

A Study of Endophytic Fungi from *Dolichandrone Spathacea* (L.F) K.Schum, and their Antibacterial Activities

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Abstract

Eight kinds of endophytic fungi were isolated from the young stems and fresh leaves of *Dolichandrone spathacea* belonging to the family Bignoniaceae. In this study, one strain showed the activity against agricultural pathogenic microorganisms. Since F-02 show more highly antibacterial activity than the others, this fungus F-02 was selected for further studies. The fungus F-02 gave the highest antibacterial activity on *Pseudomonas fluorescens*.

Keywords: Endophytic fungi

Introduction

Microorganisms have significant functions in ecosystems and are found in all kinds of habits. It is very hard to find substrata not isolated from any microbes in nature. Therefore, any substrata collected in nature are useful materials for isolating microorganisms. The typical materials are soil, living and fallen leaves, leaf litters, dung, insect, fresh water, marine water, and so on (Ando *et al.*, 2002).

Endophytes are microorganisms that are present in living tissue of various plants (root, fruit, stem, seed, leaf etc) establishing mutual relationship without apparently any symptom of diseases (Sandhu *et al.*, 2014). Endophytic fungi are a good source of antibiotics. Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful disease causing agents but not limited to phytopathogens, as well as bacteria, fungi, viruses and protozoan that affect humans and animals. It is important to explore endophytic mycoflora in the medicinal plants (Zhang *et al.*, 2006).

Many of endophytic fungi also produce antibiotic substances, which are medicinal substances to be used for treating infections caused by microbes. Microorganisms are the most potential sources for producing of natural therapeutic agents (Pannapa, 2017).

Material and Method

Collection of plant samples

The plant samples were collected from Monywa University Campus. The collected plants were identified according to outstanding characters shown in the literatures of Hooker (1885), Hundley and Chit Ko Ko (1987).

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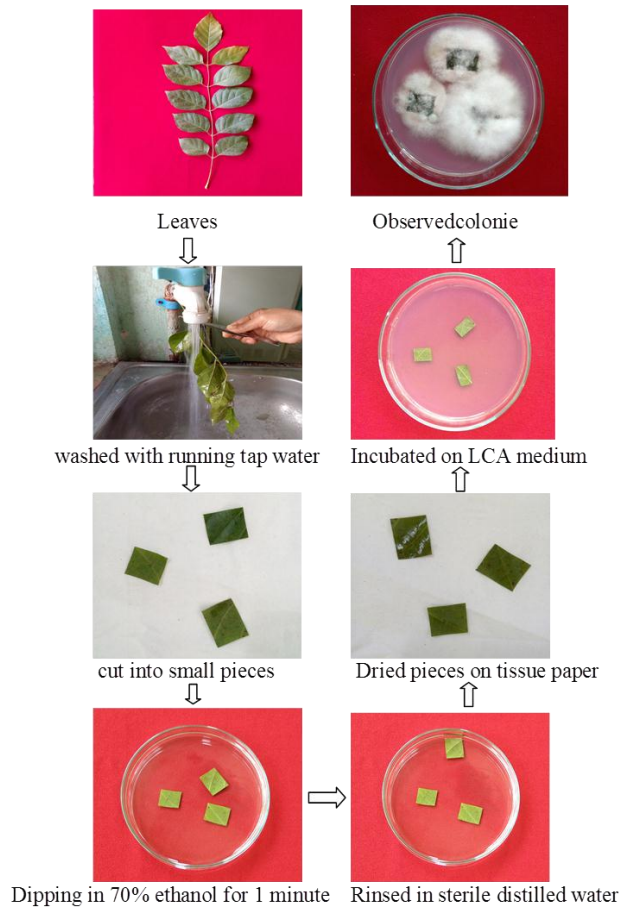


Figure.1.Surface sterilization method

Medium used for the fungi.

