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Biodiversity of Endophytic Fungi Associated with *Uncaria gambier* Roxb. (Rubiaceae) from West Sumatra

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ABSTRACT

Endophytic fungi are specific microbes that live at a specific ecosystem in nature. Various endophytic fungi have been known posses an ability to produce a broad range of biologically active substances. Totally 53 endophytic filamentous fungi were isolated from leaves, stems, fruits and roots of the two plant varieties of *Uncaria gambier* Roxb. (Rubiaceae), i.e. gambir udang and gambir nasi. Morphological observation of 53 isolated endophytic filamentous fungi was further divide into two classes, Coelomycetes and Hypomycetes. Fifteen fungi isolates are unidentified due to lack of specific morphological characters. Morphologically identified fungi at genera level are: *Aspergillus*, *Cladosporium*, *Diaporthe*, *Fusarium*, *Penicillium*, *Pestalotiopsis*, *Phoma* and *Phomopsis*. Chemotaxonomic analysis base on their TLC chromatogram patterns of the ethyl acetate extract are in agreement to morphological grouping.

Key words: endophytic fungi, *Uncaria gambier* Roxb., morphological character, chemotaxonomy.

INTRODUCTION

Microorganism live in association with plant (endophytic microbes) is commonly observed in nature (Bacon and White, 2000). They are commonly found in Coniferae, Gramineae, and Ericaceae (Okane et al., 2001a), bakau *Bruguiera gymnorrhiza* (Okane et al*.,* 2001b, 2001c), *Theobroma cacao* (Rubini et al*.,* 2005), and *Camellia sinensis* (Agusta et al., 2006). Endophytic microbes, especially endophytic fungi have been recognized as a potential source of divers array for bioactive secondary metabolites (Tan and Zou, 2001), and hence Owen and Hundley (2004) proposed they are "the chemical synthesizer inside plant". Interestingly, endpophytic fungi especially *Taxomyces andreanae* which lives in *Taxus brevifolia* is able to mimic their host metabolite, taxol (Stierle et al., 1993). More examples, an unidentified endophytic fungus isolated from the plant *Nothapodytes foetida* is also produce camptotecin under laboratory conditions (Puri et al., 2005). On the other hand, the endophytic fungus *Diaporthe* sp. isolated from *Camellia sinensis* grow in Ciawi, Bogor, West Java is selectively transform natural catechin into leucoantosianidin in a semisynthetic medium (Agusta et al., 2005). In addition, the fungus *Diaporthe* sp. is also produce two rare bisanthraquinones namely (+)-2,2'-epicytoskyrin and (+)-1,1'-bislunatin that show a high cytotoxicity effect against KB cells (Agusta et al., 2006).

Based on the above information, it is economically quite promising to explore the physiological ability of endophytic fungi to produce secondary metabolite with various interests. One important endophytic is those fungi which live in (*Uncaria gambier* (Hunter) Roxb.) which never been explored so far. This paper will report diversity of endophytic fungi which live in two varieties of *U. gambier* namely var. udang and var. nasi.

MATERIALS AND METHODS

Plant materials

The young stems of *U. gambier* var. nasi and *U. gambier* var. udang were collected from Lembah Harau, Limapuluh Kota District, West Sumatra Province, in August 2007 and taxonomically identified at the Herbarium Bogoriense, the Research Centre for Biology, Indonesian Institute of Sciences.

Isolation of the endophytic fungi

The young stems were cut into pieces (ca. 1 cm in length) and washes with tap water for 10 min. The pieces were treated with 70% aq. EtOH for 1 min,

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5.3% aq. sodium hypochlorite for 5 min, and 75 % aq. EtOH for 0.5 min, and then cut into two pieces. The cut stems were placed on *corn-meal malt agar* (CMMA) medium containing 2% gambir leaf extract and 0.05 mg/mL chloramphenicol in a Petri dish and incubated at 27^{o}C. After 3 d, individual colonies were transferred onto PDA in a Petri dish and then incubated again at 27^{o} C for several days with periodic check for the purity to obtain the 53 endopyhtic filamentous fungi. The pure culture of endopyhtic filamentous fungi were then preserved in the medium containing 10 % glycerol and 5 % trehalose and placed at -80°C (Nakagiri, 2005).

