Antibacterial eventuality of secondary metabolites produced by rhizospheric fungi isolated from diversified soil of Narmada river and forest region Jabalpur

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Abstract
There has been continuous scan for increasingly powerful antibiotic agents that can stand this emergency of drug resistance. Subsequently, there is an earnest need to research indigenous soil assets with capability of antimicrobial creation that could be utilized to deliver novel products with better viability. The present study was undertaken to test the antibacterial potentiality of rhizospheric fungi, especially found in rich diversified Narmada and Dumna, Jabalpur forest soil samples. According to their habitat, fungal members are also tacit to have healthy novel compound of pharmaceutical interest.

Thus research outputs show that rhizospheric fungi of investigated area can be used as a boon agent for highly potent antibacterial capability. All four (04) screened isolates showed vigorous and wide-ranging activity against 05 human disease causing drug resistant bacteria. 10.21±1.53 to 22.00±0.10 (mm) inhibition zone and statistically investigated error output show the wild nature of bioactive compounds present in isolates. Hence this research provides groundwork substantiation to explore the rhizospheric soil fungi for the ecofriendly drug designing.

Keywords: Rhizospheric fungi, Novel antibacterial, Narmada Soil, Forest region, Pharmaceutical effect.

Introduction

Variability comes from the biodiversity which establishes the life form of every living being on earth including animals, plants and microorganisms. Where fungi play crucial role in biodiversity, they appropriately set up the survival of other organisms. Fungi are huge spring of fresh bioactive compounds. They have achieved enormous connotation in recent subsistence; as a result, mycologists became more anxious of biodiversity of fungi and all over biologists are showing more attention in fungi.[18]

Fungi are widely distributed in soil, where maximum number of genera and species of fungi are present in soil as compared to other environmental resources. The contribution of fungi is very vast to increase soil fertility, soil structure and they are also credited for balancing the ecosystem. They have power to recycle the ecosystem credited for balancing to the ecosystem. They have power to recycle the ecosystem.[7] Relationship between plant root and microorganisms is one of the focal points of crucial and practical biology, since over a century.

Rhizospheric may be defined as the surrounding area of plant roots having microbial activity by means of plant bustle. Rhizospheric microbes are quite different from non-rhizospheric microorganisms.[19] The rhizospheric microorganisms influence the growth and they develop disease and sudden death resistance power on the plant host on the point of parasitism and pathogenicity. Rhizospheric microbes are recognized as noteworthy resource of a diversity of structurally new active secondary metabolites which can be used as a strapping antimicrobial agent.[12]

It has been established that the innate sources are crucial basis of novel dynamic agents. These types of natural compounds play a noteworthy role in the finding and expansion of remedies to cure several chronic diseases.[4] Bioactive compounds are produced by using microbial fermentation where prolific secondary metabolite producers play extensive role. Secondary metabolites contain several bioactive compounds that are traditionally used as best pharmaceutical medicine to cure contagious diseases.

Nowadays these compounds are widely used against some pathogenic bacteria as having antimicrobial activity.[1] Earth is a primary resource of fungi where they produce immense number of extracellular bioactive metabolites including immuno-suppressants, cholesterol-lowering agents and potent mycotoxins[10], antibacterial[11,16] and antifungal.[13]

The need for fresh bioactive mixes that can be utilized in pharmaceuticals, industries and agriculture is constantly escalating. Still rhizospheric soil and fungal growths are being utilized for the creation of a few bioactive mixes including anti-infection agents and different chemicals which are generally utilized as key wellspring of pharmacological specialists and modern significant mixes.[6] Rhizospheric parasites present in the rhizosphere play huge undertaking in the turn of events and biological forces.[7]

Secondary metabolites delivered by organisms are current need to build up brand new bioactive exacerbates that can assist with diminishing multi-opposition in pathogenic microorganisms. This study may turn into a positive task in the benevolence of characteristic and novel anti-microbials.[15] Our Narmada soil and Dumna forest region

soil, Jabalpur are much diversified that may be boon for the novel antimicrobials.

Material and Methods

Survey and collection of soil samples: Soil samples were collected from various region of Jabalpur (M.P.) including Hitkarini campus Dumna, Priyadarsani colony Dumna, GCF forest region and Narmada River Bheda Ghat. The samples were collected from rhizospheric zone of plants which were randomly selected, then all samples were brought to laboratory in sterilized polythene bag.

