

Isolation partial purification of fungus bio active metabolites and its antibacterial activity in pathogenic bacteria

Nelsonjoseph Lawrance¹, Manjudevi Mariappan¹, Vishnupriya Benaltraja^{*1},

¹Department of Biotechnology Kongunadu Arts and Science College, Coimbatore,
Tamilnadu, India.

Corresponding author; bvishnupriya_bt@kongunaducollege.ac.in (B. Vishnupriya)

Abstract

In the history of humanity, infectious diseases brought on by pathogenic microorganisms have been the main cause of morbidity and mortality. Few new antibiotics are being developed, despite the urgent need for them to treat infectious diseases and drug-resistant infections. Natural products are a rich source of bioactive diversity. Sample collection and purification of fungus and fungus metabolites. Identification antibacterial activity was tested against Gram-positive *Staphylococcus aureus* (MTCC 8708) and gram-negative *Proteus vulgaris* (MTCC 426), *Klebsiella pneumonia* (MTCC 4030), *Serratia marcescens* (MTCC 96). One fungi metabolite gets strong antibacterial activity against pathogenic bacteria high 30 µl of fungi metabolites produced 21 mm zone of inhibition. Selected fungi molecular identified by using ITS-1 and ITS-2 primers. Morphemically identification assess by lacto-phenol cotton blue staining.

Keywords; *Acremonium borodinense*, fungal metabolites, Antibacterial, inhibition, genotypic.

Introduction

Microbial ecologists and taxonomists relied on culturing and morphological and physiological traits to describe microbial communities and members until the late 1980s. DNA sequencing has transformed the way microbial communities are studied over the last two decades (Stahl *et al.*, 1984; Hugenholtz *et al.*, 1996). *Acremonium* is a common species. *Acremonium* sp is a fungus that produces a number of commercially and pharmaceutically important metabolites (McGee *et al.*, 1991; Januar and Molinski, 2013). Molecular phylogeny is especially useful in the study of simple organisms, which give few morphological cues to their natural affiliations (Glenn *et al.*, 1996; Summerbell *et al.*, 2007). Phylogenetic revisions of *Acremonium* species using the General Algebraic Modelling System (GAMS) revealed the presence of multiple families, for example *Acremonium sclerotigenum*, *Acremonium cucurbitacearum*, *Plectosphaerella melonis*.

White *et al.* (1990) developed the most widely used primers in fungal ecology for sequence-based fungus identification at the species level. ITS sequences from a diverse group of fungal species found in environmental soil samples (Vancov *et al.*, 2009). ITS1, ITS2, ITS3 and ITS4, were developed by Garades and Bruns in the year of 1993. ITS1F and ITS4B were designed to be fungi and basidiomycetes specific, respectively (Gardes and Bruns, 1993). Buée *et al.* (2009) microbial community characterisation at greater sequencing depth than cloning and Sanger sequencing were thought to be achievable. Researchers can now identify a huge number of organisms from environmental samples using very short DNA sequences due to a number of next-generation sequencing tools.

Fungi, on the other hand, can be found in a wide variety of ecosystems and situations. The study of fungi is difficult due to their enormous diversity and the difficulties in predicting exact numbers (Hawksworth, Lücking, 2017). One of the most important aspects of mycological investigation of identification based on morphological, phylogenetic, or ecological characteristics. The traditional methods of fungi identification, which rely on direct observation of fungi in their natural state or after growing on growth media, are still widely used (Bálint *et al.*, 2016). Despite the use of molecular approaches as more advanced current tools for fungal identification, traditional methods for studying fungal varieties still have numerous advantages. Some fungi create visible features that can be used to identify them. Because some fungi are still difficult to cultivate, molecular approaches have been shown to be highly useful in determining their taxonomic identity (Peršoh, 2015).

Traditionally, physical traits such as sexual structure have been used to identify and classify fungi. Fungi have few relevant morphological characteristics and exhibit a lot of morphological variety (Brasier 1997; Burnett 2003). Despite the fact that many fungi are anamorphic and do not reproduce sexually, they exhibit surprising genetic diversity (Kohn et al., 1995). As a result, using morphology for fungal identification and classification is very biased (Schulz *et al.*, 2022). The ability to identify species at the molecular level has increased in recent years with advanced techniques (Taylor *et al.*, 2000).

The present study explains isolation of fungus in environmental samples, isolate purification of fungus metabolites, antibacterial activity in pathogenic bacteria and its phenotypic characterization.

