ISOLATION AND ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC FUNGI FROM SIX MEDICINAL PLANTS

Theingi Aye[1], Moe Moe Aye[2] and Win Yu Yu Naing[3]

Abstract

The six plants samples were collected at Pathein Township, Ayeyarwady Region. The isolation was undertaken by Surface sterilization method using LCA medium. Thirty endophytic fungi were isolated from six different plants. These endophytic fungi were isolated by using Low Carbon Agar (LCA) medium for first culture and Potato Glucose Agar (PGA) medium for pure culture. Isolated fungi were given as temporary names TFO-01 to TFO-30. The colours of all isolated fungi rouge from white, green, black, dark green, yellowish green, pale cream, whitish gray, dark gray, whitish cream, greenish gray to gray. In the study of antimicrobial activity, fungus TFO-16 exhibited the highest activity against *Candida albicans* and TFO-15 against *Saccharomyces cerevisiae*. TFO-15 also showed antibacterial activity against *Salmonella typhimurium* and *Escherichia coli*. Among these two fungi, fungus TFO-15 was selected for further investigation.

Keywords Endophytic fungi, Antimicrobial activity

Introduction

Endophytes are microorganisms that live in the intercellular spaces of stems, petioles, roots, and leaves of plants causing no discernible manifestation of their presence and have typically gone unnoticed (Strobel and Long, 1998). An endophyte is an endosymbiont, often a bacterium or fungus, that lives within a plant for at least part of its cycle without causing apparent disease.

Most of the endophytes isolated from plants are known for their antimicrobial activity. Endophytes isolated from medicinal plants showed bioactivity for broad spectrum of pathogenic microorganisms. Most of the bioactive compounds isolated from endophytes are known to have functions of antibiotics, immunosuppressants, anticancer agents, biological control agents, and so forth.

Many endophytes have the potential to synthesize various bioactive metabolite may, directly or indirectly be used as therapeutic agents against numerous diseases. The emergence of antibiotic-resistant bacteria such as *Salmonella typhimurium* (Penicillin-resistant bacterium) calls for inventive research and developing strategies (Ezhil *et al*, 2011).

The aim and objectives of this research were to isolate endophytic fungi from medicinal plants and to study the antimicrobial activities of endophytic fungi.

[1] Department of Botany, Pathein University
[2] Department of Botany, Pathein University
[3] Department of Botany, Pathein University

Materials and Methods

Table 1 Plants samples used for the isolation of endophytic fungi

No.	Sources	Family
1.	*Mangifera indica* L.	Anacardiaceae
2.	*Averrhoa carambola* L.	Oxalidaceae
3.	*Cajanus cajan* Millsp.	Fabaceae
4.	*Talinum triangulare* (Jacq.) Willd.	Talinaceae
5.	*Sauropus androgynus* (L.) Merr.	Phyllanthaceae
6.	*Jatropha podagrica* Hook.	Euphorbiaceae

