ISOLATION AND OPTIMIZATION OF SOME FERMENTATION PARAMETERS OF THE SELECTED SOIL FUNGUS (SK-6) FROM PYAWBWE TOWNSHIP, MANDALAY REGION

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Abstract

Six different soil samples were collected from six different places in Pyawbwe Township, Mandalay Region. Thirty different soil fungi SK-1 to SK-30 were isolated from six different soil samples. Isolation of fungi SK-1 to SK-30 were undertaken by serial dilution method. The isolated fungi were cultured on Blakeslee's Malt Extract Agar (BMEA), Czapek Dox Agar (CZA), Malt Extract Agar (MEA) and Potato Glucose Agar (PGA) media. Antimicrobial activity of isolated fungal strains was evaluated by using the agar well diffusion method with seven test organisms. Among them, three fungal strains SK-1, SK-3 and SK-6 showed the antimicrobial activity against *Bacillus subtilis* and *Candida albicans* at 7 days. Especially, SK-6 gave the best antifungal activity against *Candida albicans*. Therefore, SK-6 was selected and the optimum conditions for antimicrobial metabolite production on *Candida albicans* of SK-6 were the fermentation period, suitable age and size of inoculum, different carbon and nitrogen sources.

Keywords : soil fungi, antimicrobial activity, fermentation, agar well diffusion

Introduction

Soil is the upper layer of most of the earth's surface and varies in depth from inches to over twenty feet. It is a product of weathered rock, but quite distinct in its characteristic. Soils are excellent cultural media for the growth of many types of organisms (Angelov, 2008). This includes bacteria, fungi, algae, protozoa and viruses. A spoonful of soil contains billions of microorganisms. In general the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth (Cattle, 2002). The number and kinds of organisms found in soil depend upon the nature of soil, depth, season of the year, state of cultivation, reaction, organic matter, temperature, moisture, aeration, etc. (Omalu, 2011).

Fungi have fundamental functions in terrestrial ecosystems, in degradation of organic matter and in nutrient uptake of plants through mycorrhizal interactions. From a human point of view, there are both good and bad fungi. There are quite some delicious edible fungi and other are used in productions of food like soysauce, tempe and bread. They are also a source of important drugs like the penicillins, the cholesterol-lowering lovastatin and cyclosporins, which counteracts the rejection of transplanted organs (Harayama and Isono, 2002).

Antimicrobial describes a substance that can either kill or hinder the growth of microbes such as bacteria, viruses, fungi and protozoa. It is a general term that includes antibiotics, antivirals, antifungals and antiprotozoals. Antibiotics treat bacterial infections while antivirals are specific for viral infections. Antifungals help treat such as thrush, candidiasis, athlete's foot or ringworm, while antiprotozoals specifically deal with microscopic parasites in body. Antimicrobials that kill microbes are called microbicidal, those that merely inhibit their growth are called microbiostatic (Trease and Evans,1980).

This research paper aims to investigate the isolation of soil microorganisms and to know the different soil microorganisms from various soil samples. Studies were carried out to investigate the effect of pH, temperature, static, and shaking culture on the fermentation.

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Materials and Methods

Collection of Soil Samples and Isolation of Soil Fungi

The soil samples were collected from six different locations of Pyawbwe Township, Mandalay Region, during July, 2017. Sampling sites (address, latitude and longitude) and it's condition were recorded in Table 1. The fungi were isolated by using serial dilution method, and cultured on Blakeslee's Malt Extract Agar (BMEA), Czapek Dox Agar (CZA), Malt Extract Agar (MEA) and Potato Glucose Agar (PGA).

Table 1 Six Different Soil Samples Collected from Pyawbwe Township

Soil Samples	Samples Area	Location
S.I	Lat Thae` Kyoe Village	N. 20˚ 45′ 34.209″ E. 95˚ 53′ 56.545″
S.II	War Yin Toke Village	N. 20˚ 44′ 33.441″ E. 95˚ 55′ 24.573″
S.III	Yintaw Village	N. 20˚ 42′ 43.776″ E. 95˚ 56′ 28.376″
S.IV	Chaung Magyi Village	N. 20˚ 40′ 52.574″ E. 95˚ 54′ 19.548″
S.V	Phat TawVillage	N. 20˚ 34′ 57.105″ E. 96˚ 01′ 40.497″
S.VI	Pyi Thayar	N. 20˚ 36′ 26.142″ E. 96˚ 02′ 30.760″

Serial Dilution Method

One gram of soil sample was introduced into a conical flask containing 99 mL of distilled water. The flask was then shaken for about 30 min in order to make the soil particles free from each other. This solution was then serial diluted from 10^{-3} to 10^{-7} dilution and 0.5 mL each of the above dilution was separately transferred into sterile petridishes under aseptic condition (Dubey and Maheshwari, 2002). Chloramphenicol was added to the sterilized medium for preventing bacterial growth. The sterilized medium in conical flask was cooled down to about 45 ºC and separately poured into each of the petridish containing the respective soil dilutions.

The inoculated plates were shaken by clock-wise and anticlock wise direction for about 5 min so as to make uniform distribution of the fungi inoculums. When the agar was solidified , the inoculated plates were inverted and incubated at 27º -30º C for 3 -6 days.

