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ANTI-FUNGAL ACTIVITIES OF NARDOSTACHYS JATAMANSI EXTRACT AGAINST MULTI-DRUG RESISTANT FUNGAL SPECIES

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ABSTRACT

Multi-drug resistant (MDR) fungi have become widely prevalent across the world, leading to a high morbidity and mortality due to fungal infections. Moreover, no new antifungal agents have been developed in the recent years. In this study, we explored the possible antifungal activity of a plant from higher altitude Himalayan region, *N. jatamansi* against MDR and ATCC fungal strains. Anti-fungal activities of 70% ethanolic extract of *N. jatamansi* was studied against *Candida albicans* (ATCC), *Candida parapsilosis* (ATCC), *Candida gulliermondii* (MDR) and *Candida glabrata* (MDR) along with alcohol control in two steps - at first by disc diffusion technique to check the efficiency of the extract, and then the calculation of Minimum Inhibitory Concentration (MIC) by serial dilution in Mueller Hinton broth. The extract that is under examination, showed antifungal activities with MIC varying between 2.77 and 12.24 mg/ mL for both MDR and the ATCC strains. The 70% ethanolic extract of *N. jatamansi* is effective as an antifungal agent on MDR strains and proper use of this phytochemical extract may help in saving lives of many critically ill patients.

KEYWORDS: *Nardostachys jatamansi*, MDR strains, antifungal activity, MIC value, phytochemicals, *Candida* spp., Broth dilution method.

1. INTRODUCTION

Nardostachys (Family Valerianaceae) is the small Himalayan genus, which consists of two broad range endemic species, *N. grandiflora* and *N. jatamansi* in India. According to Red Data Book of Indian Plants, *Nardostachys jatamansi* is considered as vulnerable at high altitudes. *N. jatamansi*, a significantly endangered rhizome bearing medicinal plant, is limited

to specific habitats in high altitudes of the Himalaya, from 3000 to 5000 m. The plant is collected from natural habitats for consumption and trade. The rhizome part of *N. jatamansi* is used as a replacement for valerian plant and the extracts are found to be used in over twenty six Ayurvedic preparations. The root is also used for treatment of heart diseases, high blood pressure and insomnia. The root and rhizome contain active compounds with carminative, sedative, antispasmodic and tranquilizing properties.[1] Essential oils are precious natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition.[2]

The volatile essential oils from the root of *N. jatamansi* were studied to understand the antifungal action against some common multi Drug Resistant (MDR) strains of *Candida* spp.

Infectious diseases caused by MDR strains of bacteria and fungi are the leading cause of global premature deaths, killing almost 50,000 people every day across the world.[3] The situation has become alarming in the developing as well as the developed countries due to random use of antibiotics. The rise in the prevalence of multiple drug resistance in human pathogenic organisms has necessitated a search for newer antimicrobial substances from alternative sources including plants.

Traditionally used medicinal plants produce a large number of compounds of known remedial properties.[4] The substances, which can either inhibit the growth of pathogens or kill them and have minimal or no toxicity to host cells, are considered for developing new antimicrobial drugs.

A sufficient data regarding the phytochemical analysis of the plant is available. The rhizome volatiles contain 72 components, of which 41 constituting 70% of the oils have been identified. The oil is composed of nine monoterpenes (1.7%), 25 sesquiterpenes (43.9%) and 7 non-terpenic components (24.4%). The predominant sesquiterpenes (Fig. 1-8) were: nardol (10.1%), α-selinene (9.2%), β-caryophyllene (3.3%), cubebol (2.9%), α-gurjunene (2.5%), ϒ-gurjunene (2.3%) and α-humulene (2.3%).[5]

Botanical classification of the plant[6]

Kingdom	Plantae
Division	Mangnoliophyta
Class	Mangnoliopsida
Order	Dipsacales

Family	Valerianaceae
Genus	Nardostachys
Species	Jatamansi
Botanical name	Nardostachys jatamansi
Part used	Rhizomes, rhizome oil

