EXTRACTION OF ANTIFUNGAL METABOLITE FROM ENDOPHYTIC FUNGUS ISOLATED FROM *BAUHINIA VARIEGATA* L. (SWE-DAW-NI)

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Abstract

Six medicinal plants were collected from Pathein University Campus. Ten endophytic fungi were isolated from these plants by surface sterilization method. The isolated fungal strains were given temporary names as TMO-1 to TMO-10. Antimicrobial activity of the isolated fungal strains was evaluated by the agar well diffusion assay with ten test organisms. Nearly all strains showed the antimicrobial activity. Among them, fungus TMO-4 from *Bauhinia variegata* L. (Swe-daw-ni) was screened for further investigations based on the results of antifungal activity especially against *Candida albicans.* In the fermentation conditions of this selected fungus, various carbon and nitrogen sources were utilized for the growth phase and maximum antifungal activity. These strains showed the moderate growth on glucose, glycerol and fructose. In the nitrogen source, TMO-4 exhibited the excellent growth on yeast extract (36.09 mm). The fermentation medium-1 gives the highest antifungal activity (29.45 mm) consisted of glucose and yeast extract for carbon and nitrogen source, 4 days age of culture (28.11 mm), 10 % inoculum size (30.03 mm), shaking culture (30.86 mm), temperature 25 $^{\circ}$C (30.00 mm) and pH 5 (29.93 mm). In the extraction of antifungal metabolites, fermented broth of TMO-4 was extracted by using four solvent systems. The ethyl acetate extract was the most suitable solvent system with R_f value of 0.80. Three isolated compounds A, B and C were identified by using modern spectroscopic methods such as UV, FT IR and GC-MS and found to be coumarin derivative, dibutyl phthalate and steroid derivative respectively.

Keywords: antifungal activity, surface sterilization method, MIC, metabolites, *Bauhinia variegata* L.

Introduction

Endophytes have been found in all parts of plant, including xylem and phloem. The scientific community in searching for new therapeutic alternatives has studied and found variable bioactive metabolites in

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endophytes such as antiviral, anticancer, anti-diabetic and antibacterial compounds. Natural products still remain the most important resource for the discovery of new and potential drug molecules. New approaches need to be devised to efficiently access chemical diversity for the development of new medicines, to overcome the difficulties in the treatment of infections caused by resistant bacteria pathogens (Parker, 1974).

The aim of this research was to extract the antifungal metabolite from endophytic fungi isolated from medicinal plant source (Swe-daw-ni). The objectives were to isolate the endophytic fungi from six medicinal plant sources, to evaluate antimicrobial activity of endophytic microorganisms, to optimize the fermentation condition for the antifungal activities, to extract the antifungal metabolite from endophytic fungus by using various solvent systems, to find the optimal pH, temperature of fermented broth for the best antimicrobial activity of TMO-4 against *Candida albicans*, to prepare crude extracts from fermented broth of selected fungus by using ethyl acetate solvent, to isolate some organic compounds from the ethyl acetate extract of fungus TMO-4 by using column chromatographic separation techniques, to identify the isolated fungus TMO-4, to classify and identify the isolated compounds by physicochemical tests and spectroscopic techniques such as UV, FT IR and GC-MS and to determine the Minimum Inhibitory Concentrations of isolated compounds.

Materials and Methods

Sample Collection and Isolation of Endophytic Microorganisms

Leaves from six medicinal plants were collected from Pathein University Campus (Table 1) and these plants were identified as *Polyalthia longifolia* L. (Thinbaw-te) (Annonaceae), *Oroxylum indicum* Vent. (Kyaung-sha) (Bignoniaceae), *Cassia siamea* Lam. (Mezali) (Fabaceae), *Cassia alata* L. (Thinbaw-mezali) (Fabaceae), *Bauhinia variegata* L. (Swe-daw-ni) (Fabaceae) and *Azadirachta indica* A. Juss (Tamar) (Meliaceae). In the isolation procedure of endophytic microorganisms, the leaves were washed in running tap water for 10 min and sterilized by soaking in 95 % alcohol for 15 s. Then, the leaves were cut into small pieces and dried on the sterilized tissue paper. After that, the leaves of cut pieces were incubated on nutrient

agar plate (glucose 2 %, peptone 1 %, yeast extract 0.5 %, agar 2 %-Glucose-Yeast extract-Peptone Agar medium) for 3 days to 1 week at room temperature (Figure 1).

Table 1: Endophytic Microorganism from Six Medicinal Plants

Plant	Family	Isolated endophytes (microorganisms)
Polyalthia longifolia L.(Thinbaw-te)	Annonaceae	TMO-6, TMO-7, TMO-8
Oroxylum indicum Vent. *(*Kyaung-sha)	Bignoniaceae	TMO-1
Cassia siamea Lam. (Mezali)	Fabaceae	TMO-2, TMO-3
Cassia alata L.(Thinbaw mezali)	Fabaceae	TMO-5
Bauhinia variegata L.(Swe-daw-ni)	Fabaceae	TMO-4
Az Azdirachta indica A.Juss. (Tamar)	Meliaceae	TMO-9,TMO-10
Total isolated endophytes		10