2. Materials and methods

2.1 Sample collection and isolation of fungus

A total of 40 soil samples were collected, a 20 cattle waste dumped area samples were collected and 20 samples were collected from plant leaves respectively. The samples are collected in zip lock back carefully transport to the laboratory and store at 4°C.

2.2 Molecular characterization

In this study, NCBI (National Center for Biotechnology Information) Blast and CLUSTALW were used to determine the nucleotide sequences.

2.3 Antibacterial activity of *A. borodinense* metabolites using well diffusion method

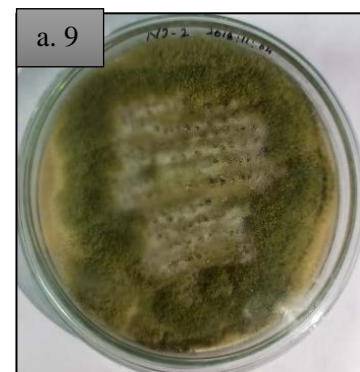
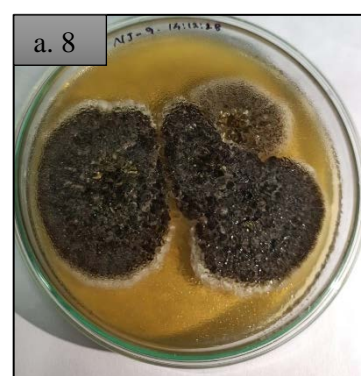
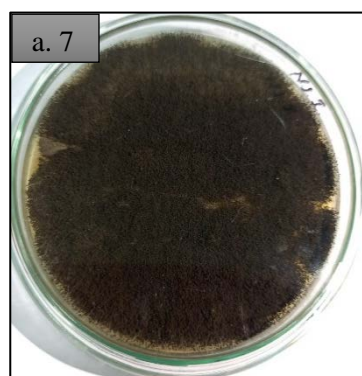
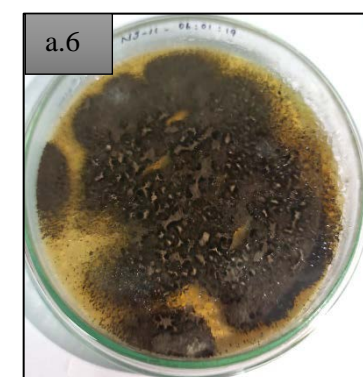
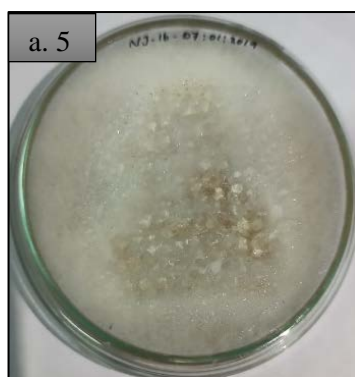
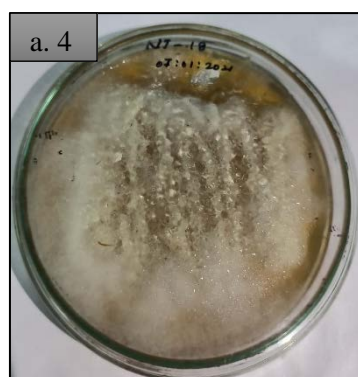
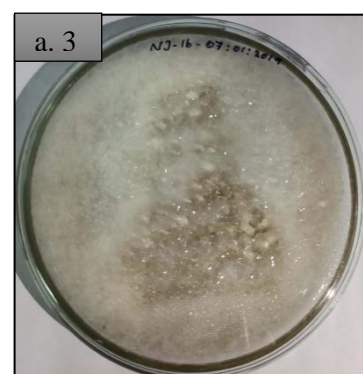
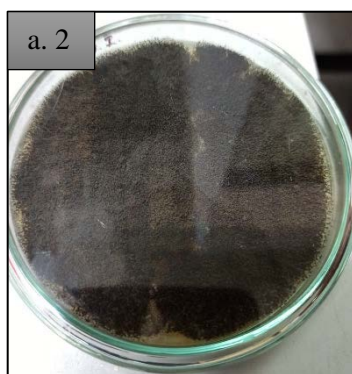
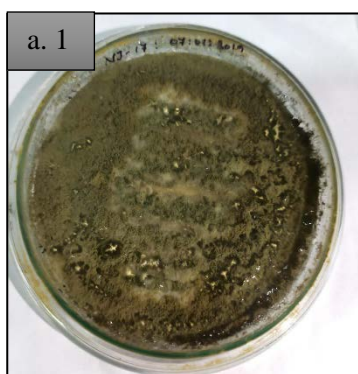
The antibacterial activity of filtered fungus metabolites was tested against the chosen bacteria: Gram-positive *Staphylococcus aureus* (MTCC 8708) and gram-negative *Proteus vulgaris* (MTCC 426), *Klebsiella pneumonia* (MTCC 4030), *Serratia marcescens* (MTCC 96). A well puncher is used to open the well in the media plate during the procedure under aseptic conditions in a laminar air flow chamber. Each bacterial strains (10^5 CFU/mL) was swabbed uniformly on the Mueller Hinton agar medium plates using sterile cotton swabs. Added (10 μ L 20 μ L 30 μ L) concentrations of fungi metabolites in respective wells, sterile water used as a control, after the plates were incubate at 37 °C for overnight at incubator after incubation to observe zone of inhibition.

