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Abstract. Endophytic fungi are a group of microorganisms that grow in plant tissue without causing symptoms of disease in host plants. Fungal endophytes have a great diversity and potential as an agent of bioactive compound and metabolites that are important in the pharmaceutical industry, agriculture, and environment. In this study isolation and identification of fungal endophytes inhabiting an assemblages of medicinal plants collected from Toba and Samosir Regency, North Sumatra, Indonesia were carried out. Fungal endophytes were isolated using surface sterilization methods with slightly modification and totally 88 selected endophytic fungal strains were isolated from roots, stems, barks, leaves, and fruits of 12 species of medicinal plants. The fungal strains identification through morphological observation showed that selected fungal endophytes were associated with host plant belonging to the taxa *Colletotrichum*, *Fusarium*, *Lasiodiplodia, Neopestalotiopsis, Phoma, Phomopsis*, *Phyllosticta*, *Schizophyllum, Trichoderma,* and *Xylaria*. In this study endophytic *Phomopsis* dominating the obtained strains where 30% (27/88) strains of them were isolated from 8 species of medicinal host plants. Preliminary exploration of fungal endophytes through isolation and identification was the first step in order to discover the potential strains were able to produce promising metabolites and bioactive materials. The results of the study are expected to be initial microbial resources and information for screening and utilization of endophytic fungi through bioprospecting.

1. Introduction

Endophytic fungi is a group of microorganisms that grow in internal and intercellular plant tissue at some of their life cycle without causing symptoms of disease in host plants [1,2]. Endophytic fungi inhabiting plant tissue has been studied for more than 15 years [1], and the study were carried out in host plants both in subtropical and tropical regions such as *Bruguiera gymnorrhiza* [3], *Theobroma cacao* [4], *Camelia sinensis* [5,6], host plant families Coniferaceae, Gramineae, and Ericaceae [7], and also 18 species from 7 families of medicinal plants from Egypt [8].

The pattern of relationships or associations of endophytic fungi with plants as their symbionts can be mutualism, commensal, saprophytic, and/or parasitic. However, endophytic fungi generally has mutual-symbiotic relationships with their host plants [9]. The benefit from mutual relationship with fungal endophytes for host plants such as improving plant growth and competitiveness, drought, diseases, and pests resistance, pollination success, and environmental stress [10,11]. The closely

relationship between both of symbionts also allows the transfer of genetic material one and another [12,13,14].

Some of the potential endophytic fungi that have been known include producing enzymes, antibiotics, and secondary metabolites including anti-cancer compounds. Various secondary metabolites can be produced from endophytic fungi including alkaloid compounds, steroids, terpenoids, diterpenes, flavonoids, phenols, aliphatic compounds, and others [9]. Fungal endophytes can produce natural metabolite products that can be useful for health, agriculture, and industrial uses such as antibiotics, anticancer, biological control agents and other beneficial bioactive compounds [9,14,15].

Every single plant may be host of one or more fungal endophytes [9]. Some of fungal endophytes have a wide range of hosts and the rest have specific hosts [16], thus fungal endophytes have a high biodiversity. It is estimated that approximately 160,000 fungal endophytes species are exist through out Indonesia with most of them not yet explored and identified [17]. The objectives of this study is to isolate and identify fungal endophytes inhabiting in an assemblages of medicinal plants were collected from Toba and Samosir Regency, North Sumatra Province, Indonesia. Medicinal plants have been recognized as a repository of endophytes with novel metabolites. Furthermore, currently fungal endophytes are viewed as potential and promising source of natural bioactive products for pharmaceutical and agriculture importance [18].

2. Materials and Methods

2.1. Sample material

Endophytic fungi were isolated from 12 species of medicinal plants collected in April 2018 from Toba and Samosir Regency, North Sumatra Province, Indonesia at 20º23'N 99º13'E and 900-1200 m above sea level. Plant samples collected from fresh material and healthy living tissue of medicinal plant consists of roots, stems, barks, leaves, and fruits.All fresh samples were marked and packed carefully and then transferred to the laboratory for isolation purposes within less than 72 hrs.