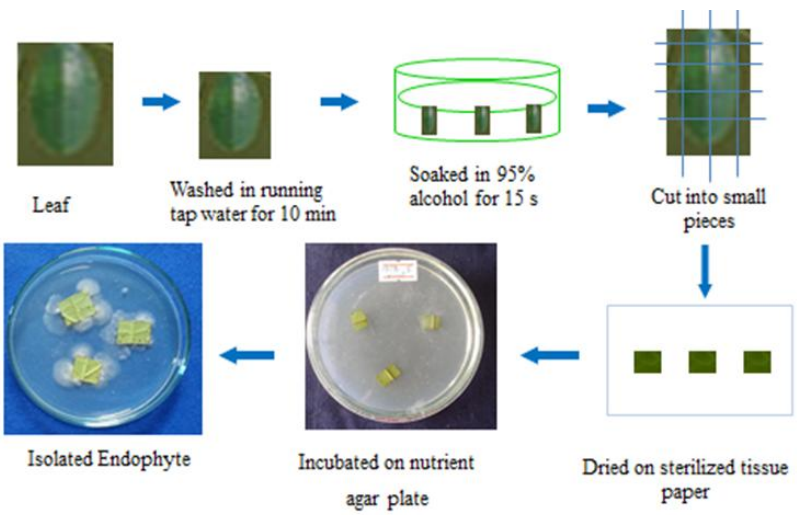


Figure 1: Procedure for isolation of endophytic microorganisms from leaf sample

Preliminary Study of the Antimicrobial Activities of the Isolated Fungi by Agar Well Method

The ten isolated fungi (TMO1-TMO10) from six medicinal plants were inoculated into the preculture medium (glucose 2 %, peptone 1 %, yeast extract 0.5 %, agar 2 % at pH 7.0) for 3 days at room temperature. After three days, the preculture (1 %) was transferred into the fermentation medium (glucose 2 %, yeast extract 0.3 %, K_2HPO_4 0.001 %, $MgSO_4$ 0.001 %, $CaCO_3$ 0.1 % at pH 6.5) and fermented for 12 days by static culture. Then, the fermented broth was used to check the antimicrobial activity by agar well method (Collins, 1965). Agar well; (8 mm in diameter) was utilized for antimicrobial activity test against ten organisms.

Fermentation Studies and Identification of Selected Fungus TMO-4 against *Candida albicans*

The effects of carbon and nitrogen sources for the growth of selected fungus (TMO-4)

In this study, carbon sources were used as glucose, sucrose, maltose, fructose, lactose, gruel, tapioca powder, glycerol, corn powder and soluble starch. Nitrogen sources such as yeast extract, peptone, potassium nitrate, ammonium chloride, ammonium nitrate, ammonium sulphate, ammonium phosphate, meat extract, peanut cake and malt extract were used. The growth phase of selected TMO-4 on various sources were recorded.

The effects of ages and sizes of inoculum for fermentation of selected fungus (TMO-4)

For the age of inoculum, seed culture (5 mL) of isolated fungus (TMO-4) was transferred into fermentation medium. After 4 days, the antimicrobial activity was tested by agar well diffusion method against *Candida albicans* (Collins, 1965). For the size of inoculum, seed cultures (5 %, 10 %, 15 %, 20 %, 25 %, 30 %) were transferred into the each flask containing 100 mL fermentation medium.

Comparison of the activities of static and shake cultures of TMO-4

In this experiment, antimicrobial activity of TMO-4 was compared on the static and shake culture (100 rpm) for 5 days fermentation.

Fermentation condition for the antimicrobial activity of TMO-4 on the six fermentation media

Various six fermentation media of selected endophytic fungus TMO-4 from *Bauhinia variegata* L. were studied for the production of antifungal metabolite.

Effect of pH for the extraction of metabolite

In the study of TMO-4, the fermented broths with pH (4, 5 and 6) were studied for the production of antifungal metabolite.

Effects of temperature on the fermentation of fungus TMO-4

The effects of temperature for the fermented broth at six different temperature (20 ˚C, 25 ˚C, 30 ˚C, 35 ˚C, 40 ˚C and 45 ˚C) were studied.

Paper chromatography

The filter paper and four solvent systems such as 20 % NH_4Cl, *n*-butanol saturated with water, *n*-butanol-acetic acid-water (3:1:1) and ethyl acetate saturated with water were used for preliminary characterization of the isolated compound.

Identification of selected fungus TMO-4

The selected fungus TMO-4 was inoculated onto PGA medium and incubated at 25 ˚C for 7 days. After incubation a positive culture was analyzed by transferring a loop of the fungal isolate onto a glass slide, then lactophenol-cotton blue stain was added before examined under light microscope 40× magnification power of the compound for microscopic characteristics including conidiophores, vesicle, hyphal and spore morphology. The selected fungus TMO-4 was identified by the morphology and microscopic character (Larone, 1995; Barnett, 1969).

Production and Extraction of Metabolite

Preparation of EtOAc extracts from the fermented broth of isolated fungus TMO-4

A modified PGA medium was inoculated with an agar block of actively growing culture of TMO-4 and incubated at 25 ˚C for 6 days. It was then centrifuged at 100 rpm for 15 min, and the supernatant was passed

through a millipore filter (0.22 μm porosity) to get a spore free filtrate. For extraction of antifungal compounds, the filtrate was treated with an equal volume of ethyl acetate (1:1). Then, the mixture was shaken in a separating funnel. The organic layer was separated and collected.