3. Results and discussion

3.1. Isolation of fungi

A total of 40 soil samples were collected, a 20 cattle waste dumped area samples were collected and 20 samples were collected from plant leaves respectively (Fig. 1,2, 3).

1.a Agriculture soil pure culture fungi plates



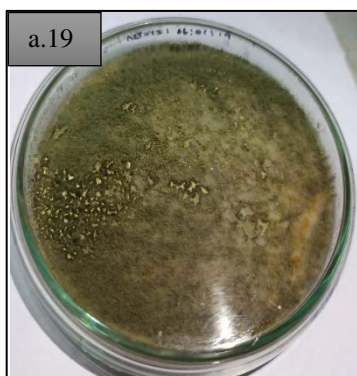
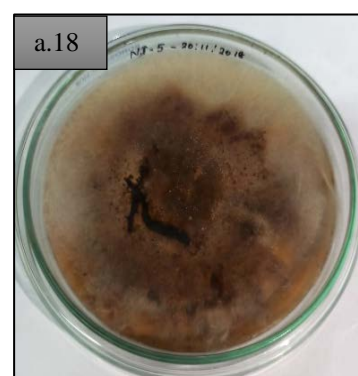
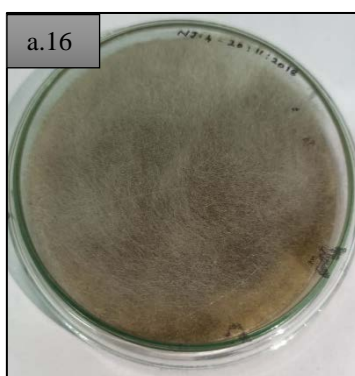
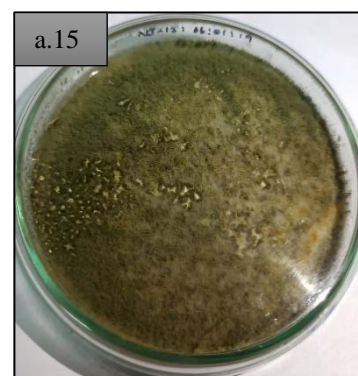
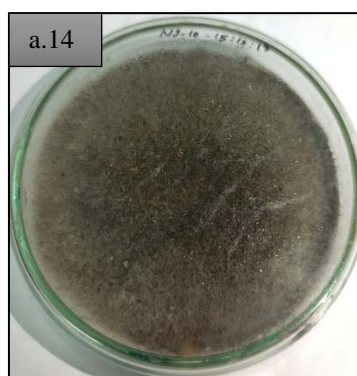
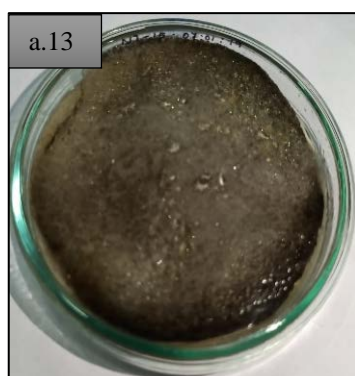
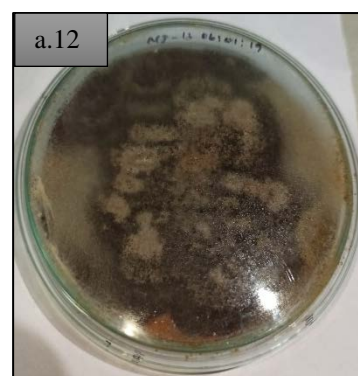
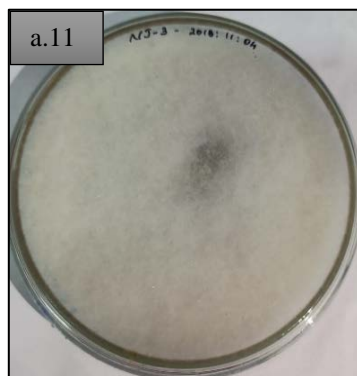
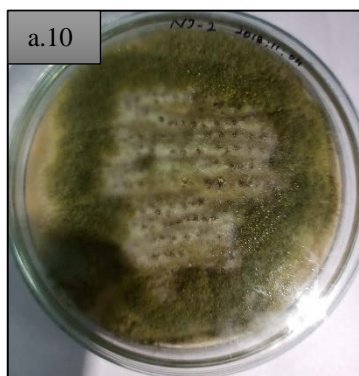
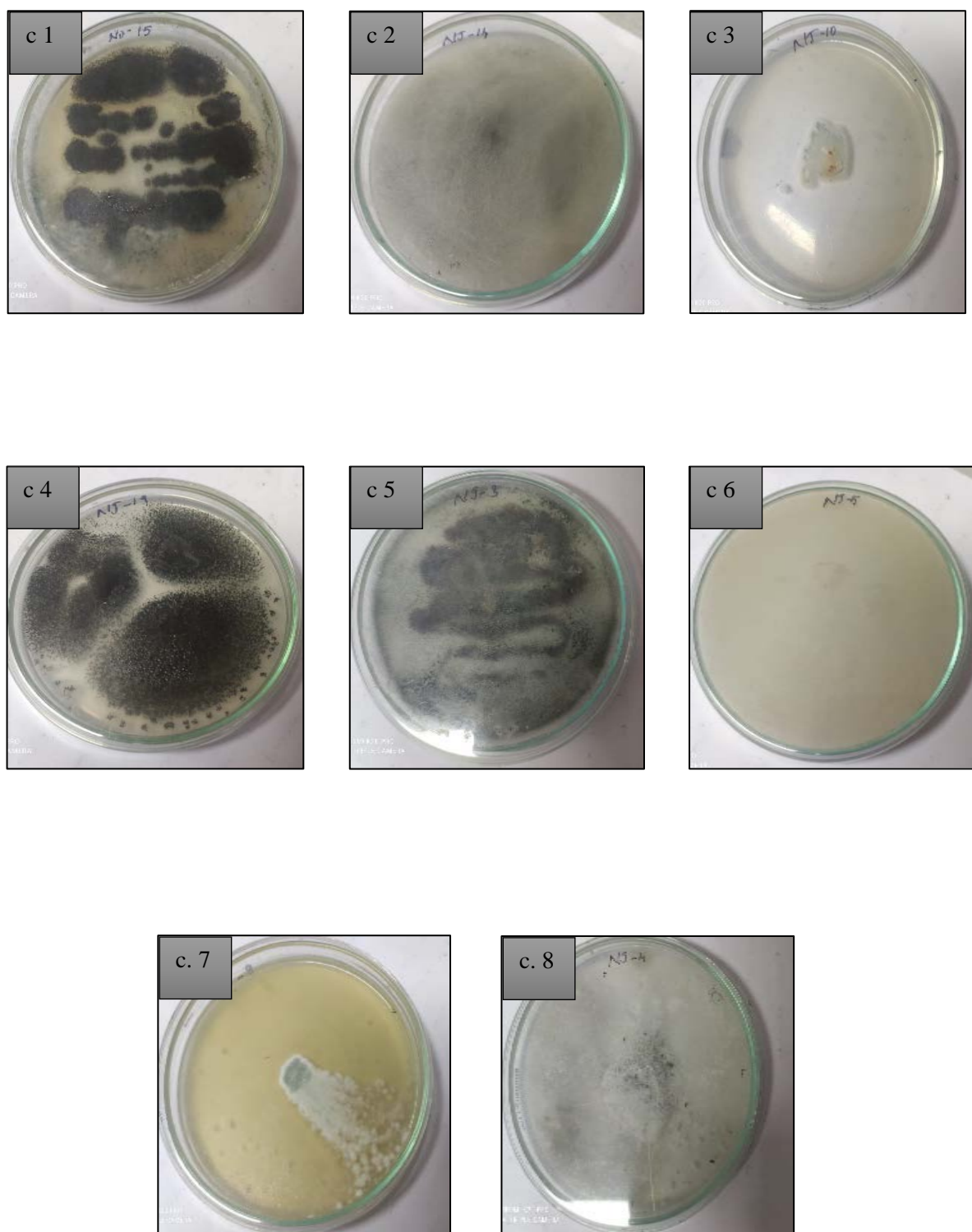


