# Screening for the Antimicrobial Activities of Endophytic Fungi from the Leaves of *Nephelium lappaceum* L.

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## Abstract

In the course of investigating antimicrobial activities, a total of seven endophytic fungal strains were isolated from the leaves of *Nephelium lappaceum* L. (Kyet-mouk). The plant samples were collected from the Boyagyi village, Kyaikhto township, Mon state. The experiment was conducted in the Microbiology Laboratory, Department of Botany, University of Yangon. The morphological and microscopical characters of isolated fungal strains were studied. Seven isolated fungal strains were given the names to KT 1-7 and three *Aspergillus* sp., two *Rhizoctonia* sp. and two *Trichosporon* sp., were identified. The screening of antimicrobial activities was carried out using paper disc diffusion assay with six test pathogenic organisms such as *Bacillus pumilus, Candida albicans, Escherichia coli, Pseudomonas fluorescens, Aspergillus flavus and Staphylococcus aureus.* Among seven fungal strains, KT7 showed the best and highest antimicrobial activity on six test organisms.

**Keyword:** *Nephelium lappaceum* L., endophytic fungal strains, antimicrobial activities

## Introduction

The investigation of novel antimicrobials is of high importance due to the increase in antibiotic resistance among pathogens (Payne *et al*., 2007). However, after many decades of exploration it is increasingly difficult to discover novel bioactive metabolites from common environments (e.g., soils) (Jiang *et al*., 2018). Endophytic microorganisms are excellent sources of bioactive natural products that can be used to satisfy the demands of pharmaceutical, medical agriculture and industries. Endophytic microorganisms live between the cells, in the intercellular spaces, or in the vascular system (Zinnel *et al*., 2002).

Endophytes can be defined by various scientists as mutualists that colonize aerial parts of living plants' tissues and do not cause symptoms of disease. There is a great biological diversity of endophytic fungi, occurring naturally in the temperate regions and tropical rainforests, where about 300,000 terrestrial host-plant species are distributed. Each plant species hosts one or more endophytic fungus species. It is estimated that over one million endophytic fungal species occur in nature (Faeth and Fagan, 2002).

Endophytic fungi have proved to be an important source for bioactive compounds that have a wide range of applications in the medical field. Endophyte plant association could also be to stimulate the production of secondary metabolites by host plants. Plants growing in adverse habitats have to be screened for the isolation of endophytes and their metabolites (Raghukumar, 2008). Endophytic fungi are also able to produce antimicrobial metabolites. In 2005, Shu *et al*., reported that some endophytic fungi may produce secondary metabolites with potential for antimicrobial or anticancer property.

The present study focuses on screening, identification, and antimicrobial activity of endophytic fungi isolated from the leaves of *Nephelium lappaceum* L. that were collected from Kyaikhto township, Mon State. There are many health benefits of *N. lappaceum.* Its fruit is rich in many vitamins, minerals and beneficial plant compounds. Some previous research reported that *N. lappaceum* has anticancer activity from fruit, antioxidant activities and antibacterial activity from peel (Carocho and Ferreira, 2013) respectively. However, only a few studies focused on the isolation of endophytic microorganisms from the leaves of *N.*

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*lappaceum.* Therefore, the present study aimed to isolate beneficial microbes from the leaves of *N. lappaceum* and determine the biological activities of the leaves.

The goal of this study is to screen endophytic fungal strains from the leaves of *N. lappaceum* to examine the morphology and microscopic characters of isolated fungal strains, and to evaluate the antimicrobial activity of isolated fungal srains.

## Materials and Methods

### Collection of plant samples

The leaves of *Nephelium lappaceum* L. were collected from Boyagyi Village, Kyaikto Township, Mon State. The morphological characters of the vegetative and reproductive parts were identified by Flora of Ceylon, Volume XII (1998).

### Surface sterilization of leaves and Isolation of endophytic fungal strains

(Lee *et al*., 1996 and Phay, 1997)

The leaves of the plant were washed in running water for 15 minutes. The leaves were cut into about 1 cm pieces. These parts were sterilized by soaking in 75 % ethanol for 2 minutes. Then, they were sterilized by soaking in 5.3 % sodium hypochloride for 1 minute. After that, these parts were sterilized by soaking in 75 % ethanol for 30 seconds. These parts were dried on sterilized paper and then they were placed on agar plates containing sucrose-yeast extract (SY) medium supplemented with chloramphenicol (100 µg/L) to suppress bacterial growth. Then, the petri-dishes were incubated at room temperature as shown in Figure 1.