Isolation of fungi: Each soil sample was refined by using blender and micro sieve tubes to get 10gm fine particles. Serial dilution was prepared up to 10^{-3} ratio. This was done by withdrawing 1 ml of stock solution and pipette it into blank test tube having 9 ml of sterilized distilled water. PDA (Potato Dextrose Agar, pH 5.5) media was prepared for the isolation of fungi. The pure cultures of isolated fungi were preserved on potato dextrose agar (PDA) slant at 4°C with proper labeling and were sub cultured time to time for further uses.

Identification of isolated rhizospheric fungi: Isolated fungal strains were identified by using macroscopic (Morphological) and microscopic (Slide culture) technique. All observations were recorded. Finally observed strains were characterized on the basis of catalogue described by Barnett and Hunter.[2]

Fermentation to produce secondary metabolites: PDB (Potato Dextrose Broth) medium was prepared for the production of bioactive compounds. All flasks were inoculated with isolated fungal strains and incubated for different incubation periods as 7, 14 and 21 days.

Extraction of MFCF (Mycelia Free Culture Filtrate): After the completion of incubation periods, all secondary metabolites were collected in separate sterile flasks by using filtration technique using Whatmann filter paper no.1 and these filtrates (MFCF) were stored at 4°C for the further uses. All secondary metabolites were tested for the potential of antibacterial activity.

Screening of antibacterial activity: Total numbers of metabolites collected from rhizospheric soil fungi were screened for their antibacterial potentiality against 05 Pathogenic bacteria i.e. *Bacillus subtilis* (B01), *Streptococcus pyogenes* (B02), *Escherichia coli* (B03), *Klebsiella pneumonia* (B04) and *Salmonella typhimurium* (B05) by using agar well diffusion method.[14] PDA plates were prepared and inoculated with overnight culture of each bacterial suspension by evenly spreading out with sterile borosil glass spreader. Agar wells were prepared by scooping out the media with a sterile cork borer (6 mm in diameter).

The wells were then filled with 80 µl, of the fungal crude extract (Fermented broth) that was already dissolved in DMSO. The plates were then incubated at 37°C for 24 hrs and the zones of inhibitions were recorded and compared with the control after the completion of incubation period. Antibacterial potential of secondary metabolites was measured by using clear zone of inhibition mapping Hi-Media scale against selected pathogenic bacteria.

Results

Survey and collection of rhizospheric soil samples: Well bio-diversified regions were selected to collect the rhizospheric soil samples. Total 08 rhizospheric soil samples were collected and refined in fine particles according to the experimental requirement. The soil sample collection site and respective sample numbers are depicted in table 1.

Isolation of rhizospheric fungi and identification: Soil samples were aseptically brought to the Fungal Biotechnology and Invertebrate Pathology Laboratory for further process. Partially dried soil particles were spread on agar media. Workup methods were used for the isolation of fungi. Sample inoculated plates were incubated at 28±1ºC for colony appearance. Observed fungi were individually transferred to the potato dextrose agar plate and allowed for full growth. Each isolate was identified by using morphological study and slide culture technique.

On the basis of their macroscopic and microscopic characteristics, fungal isolates were successfully identified as *Penicillium* sps. (HFRD-02), *Aspergillus japonicus* (GCFFR-02), *Mucor racemosus* (NRBG-01) and *Fusarium poae* (NRBG-01) shown in fig. 1 and table 2.

Antibacterial activity by agar well diffusion method: Screening of rhizospheric fungi for potent antibacterial activity was completed by using agar well diffusion method.

Table 1
Sample collection sites with sample numbers and number of isolated fungal strain.

S.N.	Rhizospheric Soil Sample Collection Site	Number of Rhizospheric Soil Sample Collected	Number of isolated fungal strain
1.	Hitkarini forest region, Dumna	2	2
2.	Priyadarshini colony, Dumna	2	2
3.	GCF forest region, Jabalpur	2	3
4.	Narmada region, Bheda Ghat	2	3

Table 2
Pure culture of potent identified isolates

S.N.	Fungal Strain Code	Identified Name
01.	HFRD-02	*Penicillium* sps.
02.	GCFFR-02	*Aspergillus japonicus*
03.	NRBG-01	*Mucor racemosus*
04.	NRBG-03	*Fusarium poae*

05 human pathogenic bacteria were used to check inhibitory activity present in fungi. Different incubated and extracted secondary metabolites were used against bacterial growth. All metabolites showed broad spectrum antibacterial activity where fungal code NRBG-03 showed maximum 22mm and 20mm clear zone of inhibition against *Salmonella typhimurium* (B05) and *Escherichia coli* (B03) respectively where isolate GCFFR-02 showed 18mm, 17mm and 15mm clear zones of inhibition against *Bacillus subtilis* (B01), *Klebsiella pneumonia* (B04) and *Salmonella typhimurium* (B05) respectively. GCFFR-02 showed maximum 18mm and 17 mm inhibition zone against *Bacillus subtilis* (B01), *Klebsiella pneumonia* (B04) respectively.

