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Antibacterial potentials of rhizophere fungi from *Hemidesmus indicus* R Br.

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Abstract
Crude extract isolated from rhizosphere fungi of *Hemidesmus indicus* R. Br. were tested against pathogenic bacteria ie. *Escherichia coli* (Gram negative), *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) using the Agar Well Diffusion (AWD) method. Antibacterial potentials of tested fungi were confirmed by measuring zone of inhibitions. Among all the tested fungi *Aspergillus nidulance* exhibits highest antibacterial activity against tested microbes.

Keywords: Antibacteial activity, rhizosphere fungi, *Hemidesmus indicus* R Br

Introduction

Microorganisms mainly resides in the rhizosphere region such as bacteria, actinomycetes, fungi, protozoa, microalgae and microfauna, such as nematodes and mites. Concentration of these microbes significantly greater in rhizosphere region than root free or non- rhizosphere soil (Subba Rao 1977) [10].

Among such microbes, most dominant group of microbe present in rhizosphere soil are fungi (Guleri, S. *et al*. 2010) [4]. Many rhizosphere fungi mostly dependant on association with plants which are regulated by root exudates (Bais, *et al*. 2006) [2]. Root exudates influence the proliferation and survival of the microorganisms.

Rich source of bioactive metabolites, are the medicinal plants. These metabolites are considered to be safe for environment as compared to the synthetic one. (Nema *et al*. 2013) [7]. The cost of production, side effects and the development of resistance by disease causing agents against synthetic chemicals, necessitate the use of chemicals derived from medicinal plants for human and animal applications. In view of these, there is a need for sustainable cultivation and continuous production of naturally available resources for variety of applications.

Materials and Methods

Hemidesmus indicus R. Br. (Family-Apocynaceae) is twining perennial shrub and commonly known as Anantmool or Indian Sarsaparilla, having thick, hard and cylindrical aromatic roots, slender, wiry and lacticiferous stems. It is extensively utilized in traditional medicine for its diverse therapeutic properties against various disease like respiratory disease, blood disease, diarrhea, eye disease, skin disease, fever, bronchitis, burning sensation, gastric disorders and rheumatism (Shete and Bodhankar, 2010) [9]. The roots are useful in leprosy, syphilis, asthma, dysentery, kidney and urinary diseases (Austin, *et al* 2008) [1].

Collection of Rhizosphere Soil Samples

Soil samples were collected from the rhizosphere regions of the selected medicinal plants during three different seasons monsoon, winter and summer following the methodology outlined by Dongmo and Oyeyiola (2008) [3]:

1. **Sampling Locations:** Samples were gathered from various locations within the Yavatmal district to capture ecological diversity.

2. **Sample Depth and Volume:** Soil was collected from a depth of approximately 5-15 cm around the root zone, ensuring samples were close to the root system where microbial activity is concentrated.

3. **Collection and Storage:** Using sterilized tools, around 100-150 grams of soil per plant were gathered in sterile polyethylene bags, labelled, and transported to the laboratory under controlled conditions to preserve microbial viability.

Isolation of Rhizosphere Fungi

The fungi were isolated from the soil samples using the serial dilution plate technique, as described by Johnson and Curl (1972) [6]:

Extraction and Isolation of Bioactive Compounds

To investigate bioactive metabolites, the isolated fungi were cultured, and metabolites were extracted using ethyl acetate extraction as per the methods of Haque *et al*. (2005) and Radji *et al*. (2011) [5, 8]:

1. **Inoculation and Cultivation:** Each fungal isolate was inoculated in a 250 ml Erlenmeyer flask containing 100 ml of Potato Dextrose Broth (PDB) supplemented with 2 g/L of geranyl pyrophosphate (GPP) to stimulate secondary metabolite production.

2. **Incubation:** Cultures were maintained at room temperature for 21 days under stationary conditions with intermittent shaking to ensure nutrient distribution.

3. **Filtration:** After incubation, cultures were filtered through Whatman filter paper to separate the fungal biomass from the culture filtrate.

4. **Extraction of Metabolites:** An equal volume of ethyl acetate was added to the culture filtrate in a separating funnel, mixed well, and left for 10 minutes to separate

the organic and aqueous layers. The ethyl acetate layer (containing active compounds) was collected, and the process was repeated twice for maximum extraction efficiency.

5. **Concentration:** The pooled ethyl acetate extracts were evaporated to dryness in a hot air oven set at 80°C, yielding crude bioactive extract. Extract residues were dissolved in 5 ml of Dimethyl Sulfoxide (DMSO) and stored at 4°C.