2.2. Fungal isolation

The fungal isolation was carried out based on surface sterilization method with slightly modification. Medicinal plant samples were sterilized by immersed in 70% ethanol for 1 min and then sterilized with 1% sodium hypochlorite (NaOCl) solution for 2 min. Samples were rinsed twice in sterile distilled water and put into sterile paper towels for 3-4 hrs to remove water from surface. Afterwards, samples aseptically cut into small segments about 5 mm^2 and then placed onto 90-mm Petri dish containing half-strength malt extract agar (MEA). On each MEA plate randomly placed 4 small segments roots, stems, barks, leaves, and fruits samples. Each plant samples was made as many as 3 replicates. Culture then incubated at 27º C for 2 weeks. The endophytic fungi growing out from samples were isolated and purified by transferred onto 60-mm Petri dish containing potato dextrose agar (PDA).

2.3. Fungal identification

Fungal identification carried out based on morphology approach. Morphological identification were made through observing both of macroscopic and microscopic phenotypic characters. Macroscopic characterization includes observation on colour, colony shape, surface, texture, exudates drop, and reverse colour. For microscopic observation, fungal mycelia were mounted in one drop of 1% lactophenol blue solution. Microscopic characterization were conducted under light microscope by observing hyphae, hyphae pigmentation, septate, clamp connection, spore, and another reproductive structures.

3. Results and Discussion

An assemblages of endophytic fungi were detected from several medicinal plant collected from Toba and Samosir Regency, North Sumatra Province, Indonesia. A total 88 strains of fungal endophytes

were isolated from12 species of medicinal plant samples (Table 1). Furthermore, fungal endophytes were morphologically identified into 10 fungal taxa member of Coelomycetes, Hypomycetes, and Basidiomycetes. According [1] most of fungal endophytes are Ascomycetes, Basidiomycetes, and imperfect fungi (Deuteromycetes).

Table 1. Selected endophytic fungi were isolated from 12 species of medicinal plants collected from Toba and Samosir Regency, North Sumatra

No	Strain no.	Plant host	Plant part	Sampling site	Fungal taxa
1	01BtTo-1	*Taxus sumatrana*	Stem	Aek Nauli	*Xylaria*
2	01BtTo-1.1	*Taxus sumatrana*	Stem	Aek Nauli	*Colletotrichum*
3	01BtTo-2.1	*Taxus sumatrana*	Stem	Aek Nauli	*Neopestalotiopsis*
4	01DnTo-1	*Taxus sumatrana*	Leaf	Aek Nauli	*Lasiodiplodia*
5	01DnTo-2	*Taxus sumatrana*	Leaf	Aek Nauli	*Colletotrichum*
6	01DnTo-3	*Taxus sumatrana*	Leaf	Aek Nauli	*Phomopsis*
7	02BtTo-1	*Styrax sumatrana*	Stem	Aek Nauli	*Phomopsis*
8	02BtTo-2	*Styrax sumatrana*	Stem	Aek Nauli	*Phomopsis*
9	02BtTo-3	*Styrax sumatrana*	Stem	Aek Nauli	*Phomopsis*
10	02BtTo-4	*Styrax sumatrana*	Stem	Aek Nauli	*Phomopsis*
11	02BtTo-5	*Styrax sumatrana*	Stem	Aek Nauli	*Lasiodiplodia*
12	02DnTo-1	*Styrax sumatrana*	Leaf	Aek Nauli	*Fusarium* cf. *solani*
13	02DnTo-2	*Styrax sumatrana*	Leaf	Aek Nauli	*Phyllosticta*
14	02DnTo-3	*Styrax sumatrana*	Leaf	Aek Nauli	*Phomopsis*
15	02DnTo-4	*Styrax sumatrana*	Leaf	Aek Nauli	*Phomopsis*
16	03BhTo-1	*Styrax benzoin*	Fruit	Aek Nauli	*Neopestalotiopsis*
17	03GhTo-1	*Styrax benzoin*	Bark	Aek Nauli	White mycelia sterilia
18	03GhTo-2	*Styrax benzoin*	Bark	Aek Nauli	*Schizophyllum*
19	04BtTo-1	*Podocarpus junghunii*	Stem	Aek Nauli	*Phomopsis*
20	04BtTo-2.1	*Podocarpus junghunii*	Stem	Aek Nauli	White mycelia sterilia
21	04BtTo-2.2	*Podocarpus junghunii*	Stem	Aek Nauli	*Phomopsis*
22	04BtTo-3.1	*Podocarpus junghunii*	Stem	Aek Nauli	White mycelia sterilia
23	04BtTo-3.2	*Podocarpus junghunii*	Stem	Aek Nauli	*Fusarium* cf. *solani*
24	04BtTo-4	*Podocarpus junghunii*	Stem	Aek Nauli	*Phomopsis*
25	04DnTo-1	*Podocarpus junghunii*	Leaf	Aek Nauli	*Xylaria*
26	04DnTo-2.1	*Podocarpus junghunii*	Leaf	Aek Nauli	Dark mycelia sterilia
27	05BtTo-1	*Podocarpus imbricatus*	Stem	Aek Nauli	*Phomopsis*
28	05BtTo-2	*Podocarpus imbricatus*	Stem	Aek Nauli	*Phomopsis*
29	05BtTo-3	*Podocarpus imbricatus*	Stem	Aek Nauli	*Xylaria*
30	05BtTo-4	*Podocarpus imbricatus*	Stem	Aek Nauli	*Phomopsis*
31	05BtTo-5	*Podocarpus imbricatus*	Stem	Aek Nauli	Dark mycelia sterilia
32	05BtTo-6	*Podocarpus imbricatus*	Stem	Aek Nauli	Dark mycelia sterilia
33	05BtTo-7	*Podocarpus imbricatus*	Stem	Aek Nauli	*Phomopsis*
34	05DnTo-1	*Podocarpus imbricatus*	Leaf	Aek Nauli	*Xylaria*
35	05DnTo-2	*Podocarpus imbricatus*	Leaf	Aek Nauli	*Phomopsis*
36	05DnTo-3	*Podocarpus imbricatus*	Leaf	Aek Nauli	*Phomopsis*