Fungal isolate NRBG-01 showed 18mm and 15mm clear inhibition zone against *Bacillus subtilis* (B01) and *Streptococcus pyogenes*. Overall inhibition zone is depicted in table 3 and graph 1 and broad spectrum activity is depicted in figure 2 and table 4. Chloramphenicol was used as positive control.

Statistical analysis: The broad spectrum data of antibacterial activities were statistically analyzed for their acceptance and rejection hypothesis. The extensive results of antibacterial activity were expressed by using average mean, standard deviation and possible errors. Minor errors showed screened potent fungal metabolites where minimum and maximum errors were 0.53514 and 0.762654 respectively. All statistics values are portrayed in graph 2.

Discussion

Secondary metabolites are traditionally natural mixes delivered from microorganisms during the change of essential metabolite union. Secondary metabolites do not show development and improvement of organisms and are normally framed in the fixed stage. Numerous among auxiliary metabolites have biological capacities which incorporate protection components, additionally work as antimicrobial operators or anti-infection agents.[8]

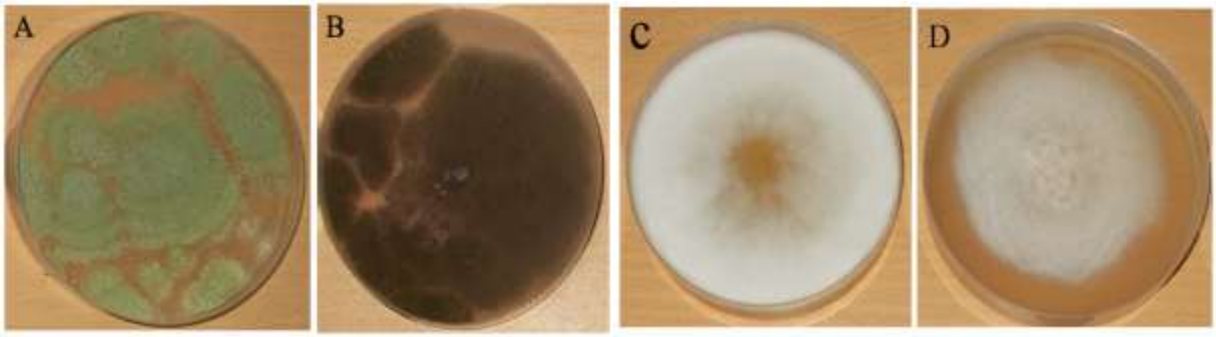
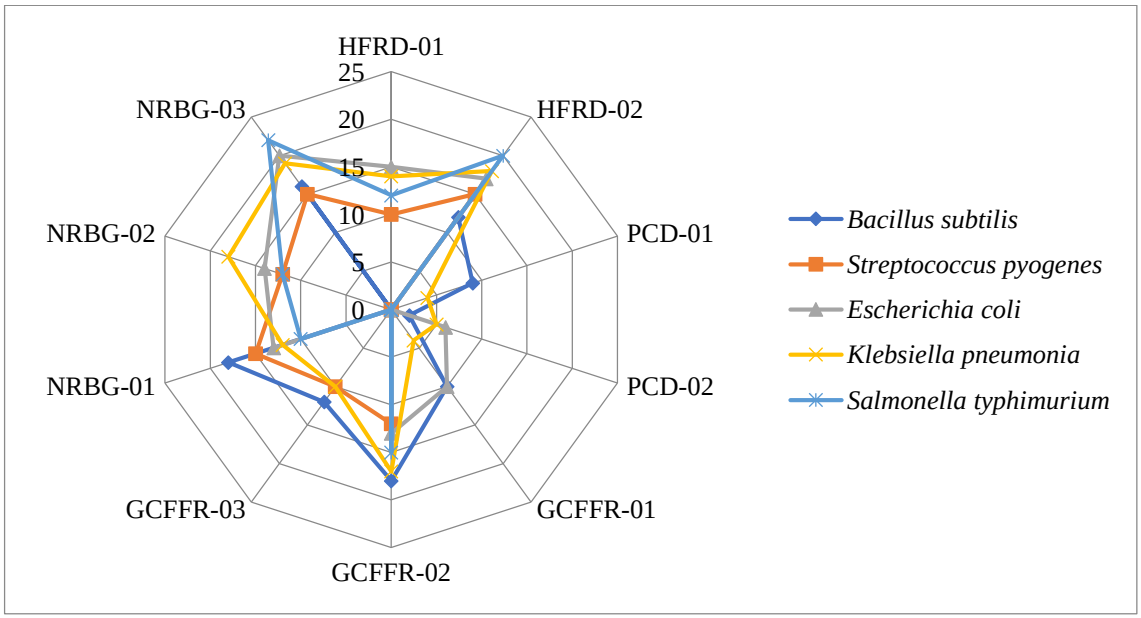


Figure 1: (A) *Penicillium* sps., (B) *Aspergillus japonicus*, (C) *Mucor racemosus* and (D) *Fusarium poae*.