Separation of ethyl acetate extracts by silica gel column chromatography

Fermented broth of TMO-4 was extracted with ethyl acetate. Pure ethyl acetate extract from fermented broth of TMO-4 was concentrated *in vacuo* and separated by using petether-ethyl acetate solvent system (19:1, 9:1, 4:1, 2:1 and 1:1). The column was packed by wet method. Two mL of each fraction was collected and examined the activity with *Candida albicans* (Simon and Gray, 1998). Finally five main fractions (F_I to F_v) were collected. The active fractions [(F_{II}) f_{21-26}, f_{41-50} and f_{54-58}; Isolated compounds A, B, and C] were concentrated *in vacuo*. Fraction F_I, F_{III}, F_{IV} and F_V were found as mixtures.

Identification of the isolated Compounds

The isolated compounds were structurally identified by modern spectroscopic techniques such as UV-visible, FT IR and GC-MS spectrometry.

Physicochemical characterization of the isolated compounds

The isolated compounds (Compounds A, B and C) from ethyl acetate extract of TMO-4 fungus were characterized by physical properties such as melting points, R_f values, solubilities in various solvents and some chemical tests.

Determination of Minimum Inhibitory Concentrations (MIC)

Minimum Inhibitory Concentration (MIC) was determined by serial dilution method (Domain, 1999; Jennifer, 2006). The concentrations were 10 μg/mL, 5 μg/mL, 2.5 μg/mL, 1.25 μg/mL, 0.625 μg/mL, 0.312 μg /mL, 0.156 μg /mL and 0.078 μg /mL respectively.

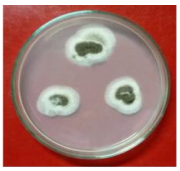
Results and Discussion

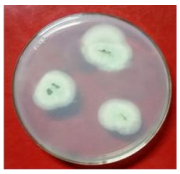
Isolation of Endophytic Microorganisms

The surface colour and reverse colour of strain TMO-4 were cream colour and TMO-1, TMO-3, TMO-7 and TMO-10 were white. The surface colour of TMO-2 was black, edge white and its reverse colour was white. Strain TMO-5 was pale pink in surface and pale yellow in reverse colour. In strain TMO-6, both the surface and reverse colour were the same pale grey. Another strain TMO-8 was reddish brown in surface and yellow in reverse colour. The last one TMO-9 was the same colour grey, edge white in both surface (Figure 2).


Surface view (TMO-1)


Reverse view (TMO-1)


Surface view (TMO-2)


Reverse view (TMO-2)


Surface view (TMO-3)

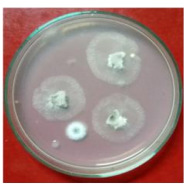

Reverse view (TMO-3)


Surface view (TMO-4)


Reverse view (TMO-4)


Surface view (TMO-5)


Reverse view (TMO-5)


Surface view (TMO-6)


Reverse view (TMO-6)

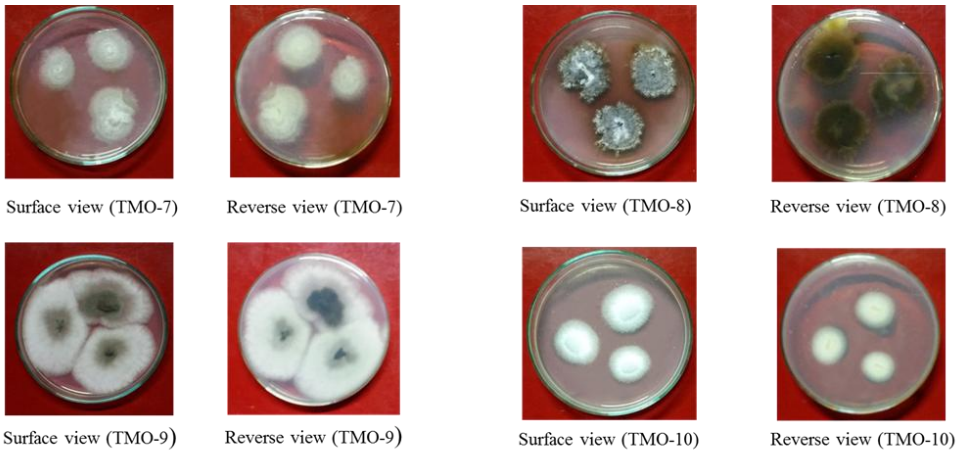


Figure 2: Morphological characters of isolated strain TMO-1 to TMO-10

Preliminary Study of the Antimicrobial Activities of Isolated Fungi by Agar Well Method

The test of antimicrobial activities of endophytic fungi (TMO-1-10) was carried for 3 to 12 days. Among them, endophytic fungus TMO-4 (28 mm) showed the highest activity in 5 days old culture against *Candida albicans* (Table 2).