37	06DnTo-1	*Saurauia bracteosa*	Leaf	Aek Nauli	*Phomopsis*
38	06DnTo-2	*Saurauia bracteosa*	Leaf	Aek Nauli	*Phomopsis*
39	06DnTo-3	*Saurauia bracteosa*	Leaf	Aek Nauli	*Colletotrichum*
40	06DnTo-4	*Saurauia bracteosa*	Leaf	Aek Nauli	*Phomopsis*

Table 1 (Continue)

No	Strain no.	Plant host	Plant part	Sampling site	Fungal taxa
41	06BtTo-1	*Saurauia bracteosa*	Stem	Aek Nauli	White mycelia sterilia
42	06BtTo-2	*Saurauia bracteosa*	Stem	Aek Nauli	*Phomopsis*
43	07DnTo-1	*Zanthoxylum acanthopodium*	Leaf	Aek Nauli	*Phyllosticta*
44	07DnTo-3	*Zanthoxylum acanthopodium*	Leaf	Aek Nauli	*Colletotrichum*
45	07DnTo-4	*Zanthoxylum acanthopodium*	Leaf	Aek Nauli	*Phomopsis*
46	07BtTo-1	*Zanthoxylum acanthopodium*	Stem	Aek Nauli	*Phomopsis*
47	07BtTo-2	*Zanthoxylum acanthopodium*	Stem	Aek Nauli	*Phomopsis*
48	07BtTo-3	*Zanthoxylum acanthopodium*	Stem	Aek Nauli	*Phomopsis*
49	07BhTo-1	*Zanthoxylum acanthopodium*	Fruit	Aek Nauli	*Colletotrichum*
50	07BhTo-2	*Zanthoxylum acanthopodium*	Fruit	Aek Nauli	White mycelia sterilia
51	07BhTo-3	*Zanthoxylum acanthopodium*	Fruit	Aek Nauli	*Schizophyllum*
52	08DnTo-1	*Artemisia vulgaris*	Leaf	Taman Eden	*Colletotrichum*
53	08DnTo-2	*Artemisia vulgaris*	Leaf	Taman Eden	*Schizophyllum*
54	08DnTo-3	*Artemisia vulgaris*	Leaf	Taman Eden	*Xylaria*
55	08AkTo-1	*Artemisia vulgaris*	Root	Taman Eden	*Trichoderma*
56	08AkTo-2	*Artemisia vulgaris*	Root	Taman Eden	*Trichoderma*
57	08AkTo-3	*Artemisia vulgaris*	Root	Taman Eden	*Colletotrichum*
58	09AkTo-1.1	*Centella asiatica*	Root	Taman Eden	*Fusarium* cf. *oxysporum*
59	09AkTo-1.2	*Centella asiatica*	Root	Taman Eden	White mycelia sterilia
60	09AkTo-2	*Centella asiatica*	Root	Taman Eden	White mycelia sterilia
61	09DnTo-1	*Centella asiatica*	Leaf	Taman Eden	*Colletotrichum*
62	09DnTo-2	*Centella asiatica*	Leaf	Taman Eden	*Colletotrichum*