Graph 1: Showing screening of antibacterial potentiality of rhizospheric fungi by radar diagram.

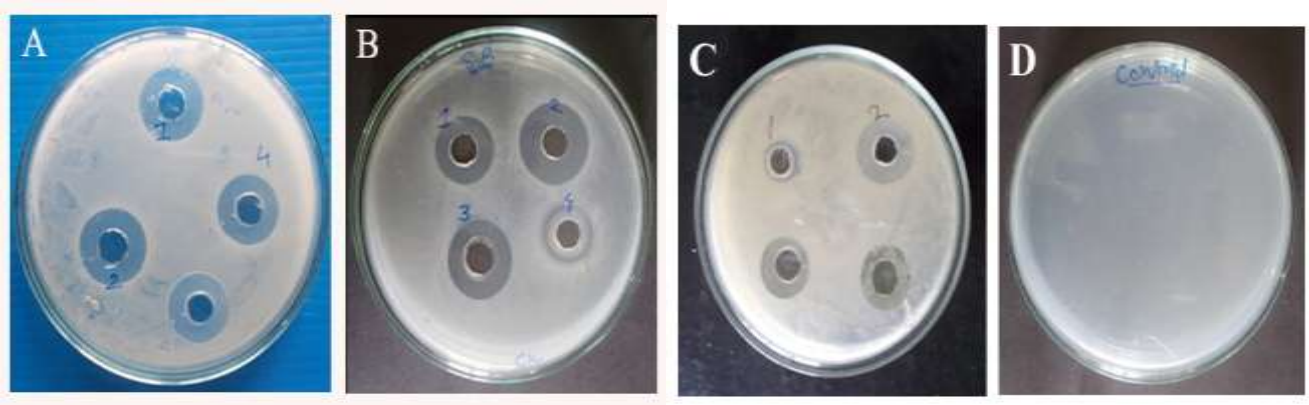
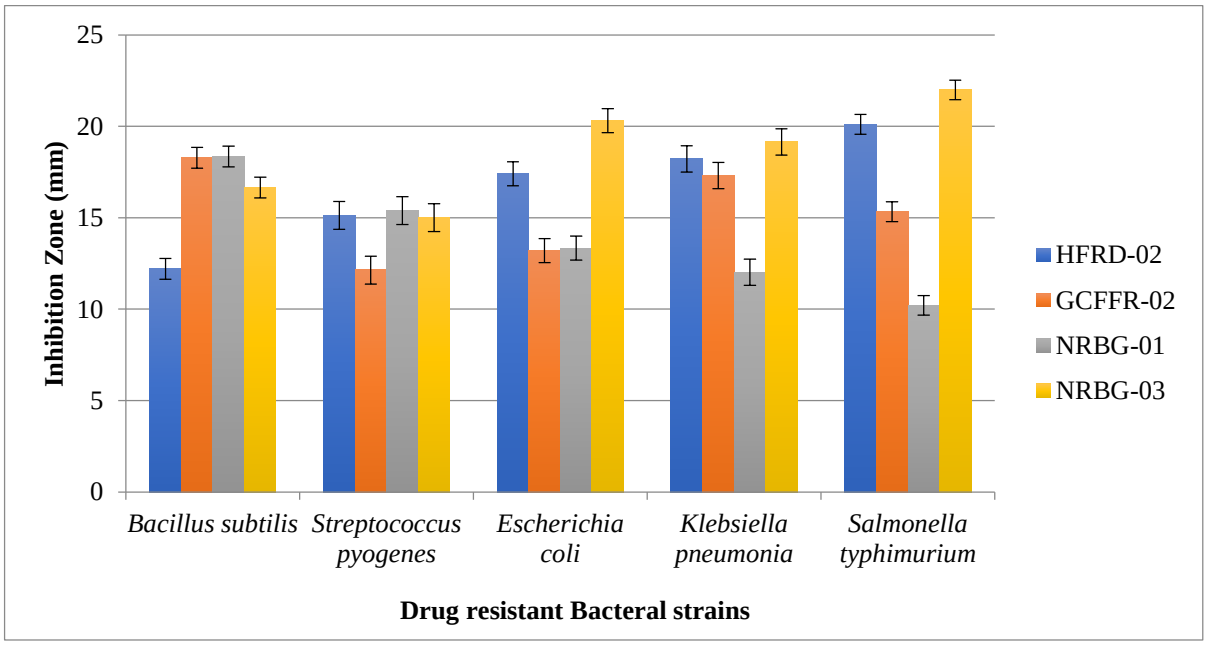


Figure 2: Broad Spectrum activity by agar well diffusion method: Showing clear zone of inhibition

Table 3
Screening of antibacterial potentiality of rhizospheric fungal isolates.