Table 2: Antimicrobial Activities of Endophytic Ten Fungal Strains (5 days old)

Test Organisms	Inhibition zone diameters (mm) of ten endophytic fungal strains									
	TMO-1	TMO-2	TMO-3	TMO-4	TMO-5	TMO-6	TMO-7	TMO-8	TMO-9	TMO-10
A.tumefaciens	24	24	24	23	24	23	24	23	24	23
A. paraciticus	21	23	22	22	24	23	22	22	21	24
B.subtilis	24	24	24	24	23	23	23	24	24	23
C.albicans	23	24	22	28	22	24	20	26	25	23
M.luteus	16	21	24	23	24	24	24	22	24	24
S.typhi	24	22	23	24	24	23	23	24	24	22

Test Organisms	Inhibition zone diameters (mm) of ten endophytic fungal strains									
	TMO-1	TMO-2	TMO-3	TMO-4	TMO-5	TMO-6	TMO-7	TMO-8	TMO-9	TMO-10
P.fluorescens	23	23	24	24	23	23	23	22	23	24
E.coli	23	24	23	24	22	24	23	20	23	22
S.aureus	24	21	24	23	23	22	24	21	24	23
S.cerevisae	24	23	23	24	21	21	22	23	23	24

10-12 mm = weak activity, > 18 mm = very high activity
13-17 mm = high activity well size = 8 mm

Fermentation Studies and Identification of Antifungal Metabolite by Endophytic Fungus TMO-4 against *Candida albicans*

On the ten carbon sources, it was found that TMO-4 showed the moderate growth on glucose, glycerol and fructose and poor growth on other carbon sources. In the nitrogen source, TMO-4 showed the excellent growth on yeast extract and good in peptone and poor growth on other nitrogen sources (Table 3).

In the fermentation conditions, the optimum age and inoculum size were found at 4 days (28.11 mm) of inoculum and 10 % (30.33 mm) size of inoculum of TMO-4 (Figure 3 and Table 4). Conti *et al.*, 2012 reported that the best time of maximum production of the bioactive metabolites for all microorganisms was 100 h. Owen and Hundley, (2004) described that the production of bioactive compounds by endophytes is stimulated by the microorganisms-plant interactions or by environmental factors.

The addition of glucose as a carbon source showed maximum activity and the inhibition zone reached (19.45 mm) in TMO-4 (Table 5). The activity was low on other carbon sources. Lactose, Maltose and Corn powder showed inactive. In the effect of nitrogen sources in the fermentation, selected strains (TMO-4) had the highest activities (36.09 mm) respectively on yeast (Table 5).

In the studies for the comparison of the activities of static and shake cultures of TMO-4, it was observed that the fermentation by Shake culture of

TMO-4 showed the inhibitory zone of 30.86 mm and the static culture showed that of 25.13 mm (Figure 4).

Among the fermentation medium, FM-1 showed the highest activity (29.45 mm) (and (Table 6 and Figure 5). The effects of pH and temperature on TMO-4 were studied and the highest antifungal activity was obtained pH 5 (29.93 mm) (Table 7 and Figure 6) and temperature at 25 ˚C (30.00 mm) (Table 8 and Figure 7).

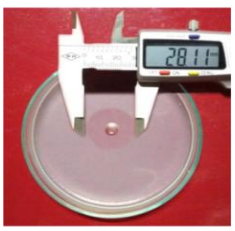
Four solvent systems-1(20 % NH_4Cl), 2 (*n*-butanol-acetic acid-water (3:1:1), 3 (*n*-butanol saturated with water), 4 (ethyl acetate saturated with water) were used for paper chromatography. According to the R_f values, it was considered that solvent systems 2, 3 and 4 were suitable for the extraction of antimicrobial metabolite. However, solvent system 4 was most suitable to extract the compound from fermented broth (Figure 8).

Table 3: Colony Characters of the Selected Endophytic Fungus (TMO-4) Isolated from *Bauhinia variegata* L. on Various Carbon and Nitrogen Sources

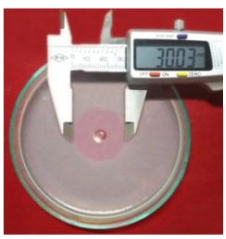
No.	Sources and Growth Phase			
	Carbon sources	**Growth phase**	**Nitrogen sources**	**Growth phase**
1	**Glucose**	**Moderate**	**Yeast extract**	**Excellent**
2	Sucrose	Poor	**Peptone**	**Good**
3	Gruel	Poor	Potassium nitrate	Poor
4	Tapioca powder	Poor	Ammonium chloride	Poor
5	**Fructose**	**Moderate**	**Ammonium nitrate**	**Moderate**
6	Lactose	Poor	Ammonium sulphate	Poor
7	Maltose	Poor	Ammonium phosphate	Poor
8	**Glycerol**	**Moderate**	Meat extract	Poor
9	Soluble starch	Moderate	Malt extract	Poor
10	Corn powder	Poor	Peanut cake	Poor

Table 4 : The Effects of Ages and Sizes of Inoculum on the Fermentation (TMO-4) against *Candida albicans*

Ages of inoculum (day)	Activity (Inhibitory Zone Diameter, mm)	Sizes of inoculum (%)	Activity (Inhibitory Zone Diameter, mm)
2	18.21	5	20.13
3	23.21	**10**	**30.03**
4	**28.11**	15	28.84
5	26.12	20	23.09
6	25.27	25	22.55
7	20.32	30	19.31



4 days (28.11 mm)



10 % (30.03 mm)

Figure 3: The ages and sizes of inoculum on the fermentation (TMO-4) against *Candida albicans*

Table 5: The Effects of Carbon and Nitrogen Sources on the Fermentation of Selected Fungus (TMO-4) against *Candida albicans*