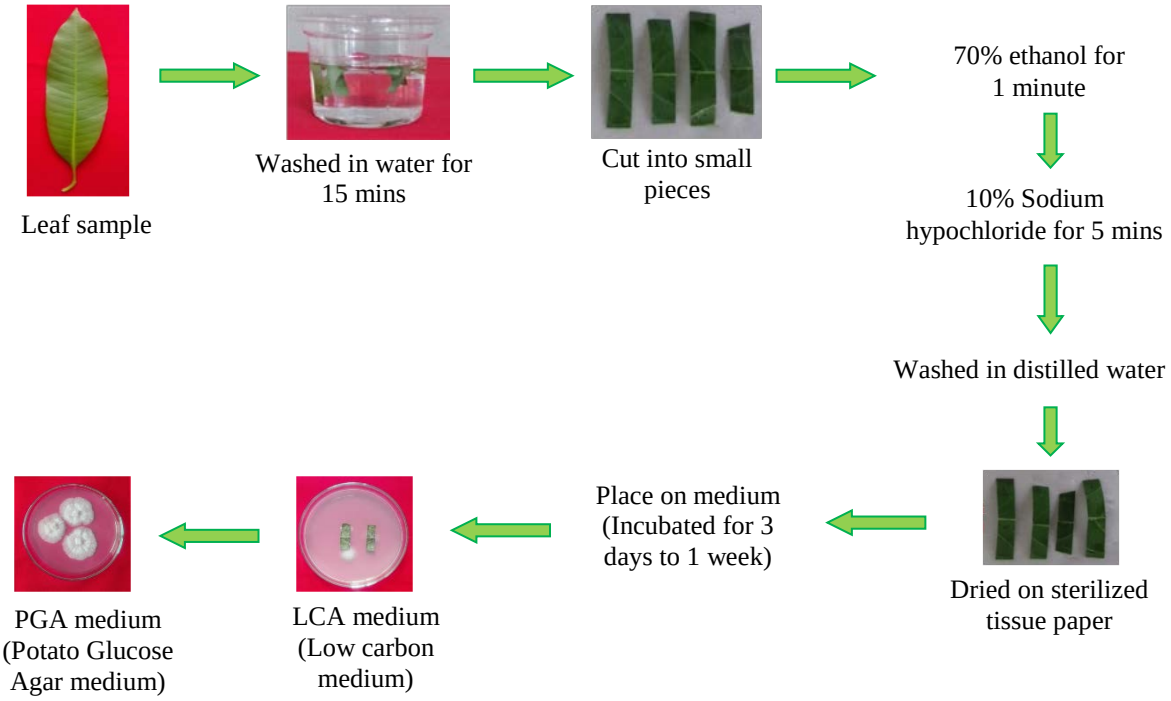


Figure 1 Procedure of Surface sterilization Method (NITE, 2004)

Medium Used for the isolation of fungi (NITE, 2004)

LCA medium

Glucose	1.0 g
Yeast extract	0.2 g
K_2HPO_4	1.0 g
$MgSO_4\,7H_2O$	0.2 g
$NaNO_3$	2.0 g
KC*l*	0.2 g
Agar	1.8 g
DW	100 mL
pH	6.5

PGA medium

(Potato Glucose Agar Medium)

Potato	20 g
Glucose	1.5 g
Agar	1.8 g
DW	100 mL
pH	6.5

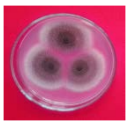
(after autoclaving chloramphenicol (20mg/L) was added to the medium)

Study on the Antimicrobial Activities

Preliminary study for antimicrobial activities by paper disc diffusion assay was carried out by the method of NITE (2004). Six kinds of test organisms *Saccharomyces cerevisiae*, *Candida albicans*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Escherichia coli* and *Salmonella typhimurium* were used in paper disc diffusion method.

Procedure for Antimicrobial Activity Test (NITE, 2004)

1. Isolated fungi were incubated for 7 days at 25ºC on PGA agar plates.



2. A piece of agar with fungi was inoculated into the tube containing 10 mL fermentation medium.

3. The tube with fungus was incubation at 25ºC for 5 days to ferment for the activity of antimicrobial metabolites.
4. Sample (20 µL) was put on paper disc and antimicrobial activities were investigated using eight kinds of test organisms.

Fermentation Medium

(NITE, 2004)

Glucose	2.0 g
glycerol	1.5 g
Yeast Extract	1.0 g
Polypeptone	0.6g
Malt Extract	0.4g
$MgSO_4.7H_2O$	0.001 g
K_2HPO_4	0.001 g
$CaCO_3$	0.1 g
DW	100 mL
pH	6.5

Assay Medium

Glucose	1.0 g
Peptone	0.3 g
Agar	1.8 g
DW	100 mL

Results

Scientific Name - *Mangifera indica* L.
Famil - Anacardiaceae
Myanmar Name - Tha-yet
English Name - Mango

Scientific Name - *Averrhoa carambola* L.
Famil - Oxalidaceae
Myanmar Name - Zaung-ya
English Name - Star fruit