Antimicrobial Assay

The antimicrobial activity of the isolated metabolites was assessed against selected pathogens *E. coli* (Gram negative), Pseudomonas aeruginosa (Gram negative) and Staphylococcus aureus (Gram positive) using the Agar Well Diffusion (AWD) method:

1. **Pathogen Preparation:** Test pathogens were cultured to an 18-24-hour growth phase and adjusted to a 0.5 McFarland standard to ensure consistent inoculum density.

2. **Plate Preparation:** Mueller-Hinton Agar (MHA) plates were prepared, and wells of 5 mm diameter were cut using a sterile cork borer, providing a well depth of 4-5 mm.

3. **Extract Application:** Each well was loaded with 30 µL of the DMSO-dissolved extract, while a DMSO-only control was added to another well.

4. **Diffusion and Incubation:** Plates were allowed to diffuse for 15 minutes at room temperature before being swabbed with pathogen cultures. Plates were then incubated at 37°C for 24 hours.

Measurement of Zone of Inhibition: After incubation, zones of inhibition around each well were measured in millimetres, indicating antimicrobial efficacy. All assays were conducted in triplicate to ensure reproducibility

Observations and Results

The crude extract of *Aspergillus terreus* (monsoon) Sample Id. H1, *Aspergillus niger* (monsoon) Sample Id. H2, *Aspergillus niger* (winter) Sample Id. H3 and *Aspergillus terreus* (winter) Sample Id. H4 isolated from *Hemidesmus indicus* R.Br tested antimicrobial activity against *E.coli* did not show any zone of inhibition at any concentration, while *Aspergillus nidulance* (monsoon) Sample Id. H5 tested antimicrobial activity against *E.coli* showed lower zone of inhibition (13) at only 100µl concentration.

The extract of *Aspergillus terreus* (monsoon) Sample Id. H1, *Aspergillus niger* (monsoon) Sample Id. H2, *Aspergillus niger* (winter) Sample Id. H3 and *Aspergillus terreus* (winter) Sample Id. H4 and *Aspergillus nidulance* (monsoon) H5 isolated from *Hemidesmus indicus* R. Br tested antimicrobial activity against *Staphylococcus aureus* did not show any zone of inhibition atany concentration.

The extract of *Aspergillus terreus* (monsoon) Sample Id. H1, *Aspergillus niger* (monsoon) Sample Id. H2, *Aspergillus niger* (winter) Sample Id. H3 and *Aspergillus terreus* (winter) Sample Id. H4 isolated from *Hemidesmus indicus* R.Br tested antimicrobial activity against *Pseudomonas aeruginosa* did not show any zone of inhibition at any concentration, only *Aspergillus nidulance* showed high (19) zone of inhibition at only 100µl conc.

Table 1: Antimicrobial sensitivity test of rhizosphere fungi isolated from *Hemidesmus indicus* R.Br. against *E. coli*

Antibacterial activity	Zone of Inhibition in mm		
EC18 against rhizosphere fungi	25µl	50 µl	100 µl
EC18-*Aspergillus terreus*	NI	NI	NI
EC18-*Aspergillus niger*	NI	NI	NI
EC18-*Aspergillus niger*	NI	NI	NI
EC18-*Aspergillus terreus*	NI	NI	NI
EC18-*Aspergillus nidulance*	NI	NI	13

Note: NI: -No Inhibition, Strain no EC18: *Escherichia coli*

Table 2: Antimicrobial sensitivity test of rhizosphere fungi isolated from *Hemidesmus indicus* R. Br. against *Staphylococcus aureus*

Antibacterial activity	Zone of Inhibition in mm		
SA16 against rhizosphere fungi	25µl	50 µl	100 µl
SA16-*Aspergillus terreus*	NI	NI	NI
SA16-*Aspergillus niger*	NI	NI	NI
SA16-*Aspergillus niger*	NI	NI	NI
SA16-*Aspergillus terreus*	NI	NI	NI
SA16-*Aspergillus nidulance*	NI	NI	NI

Note: NI: No Inhibition, Strain no SA16: *Staphylococcus aureus*

Table 3: Antimicrobial sensitivity test of rhizosphere fungi isolated from *Hemidesmus indicus* R.Br. against *Pseudomonas aeruginosa*

Antibacterial activity	Zone of Inhibition in mm		
PA13 against rhizosphere fungi	25µl	50 µl	100 µl
PA13-*Aspergillus terreus*	NI	NI	NI
PA13-*Aspergillus niger*	NI	NI	NI
PA13-*Aspergillus niger*	NI	NI	NI
PA13-*Aspergillus terreus*	NI	NI	NI
PA13-*Aspergillus nidulance*	NI	NI	19

