CHAPTER 3

RESULTS

3.1 Preliminary screening for biological activity of Antarctic fungi

3.1.1 Antimicrobial activity of Antarctic fungi using the Plug assay method

Table 3.1 showed the results of preliminary screening of antimicrobial activity using the plug assay method. Twenty strains of Antarctic fungi were screened for Antimicrobial activities. These fungi were isolated from samples collected from Dee and Barrientos Islands of Antarctica and tested against Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus,* Gram-negative bacteria; *Escherichia coli, Pseudomonas aeroginosa* and yeast: *Candida albicans.* Seven strains exhibited positive activity against the bacteria and no activity against the yeast (table 3.1). That means 35% of the strains produced antimicrobial activity. Most of strains exhibited antimicrobial activity against gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and the results indicated that 4 of these strains exhibited antibacterial activity against *Staphylococcus aureus,* 5 strains against *Bacillus subtilis,* 1 strain against *Escherichia coli,* 1 strain against *Pseudomonas aeroginosa,* and no activity detected against *Candida albicans.* The antibacterial activity result in table (3.1) was expressed as: no activity= (-), weak activity= (+), moderate activity= (++) and high activity= (+++).

Table 3.1: A activity of strains against bacteria and yeast in the preliminary study.

species	code no	*E. coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*C. albicans*
Geomyces S7	SOEDIT2/ 50-1	-	-	-	++	-
Unidentefied S1	SOEDIT2/ 75-1	+	-	-	-	-
Antarctomyces S_{10}	SOEDIT2/ 0-1	-	-	-	-	-
Geomyces S_5	SOEDIT2/ 75-4	-	-	-	-	-
Geomyces S7a	SOEDIT2/ 30-2	-	-	-	-	-
Penecillium S10	SOEDIT2/ 75-1	-	-	+	-	-
Unidentefied S8	SOEDIT2/ 80-1	-	-	-	-	-
Antarctomyces S_{14}	SOEBI/ 1-2	-	-	-	-	-
Geomyces S21	SOEBI/ 12-1	-	++	-	++	-
Geomyces S_5	SOEBI/ 3-4	-	++	-	++	-
Antarctomyces S_{14}	SOEBI/ 4-3	-	-	-	-	-
phoma S1	SOEBI/ 6-7	-	-	-	-	-
Geomyces S_5	SOEBI/ 3-1	-	++	-	-	-

Morterela S2	SOEBI/ 214	-	-	-	-	-
Geomyces S_5	SOEBI/ 146	-	++	+++	+++	-
Thelebolus S19	SOEBI/16-7	-	-	-	-	-
Penecillium S_{20}	SOEBI/ 6-7	-	-	-	-	-
Geomyces S_5	SOEBI/ 12-1	-	-	-	-	-
Deutromycete S7	SOEBI/ 20-7	-	-	-	-	-
Geomyces S_5	SOEBI/ 86	-	-	-	-	-

The diameter of plug well = 6mm Key: (-) = no activity, (+**)** = weak activity, (++) = moderate activity, (+++)= high activity.

3.2 Biological activity of extracts of selected Antarctic fungi

The bioassay was carried out only for five *Geomyces* species of selected Antarctic fungi based on their activity in the preliminary study against bacteria: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Bacillus cerus,* and yeasts: *Candida albicans, Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. The selection of these five species was based on their good activity which they exhibited in preliminary study.

3.2.1 Antimicrobial activity of extracts of selected fungi in disc diffusion assays

The selected fungi studied for disc diffusion assay were listed in table 3.2a. Chloramphenicol was used as a positive control which produced inhibition zone in mm ranged between 20mm to 34mm as given in table 3.2b. The ethyl acetate extracts of the five Antarctic fungi were tested for their antimicrobial activity using the disc diffusion assay (Table 3.2c). Not all the five strains exhibited activity against test organisms and *Geomyces* S_7 did not show any activity at all. Fungi produced active metabolites after 15 days of incubation under stationary phase. No activity produced after 10 days of incubation and the antimicrobial activity decreased after 21days incubation (data not displayed). Figure 3.1 showed photograph of inhibition zone in disk diffusion. Figure 3.2 showed photograph of the inhibition zone by chloramphenicol. dimethyle sulphpxide (DMSO) solvent was used as negative control in this study.

