

ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC FUNGI FROM *GLOBBA ARRACANENSIS* KURZ.

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Abstract

Glabba is a genus of plants in the ginger family and contains about 100 species. In this research work, the plant samples (leaves and rhizomes) of *Globba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State in September, 2023. *G. arracanensis* Kurz. is only found in Rakhine State and it is now known not to be extinct. The morphological and microscopical characters of all fungal strains were carried out at Microbiology Laboratory, Department of Botany, Dagon University. The colony appearances of isolated fungal strains were irregular, circular and rhizoid. The colony margins of isolated strains were entire, filamentous, undulate and lobate. The elevations of isolated strains were raised, flat and pulvinate. The surface and reverse colors of isolated fungal strains were white, whitish pale yellow, whitish orange, light orange, whitish green, whitish yellow, whitish grey. The colony characters and microscopic characters of isolated fungi are very closed to *Penicillium*, *Rhizopus*, *Rhizoctonia*, *Cladosporium* and *Aspergillus* species. Strains Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13 were identified as *Penicillium* species, strains Eaindra-8 and Eaindra-11 as *Aspergillus* species, strain Eaindra-3 as *Cladosporium* species, strain Eaindra-10 as *Rhizopus* species and strains Eaindra-2 and Eaindra-14 as *Rhizoctonia* species. These isolated strains possessed good antimicrobial activity on some pathogenic organisms so that this activity will be presented in the next paper.

Keywords: Endophytes of *Globba arracanensis* Kurz.

Introduction

Globba arracanensis Kurz. belongs to the genus *Globba* in the family Zingiberaceae. *Globba* is the third largest genus of the Zingiberaceae with 100-110 species in the world (Williams *et al.*, 2017). *Globba arracanensis* Kurz. was found in north of Akyab (now called Sittwe) in the Kolodyne River Valley of Arracan (now called Rakhine) State in Myanmar by Kurz in 1869.

Endophytic fungi may promote the growth of their host plant by producing phytohormones or by increasing the plant's resistance to various stresses, and they can produce pesticides to protect plants from herbivores. There are many active and biologically active substances produced grouped into different categories due to the relationship between endophytes and their hosts (Hashem *et al.*, 2023).

Several antimicrobial compounds produced by endophytic fungi are important in their effectiveness against pathogens that have developed resistance to antibiotics. Endophytic fungi produce biologically active secondary metabolites, such as terpenes, alkaloids, monoterpenoids, peptides, and polyketides. Fungal endophytes are used to control a wide range of human health issues, such as the production of antibiotics, antifungal, antiviral, anticancer, lytic enzyme, and degradation of toxins (Hashem *et al.*, 2023).

The aim of present study is isolation, characterization and identification of endophytic fungi from *Globba arracanensis* Kurz.. The objectives are to study morphology of the selected plant, to isolate endophytic fungi from the collected plant, to investigate the morphological and microscopical characters of isolated fungi and to identify the possible genera of isolated fungi.

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Materials and methods

Morphological Study of Selected Plant

The specimens of *Globba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State during the flowering period (June to November). After collection, the plants were identified with the help of literatures Wu *et al.*, (Bordelon and John Kress (2009), Hundley and Chit Ko Ko (1987) and Kress *et al.*, (2003).

Isolation of Endophytic Fungal Strains from *Globba arracanensis* Kurz .

Endophytic fungal strains were isolated from the leaves and rhizomes of *Globba arracanensis* on four different media. Isolation of endophytic fungal strains were carried out by the following schemes: (Phay, 1997).

1. The plant samples were washed under running tap water for fifteen minutes.
2. The plant samples (leaves and rhizomes) were cut into about 1.5 cm pieces.
3. These pieces were sterilized by soaking in 75% ethanol for 2 min.
4. Then, pieces of rhizomes were sterilized by soaking in 5.3% sodium hypochloride for 1 minute.
5. These parts were dried on sterilized paper and then they were placed on appropriate medium.

