

Study on the Morphological Characters and Isolation of Endophytic Fungi from Five Medicinal Plants

Khaing Thazin¹, Khin Thida Swe², Khin Min Min Phyo³

Abstract

In the isolation of the endophytic fungi, the leaf samples of five medicinal plants were collected from Patheingyi Township. A total of 19 endophytic fungi were isolated from five selected medicinal plants by surface sterilization method. They were 7 endophytic fungi from *Annona muricata* L. (du-yin-awza) belonging to Annonaceae, 4 endophytic fungi from *Annona squamosa* L.(aw-za) belonging to Annonaceae, 3 endophytic fungi from *Solanum torvum* Sw. (khaing-kazawt) belonging to Solanaceae, 2 endophytic fungi from *Morinda citrifolia* L.(ye-yo) belonging to Rubiaceae, 3 endophytic fungi from *Polyalthia longifolia* Benth.& Hook.(thinbaw-te) belonging to Annonaceae. Morphological characters of isolated endophytic microorganisms were investigated.

Keywords: *Annona muricata* L., *Annona squamosa* L., *Solanum torvum* Sw., *Morinda citrifolia* L. and *Polyalthia longifolia* Benth.& Hook., Endophytic Fungi

Introduction

Medicinal plants refer to plants used in medicine to prevent and treat diseases. All or part of medicinal plants are used in medicine and will also be used as raw materials for the pharmaceutical industry, which have a wide range of medicinal and economic uses. In recent years, researchers have gradually realized that endophytes can play an important role in affecting the quality and yield of medicinal plants through special microbe-plant interactions.

Endophytes provide more resources of new bioactive metabolites, especially alkaloids, saponins, quinones, flavonoids, terpenoids, etc., which have a lot of biological activities and have also become research hotspots in the composition and production of natural drugs. Endophytes in medicinal plants mainly include endophytic fungi, endophytic bacteria, and endophytic actinomycetes, and they are rich in species diversity. It is found that the biological functions of these endophytes have a great influence on medicinal plants, so obtaining more microbial resources, especially those with biological activity, can greatly promote the development of the medicinal plant industry (Wang *et al*, 2023).

Endophytes are the associations with diverse group of organisms throughout the plant kingdom which provide indirect defense for plants. Among the most important group of eukaryotic organisms which is fungi and very well known for producing various novel metabolites that are directly used as a drugs or functions for various bioactive products. Endophytic represent a wide diversity of microbial adaptation that have successfully evolved in environmental changes, thus it is a valuable of study especially in the field of medical, pharmacology industry and agriculture. Medicinal plants are known to nurture endophytic fungi and related with the pharmaceutical industry (Hasan *et al.*, 2019). The aim and objectives of this study are to isolate the endophytic fungi from five medicinal plants and to investigate the morphological characters of isolated endophytic fungi.

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Materials and Methods

Sample Collection

The leaves of five medicinal plants were collected from Nga Wun Kyun Thar Area, Patheingyi Township for the isolation of endophytic fungi. These medicinal plants are *Annona muricata* L. (du-yin-awza) belonging to Annonaceae, *Annona squamosa* L.(aw-za) belonging to Annonaceae, *Solanum torvum* Sw.(khayan-kazawt) belonging to Solanaceae, *Morinda citrifolia* L.(ye-yo) belonging to Rubiaceae, *Polyalthia longifolia* Benth.& Hook.(thinbaw-te) belonging to Annonaceae according to Kirtikar and Basu,1935; Ivan, 1999; Subrahmanyan,1996 and Kyaw Soe and Tin Myo Ngwe,2004.

Preparation of medium used for isolation, pure cultures and preservation of endophytic fungi

The composition of Potato glucose agar (PGA) medium was 20 g of potato, 2 g of Glucose, 2 g of Agar and 100 mL of Distilled water (Ando and Inava, 2004).

The decoction of potatoes and ingredients of PGA medium were mixed in a flask. The flask was plugged with non- absorbent cotton wool and autoclaved at 121°C for 15 minutes. After autoclaving, Chloramphenicol 250 mg/L was added for antibacterial activity. Then 20 mL of each medium was separately poured into plates. These plates were allowed to solidify and used for isolation and pure cultures. For the preservation of isolated fungi, sterilized medium was separately measured and poured into test tubes and made the agar slants.