Note: NI: No Inhibition, Strain no PA13: *Pseudomonasaeruginosa*

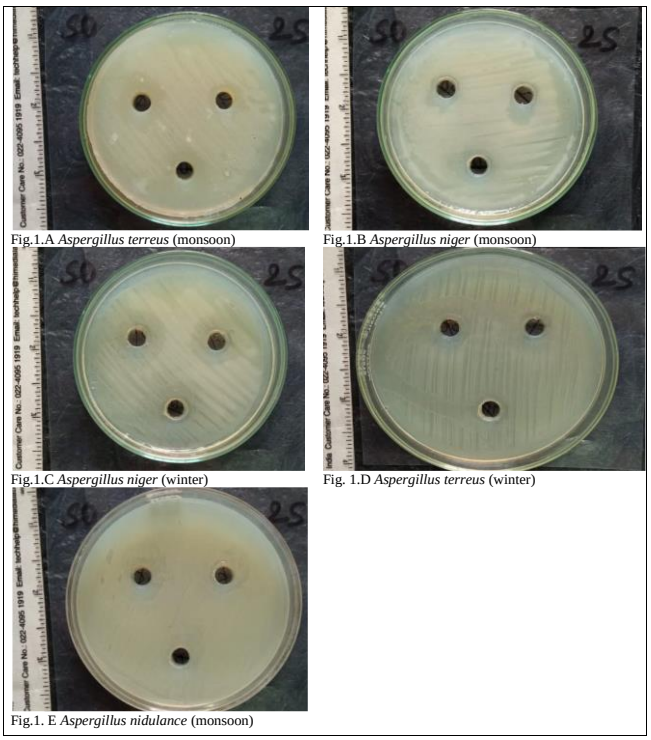

Fig.1.A *Aspergillus terreus* (monsoon)
Fig.1.B *Aspergillus niger* (monsoon)
Fig.1.C *Aspergillus niger* (winter)
Fig. 1.D *Aspergillus terreus* (winter)
Fig.1. E *Aspergillus nidulance* (monsoon)

Fig 1 A-E: Antibacterial activity of rhizosphere fungi from *Hemidesmus indicus* R.Br. against *Staphylococcus aureus* in different seasons

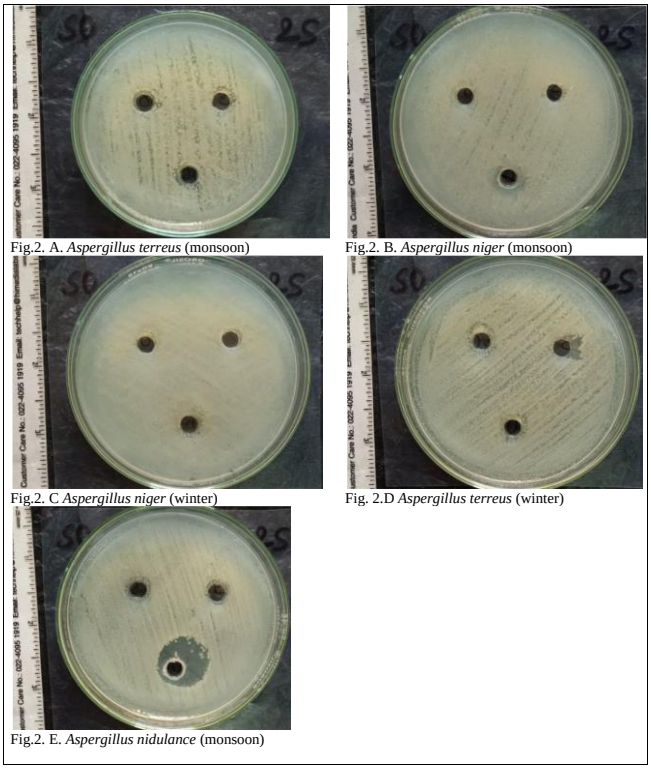

Fig.2. A. *Aspergillus terreus* (monsoon)

Fig.2. B. *Aspergillus niger* (monsoon)

Fig.2. C *Aspergillus niger* (winter)

Fig. 2.D *Aspergillus terreus* (winter)

Fig.2. E. *Aspergillus nidulance* (monsoon)

Fig 2 A-E: Antibacterial activity of rhizosphere fungi from *Hemidesmus indicus* R.Br. against *Pseudomonas aeruginosa* in different seasons