Identification of endopyhtic filamentous fungi

Taxonomic identification of the isolated endophytic filamentous fungi were done through observation of macroscopic and microscopic characters according to Barnett (1955), Domsch et al. (1980), Ellis (1971), Kobayashi (1970), Samson et al. (1995), Sutton (1980) Webster (1980), and Barnett and Hunter (1998). All of observe fungi were grown on Potato Dextrose Agar Media at 27ºC. The following features of the appearance of cultures are recorded: texture, color, surface, elevation and margin. Microscopic observation was done by microscope Olympus type BX-51 (Olympus, Japan).

Cultivation of fungi for secondary metabolite production

The endophytic filamentous fungi were inoculated into 10 mL *potato dextrose broth* (PDB) and *glucose yeast peptone* (GYP) (20 g glucose, 1 g *yeast extract*, 5 g peptone, 0.5 g K_2HPO_4, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 1.0 g $CaCO_3$, 1 L H_2O, pH 6.44) in 100 mL Erlenmeyer flask. After cultivation for 3 weeks at room temperature (26-28^{o}C), the medium including the fungus bodies were extracted with ethyl acetate and analyzed by thin layer chromatography (TLC) with dichloromethane-methanol (20:1) as developing solvent. Profiles of TLC patterns are then observed under UV 254 nm and UV 365 nm, and by using spry reagents 1% Ce_2SO_4 in 10% H_2O_4.

RESULTS AND DISSCUSION

Totally 53 fungi isolates were obtained from *U. gambier* var. udang and var. nasi. From *U. gambier* var. udang were obtained 26 isolates, i.e. 7 isolates from (GUDP-1, GUDP3 ~ GUDP-8) from leaves, 12 isolates (GUBP-1, GUBP-3, GUBP-5 ~ GUBP-15) from stems, 6 isolates (GUFP-1 ~ GUFP-6) from fruits and 1 isolate (GUAP-1) from roots (Table 1). Whereas *U. gambier* var. nasi obtained 27 isolates, i.e. 8 isolates (GNDP-1 ~ GNDP-8) from leaves, 10 isolates (GNBP-1 ~ GNBP-10) from stems, 5 isolates (GNFP-1 ~ GNFP-5) from fruits and 4 isolates (GNAP-1 ~ GNAP-4) from roots (Table 2). Based on morphological characters (Table 1 and Table 2), show that there are 12 species of 8 genera. One isolate identified until family level, one isolate identified till class level and 15 isolates are sterile-hypha and thus could not be identified by morphological characters observation.

Morphological observation show that 2 fungi isolates obtained from roots (GNAP 2 and GNAP 5) belong to member of genus *Aspergillus*. One isolate obtained from leaf of *U. gambier* var. udang (GUBP 15) is also belonging to *Aspergillus*. Detail of morphological observations (microscopic and macroscopic) shows that there are four species of *Aspergillus* (Fig. 1A-D), which clearly shows that they are belong to different species. TLC chromatogram patterns of ethyl acetate extract of fungi cultures grown in PDB and GYP are also shows different profile reinforces those preposition.

In general, *Aspergillus* is able to grow on many kinds of habitat (cosmopolite), and the genera compose of 180 species of anamorph and 70 species of teleomorph (Samson et al., 1995). *Aspergillus* is commonly observed in soil and rhizosphere of horticulture (Moreau & Moss, 1979), and recently reported some species of *Aspergillus* are also evolve to be endophyte. In addition to well known endophytic fungi *Penicillium* and *Aspergillus* are residence inside plant tissues of *Melia azedarach* (Geris-dos-Santos et al., 2003). *Aspergillus fumigatus* CY018 is endophyte of *Cynodon dactylon* (Liu et al., 2004), and *Aspergillus niger* EN-13 is endophyte in alga *Colpomenia sinuosa* (Zhang et al., 2007).