Lacto Phenol Cotton Blue Teased Mount (LPCB.TM) Staining Technique for Fungi

The needles were flamed over the burning Bunsen burner to sterile. A drop of LPCB was placed on the slide. A tiny piece of the colony was transferred into the LPCB on the slide by using a sterile needle. After staining the slide was covered with a cover slip. The wet staining was examined under the x40 objective microscope for microscopic identification.

Agar Well Method

The antimicrobial assay was performed by agar well diffusion method (Perez *et al*., 1990). Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extract. The same procedure was used in disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar

surface. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer or a tip and a volume (20 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, the plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

Preliminary Study for Antimicrobial Activity

The isolated fungi were grown on BMEA medium for 5 days. These fungi were inoculated into 25 mL seed medium and incubated at room temperature for 3 days. After 3 days, 20 mL seed culture was transferred into the 80 mL of fermentation medium and incubated at room temperature. Fermentation was carried out for 3-10 days (Ando *et al*., 2004).

One day old culture test broth (0.01 mL) was added to 25 mL of agar well diffusion assay medium and thoroughly mixed and poured into plate. After solidification, cork borer was used to make the wells (dia. T

The fermented broth (20 µL) was carefully added into the wells and incubated at room temperature for 24 to 48 h. The diameter of the zone of inhibition around each well was measured and recorded after 24 to 48 h incubation.

Effect of Fermentation Period

The fermentation period of isolated fungi was studied for 3 days to 10 days and seed culture medium was added to 100 mL of fermentation medium. The flasks were incubated at room temperature and the fermentation culture was assayed for antifungal activity by using agar well diffusion method.

The Effect of Ages of Inoculum for Fermentation

In this study, different incubation times (48, 60, 72, 84, 96, 108, 120, 132, 144 h) were used for the production of antimicrobial metabolite and the procedure of seed culture medium was also used as the previous method. And then, seed culture was transferred to 100 mL conical flask containing of fermentation medium and incubated at room temperature. The inoculum age of fermentation studied were 48, 60, 72, 84, 96, 108, 120, 132 and 144 h. Fermentation culture was kept from 48 to 144 h and antifungal activity was tested by agar well diffusion method.

The Effect of Sizes of Inoculum for Fermentation

The size of inoculum (5%, 10%, 15%, 20%, 25%, 30% and 35%) were used for the production of antimicrobial metabolite. In the investigation of size of inoculum, well sporulated selected strain SK-6 were taken and added to 250 mL conical flask containing 100 mL of seed culture medium and incubated for 3 days at room temperature. After that 3 days old seed culture were (5 %, 10 %, 15 %, 20 %, 25 %, 30 %, 35 %) transferred to 100 mL conical flasks containing 25 mL of fermentation medium respectively. The flasks were incubated at room temperature and the fermentation culture was assayed for antifungal activity by using agar well diffusion method.

Carbon and Nitrogen Utilization

In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as carrot, corn powder, glycerol, glucose, lactose, maltose, mannitol, molasses, oat, potato, rice powder, sucrose, fructose and soluble starch were used. Nitrogen sources such as casein, fish cake, gelatin, malt extract, meat extract, peanut cake, peptone, poly peptone, rice bran, soy bean, urea, yeast extract, potassium nitrate, sodium nitrate, ammonium chloride, ammonium nitrate, and ammonium sulphate were also used.

The Effects of pH on Selected Fungus SK-6

Optimum pH was studied by varying the medium pH as 4, 5, 6, 7, 8, 9 and 10. The different pH of seed medium was adjusted by using 1 M HCl and 1 M NaOH. The selected fungus SK-6 was inoculated in the optimized fermentation media and kept at room temperature. The fermentation medium was assayed for antifungal activity.

The Effects of Temperature in Selected Fungus SK-6

The selected fungus SK-6 was inoculated and incubated at different temperatures 20 °C, 25 °C, 30 °C, 35 °C and 40 °C.

The Effect of Shaking and Static Condition Upon the Secondary Metabolite

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the rotary shaker (100 rpm) for 5 days. At the same time, another fermentation medium was incubated under static condition without shaking. The antifungal activities of shaking culture and static culture were compared by using agar well diffusion assay method.

The Effect of Fermentation Media

Fermentation media were undertaken with optimized conditions of 5 % sizes, 108 h old, pH-7, temperature 20 °C, shaking culture of inoculum with thirteen different media. Fermentation media was kept for 5 days and antifungal activity test was carried out 24 h interval.

Results and Discussion

Isolation of Fungi from Soil Samples

Thirty fungi were isolated from six different soil samples of Pyawbwe Township, Mandalay Region. The results of soil samples showed that soil type S-I of Lat Thae` Kyoe Village was sandy clay loam and pH value of 9.4. War Yin Toke Village and Yintaw Village (S-II & S-III) were loamy sand soil, pH values of 10.6 and 8.8 respectively. Chaung Magyi Village (S-IV) was sandy clay soil and pH value of 8.8. Phat Taw Village (S-V) and Pyi Thayar (S-VI) were sandy loam soil and pH values of 9.2 and 8.9 respectively (Table 2).