Surface sterilization method for isolation of endophytic fungi was done by the steps. The leaves of plant sample were collected and washed on running water for 1 minute and 2 times. They were cut into about 1 cm pieces. The pieces of leaves were surface sterilized by immersing in a beaker containing 70% ethanol for 1 minute and 2 times. The pieces were then rinsed two times in sterile distilled water. The pieces were placed on sterilized filter paper and dried for 1 hour. Dried pieces were cut into smaller pieces and transferred into plates containing medium. The cultures were incubated at room temperature for 3-7days(Suto,1999).

After fungal colonies were appeared, the observed colonies were cultured separately in two mediums. Then subculture ring of isolated fungi were done 3-5 times with two mediums until the pure cultures were obtained. The subcultures were incubated at room temperature for 3 days. For the preservation of isolated fungi, 5 mL of medium was separately measured and poured into test tubes and made the agar slants. The pure strains are maintained as agar cultures in test tubes.

Results

Scientific Name	<i>Annona muricata</i> L.
Myanmar Name	duyin-awza
Family	Annonaceae

Distinguishing characters of *Annona muricata* L.

A small evergreen tree. Leaves leathery, ill-smelling, obovate- oblong to elliptic, glossy above and rusty beneath, glabrous, exstipulate. Inflorescence solitary cyme. Flowers ebracteate, ebracteolate, pedicellate, bisexual, actinomorphic, trimerous, hypogynous. Sepals 3, aposepalous, large, thick and fleshy, ovate-acute, valvate, pale yellow. Petals 6, 2-seriate, apopetalous, smaller and thinner, concave, rounded, imbricate, pale yellow. Stamens numerous, closely arranged, linear with the connective truncate, anther-cells not septate.

Carpels numerous, syncarpous, numerous locules, one ovule in each loculus, basal placentation. Fruit baccate.



Habit



Leaves

Figure 1. *Annona muricata* L.

Scientific Name - *Annona squamosa* L.

Vernacular Name- aw-za

Family - Annonaceae

Distinguishing characters of *Annona squamosa* L.

A small tree. Leaves alternate, simple, obovate, petiolate, exstipulate. Inflorescences solitary cymes. Flowers trimerous, hypogynous, sepals 3, aposepalous, ligulate, petals 6, 2-seriate, apopetalous, ligulate, pubescent, stamens numerous, spirally arranged, the filaments thick and short, the anthers ditheous, extrorse. Pistils numerous, apocarpous, each 1-carpelled, 1-loculed, the placentation parietal, the ovule 1 in the locule, the style oblong, stigma simple. Fruit an aggregate of berries, globose, fleshy.



Habit



Leaves

Figure 2. *Annona squamosa* L.

Scientific Name - *Solanum torvum* Sw.

Vernacular name- khay-an-kazawt

Family - Solanaceae

Distinguishing characters of *Solanum torvum* Sw.

Shrub, prickles scattered. Leaves simple, alternate, the base oblique, the margin sinuate, the tip acute, sparsely stellate-pubescent above, exstipulate. Inflorescences axillary, corymbose cyme. Flowers obliquely zygomorphic, bisexual, hypogynous. Sepals 5, cupular, lobes pubescent. Petals 5, white, sub-rotate, plicate. Stamens 5, epipetalous, anthers exerted, dehiscing by apical pores. Ovary bilocular, oblique, axile placentation. Fruit berry.



Habit



Leaves

Figure 3. *Solanum torvum* Sw.

Scientific name - *Morinda citrifolia* L.

Vernacular name-ye-yo

Family - Rubiaceae

Distinguishing characters of *Morinda citrifolia* L.