No.	Carbon sources	Activity (Inhibitory Zone, mm)	Nitrogen sources	Activity (Inhibitory Zone, mm)
1	**Glucose**	**19.45**	**Yeast extract**	**36.09**
2	Sucrose	-	Peptone	22.89
3	Gruel	14.70	Potassium nitrate	-
4	Tapioca powder	12.51	Ammonium chloride	-
5	Fructose	16.47	Ammonium nitrate	20.87
6	Lactose	-	Ammonium sulphate	-
7	Maltose	-	Ammonium phosphate	14.09
8	Glycerol	17.62	Meat extract	-
9	Soluble starch	17.60	Malt extract	-
10	Corn powder	-	Peanut cake	-

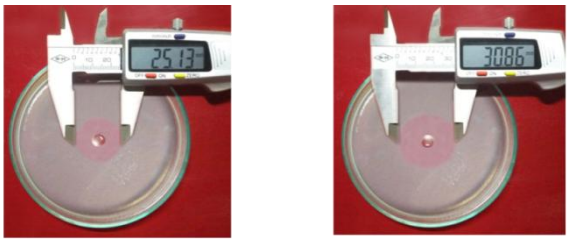


Figure 4: Comparison of the activities of static culture and shake culture of TMO-4

Table 6: Antimicrobial Activity of TMO-4 on the Six Fermentation Media

Fermentation medium	Activity (clear zone, mm)
FM-1	29.45
FM-2	25.10
FM-3	26.32
FM-4	22.54
FM-5	28.50
FM-6	29.12

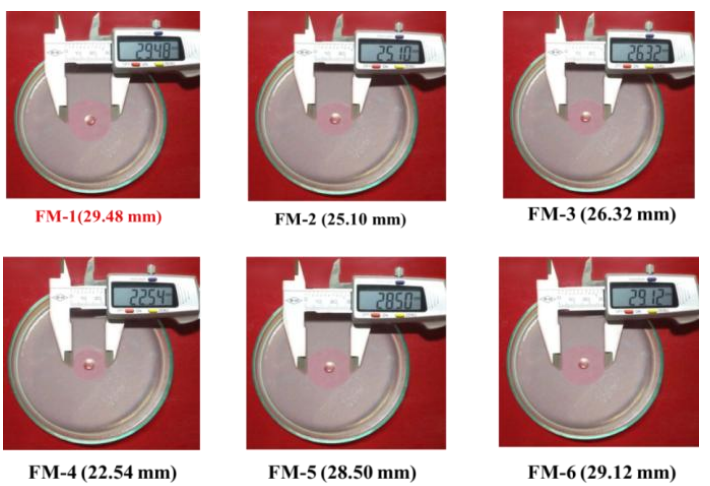
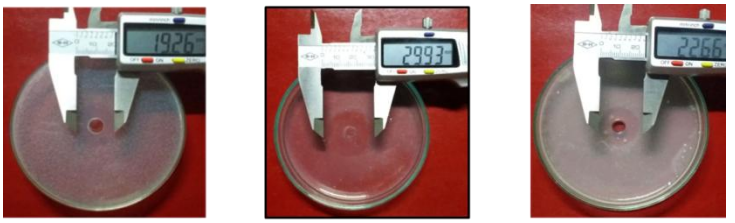


Figure 5: Antimicrobial activity of TMO-4 on the six fermentation media

Table 7: The Effect of pH on the Extraction of TMO-4

Fermented pH	Antifungal activity (clear zone, mm)
4	19.26
5	**29.93**
6	22.68



pH 4 **pH 5** pH 6

Figure 6: The effect of pH for the extraction of metabolite

Table 8: The Effect of Temperature on the Fermentation of TMO-4

Temperature (˚C)	Antifungal activity (clear zone, mm)
20	18.42
25	30.00
30	29.13
35	29.10
40	24.14
45	-

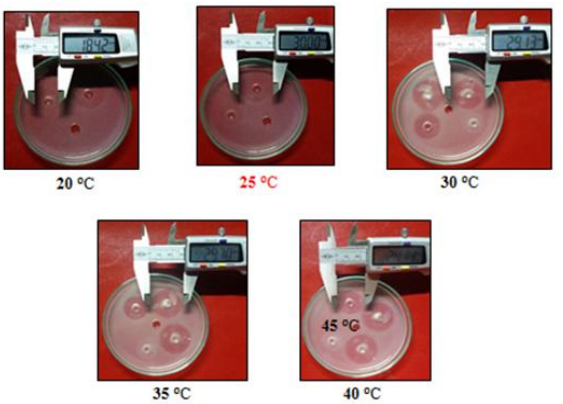
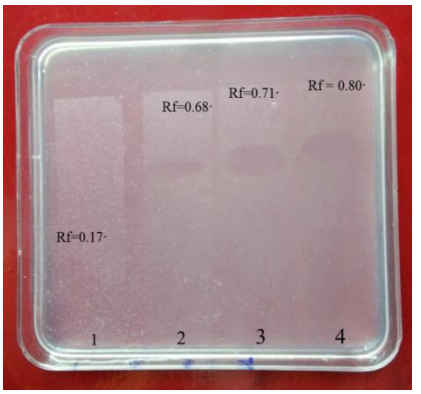