Scientific Name - *Cajanus cajan*(L.) Millsp.
Famil - Fabaceae
Myanmar Name - Pe-Zin-Gon
English Name - Pigeon-pea

Scientific Name - *Talinum triangulare* (Jacq.) Willd.
Family - Talinaceae
Myanmar Name - Unknown
English Name - Water leaf

Scientific Name - *Sauropus androgynus* (L.) Merr.
Family - Phyllanthaceae
Myanmar Name - Kyet-tha-hin
English Name - Sweet leaf

Scientific Name - *Jatropha podagrica* Hook.
Family - Euphorbiaceae
Myanmar Name - Da-bin-shwe-htee
English Name - Buddha belly plant or gout plant

Table 2 Isolated fungi from six medicinal plant samples by Surface sterilization Method

No.	Sample	Surface sterilization Method	
		Total	**Isolated No**
1.	*Mangifera indica* L.	5	TFO-01,02,03,04,05
2.	*Averrhoa carambola* L.	4	TFO-06,07,08,09
3.	*Cajanus cajan* Millsp.	5	TFO-10,11,12,13,14
4.	*Talinum triangulare* (Jacq.) Willd.	5	TFO-15,16,17,18,19
5.	*Sauropus androgynus* (L.) Merr.	6	TFO-20,21,22,23,24,25
6.	*Jatropha podagrica* Hook.	5	TFO-26,27,28,29,30

Habit Leaves Inflorescence

Figure 2 Habit of *Mangifera indica* L.


Habit


Leaves


Inflorescence

Figure 3 Habit of *Averrhoa carambola* L.


Habit


Leaves


Inflorescence

Figure 4 Habit of *Cajanus cajan* (L.) Millsp.


Habit


Leaves


Inflorescence

Figure 5 Habit of *Talinum triangulare* (Jacq.) Willd.


Habit

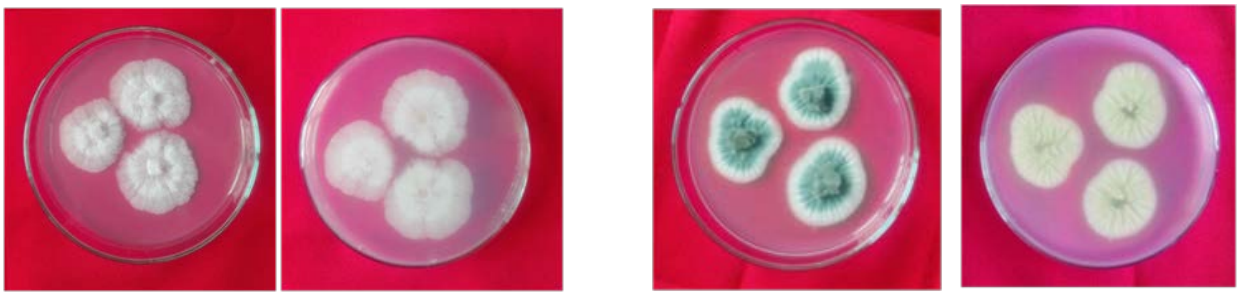

Leaves


Inflorescence

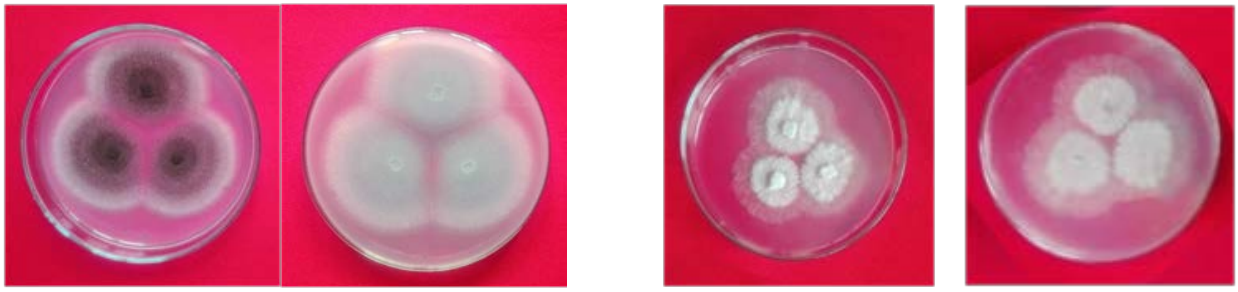
Figure 6 Habit of *Sauropus androgynus* (L.) Merr.