63	10BtTo-1	*Vitis gracilis*	Stem	Taman Eden	White mycelia sterilia
64	10BtTo-2	*Vitis gracilis*	Stem	Taman Eden	*Lasiodiplodia*
65	10DnTo-1	*Vitis gracilis*	Leaf	Taman Eden	*Colletotrichum*
66	10DnTo-2	*Vitis gracilis*	Leaf	Taman Eden	*Colletotrichum*
67	10DnTo-3	*Vitis gracilis*	Leaf	Taman Eden	*Phomopsis*
68	11AkTo-1	*Gaultheria leucocarpa*	Root	Aek Natonang	White mycelia sterilia
69	11DnTo-1	*Gaultheria leucocarpa*	Leaf	Aek Natonang	*Colletotrichum*
70	11BhTo-1	*Gaultheria leucocarpa*	Fruit	Aek Natonang	*Phyllosticta*
71	11BtTo-1	*Gaultheria leucocarpa*	Stem	Aek Natonang	White mycelia sterilia
72	11BtTo-2	*Gaultheria leucocarpa*	Stem	Aek Natonang	White mycelia sterilia
73	12DnTo-2	*Hippobroma longiflora*	Leaf	Aek Natonang	*Phoma*
74	12DnTo-3	*Hippobroma longiflora*	Leaf	Aek Natonang	*Phomopsis*
75	12DnTo-4	*Hippobroma longiflora*	Leaf	Aek Natonang	*Phomopsis*
76	12BtTo-1	*Hippobroma longiflora*	Stem	Aek Natonang	*Schizophyllum*

77	13DnTo-1	*Zanthoxylum acanthopodium*	Leaf	Aek Natonang	*Colletotrichum*
78	13DnTo-2	*Zanthoxylum acanthopodium*	Leaf	Aek Natonang	*Colletotrichum*
79	13DnTo-3	*Zanthoxylum acanthopodium*	Leaf	Aek Natonang	*Phomopsis*
80	13DnTo-4	*Zanthoxylum acanthopodium*	Leaf	AekNatonang	Dark mycelia sterilia
81	13BtTo-1	*Zanthoxylum acanthopodium*	Stem	Aek Natonang	White mycelia sterilia

Table 1 (Continue)

No	Strain no.	Plant host	Plant part	Sampling site	Fungal taxa
82	15DnTo-1	*Artemisia vulgaris*	Leaf	Sipiso-Piso	*Phoma*
83	15DnTo-2	*Artemisia vulgaris*	Leaf	Sipiso-Piso	White mycelia sterilia
84	15DnTo-3	*Artemisia vulgaris*	Leaf	Sipiso-Piso	*Colletotrichum*
85	15BtTo-1	*Artemisia vulgaris*	Stem	Sipiso-Piso	*Neopestalotiopsis*
86	15BtTo-2	*Artemisia vulgaris*	Stem	Sipiso-Piso	*Colletotrichum*
87	15BtTo-3	*Artemisia vulgaris*	Stem	Sipiso-Piso	*Phomopsis*
88	15BtTo-4	*Artemisia vulgaris*	Stem	Sipiso-Piso	*Lasiodiplodia*