S.N.	Fungal Strain Code	Clear Zone of Inhibition (mm)				
		Bacillus subtilis	***Streptococcus pyogenes***	***Escherichia coli***	***Klebsiella pneumonia***	***Salmonella typhimurium***
01.	HFRD-01	-	10	15	14	12
02.	HFRD-02	12	15	17	18	20
03.	PCD-01	09	-	-	04	-
04.	PCD-02	02	-	06	05	-
05.	GCFFR-01	10	-	10	04	-
06.	GCFFR-02	18	12	13	17	15
07.	GCFFR-03	12	10	-	10	-
08.	NRBG-01	18	15	13	12	10
09.	NRBG-02	-	12	14	18	12
10.	NRBG-03	16	15	20	19	22



Graph 2: Showing broad spectrum antibacterial potentiality with their possible standard error

Rajalakshmi and Mahesh[15] isolated fungi from rhizospheric soil sample from Kuttralam hills station and identified the potent fungi as *Aspergillus terreus* on the basis of 18S rRNA assay and phylogenetic tree similarity index. That strain showed uppermost (29mm) antibacterial action next to *Staphylococcus*. In this present study we also reported *Aspergillus japonicus*, *Penicillium*, *Mucor* and *Fusarium* sp. having far ranging antibacterial activity. *Fusarium poae* recorded highest 22mm zone of inhibition against *Salmonella typhimurium*.

Table 4
Broad Spectrum activity against 05 pathogenic bacteria

S.N.	Fungal Strain Code	Clear Zone of Inhibition (mm)				
		Bacillus subtilis	***Streptococcus pyogenes***	***Escherichia coli***	***Klebsiella pneumonia***	***Salmonella typhimurium***
01.	HFRD-02	12.21±0.21	15.13±0.15	17.41±0.29	18.22±0.18	20.11±0.57
02.	GCFFR-02	18.28±1.02	12.14±0.54	13.21±0.52	17.31±0.26	15.33±0.65
03.	NRBG-01	18.35±0.14	15.39±1.62	13.34±0.34	12.02±0.21	10.21±1.53
04.	NRBG-03	16.65±0.12	15.00±0.37	20.30±0.56	19.15±0.32	22.00±0.10

Likewise Kumar and Raj[9] collected three soil samples from Vengodu, Kanchipuram district, Tamil Nadu, India and 06 novel actinomycetes were reported for their broad spectrum antibacterial activity. Antibacterial activity was confirmed by using cross streak method. Out of six isolates, only one isolate VAS 10 showed maximum activity and that was identified as *Actinobacterium Loyola*.

According to Bizuye et al[3], fifteen soil samples were collected from waste disposal and rhizosphere (at Tewodros Campus) Gondar town, Ethiopia and three actinomycetes (Ab18, Ab28 and Ab43) were isolated. Their antimicrobial activity was checked using inhibition zones obtained from agar well diffusion method. Here we also reported that agar well diffusion method was very effective method to determine antimicrobial assay.

Conclusion

The present study was an attempt to explore the potent rhizospheric soil fungi present in Narmada soil and diversified forest soil of Jabalpur (M.P.). 04 fungal strains were found widely distributed and showed potent antibacterial activity against six frequent disease causing bacteria. Activity was authenticated with the comparison of positive controls showing maximum 17mm zone of inhibition. Very vast antibacterial activities were shown by screened strains, hence their activity was subjected to statistical analysis using standard deviation and standard error.

So in this study, we can conclude that the widely distributed and potent antibacterial rhizospheric fungal strain namely *Penicillium* sps., *Aspergillus japonicus, Mucor racemosus* and *Fusarium poae* & that showed maximum 22mm clear zone of inhibition against drug resistant bacteria. According to the outputs, rhizospheric soil fungi found in Narmada soil and Dumna forest region, Jabalpur (M.P.) India can be investigated for the isolation of natural drugs which can play vital role in pharmaceutical industries as antimicrobial agents.

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