Total of 30 fungal isolates were obtained - six strains from S-I, eight fungi from S-II, five strains from S-III, each one fungi from S-IV and S-VI and nine strains from S-V (Table 3). The soil fungi were isolated by using four different media. 17 strains were isolated from BMEA medium, 5 strains from CZA medium, other 5 strains from MEA medium and 3 fungi from PGA medium. The surface colour of SK 1 to SK10 were white, pale gray, pale brown, center green edge white, center yellow edge white and their reverse colour were cream, white, yellow, brown, pale white, pale yellow, center black edge white, center black edge brown. The surface colour of SK 11 to SK 20 were white, gray, black, greenish white , center black edge white, center greenish yellow edge white, center green edge white, center green edge cream and the reverse colour were white, yellow, brown, cream, black, center black edge white, center yellow edge white (Figure 1).

The surface colour of SK 21 to SK 30 were white, pale green, greenish white, center black edge white, center green edge white, center brown edge white and the reverse colour were cream, yellow, pale brown, pale yellow, yellowish orange, pale green and pale greenish blue respectively.

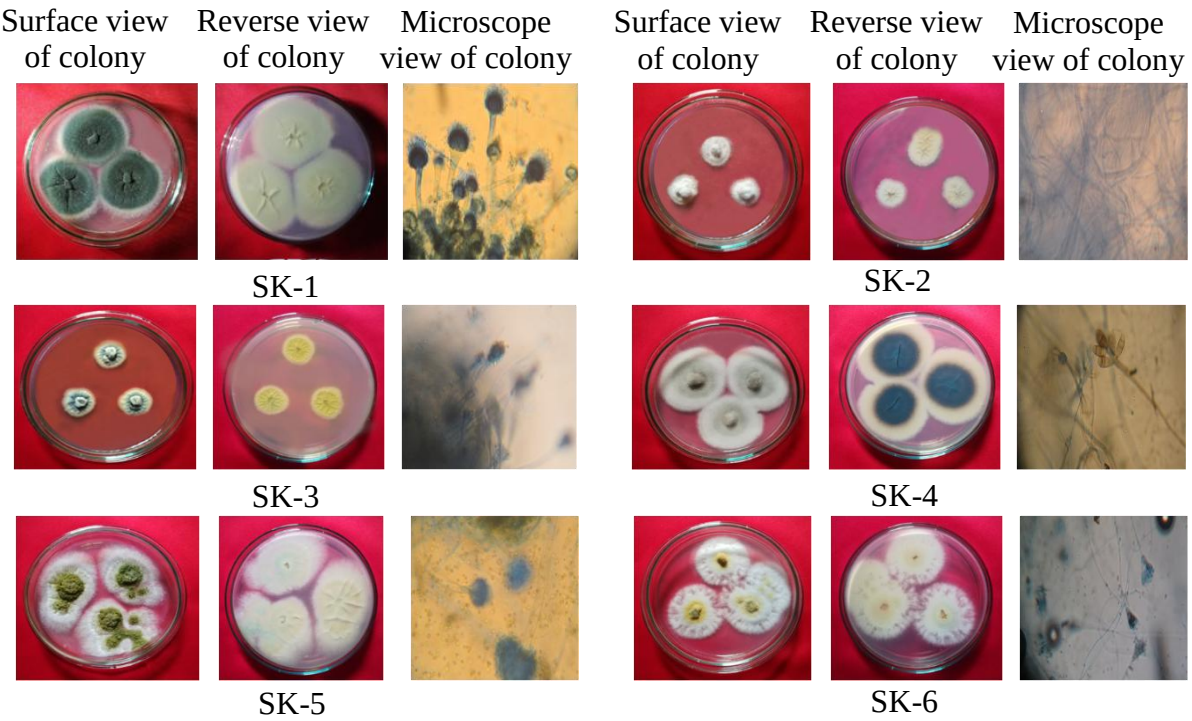
Table 2 pH and Soil Type of Soil Sample

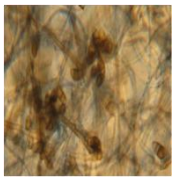
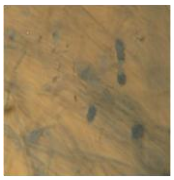
Soil Samples	pH	Soil Types
S.I	9.4	Sandy clay loam
S.II	10.6	Loamy sand
S.III	8.8	Loamy sand
S.IV	8.8	Sandy clay
S.V	9.2	Sandy loam
S.VI	8.9	Sandy loam

Table 3 Isolated Fungi from Soil Samples

Soil Samples	BMEA Medium	CZA Medium	MEA Medium	PGA medium	Total
S.I	SK-19, SK-21, SK-22, SK-27, SK-29	SK-23	-	-	6
S.II	SK-11, SK-18, SK-24	SK-17, SK-26	SK-20, SK-25, SK-28	-	8
S.III	SK-8	SK-9, SK-30	SK-12, SK-13	-	5
S.IV	SK-14	-	-	-	1
S.V	SK-1, SK-2, SK-3, SK-4, SK-5, SK-6, SK-7	-	-	SK-15, SK-16	9
S.VI	-	-	-	SK-10	1
Total	**17**	**5**	**5**	**3**	**30**

BMEA = Blakeslee's Malt Extract Agar, CZA = Czapek Dox Agar, MEA = Malt Extract Agar, PGA = Potato Glucose Agar



Surface view of colony	Reverse view of colony	Microscope view of colony	Surface view of colony	Reverse view of colony	Microscope view of colony
					