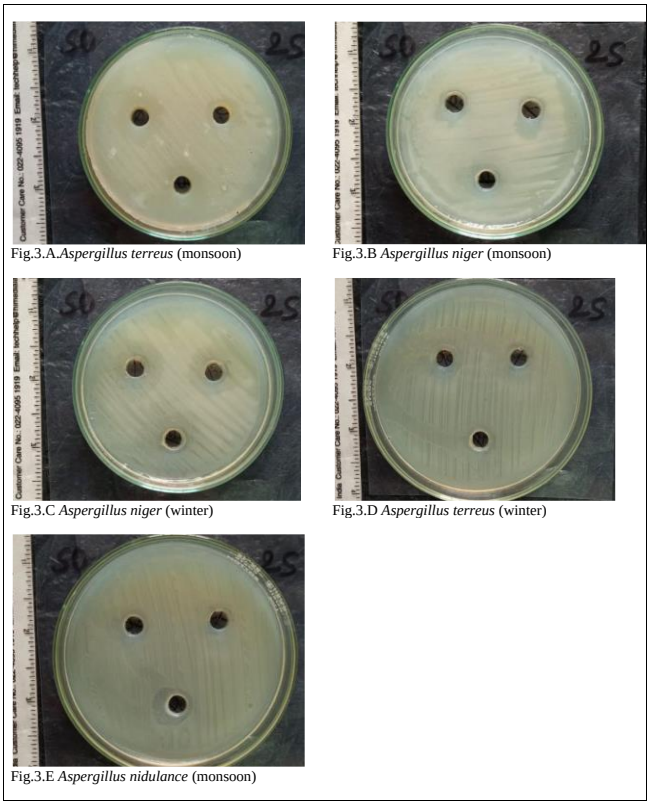

Fig.3.A.*Aspergillus terreus* (monsoon)

Fig.3.B *Aspergillus niger* (monsoon)

Fig.3.C *Aspergillus niger* (winter)

Fig.3.D *Aspergillus terreus* (winter)

Fig.3.E *Aspergillus nidulance* (monsoon)

Fig 3 A-E: Antibacterial activity of rhizosphere fungi from *Hemidesmus indicus* R.Br. against *E. coli* in different seasons

Conclusions

A key aspect of the research is the role of rhizosphere fungi in secondary metabolite production, particularly their contributions to antimicrobial, antioxidant, and plant growth-promoting properties. *Aspergillus nidulance* emerged as the most metabolically active fungal species, producing Stearic acid Hydrazide, a bioactive compound with significant antimicrobial potential. Other metabolites identified include Propanoic Acid, 3-hydroxy-hydrazide and Nonane, 3-methyl-5-propyl-, indicating that rhizosphere fungi have immense biotechnological and pharmaceutical potential.

References

1. Austin A, Rumi Herbals R and D Center. A Review on Indian Sarsaparilla *Hemidesmus indicus* R. British Journal of Biomedical Science,2008:8(1):1–12.
2. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. The role of root exudates in rhizosphere interactions. Nature Reviews Microbiology,2006:4(3):273–282.
3. Dongmo JC, Ayeyiola GP. Fusaria in the Rhizosphere and Rhizoplane of Groundnut (*Arachis hypogaea*). Journal of Agricultural Research and Development, 2008, 5(2).
4. Guleri S, Shekh NF, Sharma R. Fungal diversity and their interactions in rhizosphere soils. Indian Journal of Microbial Ecology,2010:9(2):85–93.
5. Haque MD, Mukherjee AK. An extracellular Siderophore Isolate of *Bacillus subtilis* Enhances Antibiotic Susceptibility of Multidrug-Resistant *Klebsiella pneumoniae*. FEMS Microbiology Letters,2005:244(2):273–282.
6. Johnson LF, Curl EA. Methods for Research on the Ecology of Soil-Borne Plant Pathogens. Burgess Publishing Company, 1972.
7. Nema S, Dubey NK, Kumar R. Biocontrol potential of rhizosphere fungi against soil-borne plant pathogens. Journal of Agricultural Science and Technology,2013:15(4):873–888.
8. Radji Maksum, Sitifauziah, Nurgani Aribinuko. Antibiotic sensitivity pattern of bacterial pathogen in the intensive care unit of Fatmawati Hospital, Indonesia. Asian Pac I. Trop. Biomed,2011:1(1):39–42.
9. Shete RV, Bodhankar SL. *Hemidesmus indicus*: Evaluation of its nootropic effect in mice. International Journal of Pharma and Bio Sciences,2010:1(3):1–10.
10. Subba Rao NS. Soil Microorganisms and Plant Growth. Oxford & IBH Publishing, 1977, 1–252.