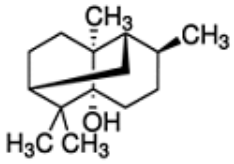

Fig. 1: Patchouli alcohol

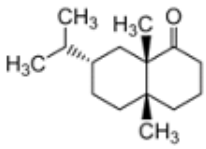

Fig. 2: Valeranol

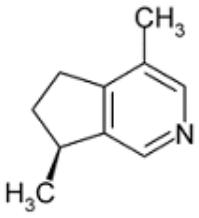

Fig. 3: Nardostachone

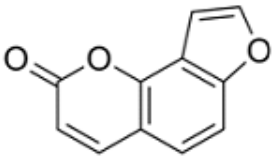

Fig. 4: Angelicin

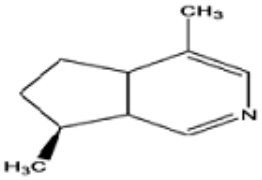

Fig. 5: Actinidine

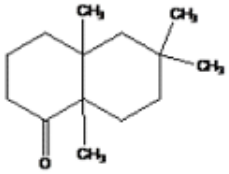

Fig. 6: Jatamansone

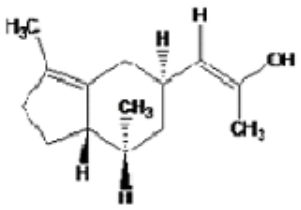

Fig. 7: Nardal

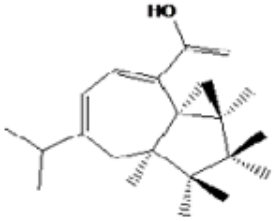

Fig. 8: Jatamansic acid

Fig. 1-8: Structures of important chemicals present in *Nardostachys jatamansi* extract.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

Plant material was purchased from a standard Ayurvedic shop, who have experience of 150 years (Adi Ambika Ausadhalaya, 2B, Shyama Prasad Mukherjee Road, Jatin Das Park,

Patuapara, Kolkata, West Bengal). An expert there identified the plant as *N. jatamansi* and the roots were collected.

2.2 Preparation of plant extract

The collected root of *N. jatamansi* was dried up in the room temperature as the root contains volatile essential oils. Then the root was carefully powdered using mortar and pestle. The powder was weighed, and 1 g of it was mixed up with 2 mL of 70% ethanol solvent to achieve the concentration of 500 mg/mL. The mixture was agitated thoroughly in vortex for about 20 minutes and kept at room temperature for around 24 hrs. After 24 hours, this mixture was again vortexed for 15-20 min before centrifugation at 3000 rpm for 10 min. After centrifugation, the supernatant was separated and collected in an aseptic vessel and stored at 4^{o}C for further use.

2.3 Tested microorganisms

The strains of *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019), as per the recommended QC strains of antifungal susceptibility testing CLSI M44, *Candida gulliermondii* (MDR), *Candida glabrata* (MDR) were used in this experiment. Their antimicrobial sensitivities are documented in tables 1-3. All the tested organisms were acquired from the patient samples of Department of Microbiology, Peerless Hospital, Kolkata, West Bengal. Before the assessment of antimicrobial sensitivity, purity of the cultures was ascertained by standard method of characterization. The antifungal sensitivity patterns of two MDR *Candida* spp. are given in Table I and II.

Table I: Antibiotics sensitivity of tested *Candida gulliermondii* (MDR) in VITEK 2 automated system

Selected Organism: *Candida gulliermondii* Source: BLOOD					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**interpretation**
Fluconazole	32	R	Micafungin	>=8	R
Voriconazole	4	R	Amphotericin B	0.5	S
Caspofungin	>=8	R	Flucytosine	>=64	R

Table II: Antibiotics sensitivity of tested *Candida glabrata* (MDR) in VITEK 2 automated system

Selected Organism: *Candida glabrata* Source: BLOOD					
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**interpretation**
Fluconazole	32	R	Micafungin	>=8	R
Voriconazole	4	R	Amphotericin B	0.5	S
Caspofungin	>=8	R	Flucytosine	>=64	R

2.4 Culture Media and Inoculums

Mueller Hinton (MH) Agar media was used for disc diffusion method and Mueller Hinton (MH) Broth was used for MIC determination. Microbial cultures were appropriately diluted in sterile normal saline to obtain the cell suspension of 0.5 McF (McFarland) by measuring in the DensiCHEK.

2.5 Disc preparation

From a sheet of filter paper, a number of 6 mm discs were cut off and were sterilized by autoclaving. The discs were then divided into two parts: one part was soaked in 70% ethanol and the other part was soaked in the about 200 µl of plant extract overnight. Then, both types of discs were dried in a laminar air flow cabinet maintaining asepsis, and the dried up discs were collected in sterilized containers and kept at 4^{o}C.