On isolate fungus (GNDP-2) obtained from leaves and one isolate (GUFP-3) obtained from fruits of *U. gambier* var. nasi shows identical morphological characters each other. Both fungi are having micro- and macro-conidia as reproductive bodies. Half-moon shaped macroconidia (*pedicellate*) is a special asexual morphological characters of *Fusarium* (Summerel et al*.,* 2003). Both fungi isolates are also have similar number of macroconidia (Fig. 1E) and identical TLC patterns of ethyl acetate extract derived from fungus culture grown in PDB and GYP reinforce that both isolates are identical each other. So that, both fungi isolates must belong to the genus of *Fusarium* (Fig. 1E).

Most member of *Fusarium* are plant pathogenic, and recently some species is evolutionary adapted to plant tissue perform endophytic growth. *Fusarium oxysporum*, a non-pathogenic fungus associate with *Cucumis sativus,* can maintain the host survival under *Pythium ultimum* infection (Rubini et al*.,* 2005). *Fusarium sambucinum* associated with plant *Aphelandra tetragona* has unique ability to detoxify benzoxazolinone (BOA), a chemical constituent of their host (Zikmundova et al., 2002). Bacon and White (2000) reported intensively association between *Fusarium* and wild plant.

Coelomycetes are commonly isolated fungi in this research; out of 53 isolates obtained 11 isolates are belonged to these taxa. Coelomycetes is well known have strong association with higher plants as pathogenic or mutual symbiotic. Close relation

between plant and fungus enable to transfer material genetic among them (Tanaka et al., 1999). Many of Coelomycetes produce asexual spore (teleomorphic phase) as well as asexual spore (anamorphic phase) in their host, but fail to do so in a synthetic medium (Paulus et al., 2003). Member of Coelomycetes which produce asexual and sexual spores in culture could be identified into genera or special level. We

Table 1. Endophytic fungi isolated from the plant of *U. gambier* var. udang.