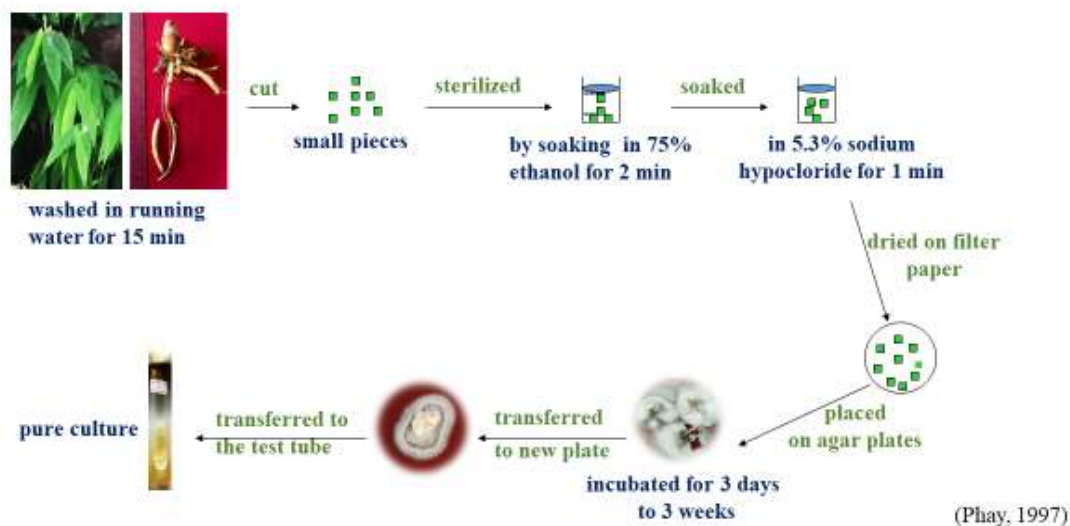


Figure.1 Isolation of endophytic fungal strain from plant

Composition of culture media (Atlas, 1993)

- | | |
|-------------------------------|---------------------------------------|
| (1) Nutrient Agar Medium (NA) | (2) Sucrose/Yeast Extract Medium (SY) |
| Nutrient Agar = 3.5 g | Sucrose = 1.0 g |
| Distilled Water = 100 ml | Yeast extract = 0.3 g |
| Agar = 1.0 | Distilled Water = 100 ml |
| pH = 7 | Agar = 2.5 g |
| | pH = 7 |

(3) Nutrient Agar Medium (NA)	(4) Sucrose/Yeast Extract Medium (SY)
Glucose = 1.0 g	Lactose = 1.0 g
Yeast extract = 0.3 g	Yeast = 0.3 g
Distilled Water = 100 ml	Distilled Water = 100 ml
Agar = 2.5 g	Agar = 2.5 g
pH = 7	pH = 7

Morphological Characters of Isolated Fungi

Isolated endophytic fungal strains grown on slant culture were transferred into the plates containing four different media. Then, these plates were incubated at 30°C for 3-7 days. The morphological and colonial characters such as colony appearance, margin, surface and reverse colours and elevation of all isolated strains were recorded as revealed in the reference of Dubey and Maheswari (2014).

Microscopical Characters of Isolated Fungi

The microscopical characters of all isolated strains *Globba arracanensis* Kurz. (GA-1 to GA-14) were carried out under light microscope with high magnification at Department of Botany, Dagon University. The main characters of hyphae, mycelia, sporangiophores, spores, color formation on upper as well as lower surface were comparatively studied. These are compared to those of fungi with available literatures such as Barnett (1969), (1998).

Results

Morphological Study of Selected Plant

Scientific Name	: <i>Globba arracanensis</i> Kurz.
Family	: Zingiberaceae
Myanmar Name	: Waso-pan
Flowering and Fruiting Period	: June to November

The native range of this species is Bangladesh to North West of Myanmar. Plant are perennial herbs with leafy pseudostem and deciduous, stem about 40-80 cm long and curving upward, rhizome branched. Leaves simple, alternate, sheathing petiole; lamina ovate-elliptic, margin entire, base slightly rounded to acute, the tip highly acuminate form a thread like, about 25-45 x 5-10 cm. Inflorescence terminal on the leafy pseudostem about 10-18 cm long, about 4-9 flowers in each cincinnus. Flower bracteate, bracts are persistent, lalic color, bracteolate, bracteoles are also persistent and light lalic color, sessile, complete, bisexual, irregular, zygomorphic, trimerous, epigynous. Sepal (2+1), synsepalous, campanulate, apex obtusely 3-lobed, persistent. Petals (1+2), tube slender, lobe oblong, lalic color. Androceium 1+ 2st + 2st, fertile stamen 1, filament white, anther ditheous, dechiscance by longitudinal slit; 2 petal like lateral staminodes, and white to lalic white color, labellum reflexed, adnate to filament to form a slender tube above lateral staminodes and corolla tube; dark purple with yellow spots at the tip and center. Carpel (3), syncarpous, tricarpeal, many ovule in the locule, parietal placentation; style long, inserted in the fertile filament, stigma capitate, ovary inferior. Fruit capsule with persistent calyx.



