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Invitro antimicrobial activity of hydrodistilled extract of *Nardostachys Jatamansi* rhizome

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Abstract:
The present study was designed to examine in vitro antimicrobial activities of the essential oil of Nardostachys Jatamansi. The major constituents of the essential oil were determined as Jatamansone (21.4%) and Isovaleric acid (13.4%). The antimicrobial activity of the oil was also tested against gram-positive and -negative bacteria and fungus using a disc-diffusion method and the minimal inhibitory concentration (MIC) values. The essential oil was tested Staphylococcus aureus, Bacillus subtilis, Corynebacterium diphteriae, Salmonella thyphi, Klebsiella pneunomonia, Proteus vulgaris, Escherichia coli and Pseudomonas aeruginosa and Candida albicans. The oil showed remarkable antimicrobial activity against all mic.

Key words: Nardostachys Jatamansi; Jatamansi oil; essential oil; antimicrobial activity

INTRODUCTION

Scientific interest in medicinal plants has proliferated in recent times specially when it comes to antimicrobial activity. The global resurgence of interest in herbal drugs, rising cost of allopathic medicines, unwanted side effects, long term adverse effects and failure of synthetic medicines to show desired effect has left scientists to look for other alternative such as herbal therapy. Essential oils have shown wide range of activity which includes against microbes such as bacteria, fungus, virus. (Chaudhary S., et al.,2015)

There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils due to this reason. Essential oils or volatile oils are extracted from different parts of plants such as flowers, leaves, bark, roots and fruits. The extract from any of these parts contributes to the beneficial or adverse effects. (Atienzar Franck A, et al.,2014)It is observed that essential oils show wide range of antimicrobial activity against bacteria and fungi for which many conventional medicines had shown resistance. Numerous essential oils can affect both Gram positive and Gram negative bacteria in addition to yeasts and fungi. Among such essential oils, the oil of commercial importance is oil extracted from *Nardostachys Jatamansi*. (Gottumukkala V. R., et al.,2011)

Nardostachys Jatamansi a member of the family Valerianaceae, is a plant that grows in the Himalayan regions of India, China and Nepal. The plant is mostly found growing in steep areas with a 25^0 to 45^0 slope. It grows well on open, stony and grassy slopes, and on the turf of glacial flats. The essential oil extracted from rhizomes of *Nardostachys Jatamansi* is known as Spikenard oil or Jatamansi oil. This essential oil possesses tremendous useful benefits both for external and internal use. For external use, it is used for improving skin complexion, antioxidant, anti-inflammatory agent, treating eczema, alopecia, imparts blackness to hair (Parekh Amit and Jadhav VM,2009). Internally, Jatamansi oil helps in digestion, act as sedative and fight insomnia, reducing hypertension, controlling cough, birth difficulties and other minor ailments. But no information is available about the anti-microbial activity of the rhizome part of this plant. The aim of the present study was to assess the *in vitro*

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antimicrobial and antifungal activities of Jatamansi oil, a hydro distilled extract from roots and rhizomes of *N. Jatamansi.(*Singh N, et al.,2006)

MATERIALS AND METHODS

Plant material

N. Jatamansi rhizomes were collected from Mumbai, India. The taxonomic identification was made and identified at the department of Pharmacognosy, Bharati Vidyapeeth college of Pharmacy, Navi Mumbai, India. The sample of dried rhizomes of *N. Jatamansi* was deposited at the same college in Pharmacognosy department.

Isolation of the essential oil

About 30gm of air-dried and finely ground roots of *N. Jatamansi* was subjected for 8 h to water distillation using a Clevenger-type apparatus. The essential oil obtained was collected in graduated receiver. The greenish oil obtained was stored in an air-tight container away from light in cool dry place and, stored at +4°C until tested and analyzed. The amount of essential oil collected was 8.2 gm (Novik Eric, et al.,2014)

Gas chromatography (GC)/EIMS analysis

GC/EIMS analyses were performed with a varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a varian saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240°C, respectively, oven temperature programmed from 60 to 240°C at 3°C /min; carrier gas helium at 1 ml/min, injection of 0.2 µl (10% hexane solution), split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings et al., 1980; Massada, 1976; Stenhagen et al., 1974; Swigar and Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI^- ionizing (Parekh Amit M, et al.,2015) (Shrirao Anil B and Perez-Castillejos Raquel,2010).

Antimicrobial activity

Microbial strains

Evaluation of antimicrobial and antifungal activities of the essential was performed by the disc diffusion method. Microorganisms investigated were 3 Gram-positive *Staphylococcus aureus* ATCC-25923**,** *Bacillus subtilis* ATCC-6633 and *Corynebacterium diphteriae* RSHM-633 and 5 Gram-negative bacteria *Salmonella thyphi* NCTC-9394, *Klebsiella pneunomonia* NCTC- 5046, *Proteus vulgaris* RSHM-96022, *Escherichia coli* ATCC-35218 and *Pseudomonas aeruginosa* ATCC-27853, 1 fungus *Candida albicans* ATCC-10231. All microbial strains were cultured overnight at 37°C in Nutrient Agar (for bacteria) and potato dextrose agar (for fungus). All the experiments were carried out in triplicate and average and standard deviation (SD) were calculated for the inhibition zone diameters. The diameter of an inhibition zone was measured in mm and disk diameter was 6 mm.