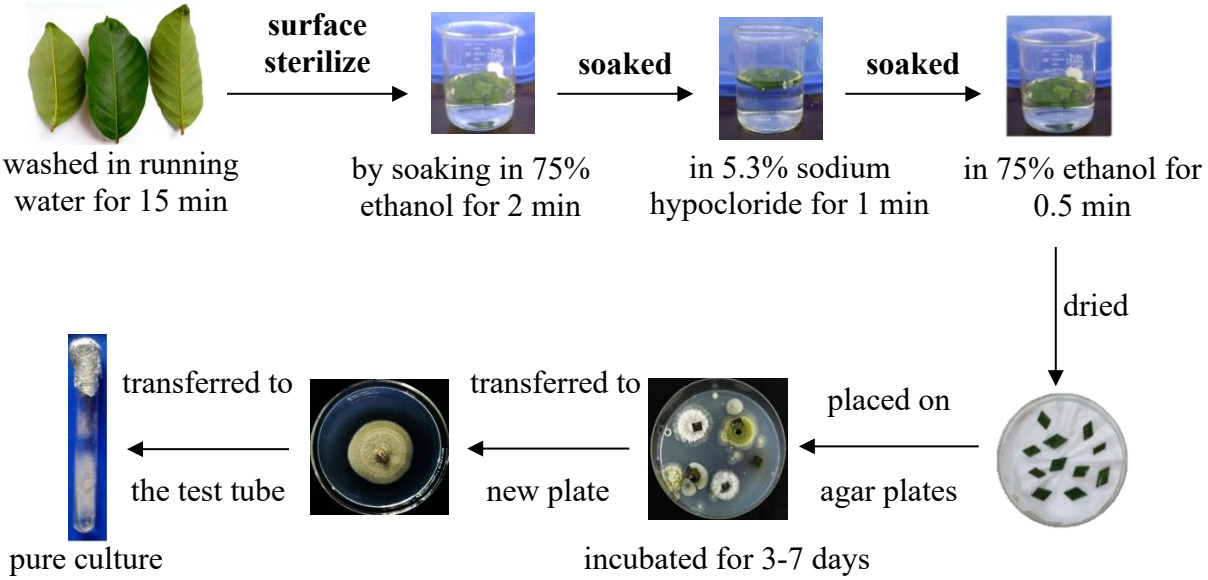



Fig 1. Surface sterilization of leaves and Isolation of endophytic fungal strains

### Morphological and microscopical characters of isolated fungal strains (Barnett, 1969)

The isolated fungal strains grown on slant culture were transferred onto culture plates containing sucrose-yeast extract (SY) medium (Atlas, 1993). In this medium, chloramphenicol was added to inhibit bacteria. And then, these plates were inoculated at 30°C for 3-7 days. Colony forms, surface and reverse pigments of isolated strains and microscopical characters were studied according to the Barnett 1969.

**Antimicrobial Activity of Isolated Endophytic Fungal Strains**

Antimicrobial activities by paper disc diffusion assay medium of isolated fungi were investigated by the method of Phay (1997) and Tomita (1998). The isolated fungal strains grown on slant culture were inoculated into 50 ml conical flasks containing 25 ml of SY medium (sucrose 1.0 g, yeast extract 0.3 g, NaCl 0.5 g, agar 2.0 g, distilled water 100 ml, pH 7.0) and incubated at room temperature for 3 days (Strobel and Sullivan, 1999). The paper disc diffusion method was used to check the antimicrobial activity of isolated strains. Test organisms (50 µl) were added to 100 ml of assay medium (SY medium), then poured into plates (Ogstan, 1984; Ryan and Ray, 2004). After solidification, paper discs were impregnated with broth samples (10 µl) and dried. Then, they were applied to the agar plates containing test organisms and incubated at room temperature for 24 hours. After 24 hours, clear zones (inhibitory zones) surrounding the test discs (6 mm) were measured. These zones indicate the presence of antimicrobial activities which inhibit the growth of selected tested pathogens.