A small tree. Leaves opposite, simple, elliptic to obovate, the base acute to cuneate, the margins entire, undulate, the tips acute, glabrous. Inflorescences axillary, dichasial cymes, condensed and aggregate into heads. Flowers 5-merous, epigynous, sepals 5, synsepalous, the tube adnate to the ovary, basally adnate to the other tubes. Petals 5, synpetalous, white. Stamens 5, epipetalous, pistil 1, 2-carpelled, syncarpous, 2-loculed, axile-basal placentation, one ovule in each locule, style slender, stigma 2-fid. Fruits berries.



Habit



Leaves

Figure 4. *Morinda citrifolia* L.

Scientific Name - *Polyalthia longifolia* Benth.& Hook.

Vernacular Name - thinbaw-te

Family - Annonaceae

Distinguishing characters of *Polyalthia longifolia* Benth. & Hook.

Trees. Leaves simple, spirally arranged, the base obtuse, the margin undulate, the tip acute, petiolate, exstipulate. Inflorescences cymose clusters. Flowers bracteates, ebracteolate, trimerous, hypogynous, greenish yellow, perianth 3 whorls of 3 segment each, polyphyllous, inner whorl larger, valvate, stamens numerous, polyandrous, spirally arranged on convex torous, anther ditheous, extrorse, carpels numerous, apocarpous, unilocular, basal placentation, style terminal, stigma simple. Fruits etaerio of free berries.



Habit



Leaves

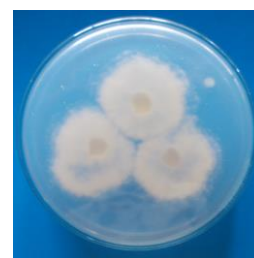
Figure 5. *Polyalthia longifolia* Benth. & Hook.

Table 1. List of endophytic fungi isolated from leaves of *Annona muricata* L.

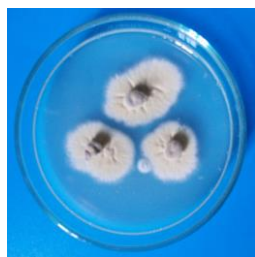
No.	Isolated fungi	Colony colors	
		Surface view	Reverse view
1	KTZ-01	Grey	Black
2	KTZ-02	White, fluffy	White
3	KTZ-03	Yellow, compact	Yellow
4	KTZ-04	Pinkish purple	Yellow
5	KTZ-05	Cream, folded	Centre yellow, white periphery
6	KTZ-06	White cottony	Orange
7	KTZ-07	White, compact	Pale yellow, folded



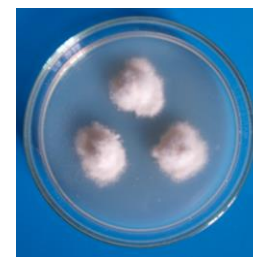
Surface view **KTZ-1** Reverse view



Surface view **KTZ-2** Reverse view



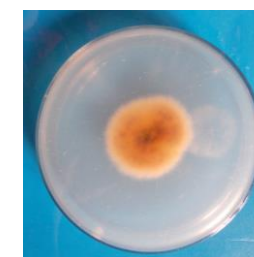
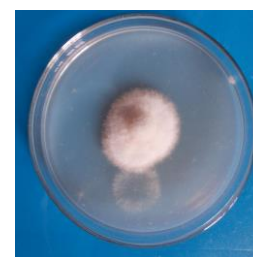
Surface view **KTZ-3** Reverse view



Surface view **KTZ-4** Reverse view



Surface view **KTZ-5** Reverse view



Reverse view **KTZ-6** Surface view

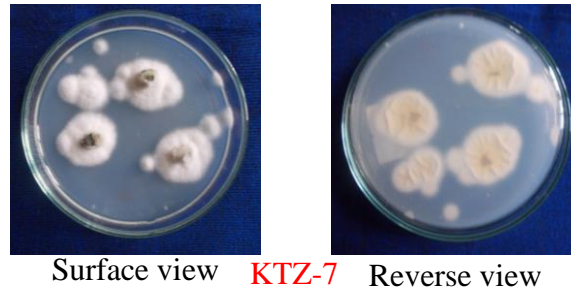


Figure 6. The endophytic fungi (KTZ-1 to KTZ-7) isolated from leaves of *Annona muricata* L.

Table 2. List of endophytic fungi isolated from the leaves of *Annona squamosa* L.