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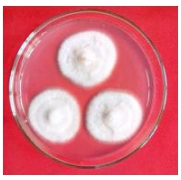		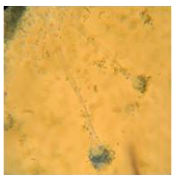	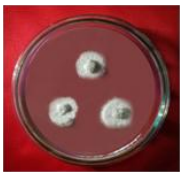		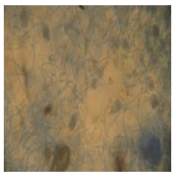
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	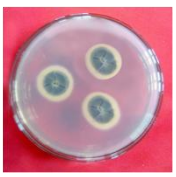				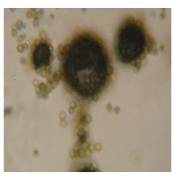
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			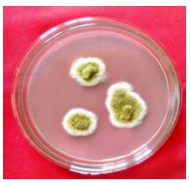		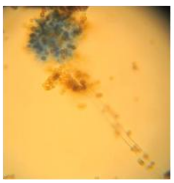
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		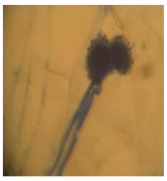			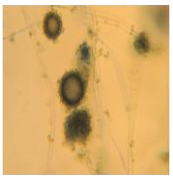
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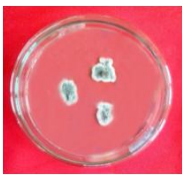			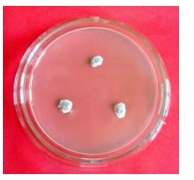	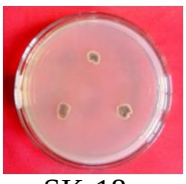	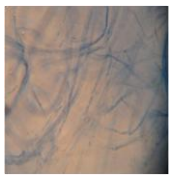
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		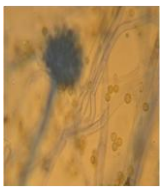			
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SK-21			SK-22		