2.6 Antifungal activity

The antifungal activity of 70% ethanolic extract of the plant was carried out by two methods-

1. Disc diffusion method

The agar disc diffusion method was used for determination of the effectivity of the root extract against the selected strains. In this method, uniform suspensions of 0.5 mcf were prepared for the four different kinds of fungal strains. These suspensions were spread on the Mueller Hinton Agar plates using sterile swabs. The pre-prepared discs with 70% ethanol and the plant extract discs were placed accordingly. The discs were placed in such a way so that there was sufficient distance from the edge of the plate. All the plates were kept for incubation at 37^{o}C overnight. The zones of inhibition were then measured.

2. Micro-dilution test

MIC determination

The 96-well sterile micro-titer plate was used for determination of minimum inhibitory concentration (MIC). 100 µL of MH broth was pipetted in each of the well. The 100 µL extract was then added in the first well and thoroughly mixed, then serially diluted in successive wells in double dilutions. Then 10 µL of 0.5 mfu fungal suspensions were pipetted in each well, Control experiment was also done similarly using only 70% ethanol. The sample was analyzed with Thermo MULTISKAN EX micro plate reader. MIC values were detected at 620 nm at zero hour and 24 hours. During incubation periods, plates were incubated under normal atmospheric conditions at 37°C for 24 h. The MIC value was determined as the lowest concentration of the extract in the broth medium that inhibits the growth of the test microorganism.

3. RESULTS

The antimicrobial activity was determined by the agar disc diffusion method followed by the broth dilution method, Results are given in Table III. The ethanolic extract of *N. jatamansi* showed antifungal activities against multi-drug Resistant (MDR) fungal species. *N. jatamansi* extract inhibited the growth of all tested microorganisms - *Candida albicans* (ATCC), *Candida parapsilosis* (ATCC), *Candida gulliermondii* (MDR), *Candida glabrata* (MDR) plates, with mild zones of inhibition around the discs.

Minimum inhibitory concentrations (MIC) values showed a similar pattern of growth inhibition against all tested microorganisms. These results are shown in the graphs (Fig.9-12) depicting differences between the absorbance values in the zero h and 24 h *versus* the concentrations of the plant extract. Almost similar inhibition patterns were observed against MDR strains. MIC of the extract against ATCC *C. albicans* and MDR *C. gulliermondii* were found 2.77 mg/mL, whereas, that against the ATCC *C. parapsilosis* and MDR *C. glabrata* were 5.83 and 12.24 mg/mL respectively.

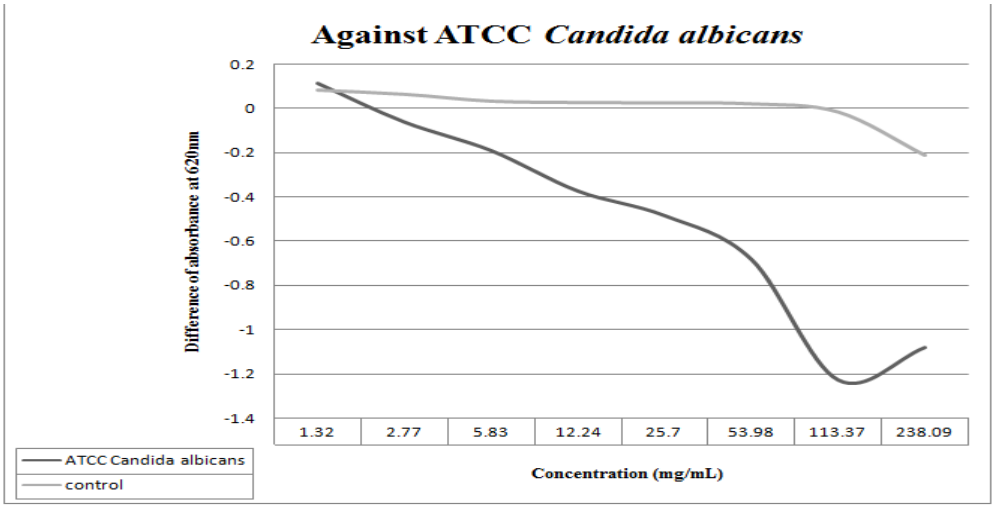

Fig. 9: Antifungal ativity of root extract from *N. jatamansi* against *C. albicans* (ATCC)