Habit Leaves Inflorescence

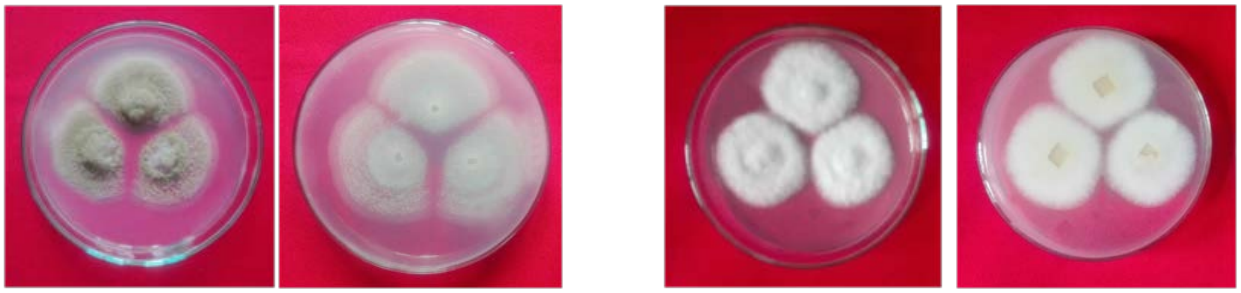
Figure 7 Habit of *Jatropha podagrica* Hook.