No.	Isolated fungi	Colony colors	
		Surface view	Reverse view
1	KTZ-08	Cream, spread	Cream
2	KTZ-09	Centre white, light periphery	Cream
3	KTZ-10	White, woolly	Pale yellow
4	KTZ-11	White, smooth, raised	White

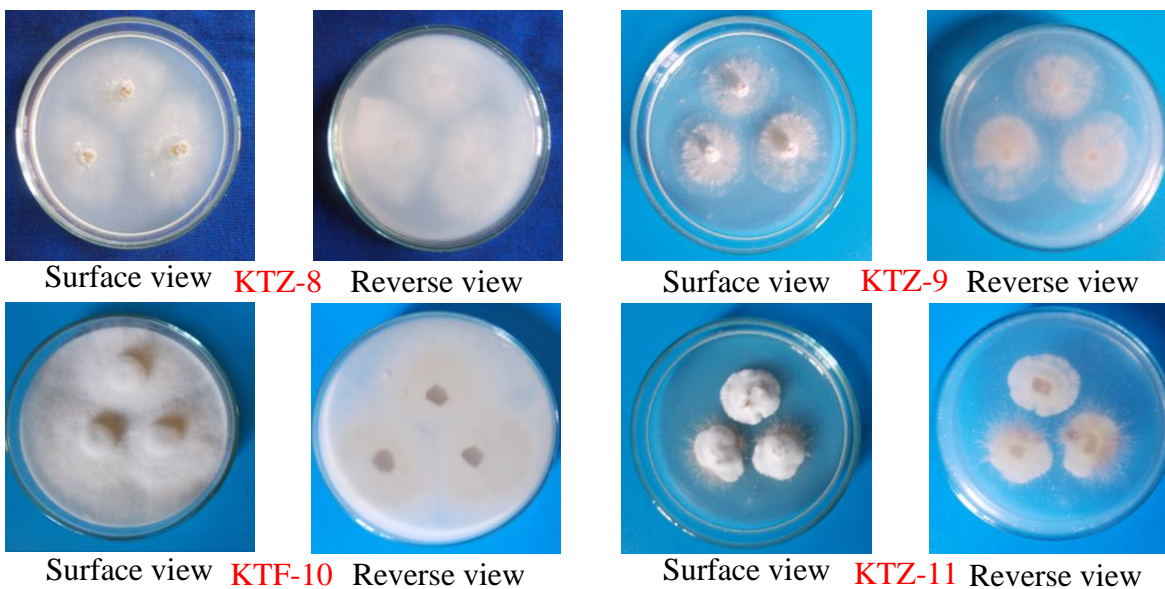


Figure 7. The endophytic fungi(KTZ-8 to KTZ-11) isolated from leaves of *Annona squamosa* L.

Table 3. List of endophytic fungi isolated from leaves of *Solanum torvum* Sw.

No.	Isolated fungi	Colony colors	
		Surface view	Reverse view
1	KTZ-12	Centre light, white periphery	Cream
2	KTZ-13	White, cottony	White
3	KTZ-14	Purple, felt like	Pale purple