LCA medium

(Low Carbon Agar Medium)

| | |
|--------------------------------------|--------|
| Glucose | 0.2 g |
| Sucrose | 0.2 g |
| K ₂ HPO ₄ | 0.1 g |
| Mg SO ₄ 7H ₂ O | 0.05 g |
| KNO ₃ | 0.1 g |
| KCL | 0.05 g |
| Agar | 1.8 g |
| DW | 100 mL |
| pH | 6.5 |

PGA medium

(Potato Glucose Agar medium)

| | |
|-----------------|--------|
| PGA | 3.9 g |
| Distilled water | 100 mL |
| pH | 6.5 |

WGA medium (Water

Glucose Agar Medium)

| | |
|-----------------|--------|
| Glucose | 0.2g |
| Agar | 1.8g |
| Distilled water | 100 mL |

(After autoclaving chloramphenicol 0.8 g was added to the medium)

Preliminary study on Antibacterial activity of isolated fungi by Paper Disc Diffusion Assay (Tomita, 1988).

The isolated endophytic fungi were grown at 25°C for 7 days on PGA medium. These fungi were inoculated on seed medium and incubated 25°C for 3 days. Ten mL of seed culture was transferred into the fermentation medium (40 mL) and incubated at 25°C for 3 days. The fermented broth (20µL) was used to check the antimicrobial activity against test organisms by paper disc diffusion assay. Paper discs having eight millimeter diameter (Adavantec, Toyo Roshi Kaisha Co; Ltd; Japan) were utilized for antimicrobial assays.

The assay medium (Glucose 1%, Polypeptone 0.3%, Agar 1.8%, Distilled water 100 mL, pH 6.5 – 7.0) was used for the antimicrobial activity test. One percent (1.5×10^8 / mL of spore suspension) test organism was added to assay medium, then poured into plates. After solidification, paper discs impregnated with samples (fermented broth) were applied on the agar plates and the plates were incubated for 24 – 36 hours at 28° to 30°C. Clear zones (inhibitory zones) surrounding, the test disc indicated the present of bioactive metabolites which inhibit the growth of test organisms.

Seed Medium NITE (2004) Fermentation Medium NITE (2004)

| | | | |
|-------------------------------------|---------|-------------------------------------|--------|
| Glucose | 1.5 g | Glucose | 2.0g |
| Yeast Extract | 0.8 g | Yeast Extract | 0.8g |
| Polypeptone | 0.4 g | Polypeptone | 0.6g |
| K ₂ HPO ₄ | 0.001 g | MgSO ₄ 7H ₂ O | 0.001g |
| MgSO ₄ 7H ₂ O | 0.001 g | K ₂ HPO ₄ | 0.001g |
| Distilled Water | 100 mL | CaCO ₃ | 0.1g |
| pH | 6.5 | Distill Water | 100 mL |
| | | pH | 6.5 |

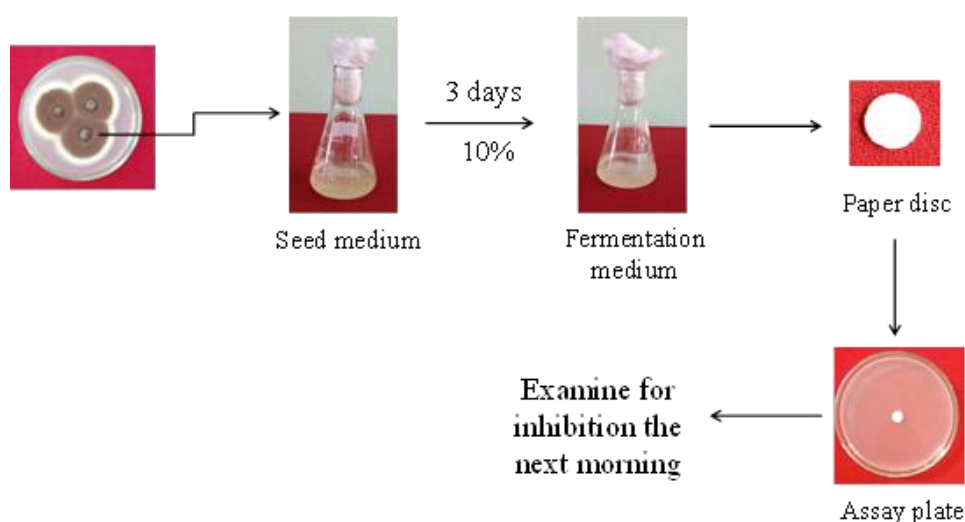


Figure.2. Procedure for preliminary study of antimicrobial activity test.