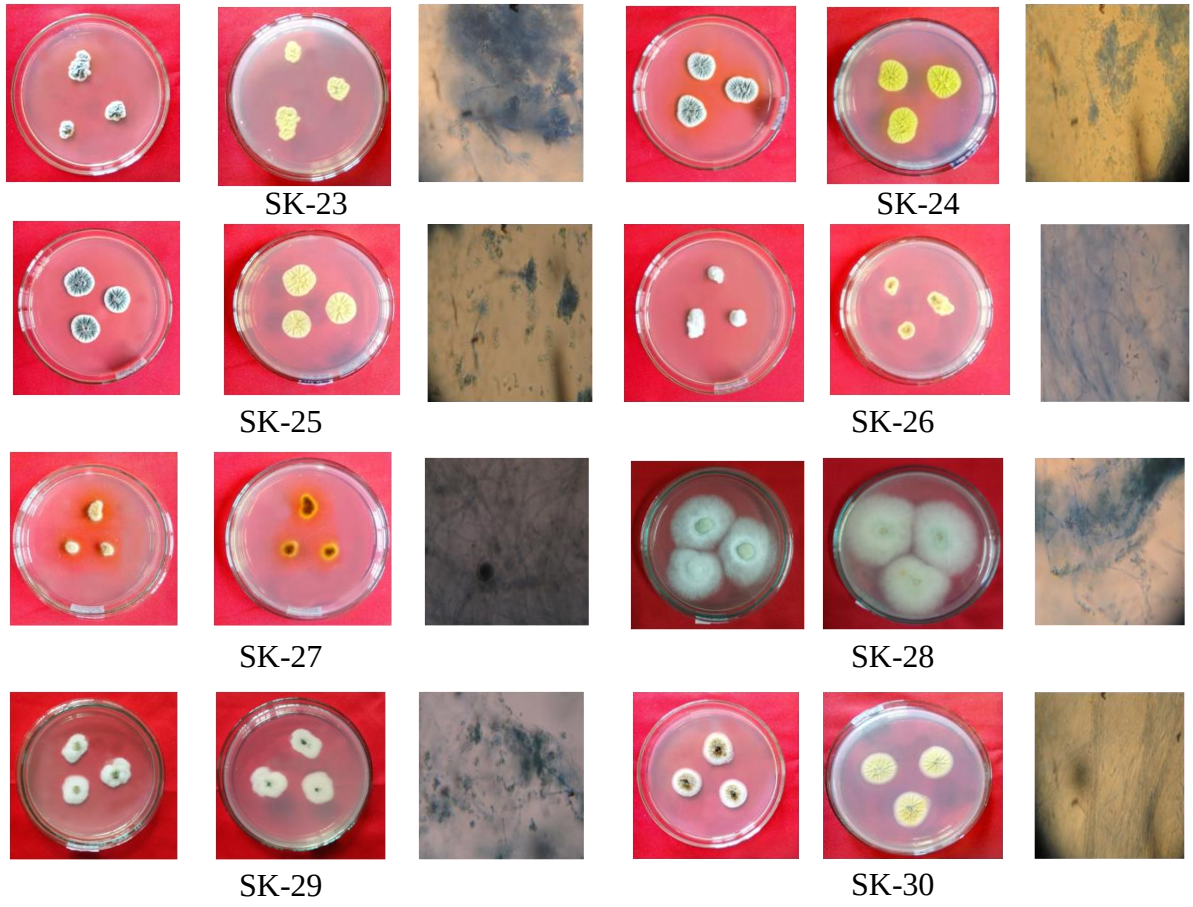

Figure 1 Morphological character of isolated fungi SK-1 to SK-30

Antimicrobial Activity of Isolated Fungi

The antimicrobial activity of all fungal strains were tested by using seven test organisms. Among them, three selected fungi- SK 1, 3 and 6 were moderate antimicrobial activity on *Bacillus subtilis* and *Candida albican*s (Table 4 and Figure 2). SK-6 showed strong antifungal activity against *Candida albicans* (28.59 mm) followed by SK-3 (28.56 mm) and SK-1 (27.78 mm). And then, SK-6 exhibited the moderate antibacterial activity against *Bacillus subtilis* (21.56 mm), SK-1 (20.28 mm) and SK-3 (18.98 mm) at 5 days fermentation period respectively. According to these results, SK-6 was selected for further investigations.

Table 4 Antimicrobial Activity of Isolated Three Fungus against *B. subtilis* and *C. albicans*

Isolated Fungi	Inhibition zone diameter	
	Bacillus subtilis	***Candida albicans***
SK-1	20.28	27.78
SK-3	18. 98	28.56
SK-6	21.56	28.59

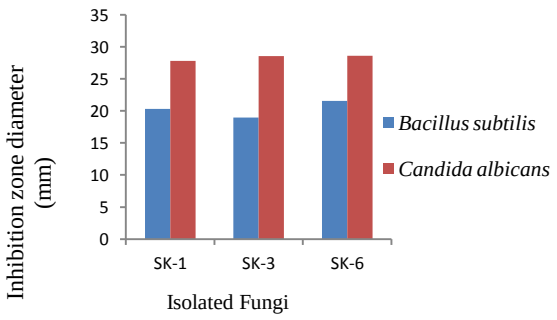

Figure 2 Antimicrobial activity of isolated fungi against *B. subtilis* and *C. albicans*

The Fermentation Period of Selected Fungus (SK-6) against *Candida albicans*

SK-6 reached the highest activities (25.77 mm) in 5 days fermentation period on *Candida albicans* (Table 5 and Figure 3).

Table 5 Antifungal Activity the Fermentation Period of Selected Fungus (SK-6) against *Candida albicans*

Fermentation period (Days)	Inhibition zone diameter (mm)
3	16.97
4	21.10
5	25.77
6	25.73
7	23.02
8	22.78
9	18.20
10	15.76

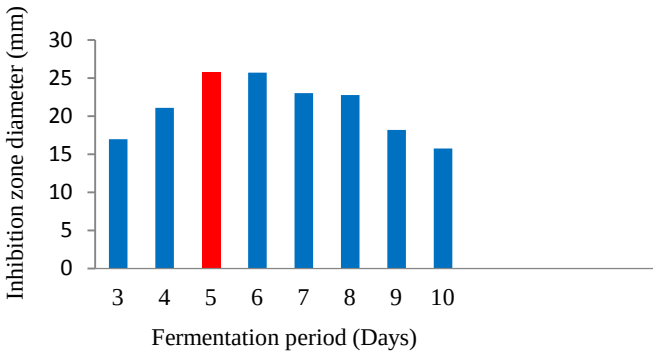

Figure 3 Antifungal activity on the fermentation period of selected fungus (SK-6) against *Candida albicans*

The Effects of Ages of Inoculums of SK-6 against *Candida albicans*

In this research work, the effect of ages of inoculum, SK-6 was examined by using 48, 60, 72, 84, 96, 108, 120, 132 and 144 h old culture age of inoculums. The results showed that 108 h age of inoculum gave the highest activities (25.02 mm), followed by (24.77 mm) at 120 h and (22.96 mm) at 132 h age of inoculum. These results are shown in Table 6 and Figure 4.

Table 6 The Effect of Ages of Inoculums of SK-6 against *Candida albicans*

Sr. No	Age of Inoculum (h)	Inhibition zone diameter (mm)
1	48	18.78
2	60	21.28
3	72	21.02
4	84	21.47
5	96	21.46
6	**108**	**25.02**
7	120	24.77
8	132	22.96
9	144	20.19

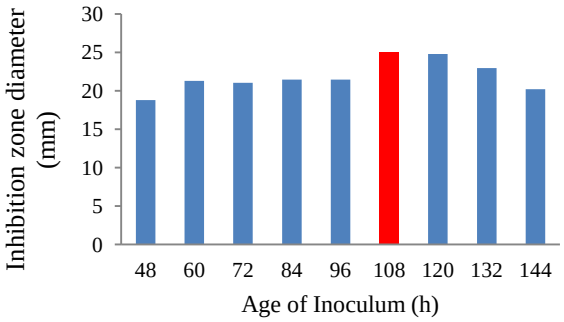

Figure 4 The effect of ages of inoculums of SK-6 against *Candida albicans*

The Effects of Sizes of Inoculums of SK-6 against *Candida albicans*

The effect of sizes of inoculums was investigated by using 5 %, 10 %, 15 %, 20 %, 25 %, 30 % and 35 % inoculums (Table 7). The use of 5 % inoculums exhibited higher activity (33.03 mm) than others, followed by 15 % and 30 % (24.22 mm and 24.16 mm) respectively in Figure 5.