Table 3.2a: List of selected fungi studied for disc diffusion assay method

Species	**Code number**
Geomyces S_7	SOEDIT2/50-1
Geomyces S_5	SOEBI/3-4
Geomyces S_{21}	SOEBI/12-1
Geomyces S_5	SOEBI/3-1
Geomyces S_5	SOEBI/146

Table 3.2b Activity of Chloramphenicol, the positive control in the disk diffusion assay

Antibiotic	Diameter of inhibition zone				
Bacteria tested	*E. coli*	*B. subtilis*	*S. aureus*	*B. cereus*	*P. aeroginosa*
Chloramphenicol	34	24	33	30	20

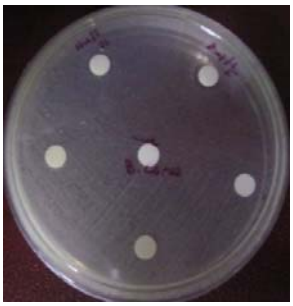
Table 3.2c Antimicrobial activity of ethyl acetate extracts of selected Antarctic fungi in agar disk diffusion assay

species name	**incubation period**	***E. coli***	***S. aureus***	***P. aeroginosa***	***B. subtilis***	***B. cereus***	***S. pombe***	***S.cerevisiae***	***C .albicans***
Geomyces 3-1/sp_5	15 days	NA	10mm	NA	NA	11mm	NA	NA	NA
Geomyces 12-1/sp_{21}	15 days	NA	NA	NA	NA	9mm	NA	NA	NA
Geomyces 3-4/sp_5	15 days	NA	10mm	NA	11mm	NA	NA	NA	NA
Geomyces146/ sp_5	15 days	NA	13mm	16mm	18mm	NA	NA	NA	NA
Geomyces50-1 sp_7	15 days	NA	NA	NA	NA	NA	NA	NA	NA

The diameter of disc=6mm. Key: NA=no activity, *Escherichia coli* = E.coli; *Bacillus subtilis* = B.subtilis; *Staphylococcus aureus* = S.aureus; *Candida albicans* = C. albicans , *Pseudomonus aeroginosa P. aeroginosa.*

9mm-11mm=weak activity, 13mm-16mm= moderate activity and > 16 mm= good activity

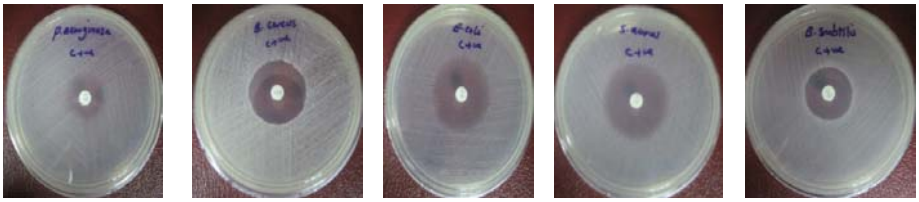
B. subtilis *P. aeruginosa*



S. aureus *B. cereus*

Figure 3.1 photograph of inhibition zone in disk diffusion assay. The photos shows the EtOAc extracts of the five species that exhibited positive results in four types of bacteria namely; *Bacillus subtilis*, *Pseudomonas aeruginosa, Bacillus cereus* and *Staphylococcus aureus.*

Chloramphenicol (positive control)



P. aeruginosa. *B. cereus* *E. coli* *S. aureus* *B. subtilis*

Figure 3.2 Photograph of inhibition zone by Chloramphenicol. The diameter of disk=6mm with 30 mg concentration.

3.3 Quantitative assay- Minimum Inhibitory Concentration (MIC)

The lowest concentration that result in complete inhibition of the growth of microorganism was determined as blue colour or the colour before pink in the microtitre plates after addition of resuzarin dye indicator (Figure 3.3). Resazurin is an oxidation–reduction indicator used for the evaluation of cell growth, particularly in various cytotoxicity assays. It is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. Resorufin is further reduced to hydroresorufin (uncoloured and nonfluorescent) (Drummond and Waigh, 2000). This value was taken as minimum inhibitory concentration (MIC). Figure 3.3 showed microtitre plate of *Geomyces* 146/S_5 against *P. eorogenosa.*

The (MIC) values of the active extract were recorded in table (3.3a), which was carried out against; *B. subtilis, S. aureus, B. cerus* and *P. aeroginosa.* The MIC values ranged from 6.25 mg ml^{-1} to 25 mg ml^{-1}, *B .subtilis* and *P. aeruginosa* showed lower MIC value of 6.25 mg ml^{-1} with *Geomyces* 146/S_5. However, the result indicated that only *Geomyces* 146/S_5 has stronger activity against *B. subtilis* and *P. aeruginosa ,* while the rest of the species showed weak activities against these test microorganisms.