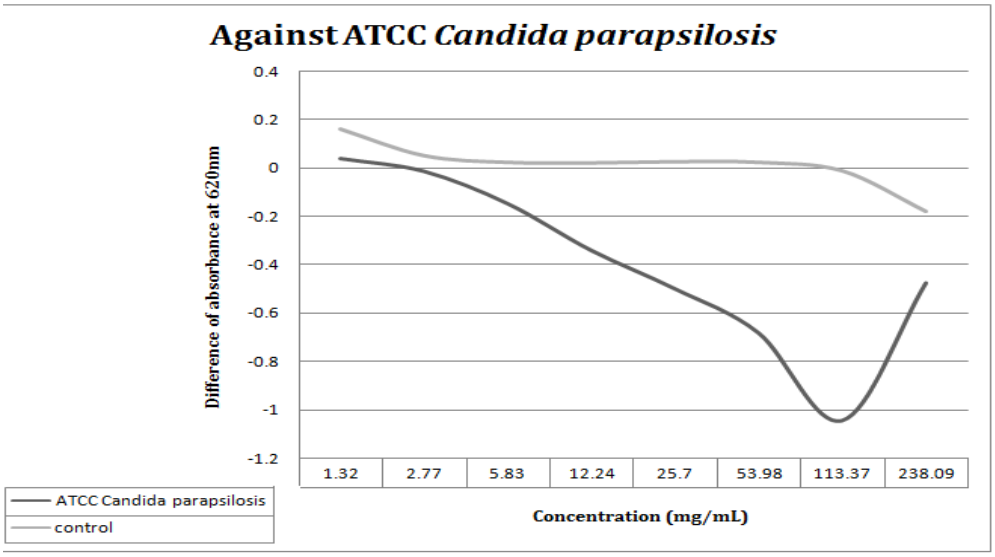

Fig. 10: Antifungal activity of the root extract from *N. jatamansi* against *C. parapsilosis* (ATCC)

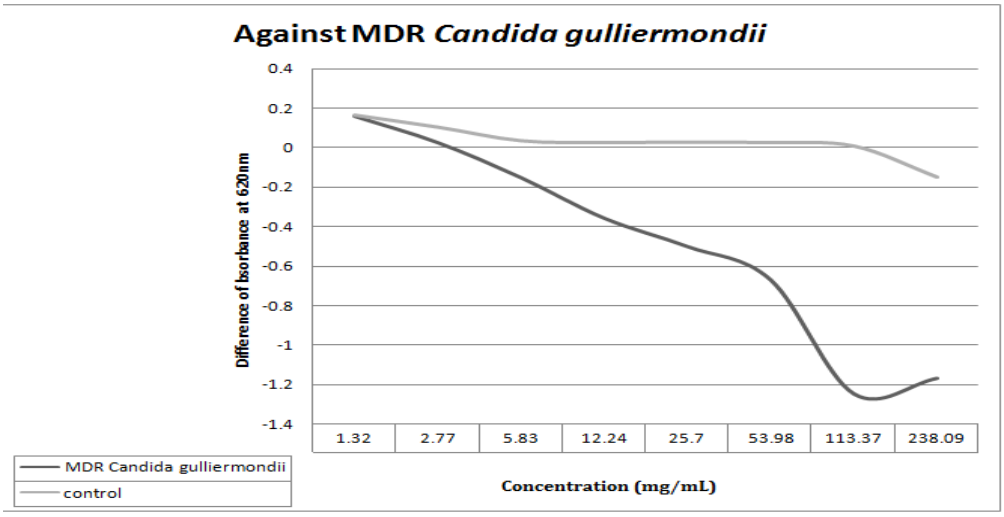

Fig. 11: Antifungal activity of the root extract from *N. jatamansi* against *C. gulliermondii* (MDR)