Table 7 The Effects of Sizes of Inoculums of SK-6 on *Candida albicans*

Size of inoculum (%)	Inhibition zone diameter (mm)
5	**33.03**
10	24.09
15	24.22
20	21.23
25	18.92
30	24.16
35	21.11

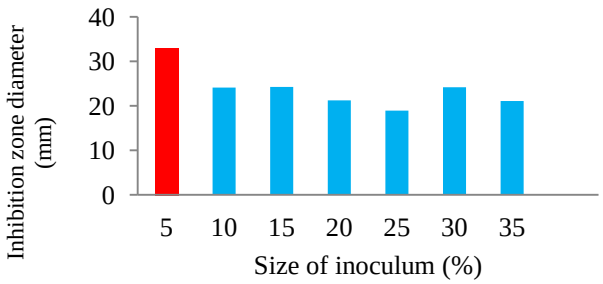

Figure 5 The effects of sizes of inoculums of SK-6 on *Candida albicans*

Antifungal activity on carbon utilization of SK-6

During these experiments, the results displayed that the different levels of antifungal activity were found when the different carbon sources were added into the fermentation medium. The inclusion of different carbon sources showed the highest antifungal activities on glucose (30.24 mm) followed by glycerol (24.15 mm), corn powder (22.89 mm), molasses (22.67 mm) and then rice powder (21.50 mm), sucrose (21.34 mm), potato (21.25 mm), carrot (21.07 mm), lactose (20.92 mm), maltose (20.48 mm), mannitol (19.89 mm) and oat (19.09 mm). These results are shown in Table 8 and Figure 6.

Table 8 Antifungal Activity on Carbon Utilization of SK-6

Sr. No	Carbon Sources	Inhibition zone diameter (mm)
1	Carrot	21.07
2	Corn Powder	22.89
3	Glycerol	24.15
4	**Glucose**	**30.24**
5	Lactose	20.92
6	Maltose	20.48
7	Mannitol	19.89
8	Molasses	22.67
9	Oat	19.09
10	Potato	21.25
11	Rice Powder	21.50
12	Sucrose	21.34
13	Fructose	15.11
14	Soluble Starch	16.18

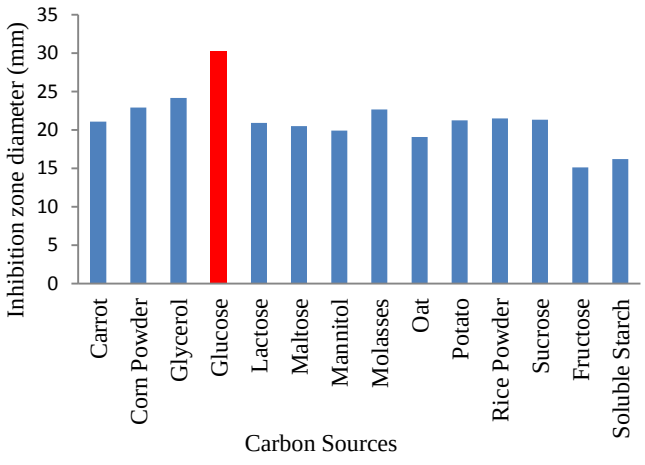

Figure 6 Antifungal activity on carbon utilization of SK-6

Antifungal Activity on Nitrogen Utilization of SK-6

There were variations in the level of antifungal activity when the different nitrogen sources were tested in the fermentation medium. When the various nitrogen sources, the significant inhibition zones were obtained in ammonium nitrate (59.21 mm), sodium nitrate (47.28 mm), potassium nitrate (43.79 mm) and malt extract (30.19 mm) respectively. Moderate inhibition zones were also found in peptone (29.77 mm), ammonium sulphate (29.19 mm), yeast extract (28.85 mm), ammonium chloride (27.95 mm), urea (26.88 mm), fish cake (26.49 mm), gelatin (26.26 mm), polypeptone (26.11 mm), meat extract (25.95 mm), casein (25.09 mm), peanut cake (24.25 mm), rice bran (23.22 mm) and soy bean (22.35 mm) (Table 9 and Figure 7).

Table 9 The Effect of Nitrogen Sources on SK-6

Sr. No	Nitrogen Sources	Inhibition zone diameter (mm)
1	Casein	25.09
2	Fish cake	26.49
3	Gelatin	26.26
	KNO_3	43.79
5	Malt extract	30.19
6	Meat extract	25.95
7	$NaNO_3$	47.28
8	NH_4Cl	27.95
9	**NH_4NO_3**	**59.21**
10	$(NH_4)_2SO_4$	29.19
11	Peanut cake	24.25
12	Peptone	29.77
13	Poly peptone	26.11
14	Rice bean	23.22
15	Soy bean	22.35
16	Urea	26.88
17	Yeast extract	28.85