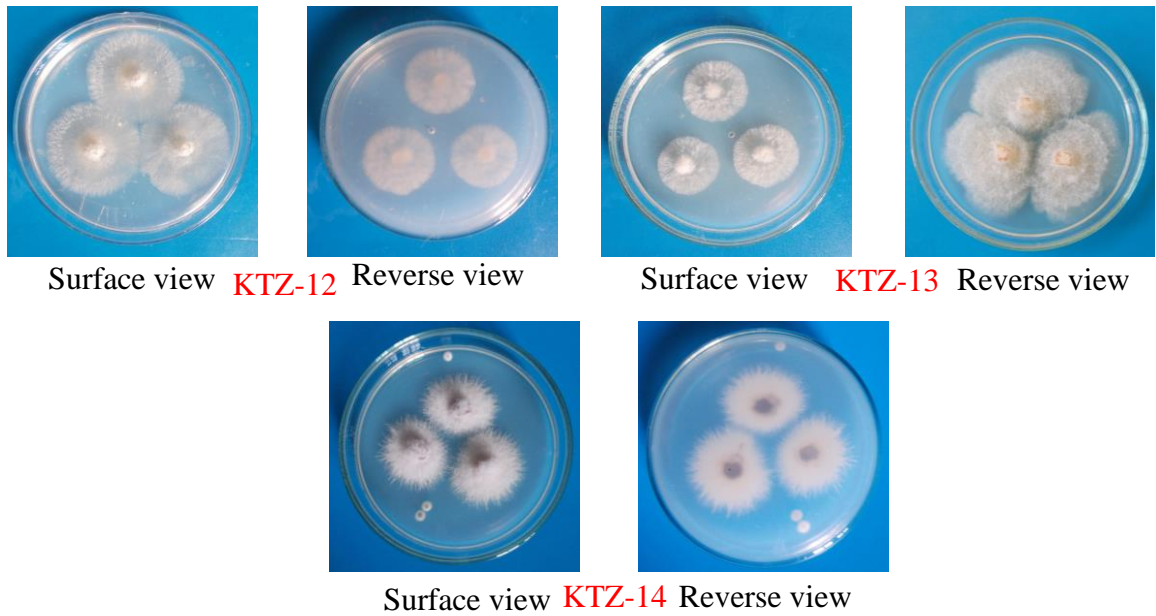


Figure 8. The endophytic fungi (KTZ-12 to KTZ-14) isolated from leaves of *Solanum torvum* Sw.

Table. 4. List of endophytic fungi isolated from leaves of *Morinda citrifolia* L.

No.	Isolated fungi	Colony colors	
		Surface view	Reverse view
1	KTZ-15	Grey	Grey
2	KTZ-16	Centre green, white periphery	Cream

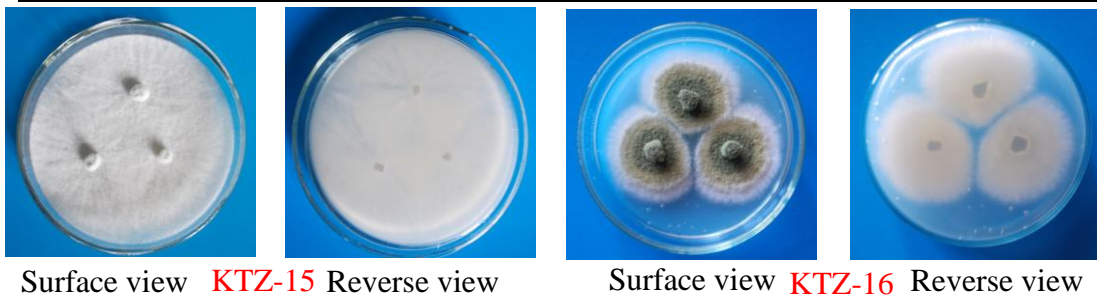


Figure 9. The endophytic fungi (KTZ-15 to KTZ-16) isolated from leaves of *Morinda citrifolia* L.

Table 5. List of endophytic fungi isolated from leaves of *Poltalthia longifolia* Benth & Hook.

No.	Isolated fungi	Colony colors	
		Surface view	Reverse view
1	KTZ-17	Centre black, periphery white	Cream
2	KTZ-18	Centre white, periphery yellow	Bright yellow
3	KTZ-19	Pink, compact, raised	Cream