Table 1 shows that from total 88 endophyte strains, based on the morphological characteristics could be identified into 10 genera and 2 groups sterile strain were identified as dark and white mycelia sterilia. Higher vascular plants are dynamic and complex habitats which where various factors will affect the structure and composition of endophytes that occupy the niches of roots, stems, branches and leaves of plants [4]. Fungal endophytes member of Coelomycetes and Hypomycetes were dominant in the acquisition of isolates. Previous study reported that endophytic Coelomycetes and Hypomycetes are known have strongly associations with higher vascular plants [13]. Many endophytic Hypomycetes and Coelomycetes are capable for sporulating in their natural or host plant habitat, but turn to be sterile or fail to sporulating during subculturing onto artificial media [19].

Table 1 also shows that 59 % (52/88) strains are member of Coelomycetes and furthermore they were morphologically identified into 5 genera level, i.e. *Colletotrichum, Neopestalotiopsis, Phoma, Phomopsis*, and *Phyllosticta*. A total 15% (14/88) strains of *Colletotrichum* (Figure 1A) were isolated from 7 species of host plants. Fungal taxa *Colletotrichum* are among the most commonly occurring pathogens and foliar endophytes of terrestrial plants and have been recorded from approximately 2.200 plant species [20]. Endophytic *Neopestalotiopsis* (previously *Pestalotiopsis*) (Figure 1D) is the most studied and potentially known of endophytic fungi. A total 3 endophytic strains of *Neopestalotiopsis* were isolated from 3 medicinal plant, i.e. *Taxus sumatrana* (01BtTo-2.1), *Styrax benzoin* (03BhTo-1), and *Artemisia vulgaris* (15BtTo-1). Previous study on endophytic *Pestalotiopsis microspora* were isolated from host plant *Taxus wallichiana* has been known producing taxol compounds that have potential as anti-cancer [14]. Endophytic *Phoma* (Figure 1F) with anamorphic phase is characterized by the formation of conidia or phialospores in the picnidia [21]. A total 2 strains of *Phoma* were isolated from host plant *Hippobroma longiflora* (12DnTo-2) and *Artemisia vulgaris* (15DnTo-1). Previous study reported that *Phoma* has been successfully isolated from various host plant such as beet, Leguminoseae, and Cruciferae [22]. Two strains of *Phyllosticta* (Figure 1E) were isolated from medicinal plant *Styrax sumatrana* (02DnTo-2) and *Gaultheria leucocarpa* (11BhTo-1). *Phyllosticta* species have often been reported as endophytes, plant pathogens or saprobes [23,24].

Fungal endophytes strains were obtained wasdominated by genera *Phomopsis* where 30% (27/88) of them were isolated from 8 species of medicinal host plants. Endophytic *Phomopsis* (Figures 1G and 1H) are endophytic fungi that have a wide range of hostplants, especially in tropical regions. *Diaporthe* is the sexual phase (teleomorph) which is mostly asexual phase (anamorphous phase) in the form of the genus *Phomopsis* [25]. One of the potential use of the endophytic *Phomopsis (Diaporthe)*

is as a producer of bioactive compounds namely (+) – epiepoxidone, and the strains has been isolated from tea plant *Camillia sinensis* [5,6,17].

Totally 3 strains of *Fusarium* were isolated from 3 different host plants, i.e. *Styrax sumatrana* (02DnTo-1), *Podocarpus junghunii* (04BtTo-3.2), and *Centella asiatica* (09AkTo-1.1). The genera *Fusarium* (Figure 1B) has been described as endophytes and also pathogens in various species and cultivar of vascular plants. Genera *Fusarium* are known as one of the parasitic fungi in plants because they can cause wilt and rot in many plant species [29,30,31]. However, in some plants such as cucumber, non-pathogenic *Fusarium oxysporum* can help increase the resistance of host plants to *Phytium ultimum* by a combination of the effects of antibiosis and mycoparasites [4]. Two strains of *Trichoderma* were isolated from root sample of medicinal plants *Artemisia vulgaris* (Figure 1J). *Trichoderma* are the most prevalent soil fungi and they have beneficial effects on plants as a biological controls against plant pathogenic fungi such as *B. cinerea*, *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. [24, 32].