Results

| | |
|----------------|---|
| Botanical Name | - <i>Dolichandrone spathacea</i> (L.f) K. Schum; Fl. Kais. Wilh. Lad. 123. 1889. - <i>Bignonia spathacea</i> L.f. |
| Myanmar Name | - Hingut; Thakut |
| English Name | - Mangrove trumpet tree |
| Family | - Bignoniaceae. |

Outstanding Characters

It is a perennial tree, up to 20.0 m high; often branching from near the base. Leaves are odd-pinnate compound, imparipinnate, opposite; exstipulate; petiole cylindrical, channelled above; leaflet 9-15, subopposite, ovate-lanceolated, acute to oblique at the base, entire along the margin, acuminate at the apex, glabrous on both surfaces. Inflorescences are axillary cyme, 2-8 flowered, peduncle stout. Flower bisexual, actinomorphic, hypogynous, white, fragrant. Calyx is spathaceous, glabrous, hooked at tip. Corolla is 5-lobed, white, cylindrical portion of tube, lobes rounded, much crisped and crenate on margins. Stamens are 4, didynamous, equally inserted at base of swollen portion of tube; anther dithecous, basifixed, oblong, longitudinal dehiscing. Ovary is superior, linear oblong, bilocular with one ovule in each locule on the marginal placenta; style slender, stigma simple. Capsules are of variable length, value thin, semiwoody, bluntly pointed, smooth or obscurely ribbed. Seeds are corky-winged, oblong.



Habit



Inflorescence

Figure.3. *Dolichandrone spathacea* (L.f) K. Schum

Isolation of Endophytic fungi

A total of 8 fungal endohyptes were isolated from the leaves and stems of plant *Dolichandrone spathacea* (Thakut) according to their colony colours (Table 1 and Figure 4).

Table.1. Isolated endophytic fungi from *Dolichandrone spathacea*

| No | Part used | Numbers | Isolated Endophytic Fungi |
|----|-----------|---------|---------------------------|
| 1 | Stem | 5 | F-01, 02, 03, 04, 05 |
| 2 | Leaves | 3 | F-06, 07, 08 |



F-01 (Front view)

F-01 (Reverse view)



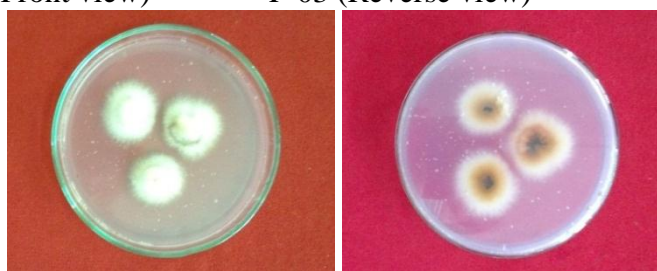
F-02 (Front view)

F-02 (Reverse view)



F-03 (Front view)

F-03 (Reverse view)



F-04 (Front view)

F-04 (Reverse view)



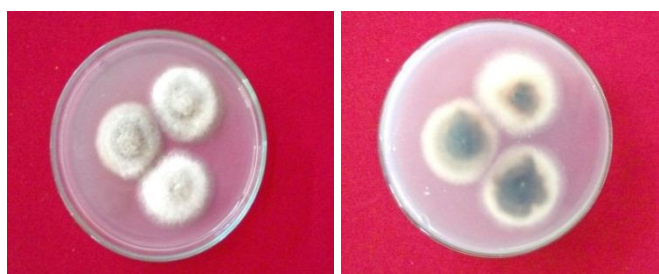
F-05 (Front view)

F-05 (Reverse view)



F-06 (Front view)

F-06 (Reverse view)



F-07 (Front view)

F-07 (Reverse view)



F-08 (Front view)

