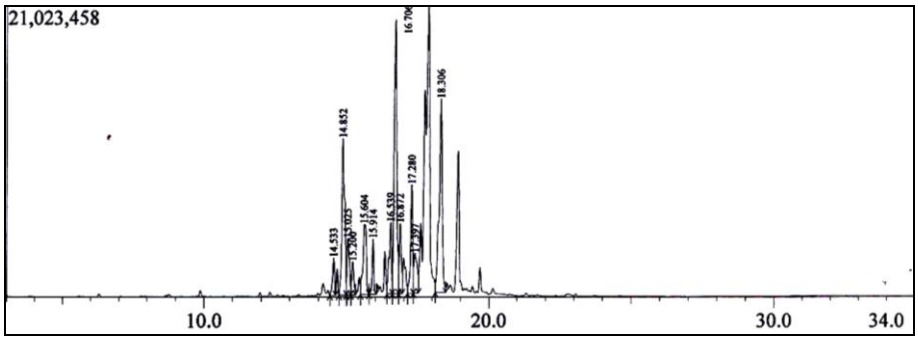

Fig 1: Chromatogram of essential oils of *N. jatamansi*

The major constituents present in the essential oils sample were Ledol (23.82%), Sativen (20.00%), *β*-Gurjunene (13.82%) and Valencene (7.75%). Constituents of essential oils of *N. jatamansi* are tabulated as follows.

Table 1: List of compounds in essential oils of *N. jatamansi*

S.N.	Name of the compounds	Molecular Formula	Molecular Weight	Retention Time	Area %	Height %
1.	*α*-Gurjunene	$C_{15}H_{24}$	204	14.533	2.48	3.23
2.	*β*-Gurjunene	$C_{15}H_{24}$	204	14.852	13.82	13.40
3.	Methandienone	$C_{20}H_{28}O_2$	300	15.025	4.41	4.86
4.	Aromadendrene	$C_{15}H_{24}$	204	15.200	2.35	2.86
5.	Valencene	$C_{15}H_{24}$	204	15.604	7.75	6.01
6.	*β*-Gurjunene	$C_{15}H_{24}$	204	15.914	3.15	4.75
7.	*γ*-Eudesmol	$C_{15}H_{26}O_2$	222	16.539	5.74	6.11
8.	Ledol	$C_{15}H_{26}O_2$	222	16.706	23.82	23.53
9.	Viridiflorol	$C_{15}H_{26}O_2$	222	16.872	4.80	6.01
10.	Ledol	$C_{15}H_{26}O_2$	222	17.280	7.46	9.26
11.	Viridiflorol	$C_{15}H_{26}O_2$	222	17.397	4.22	3.43
12.	Sativen	$C_{15}H_{24}$	204	18.306	20.00	16.56
					100.00	100.00

Antibacterial activity

Table 2: Antibacterial activity of *N. jatamansi*

Sample	MIC values			MBC Values		
	EC	MRSA	KP	EC	MRSA	KP
Neomycin*(µg/ml)	0.156	0.156	0.0195	0.625	5	0.156
Oil(mg/ml)	1.56	0.048	0.048	3.125	3.125	3.125

*Control Antibiotics

EC = *Escheriachia coli* (ATCC) 25922

MRSA = Methicillin resistance *Staphalococcus aureus* (MRSA)

KP = *Klebsiella pneumoniae* (MDR)

Conclusion

GC-MS analysis of essentials oil shows the presence of 10 different compounds. Among them Ledol (23.82%), Sativen (20.00%), *β*-Gurjunene (13.82%) and Valencene (7.75%) are the major constituents. Extract of plant shows antibacterial activity against *Escheriachia coli* (ATCC) 25922, Methicillin resistance *Staphalococcus aureus* (MRSA), *Klebsiella pneumoniae* (MDR).

Acknowledgement

Authors are thankful to Mr. Santosh Basnet, Kathmandu Valley School and College, Chhauni, Kathmandu for providing the research lab, Dr. Bishnu Marasini Department of Biotechnology, National College, Kathmandu for bioactivity test and Mr. Tayer Mohamad Miya, Department of Food Technology and Quality Control, Nepal Government for GC-MS analysis.

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