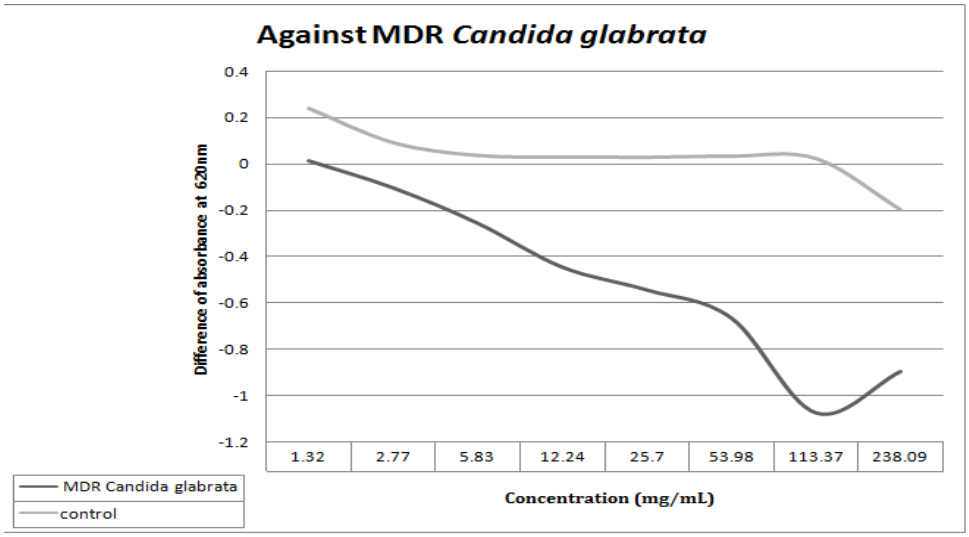

Fig. 12: Antifungal activity of the root extract from *N. jatamansi* against *C. glabrata* (MDR)

Table III: Antifungal activity exhibited by roots of *Nardostachysjatamansi* against tested microorganisms

Name of organisms	Zone of Inhibition; dia (mm)		
	Control (70% ethanol)	Plant extract	
		Disc I	Disc II
ATCC *Candida albicans*	6	7	7
ATCC *Candida parapsilosis*	6	7	6.5
MDR *Candida gulliermondii*	6	6.5	7
MDR *Candida glabrata*	6	7	8

4. DISCUSSION

From this experiment we have established that *N. jatamansi* root extract has antifungal activities against different *Candida* spp. like *Candida albicans* (ATCC), *Candida parapsilosis* (ATCC), *Candida gulliermondii* (MDR), *Candida glabrata* (MDR). Many studies have highlighted the antimicrobial activities of *N. jatamansi* against different micro-organisms, but there is no study reflecting the use of this plant extract against MDR *Candida* strains. In an important study done by Rachotimath *et al*,[7] 16 - 40% Candidacidal activities were shown by the different extracts of this plant. However, in this study, we are highlighting its action against MDR *Candida* spp., which has important clinical implications. Our study results thus show a ray of hope in future applications of this extract to win the battle against

MDR strains of *Candida* spp. The present work requires further attention and in-depth studies are needed in this direction to develop life saving antifungal agents for humans.

5. CONCLUSION

Ethanolic extract of *N. jatamansi* shows antifungal activities against ATCC *C. albicans*, MDR *C. gulliermondii*, ATCC *C. parapsilosis*, MDR *C. glabrata* with MIC values varyimg from 2.77 to 12.24 mg/mL.

6. ACKNOWLEDGEMENT

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7. ABBREVIATIONS

MDR - Multi Drug Resistance

ATCC – American Type Culture Collection

MIC – Minimal Inhibitory Concentration

MH - Mueller Hinton

8. BIBLIOGRAPHY

1. S. Airi, R. S. Rawal, U. Dhar, A. N. Purohit; *Current Science*, 2000; 79(10): 1467-1471.
2. G. Buchbaur; Perfumer and Flavorist, 2000; 25: 64-67.
3. Robin C, Desprez-Loustau ML. Testing variability in pathogenicity of *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, 1998; 104: 465– 475.
4. Harborne J B and Baxter H. Phytochemical Dictionary: a Handbook of Bioactive Compounds from Plants, Taylor & Francis, 1995.
5. Deyuan Hong, Fred R. Barrie & Charles D. Bell. In Wu, Z. Y., P. H. Raven & D. Y. Hong, eds. Flora of China. (Cucurbitaceae through Valerianaceae, with Annonaceae and Berberidaceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis, 2011; 19.
6. SAHU *et al.*, *Orient. J. Chem.*, 2016; 32(2): 859-866.

7. Rachotimath B, Gavimath M S, Uppar V K et al. Anti-inflammatory, anti-candidal activity and in silico prediction of pharmacokinetic properties of Nordostachys jatamansi, IJPSR, 2021; 12(9): 4972-4978.