Table 1. Test organisms and diseases

| **Test organisms** | Code | **Diseases** |
|---|---|---|
| *Aspergillus flavus* | JAP- 0225025 | Bronchitics |
| *Bacillus pumilus* | IFO-905771 | Wound and burn infection |
| *Candida albicans* | NITE-09542 | Skin infection, vaginal candidasis, alimentary tract infection urogenital infection. |
| *Escherichia coli* | AHU-5436 | Cholera, diarrhea and vomiting, urinary tract infections |
| *Salmonella typhi* | ST-3/JEP-69 | Typhoid, strong fever. |
| *Staphylococcus aureus* | ATCC-12877 | Skin disease, food poison, wound infection, burns, abscesses, blood stream infection, staphylococcal pneumonia. |

## Results

**Scientific classification**

Scientific name *Nephelium lappaceum* L.

Common name Rambosteen

Myanmar name Kyet-mouk

Family name Sapindaceae

**Outstanding characters**

Evergreen tall trees, with buttresses up tp 1.5 m high; branches puberulous, early glabrescent or persistant hairy. Leaves 1-foliate to 5-jugate, simple, alternate; petioles 1.5-10 cm long; leaflets ovate to obovate, usually widest in the middle, above glabrous, beneath pale green, sparsely hairy on the midrib; petiolules broadly and shallowly grooved with a strong median rib. Inflorescences axillary and sub-terminal, cymes. Flowers obliquely pentamerous, polygamous or polygamdioecious; honey-scented. Sepals 4-5 usually slightly connate below, 1-2 mm long, outside thin-densely appressed short hairy, inside usually densely long hairy.

Petals usually absent, sometimes 4, reduced, clawed, margin infolded, outside glabrous, margin ciliate. Disk hairy, sometimes glabrous. Stamens 5-8. Ovary 2 (-3) celled. Fruit ellipsoid to subglobular, orange-yellow-red, with bulbous or broad-based, tapering to strap-shaped or filiform; wall thin**.**

Fig 2. The plant and leaves of *Nephelium lapaceum* L.

**Isolation of endophytic fungal strains from plant sample**

Seven endophytic fungal strains were isolated from the leaves of *N. lappaceum* L. and were given the names as KT1 to KT7.

**Morphological and microscopical characters of isolated fungal strains**

The morphological and microscopical characters of seven isolated fungal strains including surface and reverse color of each strains were shown in Table 1 together with the possible genus of each strain.

Table 2. Morphological characters of seven endophytic fungal strains on Sucrose Yeast (SY) medium

| Strains | Cultural characters | | Possible genus |
|---|---|---|---|
| | Surface color | Reverse color | |
| KT1 | Green | White | *Aspergillus* sp. |
| KT2 | White | Cream | *Rhizotonia* sp. |
| KT3 | White | Yellowish white | *Trichosporon* sp. |
| KT4 | Yellow to white | Yellow to white | *Aspergillus* sp. |
| KT5 | Yellowish white | Brownish white | *Rhizotonia* sp. |
| KT6 | Yellowish green | Yellow | *Trichosporon* sp. |
| KT7 | Greenish white | White | *Aspergillus* sp. |

**Morphological and microscopical characters of KT1**

In morphology character, the surface color of KT1 was green. Its reverse color was white. The microscopical character of KT1 is that the conidiophores are enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped

phialides which may develop directly on the vesicle (uniseriate form). The phialides produce chains that are mostly round. So, it may be *Aspergillus* sp. shown in Figure 3.

**Morphological and microscopical characters of KT2**

In morphological character, the surface color of KT2 was white and its reverse color was cream. The microscopical character of KT2 was cell of mycelium long, septa of branches usually set off from the main hyphae. Conidia are absent, sporodochium-like bodies and chlamydospore-like cells in chains. This fungus was assumed to be *Rhizoctonia* sp. in Figure 4.

**Morphological and microscopical characters of KT3**

In morphological character, the surface color of KT3 was white. Its reverse color was yellowish white. The microscopical character of KT3 was that it produced abundant and well-developed pseudohyphae and hyphae. Blastoconidia are unicellular and variable in shape. The most typical microscopic feature of this genus is the production of anthroconidia. These anthroconidia are unicellular and usually cubical, barrel or elongate in shape. Therefore, this fungus was assumed to be *Trichosporon* sp. as shown in Figure 5.

**Morphological and microscopical characters of KT4**

In morphology character, the surface color of KT4 was green. Its reverse color was white. The microscopical character of KT4 is that the conidiophores are enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped phialides which may develop directly on the vesicle (uniseriate form). The phialides produce chains that are mostly round. So, it may be *Aspergillus* sp. shown in Figure 6.