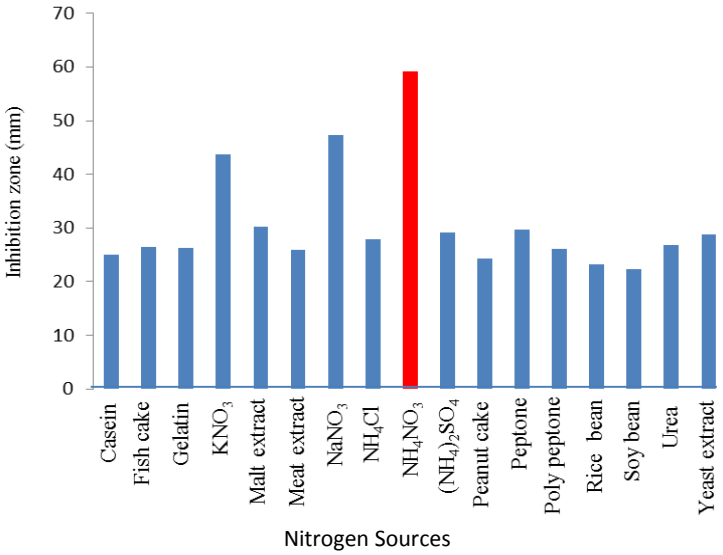

Figure 7 The effect on nitrogen sources of SK-6

The effect of pH on antifungal activity of SK-6

Effect of pH was assessed in the range of pH 4 to 10. The best antifungal activity was found at pH-7 (28.12 mm) (Table 10 and Figure 8).

Table 10 The Effect of pH on Antifungal Activity of SK-6

pH	Inhibition zone diameter (mm)
4	25.22
5	25.48
6	27.60
7	**28.12**
8	27.82
9	27.10
10	26.08

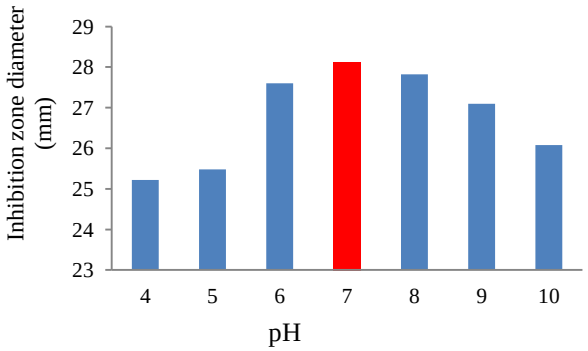

Figure 8 The Effect of pH on Antifungal Activity of SK-6

The Effect of Temperature on Antifungal Activity of SK-6

Effect of temperature was studied by changing temperature 20 °C, 25 °C, 30 °C, 35 °C and 40 °C. The maximum antifungal activity was obtained at 20 °C (27.31 mm) (Table 11 and Figure 9).

Table 11 The Effects of Temperature on Antifungal Activity of SK-6

Temperature (°C)	Inhibition zone diameter (mm)
20	**27.31**
25	27.25
30	26.76
35	25.89
40	16.17

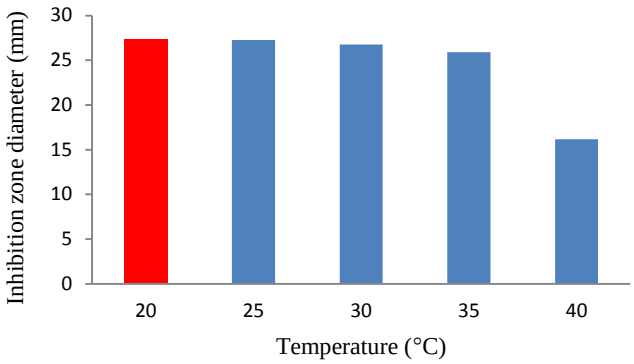

Figure 9 The effects of temperature on antifungal activity of SK-6

The Effect on Static and Shaking Culture of SK-6 against *C. albicans*.

In the comparison of shaking and static culture, the antifungal activity of shaking culture (30.60 mm) was more than that of static culture (21.90 mm) (Table 12 and Figure 10).

Table 12 Comparison on Antifungal Activity of SK-6 by using Shaking and Static Culture

Agitation and aeration condition	Inhibition zone diameter (mm)
Shaker culture	**30.60**
Static culture	21.90

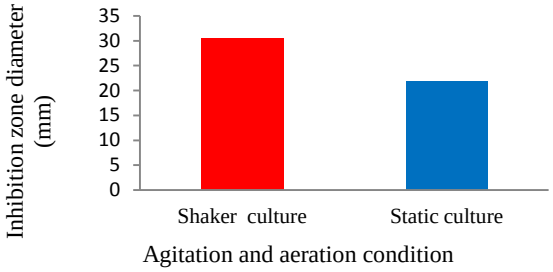

Figure 10 Comparison on antifungal activity of SK-6

Antifungal Activity of SK-6 on Various Fermentation Media

In the fermentation medium (FM), the best antifungal activity was obtained by using FM-1, (glucose and ammonium nitrate, 40.75 mm) followed by FM-2, (glucose and sodium nitrate, 40.49 mm), FM-8, (ammonium nitrate and glycerol, 32.06 mm) and FM-3, (glucose and potassium nitrate, 31.93 mm) respectively (Table 13 and Figure 11).