Front view TFO-01 Reverse view Front view TFO-02 Reverse view



Front view TFO-03 Reverse view Front view TFO-04 Reverse view



Front view TFO-05 Reverse view Front view TFO-06 Reverse view

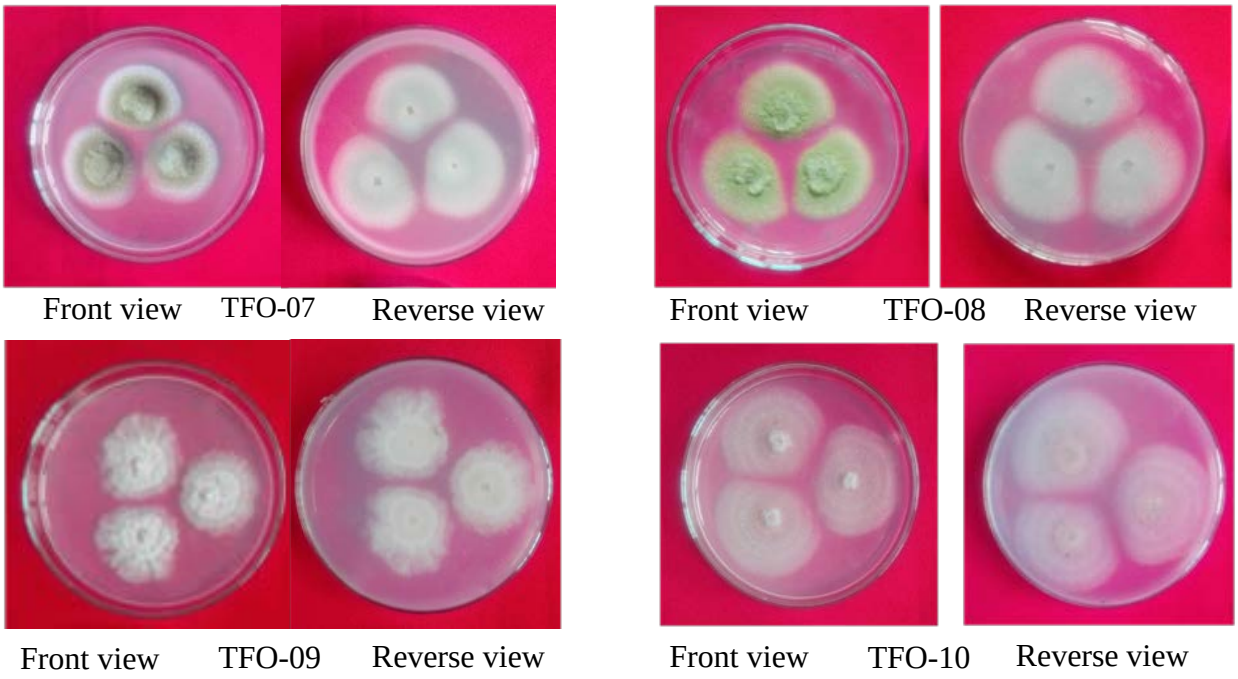
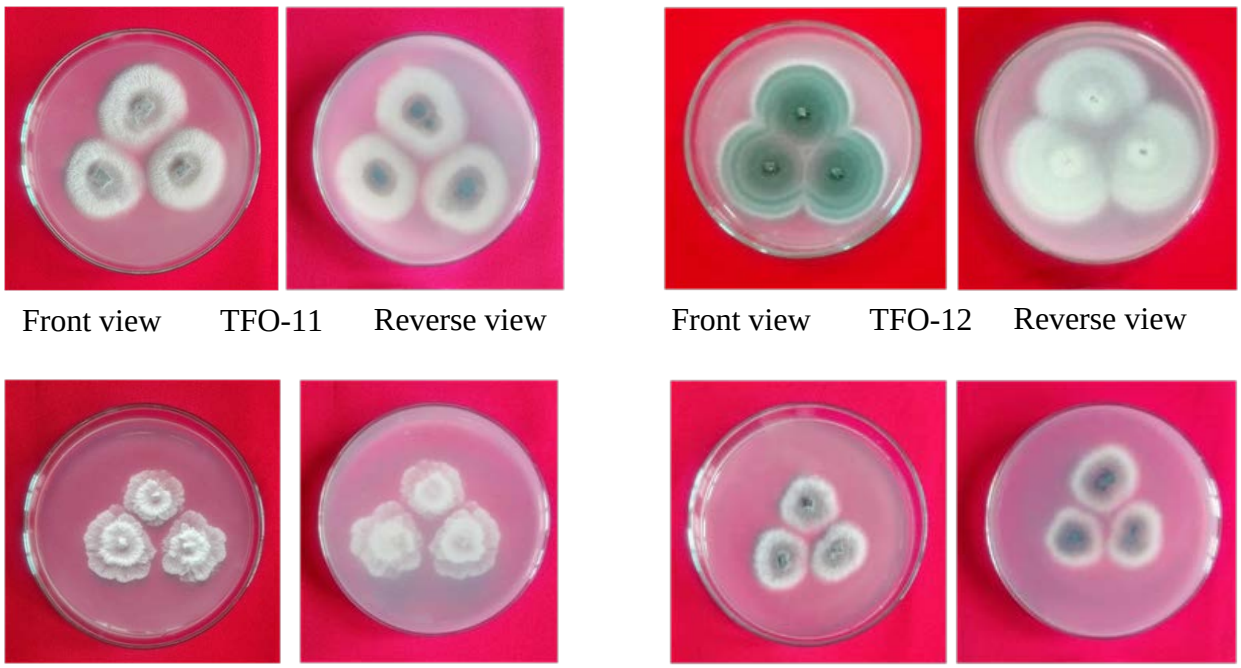
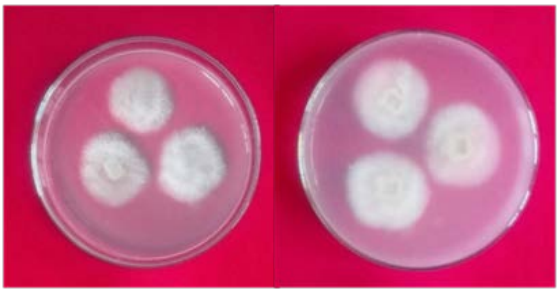