**Morphological and microscopical characters of KT5**

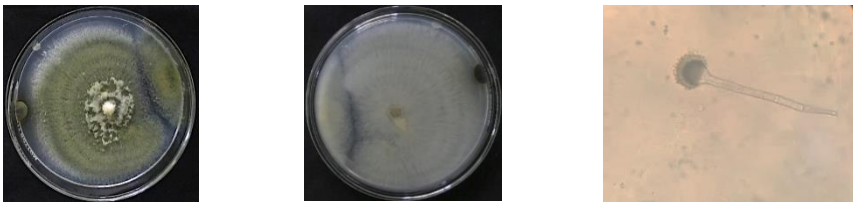
In morphological character, the surface color of KT5 was white and its reverse color was cream. The microscopical character of KT5 was that it was a cell of mycelium long. The septa of branches are usually set off from the main hyphae. Conidia are absent, sporodochium-like bodies and chlamydospore-like cells in chains. This fungus was assumed to be *Rhizoctonia* sp. in Figure 7.

**Morphological and microscopical characters of KT6**

In morphological character, the surface color of KT6 was white. Its reverse color was yellowish white. The microscopical character of KT6 was that it produces abundant and well-developed pseudohyphae and hyphae. Blastoconidia are unicellular and variable in shape. The most typical microscopic feature of this genus is production of anthroconidia. These anthroconidia are unicellular and usually cubical, barrel or elongate in shape. Therefore, this fungus was assumed to be *Trichosporon* sp. as shown in Figure 8.

**Morphological and microscopical characters of KT7**

In morphology character, the surface color of KT7 was green. Its reverse color was white. The microscopical character of KT7 is that the conidiophores are enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped phialides which may develop directly on the vesicle (uniseriate form). The phialides produce chains that are mostly round. So, it may be *Aspergillus* sp. shown in Figure 9.

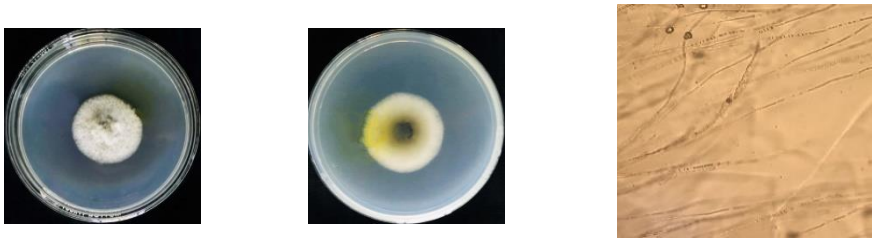


Surface View Reverse View *Aspergillus* sp.

Fig 3. Morphological and microscopical characters of strain KT1 X 400

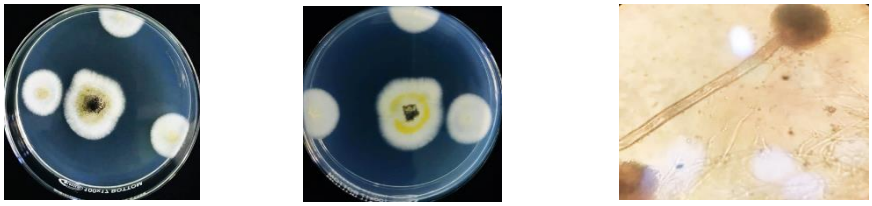
Surface View Reverse View *Rhizoctonia* sp.

Fig 4. Morphological and microscopical characters of strain KT2 X 400



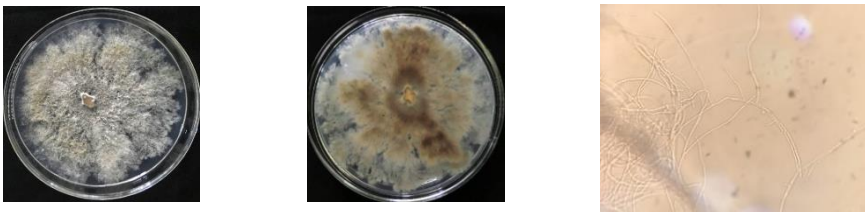
Surface View Reverse View *Trichosporon* sp.