Figure (2)a *Globba arracanensis* Kurz.

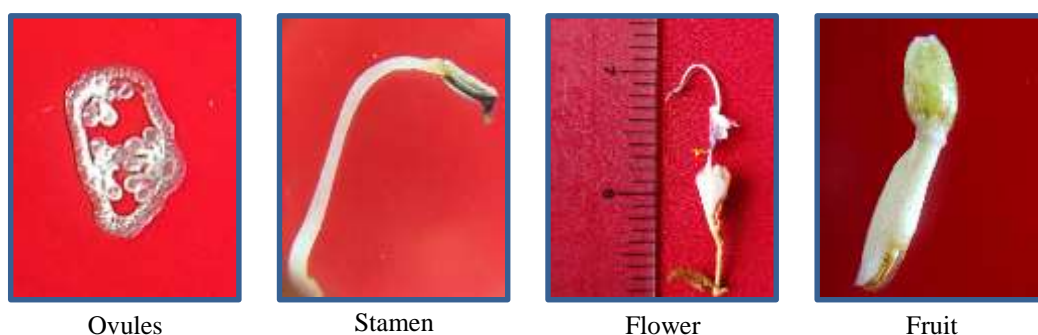


Figure (2)b *Globba arracanensis* Kurz.

Isolation of Endophytic Fungi

In the present works, fourteen isolated strains were maintained into the pure culture and designated as *Globba arracanensis* Kurz. Eaindra-1 to Eaindra-14.

Table (1) Isolation of fungi from leaves and rhizomes of *Globba arracanensis* Kurz.

Strain from leaves	Strain from rhizomes	Culture medium
Eaindra-1	Eaindra-8 and Eaindra-9	Nutrient Agar
Eaindra-2	Eaindra-10 to Eaindra-12	Sucrose/Yeast Extract Medium
Eaindra-3	Eaindra-13	Glucose/Yeast Extract Medium
Eaindra-4 to Eaindra-7	Eaindra-14	Lactose/Yeast Extract Medium

Morphological and Microscopical Characters of Isolated Fungi

The morphological characters of isolated fungal strains were carried out at Microbiology Lab, in the Department of Botany, Dagon University. The colony appearances of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-5, Eaindra-6, Eaindra-7, Eaindra-8, Eaindra-9, Eaindra-11, Eaindra-12 and Eaindra-13 were irregular, strains Eaindra-3, Eaindra-4 and Eaindra-10 were circular, strain Eaindra-14 was rhizoid.

The margin of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-3, Eaindra-6, Eaindra-7, Eaindra-10 and Eaindra-14 were filamentous, strains Eaindra-4, Eaindra-5 and Eaindra-13 were entire, strains Eaindra-8, Eaindra-9 and Eaindra-11 were lobate and strain Eaindra-12 was undulate.

The elevation of isolated fungal strains Eaindra-1, Eaindra-2, Eaindra-3, Eaindra-4, Eaindra-8, Eaindra-9, Eaindra-11, Eaindra-12 and Eaindra-13 were raised, strains Eaindra-5, Eaindra-6, Eaindra-7 and Eaindra-14 were flat and strain Eaindra-10 was pulvinate.

The surface and reverse colors of isolated fungal strains Eaindra-4, Eaindra-5, Eaindra-6, Eaindra-12 and Eaindra-13 were white and whitish pale yellow. Strains Eaindra-2 and Eaindra-7 were white and whitish orange and strain Eaindra-1 was white and light orange. The colors of strain Eaindra-10 was white and strain Eaindra-14 was whitish grey. Strain Eaindra-11 were white and whitish grey. Strain Eaindra-3 was white and whitish yellow. The colors of strain Eaindra-8 was whitish green and whitish orange and strain Eaindra-9 was whitish green and whitish pale yellow.

Microscopical Characters of Isolated Fungi

The microscopical characters of isolated mangrove fungi are the same to the genera of *Penicillium*, *Aspergillus*, *Rhizopus* and *Rhizoctonia*. These strains were identified as strains Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13 as *Penicillium* species, strains Eaindra-8 and Eaindra-11 as *Aspergillus* species, strain Eaindra-3 as *Cladosporium* species, strains Eaindra-2 and Eaindra-14 as *Rhizoctonia* species and strain Eaindra-10 as *Rhizopus* species. Strains Eaindra-1, Eaindra-4 and Eaindra-7 were being unable to identify their genus level. So, they were assumed as unidentified isolates.