F-08 (Reverse view)

Figure.4.Morphological characters of Fungi F-01 to F-08

Antimicrobial Activity of isolated fungi

Table 2. Antimicrobial activities of isolated endophytic fungi
(At 5 days fermentation)

| Isolated Fungi | <i>Pseudomonas fluorescens</i> |
|----------------|--------------------------------|
| TF-01 | No activity |
| TF-02 | 22.77 mm |
| TF-03 | No activity |
| TF-04 | No activity |
| TF-05 | No activity |
| TF-06 | No activity |
| TF-07 | No activity |
| TF-08 | No activity |

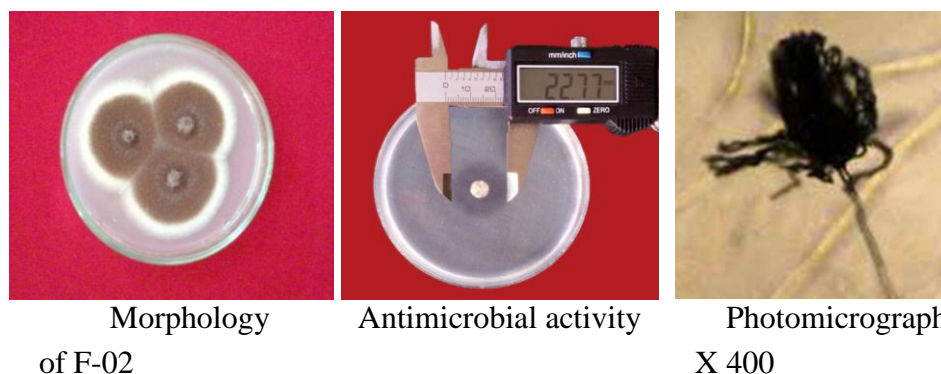


Figure 5. Morphology and Photomicrograph of fungus F-02 on *Pseudomonas fluorescens*

Discussion and Conclusion

In this study, endophytic fungi were isolated from *Dolichandrone spathacea* belonging to family Bignoniaceae by surface sterilization method. A total of eight endophytic fungi were isolated from the leaves and young stems. Five fungi were isolated from the young stems of plant sample and three fungi were isolated from fresh leaves. Strain F-02 was selected for studies because it showed more selective antibacterial activity against *Pseudomonas fluorescens*.

According to Barnett (1956), the conidiophores which were upright, simple, terminating in clavate swelling, conidia 1-celled, globose, radiating from the entire surface were observed in F-02. It was identified as *Aspergillus* spp.

It is hoped that this study will provide high potential to discover useful antibiotic producing endophytic fungi isolated from the young stems of high potential for the production of antibacterial drug especially in the treatment of fever, chills, contusion, nausea and vomiting, and rapid heat rate.

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References

- Ando, K., S. Inaba. 2002. **Washing methods for the isolation of endophytes.**
- Barnett, H. L. 1956. **Illustrated genera of imperfect fungi.** United States of America.
- Hooker, J.D. 1885. **Flora of British India Vol. 3.** L. Reeved Co. Ltd. London.
- Hundley, H.G and Chit Ko Ko. 1987. **List of Trees, shrubs, Herbs and principal Climbers etc.** 4th ed. Printing and stationary, Burma.
- NITE (National Institute of Technology and Evaluation) 2004. **Media for fermentation to produce the metabolite.**
- Pannapa P, Pattra S. 2017. **Antimicrobial and enzyme activity produced by Bacillus.** Sp. Isolated from soil. Int J Pharm Sci 9 (S): 205-10.
- Sandhu SS, Suneel K, Aharwal RP. 2014. **Endophytic Fungi: As A Source of Antimicrobials Bioactive Compounds.** World J Phar Pharmac Sc: 3 (2): 1179-1197.
- Tomita, S., 1988. **Microbial growth kinetics and secondary metabolites.** J. fermentation Technology, 46: 134-140.
- Zhang HW, Song YC and Tan RX. 2006. **Biology and chemistry of endophytes.** Nat Pro Rep; 23: 753.