Figure 2 Cattle Waste Dumped Area pure culture fungi plates

Figure 3 Plant Leaves pure culture fungi plates

3.2. Molecular identification

A phylogenetic relationship between fungal neighbour-joining tree based on ITS gene sequences was used to visualise closely related strains obtained from NCBI GenBank (Fig.3.2.2). The fungal sequence is 100 % matched with *Acremonium borodinense* (MH424154.1).

3.3. Antibacterial activity of *A. borodinense* metabolites by the well diffusion method

A. borodinense filtered metabolites were tested for antibacterial activity against pathogens. A total of 37 fungal metabolites antibacterial profiles Table 3.5 shows the zone of inhibition in bacterial culture plates. *A. borodinense* fungal metabolite tested against pathogenic bacteria at three distinct concentration (10, 20, and 30 μ L). Table 3.4 depicts the zone of inhibition in four different bacterial cultures (*Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus* and *Klebsiella pneumonia*). A volume of 30 μ L of *A. borodinense* fungal metabolite showed a 12 mm zone of inhibition against *Proteus vulgaris*, 5 mm zone of inhibition against *Serratia marcescens*, 6 mm zone of inhibition against *Staphylococcus aureus*. The filtered metabolites of *A. borodinense* do not inhibit the pathogenic bacteria *Klebsiella pneumonia* (Fig 4).

Figure 4. Antibacterial activity of crude *A. borodinense* metabolites: (a) *Proteus vulgaris*, (b) *Klebsiella pneumonia*, (c) *Staphylococcus aureus*, (b) *Serratia marcescens*.