Scientific classification

<i>Penicillium</i> Kingdom : Fungi Division : Ascomycota Class : Eurotiomycetes Order : Eurotiales Family : Trichocomaceae Genus : <i>Penicillium</i>	<i>Aspergillus</i> Kingdom : Fungi Division : Ascomycota Class : Eurotiomycetes Order : Eurotiales Family : Aspergillaceae Genus : <i>Aspergillus</i>
<i>Rhizoctonia</i> Kingdom : Fungi Division : Basidiomycota Class : Agaricomycetes Order : Cantharellales Family : Ceratobasidiaceae Genus : <i>Rhizoctonia</i>	<i>Rhizopus</i> Kingdom : Fungi Division : Zygomycota Class : Zygomycetes Order : Mucorales Family : Mucoraceae Genus : <i>Rhizopus</i>
<i>Cladosporium</i> Kingdom : Fungi Division : Ascomycota Class : Dothideomycetes Order : Capnodiales Family : Davidiellaceae Genus : <i>Cladosporium</i>	

Morphological and Microscopical Characters of All Fungal Strains



Figure (3) Morphological and microscopical characters of isolated endophytic fungus Eaindra -(1)

Most mycelia are thin without septate. They are highly branched at the tip. Conidiophores are absent. Conidia are not produced. This fungal strain (Eaindra-1) was not identified as shown in Figure (3).



Figure (4) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(2)

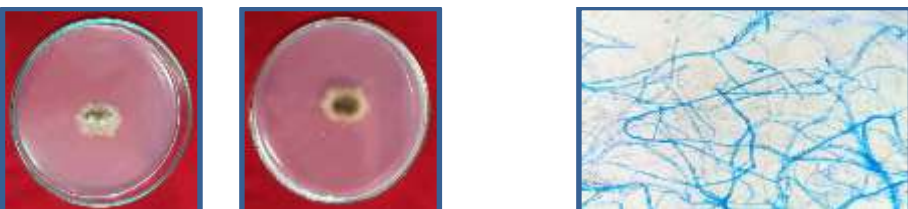


Figure (5) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(14)

Mycelium white with long cells, septa of branch (right angle) set off from main hypha. Conidiophores are connected by mycelial threads, and spores lacking. These strains (Eaindra-2 and Eaindra-14) were identified as *Rhizoctonia* species as shown in Figures (4 and 5).



Figure (6) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(3)

Mycelia are thin with septa. Conidiophores are long, branched variously near the apex. Conidia are light, various shapes (oval, cylindrical, irregular, spherical) and sizes. This fungal strain (Eaindra-3) was identified as *Cladosporium* species as shown in Figure (6).



Figure (7) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(4)

Eaindra Mycelia are thin, and some are branched but some are not branched at the tip. Conidiophores are continuous with mycelium. Conidia are long oval shape at the tip of mycelium. This fungal strain (Eaindra-4) was not identified as shown in Figure (7).



Figure (8) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(5)



Figure (9) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(6)



Figure (10) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(9)



Figure (11) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(12)



Figure (12) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(13)

Conidiophores arising from the hypha singly at the apex, ending in a group of phialides; conidia Hyaline, 1- celled, mostly globose, in chains. These four strains (Eaindra-5, Eaindra-6, Eaindra-9, Eaindra-12 and Eaindra-13) were identified as *Penicillium* species as shown in Figures (8, 9, 10, 11 and 12).



Figure (13) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(7)

Mycelia are highly branched with septate, some are globose at the tip. Conidia are not produced. This fungal strain (Eaindra-7) was not identified as shown in Figure (13).



Figure (14) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(8)



Figure (15) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(11)

Conidiophores long, upright, terminating in a large globose swelling, bearing phialides which are radiating from entire surface. Conidia: 1- celled, globose, produced basipetally. These strains (Eaindra-8 and Eaindra-11) may be identified as *Aspergillus* species as shown in Figures (14 and 15).



Figure (16) Morphological and microscopical characters of isolated endophytic fungus Eaindra-(10)

Broad have no or very few, septa, usually are unbranched. Conidiophores: round sporangiophores are long, many oval, colorless spore. This strain (Eaindra-10) may be identified as *Rhizopus* species as shown in Figure (16).

Discussion and conclusion

Glomba is a genus of plants in the ginger family and contains about 100 species including about 17 species in Myanmar. In this research work, the plant samples (leaves and rhizomes) of *Glomba arracanensis* Kurz. were collected from Nat-Taung-Pyin Hill, Pauktaw Township in Rakhine State in September 2023.