Disc diffusion method

Agar disc diffusion method was used for the determining antimicrobial activities of the essential oil. The microbial inoculum about 0.1ml with the density of 10^8 cells per ml was uniformly spread on a sterile petri dish 100mm having solid agar. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of the oil and placed on the inoculated plates. These plates, after staying at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. (Shrirao Anil B, et al.) The diameters of the inhibition zones were measured in millimeters. Gentamycin and Nystatin was used as positive control for antibacterial and antifungal activities in the disc diffusion test.(Kordali S., et al.,2005)

Microdilution test

To determine minimum inhibitory concentration (MICs) 96- well microtiter plate was used. Different amount of Jatamansi oil was mixed with the agar medium (100uL) with bacterial inoculum (1.0 x 104 cfu per well) to achieve the wanted concentration of

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(0.2–50.0 µg/mL) using dimethyl sulfoxide (DMSO) as solvent. Plates were incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The lowest concentrations without visible growth (at the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MIC of DMSO (negative control), Gentamycin and Nystatin as positive control were individually determined in parallel experiments in order to control the sensitivity of the test organisms(Takarada K., et al.,2002). Bacterial growth was indicated by the presence of a white "pellet" on the well bottom.(Calabrese V., et al.,1999)

RESULTS AND DISCUSSION

Chemical composition of the essential oil

According to gas chromatography (GC)/EIMS analysis, 29 (55.33%) compounds were identified in *N. Jatamansi* essential oil (Table 1). Among the constituents identified, Jatamansone (21.1%) and Carotel (10.4%) were the major ones.

No	Compounds	Composition(%)
1	4-(1,-Dimethylethyl)-benzenemethanol	0.52
2	α-Gurjunene	1.46
3	Carotol	10.4
4	Aristolenone	8.1
5	Jatamansone	21.4
6	α-cadinol	0.05
8	*Isovaleric acid*	13.4
Total		**55.33**

Table 1. Chemical composition of the essential oil of *N Jatamansi*

Antimicrobial activity

The in vitro antimicrobial activity carried out by the agar disc diffusion method and minimum inhibitory concentration (MIC) values of the essential oil extracted from rhizomes of *N. Jatamansi* resulted in a range of growth inhibition pattern against pathogenic micro-organisms Table 2.

Micro-organisms	DD(Jat)	DD(Gen)	DD(Nys)
	millimiters (mm)		
S. aureus	32 ± 1.25	36 ± 2.06	-
E. coli	22 ± 0.76	19 ± 1.86	-
P. aeruginosa	15 ± 0.30	28 ±1.16	-
S. thyphi	4 ± 1.26	13 ± 0.25	-
K. pneumonia	11 ± 1.16	16 ± 0.90	-
P. vulgaris	34 ± 1.08	26 ± 1.10	-
B. subtilis	15 ± 1.01	21 ± 1.85	-
C. diphteriae	19 ± 0.85	19 ± 0.90	-
C. albicans	21. ± 1.85	-	33 ± 0.80

Table 2. Antimicrobial and antifungal activity of the essential oil extracted from rhizomes of *N Jatamansi* using agar disc diffusion method.

In Table 2, DD (Jat) is agar disc diffusion method using Jatamansi oil, DD (Gen) is agar disc diffusion method using Gentamycin (positive control), DD(Nys) is agar disc diffusion method using Nystatin(antifungal).

In the present study the result showed that *N. Jatamansi* extracted essential oil inhibited the growth of all the microbes tested. In the case of minimal inhibitory concentrations (MIC) as mentioned in Table 3, essential oil of *N. Jatamansi* rhizomes showed more or less antimicrobial activity against all pathogenic bacteria and a pathogenic yeast. Sensitive microorganisms were *K.pneunomonia*, *S. aureus* and *Candida albicans* in decreasing sensitivity, respectively. The weakest activity was observed against *E. coli*. Among the test micro-organisms, the most resistant was *P. aeruginosa*. (Parekh Amit M, et al.,2015)

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Micro-organisms	MIC (Jat)	MIC (Gen)	MIC (Nys)	MIC (DMSO)
	µg/ml			
S. aureus	9.14	4.2	-	-
E. coli	11.6	3.6	-	-
P. aeruginosa	14.5	5.8	-	-
S. thyphi	116.5	10.6	-	-
K. pneumonia	21.4	9.5	-	-
P. vulgaris	18.9	7.5	-	-
B. subtilis	16.7	3.2	-	-
C. diphteriae	23.1	11.5	-	-
C. albicans	6.2	-	3.6	-

Table 3. Minimum inhibitory concentration (MIC) of Jatamansi oil extracted from rhizomes of *N. Jatamansi* on microbial strains.

In Table 3, MIC(Jat) is minimum inhibitory concentration using Jatamansi oil, MIC(Gen) is minimum inhibitory concentration using Gentamycin (positive control), MIC(DMSO) is minimum inhibitory concentration using Dimethyl sulfoxide (DMSO) (negative control) and MIC(Nys) is minimum inhibitory concentration using Nystatin.

This study could be assumed as the first report on the antimicrobial activity of the essential oil extracted from rhizomes of *N. Jatamansi*. Due to the respectable antimicrobial activity results, if combined with other plants having similar or better activity can show significant synergistic effect. We hope that our results will provide a starting point for investigations designed new natural antimicrobial essential oil of this plant species.

CONCLUSIONS

Jatamansi oil extracted from *N. Jatamansi* rhizomes exhibited good antibacterial and antifungal activity against all the tested organisms that are known human pathogens. Jatamansi oil was observed to show maximum activity against *P. vulgaris*, *Staphylococcus aureus* and *C. albicans*. Future research needs focus on evaluation of antimicrobial and toxicity studies of Jatamansi oil on animals and Humans.

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