Figure 8 Morphology of fungi TFO-01 to TFO-10 (7 days old culture on PGA medium)

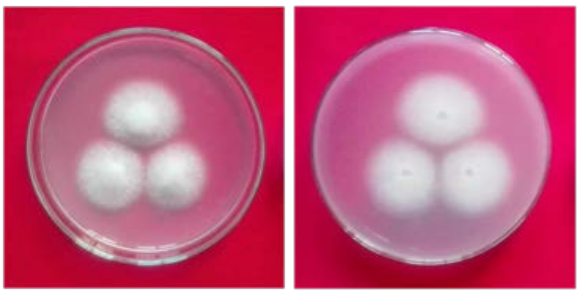




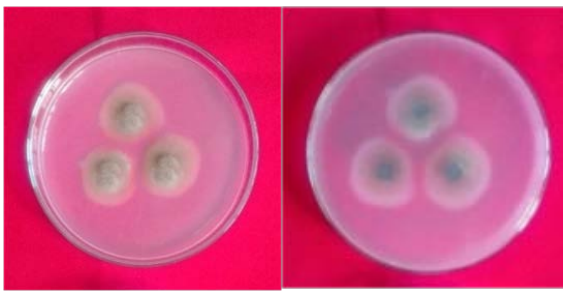
Front view TFO-15 Reverse view

Front view TFO-16 Reverse view

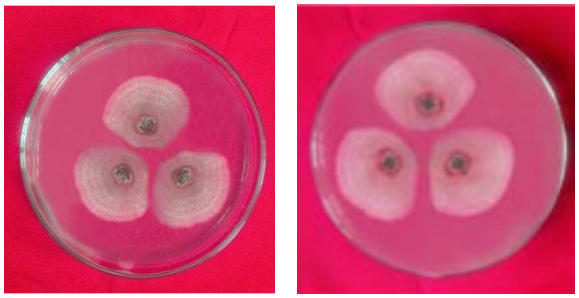
Front view TFO-17 Reverse view



Front view TFO-18 Reverse view



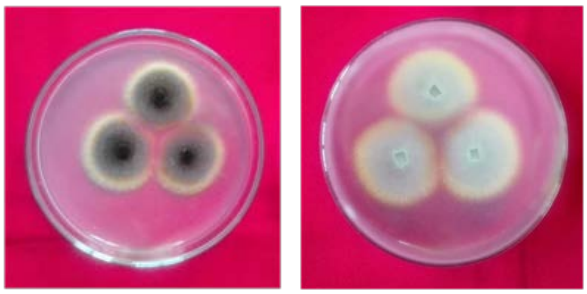
Front view TFO-19 Reverse view



Front view TFO-20 Reverse view

Figure 9 Morphology of fungi TFO-11 to TFO-20 (7 days old culture on PGA medium)