Figure 7: The effect of temperature on the fermentation of TMO-4



1. 20 % NH_4Cl (R_f = 0.17)
2. *n*-butanol-acetic acid–water (3:1:1) (R_f = 0.68)
3. *n*-butanol saturated with water (R_f = 0.71)
4. ethyl acetate saturated with water (R_f = 0.80)

Figure 8: Bioautography of paper chromatography with four solvent Systems

Identification of the selected fungus TMO-4

In the identification of TMO-4, the surface and reverse colour of colony was cream. Colony diameter was 40 mm on Potato Glucose Agar medium. The cream colour parts had scattered conidia. They produced small conidia heads. TMO-4 had uniseriate heads. Hyphae were septate. Mycelia was cream colour and formed a mat beneath the colonies, The hyphal threads were very conspicuous. Conidia heads were radiate with loosely attached phialides. These results were in agreement with descriptions of Larone (1995) and Barnett (1969). Therefore, selected fungus TMO-4 was identified as *Aspergillus sp.* (Figure 9). The production and quality of bioactive compounds from endophytic fungi depend on natural conditions of the association and the nature of the synthetic medium used (Strobel, 2003; Daisy, 2003).

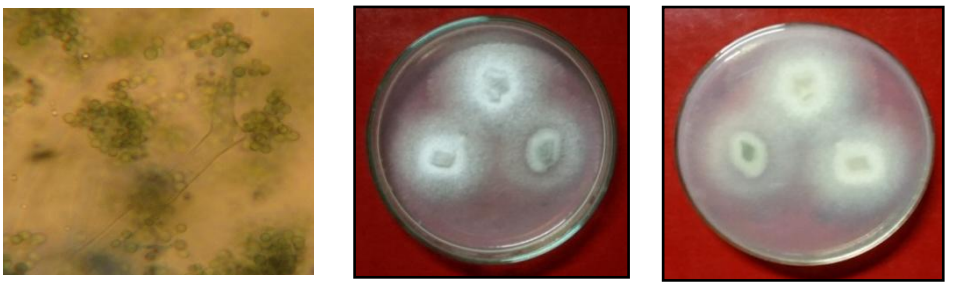
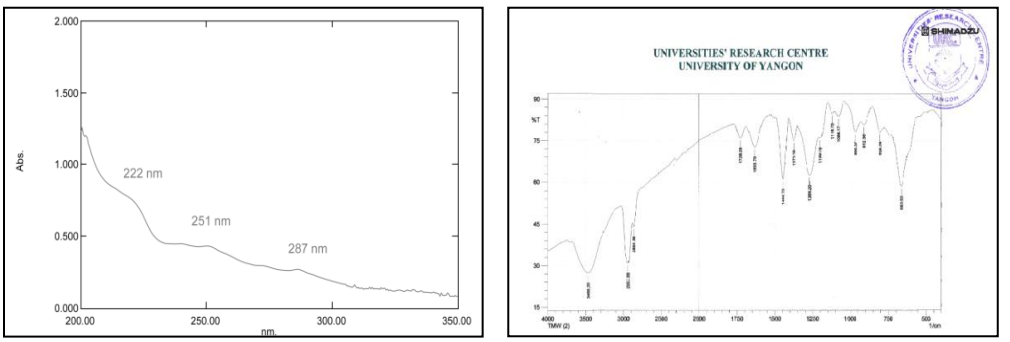


Figure 9: Morphological and microscopically character of fungus TMO-4 (*Aspergillus* sp.)

Identification of the isolated compounds (A, B, and C)

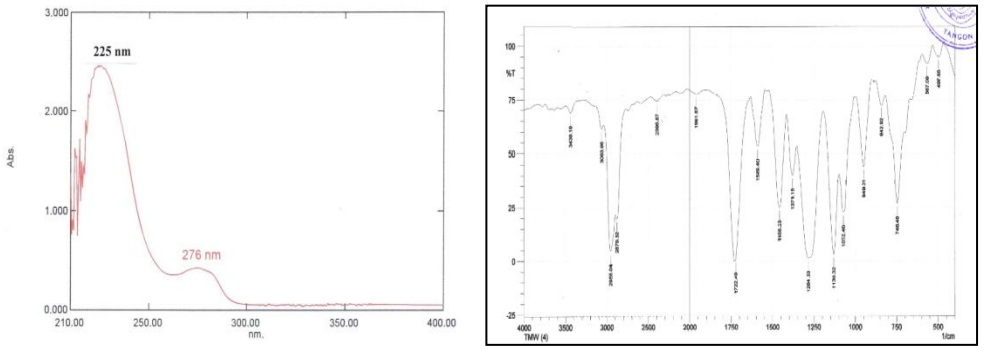
The isolated compounds (A, B and C) were identified by modern spectroscopic techniques such as UV, FT IR and specifically B was identified by GC-MS spectrometry (Figures 10, 11, 12).