Fig 5. Morphological and microscopical characters of strain KT3 X 400



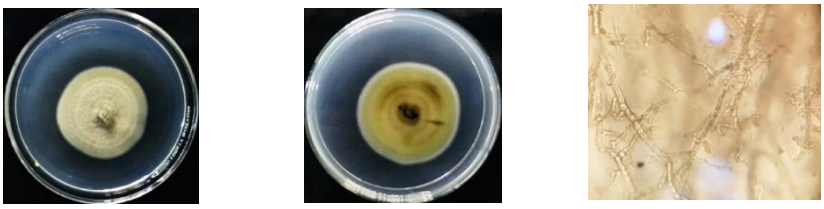
Surface View Reverse View *Aspergillus* sp.

Fig 6. Morphological and microscopical characters of strain KT4 X 400



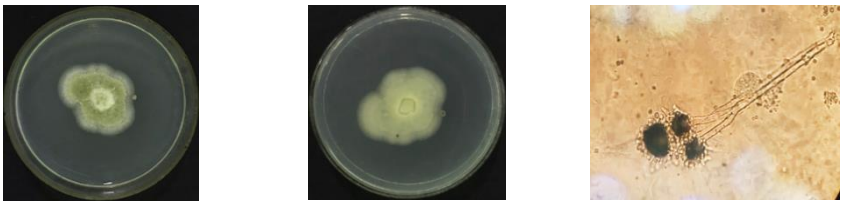
Surface View Reverse View *Rhizoctonia* sp.

Fig 7. Morphological and microscopical characters of strain KT5 X 400



Surface View Reverse View *Trichosporon* sp.

Fig 8. Morphological and microscopical characters of strain KT6 X 400

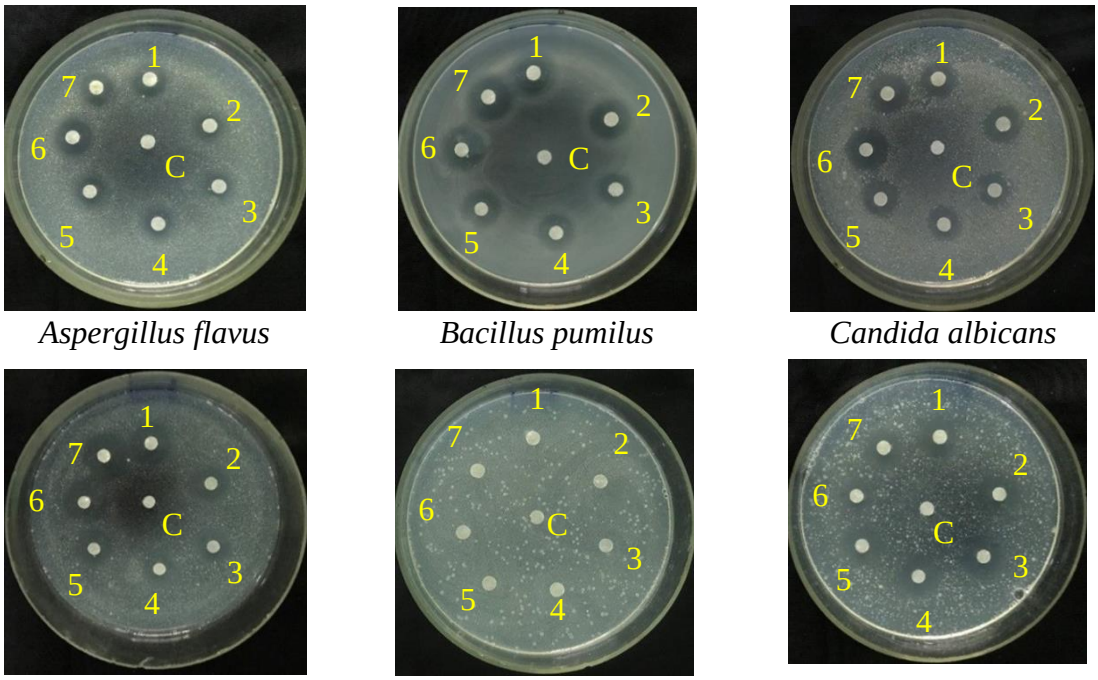


Surface View Reverse View *Aspergillus* sp.