Front view TFO-11 Reverse view



Front view TFO-12 Reverse view

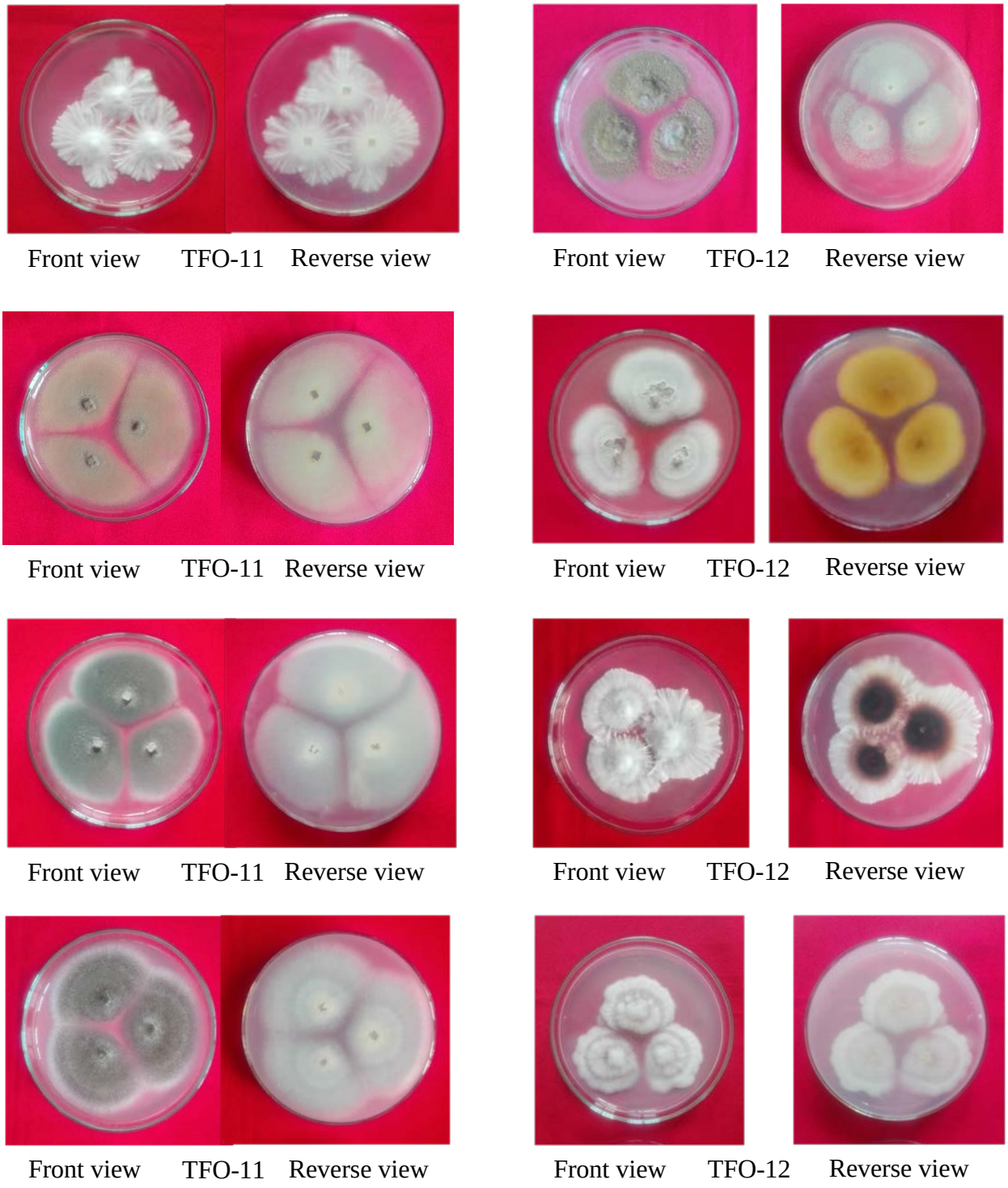


Figure 10 Morphology of fungi TFO-21 to TFO-30 (7 days old culture on PGA medium)

Table 3 Antimicrobial activities of isolated fungi (At 7 days fermentation)

Isolated fungi	Test Organisms and Inhibitory Zone (mm)					
	1	**2**	**3**	**4**	**5**	**6**
TFO-01	17.60	-	16.94	13.40	-	
TFO-02	18.16	13.06	17.14	-	-	-
TFO-03	17.48	20.88	14.17	-	-	-
TFO-04	16.42	-	16.46	-	-	-
TFO-05	14.03	13.35	16.33	-	-	-
TFO-06	20.65	11.09	16.46	-	-	-
TFO-07	15.05	-	15.67	12.36	-	19.38
TFO-08	22.86	12.75	17.14	-	15.25	20.25
TFO-09	23.65	15.75	14.17	-	22.06	23.73
TFO-10	24.64	-	16.46	-	-	21.00
TFO-11	24.63	-	16.33	-	12.00	20.40
TFO-12	21.82	12.05	15.31	-	15.12	21.15
TFO-13	13.36	-	-	-	-	-
TFO-14	20.63	21.32	-	-	18.15	-26.40
TFO-15	24.35	22.85	-	-	26.14	26.40