In this study, fourteen endophytic strains were isolated from Rhizomes of *Glomba arracanensis* Kurz. (Family Zingibraceae). Lutfia and Munir (2019) have isolated endophytic fungi from rhizome of *Glomba pendula* Roxb. Soe Soe Yu Hnin (2018) isolated endophytic fungal strains

from rhizomes of the selected plant from the family Zingibraceae. Saw Ohnmar *et al.* (2020) studied morphology and phytochemical and antimicrobial activity of *Globba arracanensis* Kurz. But, there was no the previous record for endophytes from *Globba arracanensis* Kurz. in Myanmar.

In this study, the morphological characters of *Globba arracanensis* Kurz. were mentioned, and the colony appearance of isolated fungal strains were circular, irregular and rhizoid. The margins of isolated fungi were entire, filamentous, undulate and lobate.

In this study, the elevation of isolated fungal strains was raised, flat and pulvinate. These characters were in agreement with the statements of Dubey and Maheshwari (2014). The surface and reverse colors of isolated fungal strains were the same to the statement reported by Kyawt Kyawt Aung (2014), Soe Soe Yu Hnin (2018) and Yee Yee Thu *et al.*, (2016).

In this study, five *Penicillium* spp., two *Aspergillus* spp., two *Rhizoctonia* spp., one *Cladosporium* spp., one *Rhizopus* sp., and three unknown species were isolated from *Globba arracanensis* Kurz. Many researchers have isolated bioactive fungi such as *Penicillium* sp., *Aspergillus* sp., *Cladosporium* spp., *Rhizoctonia* sp., *Rhizopus* sp., etc. The microscopical characters of isolated endophytic fungi were agreed with the statements of Barnett (1969).

Barnett *et al.* (1998) have stated that conidiophores of *Penicillium* are arising from the hypha singly at the apex, ending in a group of phialides; and conidia are hyaline, 1- celled, mostly globose or ovoid, in chains. Ariantari *et al.*, (2019) have stated that endophytic *Penicillium* species were isolated from roots of the medicinal plant *Zingiber officinale* to produce indole diterpenoids. Urooj *et al.*, (2021) have reported endophytic *Penicillium* species and *Pseudomonas monteilii* in inducing the systemic resistance in okra against root rotting fungi and their effect on some physiochemical properties of okra fruit.

Otero *et al.*, (2002) have reported that 108 *Rhizoctonia* like fungi were isolated from nine Puerto Rican orchids. Ma *et al.*, (2004) have isolated endophytic *Rhizoctonia* sp. from *Cyanodin dactylon* to produce Anti-Helicobacter pylori metabolites. The genus *Rhizoctonia* is comprised of a highly divergent group of sterile fungi that still share similar characteristics in their anamorphic (asexual) state, namely they remain vegetative, producing no asexual spores (Harveson, 2013).

Barnett *et al.* (1998) have stated that conidiophores of *Aspergillus* spp. are long, upright, terminating in a large globose swelling, bearing phialides which are radiating from entire surface, and conidia are 1- celled, globose, produced basipetally. Hartanto *et al.*, (2019) isolated *Aspergillus* species from rhizome of *Alpinia* sp. (Zingiberaceae) in Hutan Sibayak, North Sumatera. Liu *et al.*, (2019) have mentioned that an endophytic *Aspergillus flavus* was isolated from a toxic medicinal plant, *Tylophora ovata* to produce sesquiterpene.

Berisch *etal.*(2012) stated that the vegetative hyphae were erect, straight, branched or unbranched. Some conidia were produced in branched acropetal chains in *Cladosporium* sp. Alvaro *et al.* (2022) isolated endophytic fungus *Cladosporium* sp. (AC-1) from the leaves of *Annona cacans* L.

El-Zawawy *et al.* (2023) have isolated a new endophytic fungus *Rhizopus oryzae* AUMC14899 for the production of L-tyrosine and its biomedical applications. They investigated that the genus *Rhizopus* exhibited strong antibacterial and anti-biofilm activities against multidrug-resistant Gram-negative and Gram-positive bacteria.

In conclusion, fungi naturally produce antibiotics to kill or inhibit the growth of microbial diseases. Endophytic fungi have been known as excellent source of antimicrobial agents. Endophytic fungi have antibiotic activity as they can produce the bioactive compounds. In this research, some of fungal strains isolated from leaves are the same genera to fungal strains from rhizomes of *Globba arracanensis* Kurz. All endophytic strains (six *Penicillium* species, two *Aspergillus* species, two *Rhizoctonia* species, one *Cladosporium* sp., one *Rhizopus* species and three unknown species) possessed good antimicrobial activity. This activity will be presented in the next paper.

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