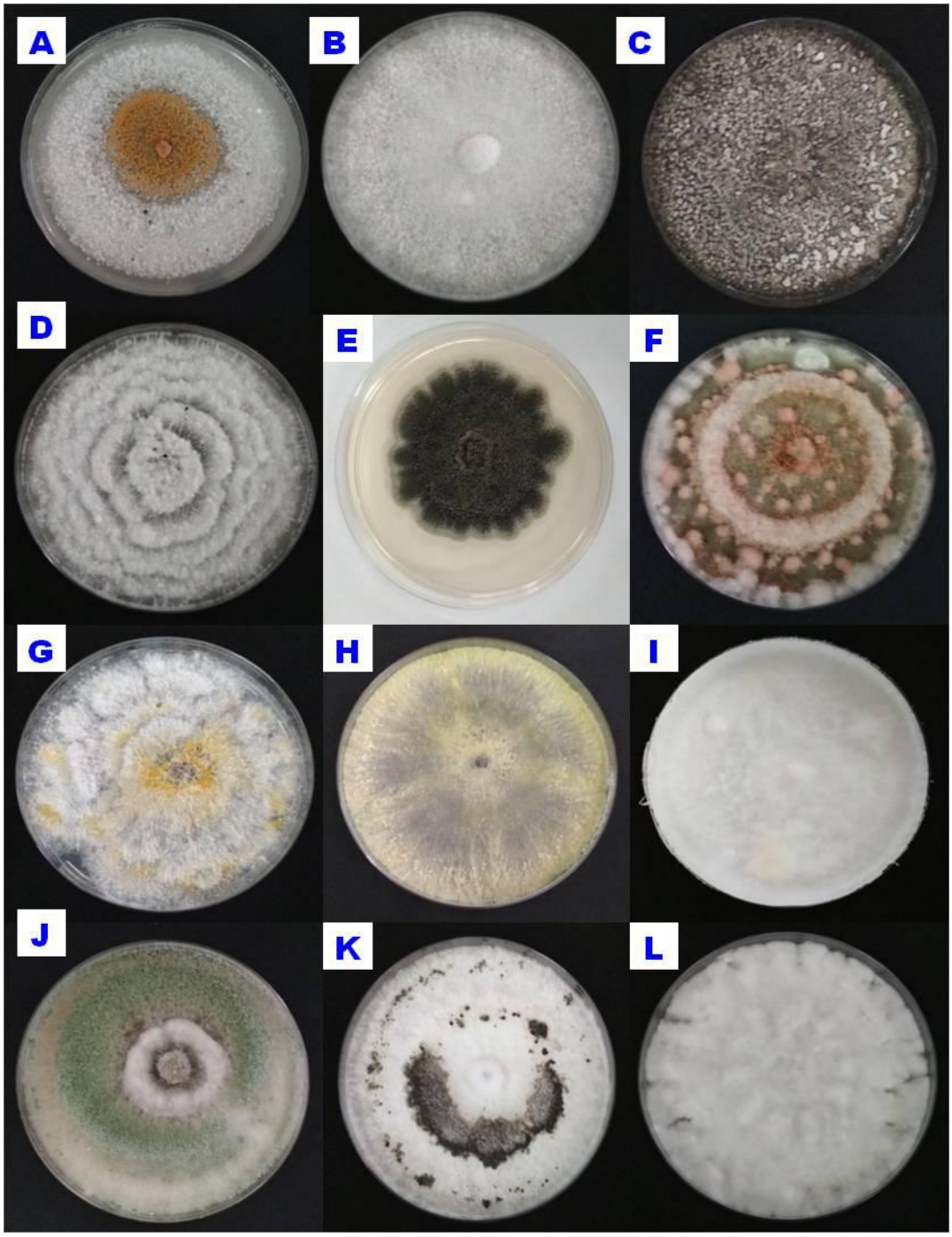

Figure 1. Macroscopic view of representatives fungal endophyte inhabiting medicinal plants collected from Toba and Samosir, North Sumatra growth on PDA, 7-10 days incubation at T 27°C: (A) *Colletotrichum* 08AkTo-03 (B) *Fusarium* 09AkTo-1.1 (C) *Lasiodiplodia* 15BtTo-4 (D) *Neopestalotiopsis* 01BtTo-2.1 (E) *Phyllosticta* 07DnTo-1 (F) *Phoma* 15DnTo-1 (G) *Phomopsis* 05BtTo-4 (H) *Phomopsis* 02BtTo-4 (I) *Schizophyllum* 07BhTo-03 (J) *Trichoderma* 08AkTo-1 (K) *Xylaria* 04DnTo-1 and (L) *Xylaria* 08DnTo-3