Table 13 The Antifungal Activity of SK-6 on Various Fermentation Media

Fermentation Media (FM)	Inhibition zone diameter (mm)
FM 1	**40.75**
FM 2	40.49
FM 3	31.93
FM 4	25.97
FM 5	25.17
FM 6	24.88
FM 7	21.80
FM 8	32.06
FM 9	29.13
FM 10	25.42
FM 11	23.83
FM 12	23.35
FM 13	21.20

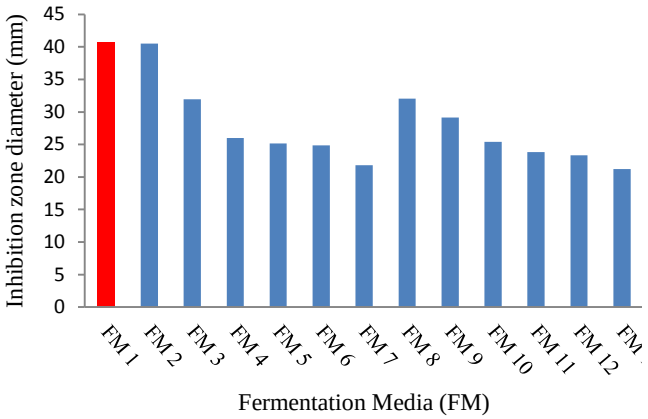

Figure 11 The antifungal activity of SK-6 on various fermentation media

Conclusion

Six different soil samples were collected from six different places at Pyawbwe Township, Mandalay Region. Soil fungi were isolated by serial dilution method.

A total of 30 strains fungal were isolated from six different soil samples of Pyawbwe Township by using four different media including BMEA, CZA, MEA and PGA medium and incubated for 3-7 days at room temperature. Pure colonies were inoculated into slant culture containing BMEA medium.

Six fungi SK-19, 21, 22, 23, 27 and 29 were isolated from soil sample S-I, SK-11, 17, 18, 20, 24, 25, 26 and 28 from sample S-II, SK-8, 9, 12, 13 and 30 from sample S-III, SK-14 from sample S-IV, SK-1, 2, 3, 4, 5, 6, 7, 15 and 16 from sample S-V and fungal strains SK 10 was isolated from sample S-VI.

A total of seventeen fungal colonies were isolated from Blakeslee's Malt Extract Agar (BMEA) medium and five fungal strains were isolated from Czapek Dox Agar (CZA) and Malt Extract Agar (MEA) respectively. Three colonies were isolated from Potato Glucose Agar (PGA) medium.

In the investigation of antimicrobial activities, ten fungi were tested by using Agar Well diffusion assay with seven test organisms. SK17 showed the highest antibacterial activities (30.28 mm) at 5 days on *Bacillus pumilus*. SK 19 exhibited the moderate antibacterial activities (24.23 mm) at 3days on *Bacillus subtilis.* Some isolated fungi did not have the antimicrobial activity on *Escherichia coli*. Mostly soil fungi were against *Bacillus pumilus*, *Bacillus subtilis* and *Candida albicans*. Especially, SK 6 showed the moderate antimicrobial activity against all test organisms.

From the research work, three fungal strains SK-1, SK-3 and SK-6 showed antimicrobial activity against on *Bacillus subtilis* and *Candida albicans* while SK-6 showed significant antimicrobial activity on *Candida albicans*. Thus, SK-6 was selected for the best fermentation conditions. The antifungal activity of SK-6 was highest against *Candida albicans* (28.59 mm) in 5 days fermentation period.

To study the optimization of inoculums age, incubation time (48, 60, 72, 84, 96, 108, 120, 132 and 144 h) were used and the maximum antifungal activity (25.02 mm) was found at 108 h.

In the appropriate size of inoculums, 5 % was the foremost suitable and the highest activities (33.03 mm) in SK-6 followed by 15 % and 30 % respectively.

In the carbon source, the colony of SK-6 was good growth on lactose, soluble starch and oat. The antifungal activity of SK-6 were affected by addition of glucose and showed the highest activity (30.24 mm), followed by glycerol and corn powder.

The nitrogen source has remarkable effect on the production of antifungal metabolite in SK-6. Especially SK-6 showed excellent growth on casein, followed by yeast extract, gelatin, ammonium chloride and peptone. Maximum antifungal metabolite of SK-6 was found in the ammonium nitrate (59.21 mm) followed by sodium nitrate, potassium nitrate respectively as nitrogen sources.

Effect of pH was investigated by varying from pH 4, 5, 6, 7, 8, 9 and 10. The highest antifungal activity was found at pH 7 (28.12 mm). Effects of temperature was observed by varying from 20 °C, 25 °C, 30°C, 35 °C and 40 °C. The best antifungal activity for temperature was found at 20 °C (27.31 mm) followed by 25 °C (27.25 mm), 30 °C (26.76 mm) respectively.

The results of static and shaking culture condition were compared, the best antifungal activity was found at shaking culture (30.60 mm).

In the fermentation medium (FM), thirteen kinds of FM were used and FM-1 gave the highest antifungal activity (40.75 mm) by using glucose and ammonium nitrate. The best production of drug by exploting sorbose in their fermentation media while others found glucose and yeast extract to be best carbon and nitrogen sources.

Thus, the results of the best fermentation conditions were investigated that antifungal metabolite of SK-6 were obtained by optimally 5 days fermentation period, 108 h age of inoculums, 5 % inoculums size, lactose and glucose in the carbon source, casein and ammonium nitrate in the nitrogen source, pH-7 and temperature 20 °C, shaking culture and fermentation medium FM-1.

It was concluded that the present research work was to study the antimicrobial properties of three isolated fungi, to investigate the fermentation conditions of selected fungus SK-6. The isolated strain will be studied for further investigation such as identification of isolated fungi and extraction of their antimicrobial compound.

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