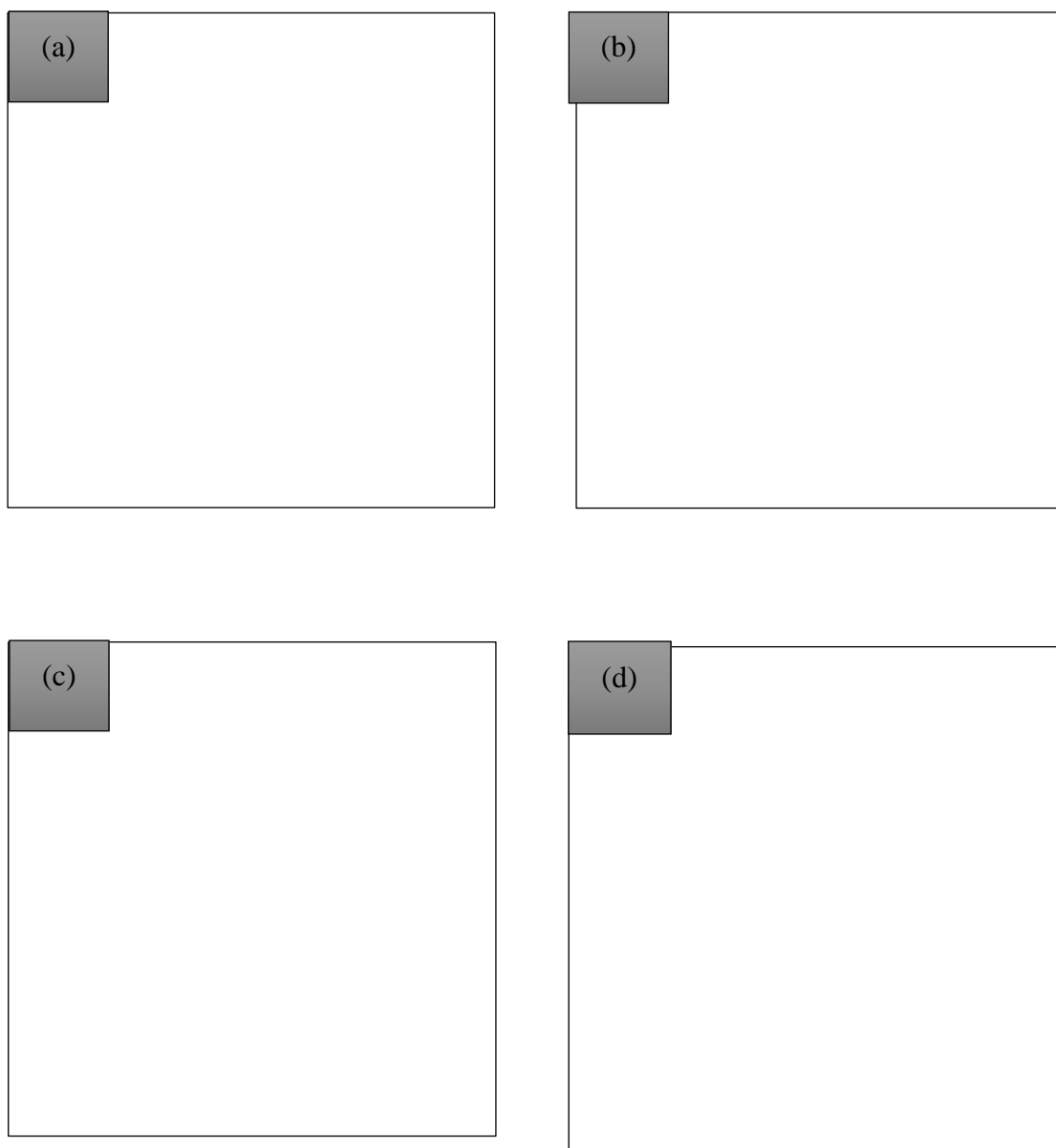


Table. 1. Antibacterial activity of fugal metabolites 37 fugus samples

Isolates. NO	<i>Staphylococcus aureus</i> (MTCC 8708)	<i>Proteus vulgaris</i> (MTCC 426)	<i>Klebsiella pneumonia</i> (MTCC 4030)	<i>Serratia marcescens</i> (MTCC 96)
F.NJ. 1	–	–	–	–
F.NJ. 2	–	–	–	–
F.NJ. 3	–	–	–	–
F.NJ. 4	–	–	–	–
F.NJ. 5	+	+	+	+
F.NJ. 6	–	–	–	–
F.NJ. 7	–	–	–	–
F.NJ. 8	–	–	–	–
F.NJ. 9	–	–	–	–
F.NJ. 10	–	–	–	–
F.NJ. 11	–			
F.NJ. 12	–	–	–	–
F.NJ. 13	–	–	–	–
F.NJ. 14	–	–	–	–
F.NJ. 15	–	–	–	–
F.NJ. 16	–	–	–	–
F.NJ. 17	–	–	–	–
F.NJ. 18	–	–	–	–
F.NJ. 19	–	–	–	–
F.NJ. 20	–	–	—	–
F.NJ. 21	–	–	–	–
F.NJ. 22	–	–	–	–
F.NJ. 23	–	–	–	–
F.NJ. 24	–	–	–	–
F.NJ. 25	–	–	–	–
F.NJ. 26	–	–	–	–
F.NJ. 27	–	–	–	–
F.NJ. 28	–	–	–	–
F.NJ. 29	–	–	–	–
F.NJ. 30	–	–	–	–
F.NJ. 31	–	–	–	–
F.NJ. 32	–	–	–	–
F.NJ. 33	–	–	–	–
F.NJ. 34	–	–	–	–
F.NJ. 35	–	–	–	–
F.NJ. 36	–	–	–	–
F.NJ.37	–	–	–	–

Table. 2. Zone of inhibition of crude *Acremonium borodinense* metabolites.

S.NO	Fungi metabolites	Zone of Inhibition(mm)			
		Gram-positive Bacteria		Gram-negative Bacteria	
		<i>S. aureus</i>	<i>P. vulgaris</i>	<i>S. marcescens</i>	<i>K. pneumonia</i>
1.	10 μ L	03	07	02	-
2.	20 μ L	04	09	03	-
3.	30 μ L	06	12	05	-

Conclusion

The present study attempts to isolate fungus metabolites and their antibacterial activity of the 37 fungal metabolites tested in antibacterial activity only one fungal metabolite got an antibacterial for screening of fungus that was molecular cauterized by sequencing 100% matched with *Acremonium borodinense*. *Acremonium borodinense* fungi metabolites given strong antibacterial activity it havens alternative drug against pathogenic multi drug resistant bacteria.

Conflict of interest

None

Reference

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