A total 4 strains of Basidiomycetous fungal strain *Schizophyllum* were isolated from 4 species of medicinal plant hosts, i.e. *Styrax benzoin* (03GhTo-2), *Zanthoxylum acanthopodium* (07BhTo-3), *Artemisia vulgaris* (08DnTo-2), and *Hippobroma longiflora* (12BtTo-1). Previous study by [33,34]

reported endophytic *Schizophyllum* were isolated from quina plant *Cinchona ledgeriana* and the endophytic fungi also shows their ability to produce *Cinchona* alkaloids in artificial medium such as potato dextrose agar and synthetic liquid medium. Five strains of *Xylaria* (Figure 1K & 1L) from 3 species of medicinal plants were isolated. *Xylaria* are common endophytes in many tropical plants, including palms, orchids, bromeliads, orchids, and ferns [35].

All of the selected endophytic fungi were isolated from 12 species medicinal host plants are belong to the Ascomycetes, Basidiomycetes, and imperfect fungi of the Deuteromycetes. In many cases, endophytic fungi Ascomycetes, Deuteromycetes, and Basidiomycetes are found and are able to grow well as endophytes in plant tissue [1]. Some of isolated endophytic taxa above such as the *Fusarium, Colletotrichum, Neopestalotiopsis, Phoma* and *Phomopsis* are generally expressed as saprophytic and weak parasitic [28,29,30]. However, some strains of them could be mutualistic endophytes depend on the fungal strains and host plants [31].

Based on morphological identification, some of fungal endophytes could be identified until genus level. However, some isolates cannot be identified as they were sterile, did not produce any reproductive structure during incubation period. According their macroscopic characters during culturing in PDA, they were divided into two groups, i.e. dark mycelia sterilia and white mycelia sterilia. Totally 18% (16/88) strains of sterile isolates consists of 4 stains of dark mycelia sterilia and 12 strains of white mycelia sterilia were isolated from 8 species of medicinal host plants. Sterile endophytic fungi are prevalent in many endophytic studies [36] and these lead to problem in the morphological identification of endophytes [37, 38].

Study on isolation and identification of endophytic fungi was carried out as the first step in order to searching and exploring the potentialendophytes which capable in producing important metabolites and bioactive compounds. Research on endophytes is generally intended to isolate and identify bioactive materials produced by endophytic fungi [12,18,39]. Further research on selected fungal endophyte needs to be done for screening of isolates potentially as secondary metabolite and potential bioactive compounds.Furthermore, the research was carried out for screening potential isolates capable of producing antibiotic, antiviral, anticancer, immunomodulatory and antioxidant compounds [9].

4. Conclusions

Totally 88 selected endophytic fungal strains were isolated from roots, stems, barks, leaves, and fruits of 12 species of medicinal plants collected from Toba and Samosir Regency, North Sumatra Province, Indonesia. The fungal strains identification through morphological observation showed that selected fungal fungal endophytes were associated with host plant belonging to the taxa *Colletotrichum*, *Fusarium*, *Lasiodiplodia, Neopestalotiopsis, Phoma, Phomopsis*, *Phyllosticta*, *Schizophyllum, Trichoderma,* and *Xylaria*. In this study, endophytic *Phomopsis* dominating the obtained strains where 30% (27/88) strains of them were isolated from 8 species of medicinal host plants.The results of the study are expected to be initial microbial resources and information for screening and utilization of endophytic fungi through bioprospecting. Further bioprospection based research should be performed for screening of isolates potentially as secondary metabolite and potential bioactive compounds.

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