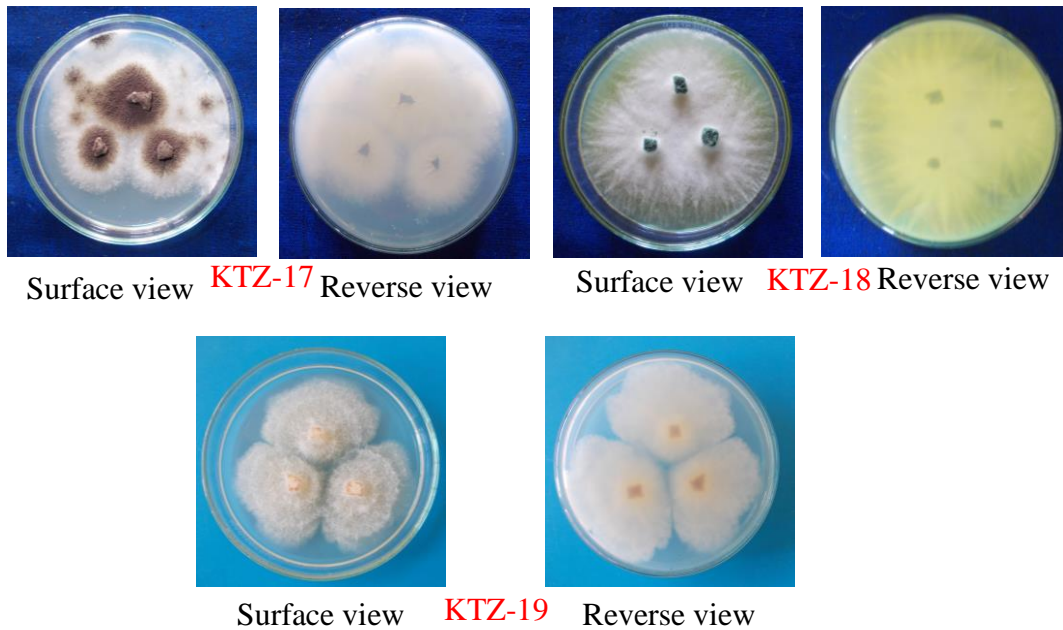


Figure 10. The endophytic fungi (KTZ-17 to KTZ-19) isolated from leaves of *Poltalthia longifolia* Benth & Hook.

Discussion and Conclusion

Endophytes live in medicinal plants and have the functions of promoting the growth of host plants, enhancing the stress resistance of host plants, and regulating the synthesis of secondary metabolites and being able to metabolize medicinal compounds. The secondary metabolites of endophytes from most medicinal plants have medicinal activities, which have great potential in the development of new drugs (Wang *et al.*,2023).

The nine isolates of endophytic bacteria and 3 isolates of endophytic fungi obtained from soursop leaves (Aisyah *et al.*, 2023).Seven endophytic fungi were isolated from *Annona muricata* L. (duyin-awza) belonging to Annonaceae in this study.

The black and white endophytic fungi have been successfully isolated and purified from the leaves of the mature annona leaves (*Annona squamosa* L.) (Ola *et al.*, 2019). In the present study, four endophytic fungi were isolated from *Annona squamosa* L. (awza) belonging to Annonaceae.

Eight fungal endophytes were isolated from the leaves and unripe fruits of *Solanum mauritianum* (Pelo *et al.*, 2020). In this study, three endophytic fungi were isolated from *Solanum torvum* Sw.(khayan-kazawt) belonging to Solanaceae.

Six isolated endophytic bacteria were selected from *Morinda citrifolia* L. based on morphological characteristics while namely ACP1, ACP2, ACP3, ACP4, ACP5, and ACP6 (Sogandi *et al.*, 2019). Two endophytic fungi were isolated from *Morinda citrifolia* L.(ye-yo) belonging to Rubiaceae in the present study.

The endophyte *Alternaria* sp. (MT995125) was isolated from the leaves of *Polyalthia longifolia* (Sabry *et al.*,2020). Three endophytic fungi were isolated from *Polyalthia longifolia* Benth.& Hook.(thinbaw-te) belonging to Annonaceae in this research.

A total of nineteen fungi were isolated according to their morphological characters and colony colours. These pure cultures of isolated fungi were named temporarily as KTZ-01 to KTZ-19. Fungi KTZ- 01 to KTZ- 07 are isolated from *Annona muricata* L., KTZ- 08 to KTZ-

11 from *Annona squamosa* L., KTZ 12 to KTZ-14 from *Solanum torvum* Sw., KTZ -15 to KTZ-16 from *Morinda citrifolia* L. and KTZ -17 to KTZ-19 from *Polyalthia longifolia* Benth & Hook.. The morphological characters and colony colors of isolated fungi were studied and stored in the slant culture for further investigations.

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