By silica gel column chromatographic separation, compound A (a coumarin derivative, 0.66%, semisolid in yellow), compound B (dibutyl phthalate, 0.58%, semisolid in yellow), and compound C (steroid compound, 0.45%, yellow solid powder, m.pt = 78-80 ˚C) were isolated from ethyl acetate extract of fermented broth of the fungus TMO-4 from Swe-daw-ni. According to the reported data, all isolated compounds (A, B and C) have antifungal activity. GC-MS is a novel technique to identify the secondary metabolites from the fermented broth and analysis of GC-MS is very reliable to identify the compound in complex biochemical products. Moreover, dibutyl phthalate also reported as antifungal compound. Antimicrobial efficacy of dibutyl phthalate (DBP) has been reported from *Streptomyces.* DBP is also used as peroxisome proliferator which is an effective compound against demodicidosis as well as an endocrine disruptor with estrogenic activity (Marchetti *et al*, 2002), and a drug channeling agent (Nandhini 2015; Roy *et al*, 2006).



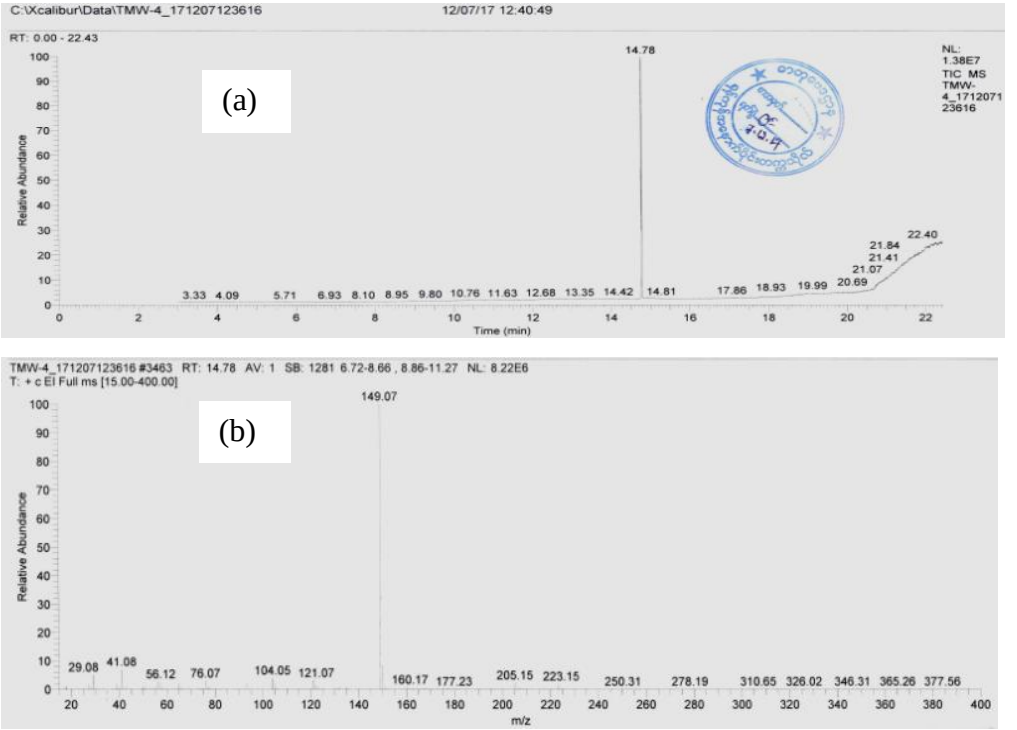
(a) (b)

Figure 10: (a) UV and (b) FTIR spectra of isolated compound A



(a) (b)

Figure 11: (a) UV and (b) FTIR spectra of isolated compound B



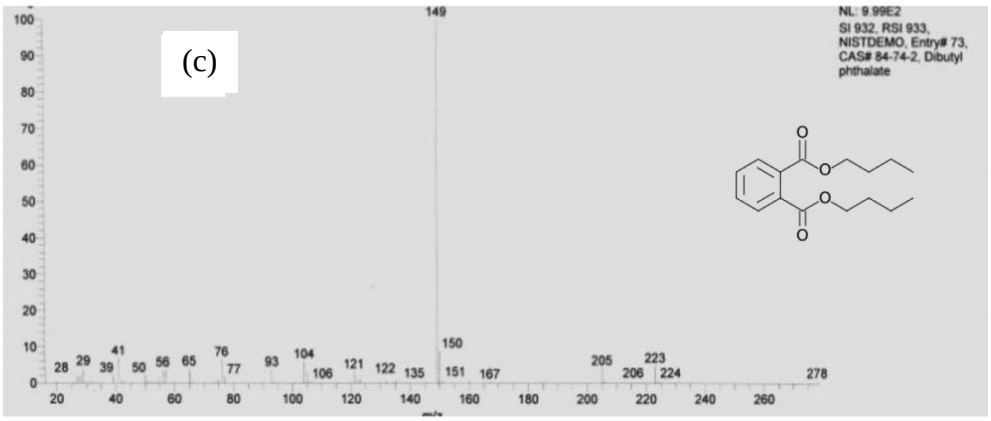
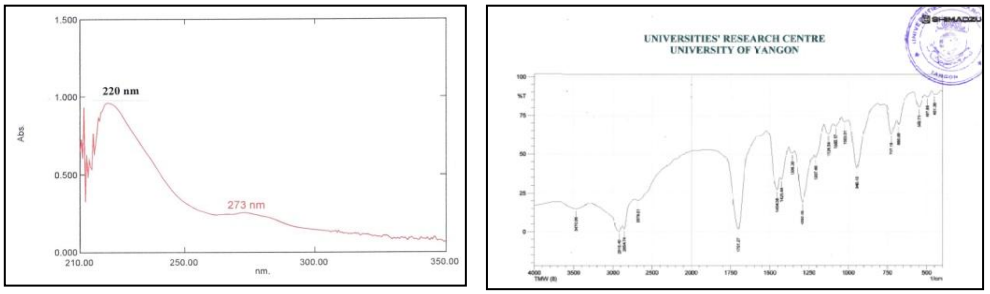