Table 3.3: shows MIC values of active extracts in mg ml^{-1}

Species name	***B. subtilis***	***S. aureus***	***B. cereus***	***P. aeruginosa***
Geomyces 3-1/S_5	-	12.5	25	-
Geomyces 3-4/S_5	12.5	12.5	-	-
Geomyces 146/S_5	6.25	12.5	-	6.25
Geomyces 12-1/S21	-	-	-	-
Geomyces50-1/S7	-	-	-	-

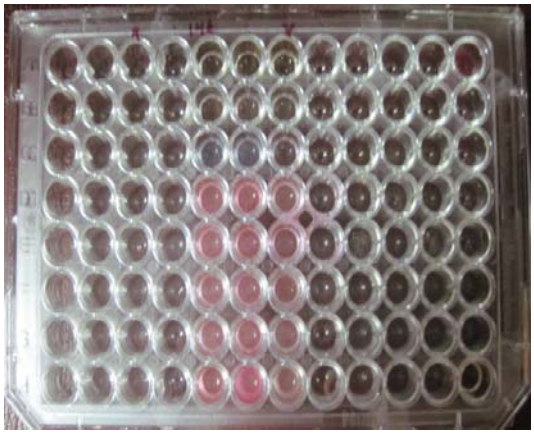


Figure 3.3 Photograph of microtitre plate of MIC test of *Geomyces* 146/S_5 against *P. aeruginosa.* The pink colour result from addition of resuzarin dye which indicated the growth of the test microorganism so, the MIC value determines as a well above which it is 6.25 mg ml^{-1}.

3.4 Quantitative assay- Minimum Bactericidal Concentration (MBC)

The lowest concentration that results in the killing of microorganism was determined after subcultured from overnight incubation of MIC microtitre plate and before addition of resuzarin dye on Luria agar plates and it is indicated as clear plate without growth (Figure 3.4). Figure 3.4 showed subcultured from microtitre plate of MIC test of *Geomyces* 146/S_5 on Luria agar against *P. aeruginosa.* This value was taken as minimum bactericidal concentration (MBC).

The (MBC) values of the active extract were recorded in table (3.3b), which was carried out against; *B .subtilis, S. aureus, B. cereus* and *P. aeruginosa.*The MBC values were range between 12.5 mg ml^{-1}- >25 mg ml^{-1}, *B .subtilis* and *p. aeruginosa* showed lower MBC value of 12.5 mg ml^{-1} with *Geomyces* 146/S_5.

Table 3.4: Minimum Bactericidal Concentration MBC values of active extracts in mg ml^{-1}

Species name	***B. subtilis***	***S. aureus***	***B. cerus***	***P. aeruginosa***
Geomyces 3-1/ S_5	-	25	>25	-
Geomyces 3-4/S_5	25	25	-	-
Geomyces 146/S_5	12.5	25	-	12.5

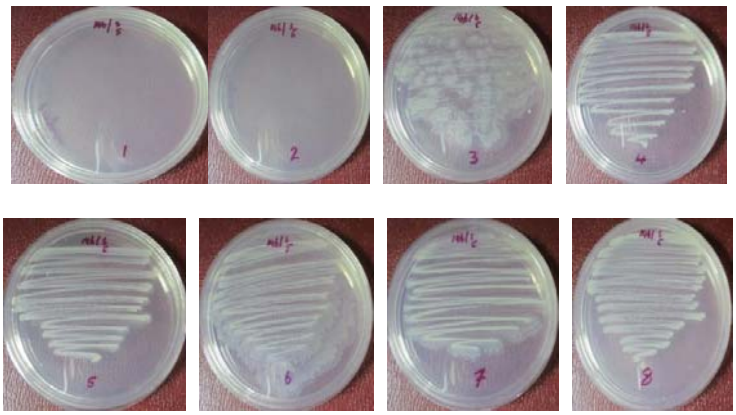


Figure 3.4 Photograph shows the subculture from microtitre plate of MIC of *Geomyces* 146/S_5 on Luria agar plates against *P. aeruginosa a,* the growth appeared on the third plate result in MBC value of 12.5 mg ml^{-1}**.**