Table 4 Antimicrobial activities of isolated fungi (At 7 days fermentation)

Isolated fungi	Test Organisms and Inhibitory Zone (mm)					
	1	**2**	**3**	**4**	**5**	**6**
TFO-16	20.57	26.15	-	-	20.00	-
TFO-17	19.86	21.45	-	13.26	16.35	-
TFO-18	17.34	20.92	18.03	-20.08	15.40	-
TFO-19	15.75	15.05	-	-	14.35	-
TFO-20	16.30	-	19.36	-	15.00	-
TFO-21	20.36	-	18.37	-	13.65	-
TFO-22	20.18	-	17.03	12.85	13.00	-
TFO-23	27.65	-	18.63	-	15.15	-
TFO-24	18.07	-	18.03	-	16.52	-
TFO-25	12.07	23.54	17.16	14.50	-	-
TFO-26	-	-	16.82	24.00	-	-
TFO-27	-	-	19.46	27.30	-	-
TFO-28	-	-	19.70	24.50	-	20.05
TFO-29	-	-	19.45	13.00	-	-
TFO-30	10.00	-	18.35	25.05	-	-

Table 5 Higher Antimicrobial activities of isolated fungiTFO-15 and TFO-16

Ser. No	**Isolated fungi**	***S. cerevisiae***	***E. coli***	***S. typhimurium***	***C. albicans***
1.	TFO-15	26.14 mm	24.35 mm	26.40 mm	
2.	TFO-16	-	-	-	26.15 mm

1 = *Escherichia coli* 2 = *Candida albicans*

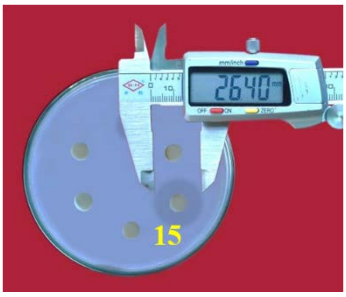
3 = *Micrococcus luteus* 4 = *Pseudomonas fluorescens*

5 = *Saccharomyces cerevisiae* 6 = *Salmonella typhimurium*

(Paper disc = 8 mm)

Against
Saccharomyces cerevisiae

Against
E. coli



Against
Salmonella typhimurium

Figure 11 Antimicrobial activities of fungus TFO-15

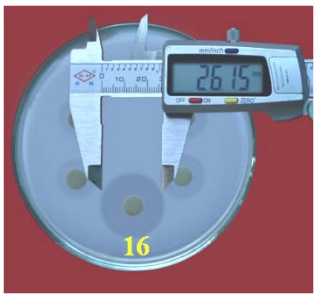


Figure 12 Antifungal activities of fungus TFO-16 on
C. albicans

Discussion and Conclusion

In the isolation of fungi, six plants were collected at Pathein Township. Endophytic fungi were isolated by the method of Surface sterilization Method (NITE, 2004). In this study thirty endophytic fungi were isolated from six different plants.

Five fungi were isolated from *Mangifera indica*, four fungi were isolated from *Averrhoa carambola*, five fungi were isolated from *Cajanus cajan*, five fungi were isolated from *Talinum triangulare* (Jacq.) Willd. Six fungi were isolated from *Sauropus androgynus*, five fungi were isolated from *Jatropha podagrica*.

In the study of antimicrobial activities, it was found that fungus TFO-16 exhibited the highest activity against *Candida albicans* and TFO-15 against *Saccharomyces cerevisiae*. TFO-15 also showed antibacterial activity against *Salmonella typhimurium* and *E. coli.*

Therefore, fungus TFO-15 was selected for further investigations such as identification, fermentation and purification of antibacterial metabolite.

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