Fig 9. Morphological and microscopical characters of strain KT7 X 400

**Antimicrobial activity of isolated endophytic fungal strains**

In this study, the antimicrobial activities of seven isolated fungal strains, namely: KT1 to KT7 were evaluated on six test organisms. Among them, KT7 showed the best antimicrobial activity against six tested pathogens compared with other strains. The highest activity was observed on *Bacillus pumilus*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*, respectively, as shown in Table 3 and Figure 10.




*Aspergillus flavus* *Bacillus pumilus* *Candida albicans*

*Eschericheria coli* *Salmonella typhi* *Staphylococcus aureus*

Fig 10. Inhibitory effect of islolated strains on six test organisms

Table 3. Antimicrobial activity of isolated endophytic fungal strains (disc size- 6mm)

| Strains / Test organisms | KT1 | KT2 | KT3 | KT4 | KT5 | KT6 | KT7 |
|---|---|---|---|---|---|---|---|
| *Aspergillus flavus* | 14 | 13 | 14 | 12 | 12 | 13 | 13 |
| *Bacillus pumilus* | 20 | 18 | 15 | 15 | 19 | 16 | **21** |
| *Candida albicans* | 15 | 14 | 14 | 13 | 17 | 14 | **21** |
| *Escherichia coli* | 20 | 19 | 16 | 15 | 17 | 16 | **22** |
| *Salmonella typhi* | 20 | 20 | _ | _ | 20 | _ | 15 |
| *Staphylococcus aureus* | 15 | 17 | 15 | 14 | 15 | 15 | **23** |

## Discussion and Conclusion

Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease (Wilson, 1995). In 2005, Berdy reported that more than 20,000 bioactive metabolites are of microbial origin. Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites that are directly used as drugs or function as lead structures for various bioactive products.

The research work related to the endophytic fungi from the leaves of *Nephelium lappaceum* was very poor and most of the reported studies were on the endophytic bacteria and extracts from the other plant parts of *N. lappaceum*. In the present study, seven endophytic fungal strains were isolated from the leaves of *Nephelium lappaceum* and the antimicrobial activity of each was evaluated. According to the results of antimicrobial activity, fungal strain KT7 showed the highest inhibition activity on different test organisms and the highest inhibition zone was observed on *Bacillus pumilus, Candida albicans*, *Escherichia coli,* and *Staphylococcus aureus.*

Similar to the results reported by Dalee *et al*. (2015), the antimicrobial activity of endophytic fungi from the leaves of rambutan against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus cereus* as well as *Candida albicans*. In 2011, Tadtong *et al.* reported that the antimicrobial activities of the extracts of whole fruit, skin, peel and seed of Rambutan were against on *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Pseudomonas aeruginosa* and *Aspergillus niger*. Malini and Maheshkumar (2013) have disclosed significant antimicrobial activity of *N. lappaceum* fruit sap extracts towards *Pseudomonas aeruginosa* while Bhat and Al-daihan (2014) revealed antibacterial activities of *N. lappaceum* seed extracts against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.*

All of the fungal strains were identified by the Illustrated Genera of Imperfect Fungi (Barnett, 1969) and Medically Important Fungi (Davise, 1995). Strains KT1, 4, and 7 were identified as *Aspergillus* sp., strains 2 and 5 were *Rhizoctonia* sp., and strains 3 and 6 were identified as *Trichosporon* sp. respectively. *Aspergillus* is one of the oldest genera of fungi described by Micheli in 1729 (Ross, 1951). They are generally found in soils, including forest humus layers and agricultural. Although some species can cause infections in humans, other

species are important in commercial microbial fermentation such as Japanese sake, which is often made from rice, other starch ingredients, grapes of malted Harley (Klich, 2002). *Rhizoctonia* is a plant pathogenic fungus with a wide range and worldwide distribution (Sneh *et al*, 1991). *Trichosporon* sp. was first described by Beigel in 1865 as the agent responsible for a benign hair infection called white *piedra* (meaning "stone" in Spanish) (Montoya and Gonzalez, 2014).

In conclusion, the present study obtained the three endophytic fungal strains such as *Aspergillus* sp., *Rhizotonia* sp., and *Trichosporon* sp. from the leaves of *Nephelium lappaceum* L. A selected fungal strain *Aspergillus* sp. indicated the highest antimicrobial activity on tested pathogenic organisms. However, further studies are still needed to investigate fermentation studies and to conduct preliminary characterization of bioactive compounds from active strains in order to discover new drugs with pharmaceutical application.

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