Figure 12: (a) GC-MS chromatogram; (b) EI-MS spectrum of isolated compound B (c) EI-MS spectrum of dibutyl phthalate (Library)



(a) (b)

Figure 13: (a) UV and (b) FTIR spectra of isolated compound C

Minimum Inhibitory Concentrations (MIC)

In the Minimum Inhibitory Concentrations (MIC) of endophytic fungus (TMO-4), in the best antifungal action was found with 0.312 µg/mL (Table 9 and Figure 14).

Table 9: Minimum Inhibitory Concentrations of Secondary Metabolites from TMO-4 (Compound B) on *Candida albicans*

MIC values of ethyl acetate extract (µg/mL)	Antifungal activity (clear zone, mm)
Control	-
10.000	30.78
5.000	29.76
2.500	29.45
1.250	27.95
0.625	26.25
0.312	13.17
0.156	-
0.078	-

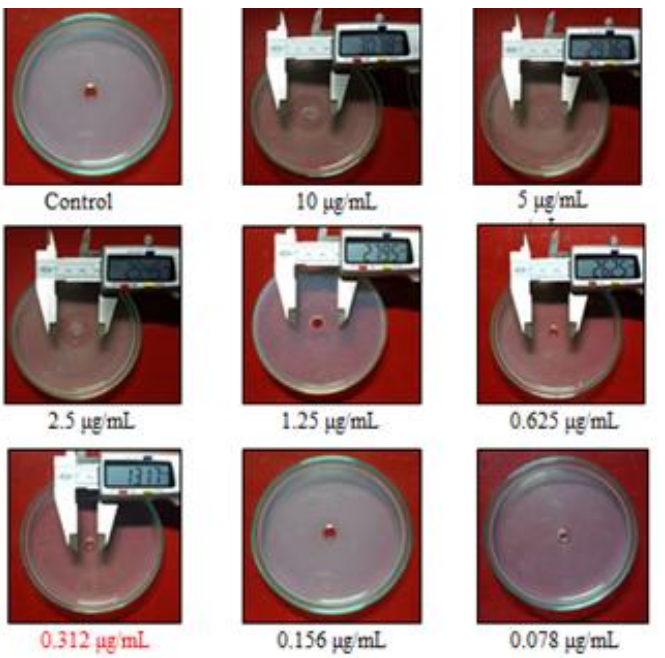


Figure 14: Minimum inhibitory concentration of secondary metabolites from TMO-4 (compound B) on *Candida albicans*

Conclusion

In this study, ten fungi were isolated from six medicinal plants collected from Pathein University Campus. As the preliminary test, isolated fungal strains were tested by using ten tested organisms. Endophytic fungus TMO-4 (28 mm) which possessed the highest activity in 5 days old culture was selected for further investigation. Selected fungus TMO-4 was obtained from the leaves of *Bauhinia variegata* L. TMO-4 showed the moderate growth on glucose, glycerol and fructose and poor growth on other carbon sources and TMO-4 showed the excellent growth on yeast extract and good in peptone and poor growth on other nitrogen sources. The addition of glucose as a carbon source showed the maximum activity and the inhibition zone reached (19.45 mm) in TMO-4. In the fermentation conditions, the optimum age and inoculum size were found at 4 days (28.11 mm) of inoculum and 10 % (30.33 mm) size of inoculum of TMO-4. Effect of nitrogen sources in the fermentation, showed selected strains (TMO-4) had the highest activities (36.09 mm) respectively on yeast. In the studies for the comparison of the activities of static and shake cultures of TMO-4, it was observed that the fermentation by shake culture of TMO-4 showed the highest activity of 30.86 mm. Among the fermentation media, FM-1 showed the highest activity (29.45 mm). The effects of pH and temperature on TMO-4 were studied and the highest antifungal activity was obtained at temperature 25 ˚C (30.00 mm) and pH 5 (29.93 mm). Solvent system 4 was most suitable to extract the compound from fermented broth. The selected fungus TMO-4 was identified as *Aspergillus sp.*

By silica gel column chromatographic separation, compound A (a coumarin derivative), compound B (dibutyl phthalate), and compound C (steroid compound) were isolated from ethyl acetate extract of fermented broth of the fungus TMO-4 from Swe-daw-ni. The isolated compounds were identified by chemical reagent tests and modern spectroscopic methods such as UV, FT IR and GC-MS. From current study, the results indicate that endophytic fungi, isolated from *B. variegata* L. (Swe-daw-ni) could be a potential source for bioactive compounds. In the Minimum Inhibitory Concentrations (MIC) of endophytic fungus (TMO-4), in the best antifungal action was found with 0.312 μg/mL.

This research indicated that endophytic fungi isolated from leaves of *Bauhinia variegata* L. (Swe-daw-ni) had bioactive compounds with antifungal potential. This may be due to the fact that endophytic microorganisms produced bioactive secondary metabolites.

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