Plant organ	Isolates code	Taxon	Morphology description (*)
Root	GUAP-1	Dematiaceae	Colonies: effuse, greyish black, sterile. Mycelium: immersed and partly superficial, branched, septate, and dematiaceous.
Stem	GUBP-1	Dematiaceae	Colonies: effuse, dark grey to black, sterile. Mycelium: immersed, branched, septate, dematiaceous.
	GUBP-3	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli: dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
	GUBP-5	nd	Colonies: hairy, creamish, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUBP-6	Coelomycetes	Colonies: thick-cottony, creamish to pale green. Mycelium: immersed, branched, septate, hyaline. Sclerotia flattened, black, tuberculate.
	GUBP-7	nd	Colonies: thick-cottony, white creamish, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUBP-9	Coelomycetes	Colonies: cottony, greyish-brown. Mycelium: immersed, branched, septate, hyaline. Sclerotia: numerous, flattened, black, tuberculate.
	GUBP-10	Coelomycetes	Colonies: thick-cottony, creamish to pale green. Mycelium: immersed, branched, septate, hyaline. Sclerotia: flattened, black, tuberculate.
	GUBP-11	Coelomycetes	Colonies: cottony, greyish-brown. Mycelium: immersed, branched, septate, hyaline. Sclerotia: numerous, flattened, black, tuberculate.
	GUBP-12	Dematiaceae	Colonies: effuse, dark brown to black, sterile. Mycelium: immersed, branched, septate, dematiaceous
	GUBP-13	*Phoma* sp.	Colonies: cream grey, dense aerial mycelium. Pycnidia abundant, formed in concentric dark grey zone. Conidia hyaline, ellipsoid to cylindrical.
	GUBP-14	Coelomycetes	Colonies: cottony, white with distinct dark grey zonation, produce orange exudates, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUBP-15	*Aspergillus* sp.	Colonies: hairy-powdery, yellow. Conidial head dark yellow to orange, globose or radiate, uniseriate. Conidiophore smooth walled, colourless. Conidia smooth, globose or subglobose.
Leaf	GUDP-1	*Phomopsis* sp.	Mycelium: immersed, branched, septate, hyaline. Conidiomata: immersed, dark brown to black. α-conidia hyaline, fusiform, aseptate. β-conidia filiform, hyaline, aseptate.
	GUDP-3	Coelomycetes	Colonies: thick-cottony, white-creamish. Mycelium: immersed, branched, septate, hyaline. Sclerotia: flattened, black, tuberculate.
	GUDP-4	*Phoma* sp.	Colonies: cream grey with aerial mycelium, produce orange exudates. Pycnidia black. Conidia: hyaline, ellipsoid to cylindrical.
	GUDP-5	Dematiaceae	Colonies: effuse, dark grey to black, sterile. Mycelium: immersed, branched, septate, dematiaceous. Sclerotia: black, superficial.
	GUDP-6	nd	Colonies: hairy, white-creamish, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUDP-7	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli: dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
	GUDP-8	Coelomycetes	Colonies: cottony, white-greyish. Mycelium: immersed, branched, septate, hyaline. Sclerotia: numerous, flattened, black, tuberculate.
Fruit	GUFP-1	nd	Colonies: slow growing, cottony, white, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUFP-2	Coelomycetes	Colonies: cottony, grey-dark brown. Mycelium: immersed, branched, septate, hyaline. Sclerotia: black, tuberculate.
	GUFP-3	*Fusarium* sp.	Colonies: cottony, white, formed pink concentric zone. Macroconidia: hyaline, several celled, curved or bent at pointed ends, with pedicellate. Microconidia: hyaline, 1-celled, ovoid or oblong.
	GUFP-4	nd	Colonies: hairy, white, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUFP-5	nd	Colonies: hairy, pale brown, sterile. Mycelium: immersed, branched, septate, hyaline.
	GUFP-6	*Cladosporium* sp.	Colonies: effuse, velvety, dark olivaceous. Conidiophores dark, branched variously near apex, clustered or single. Conidia dark 1 or 2-celled, ovoid to cylindrical, in acropetalous chains.

Table 2 . Endophytic fungi isolated from the plant of *U. gambier* var. nasi.

Plant organ	Isolates code	Taxon	Morphology description (*)
Root	GNAP-2	*Aspergillus* sp.	Colonies: hairy-powdery, reverse colony brown-red. Conidial: head blue-green, radiate, uniseriate. Conidiophore: slightly granular, colourless. Conidia smooth, globose or subglobose
	GNAP-3	*Penicillium* sp.	Colonies: powdery, blue-grey. Conidiophore: simple branch, singly or less often in synnemata. Conidia: hyaline or light grey in mass, 1-celled, globose or ovoid.
	GNAP-5	*Aspergillus niger*	Colonies: hairy-powdery, black. Conidial head black, radiate. Conidiophore: smooth-walled or slightly ornamented, brown near apex. Conidia: ornamented, brown, globose to subglobose.
	GNAP-6	*Penicillium* sp.	Colonies: powdery, dark grey. Conidiophore: simple branch, singly or less often in synnemata. Conidia: hyaline or light grey in mass, 1-celled, smooth, globose or ovoid.
Stem	GNBP-1	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
	GNBP-2	nd	Colonies: thick cottony, pale brown, forming fruiting-body like, sterile. Mycelium: branched, septate, no clamp connection, hyaline.
	GNBP-3	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli: dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
	GNBP-4	*Phoma* sp.	Colonies: cream grey with aerial mycelium, formed dark and light grey zonation, produce orange exudates. Pycnidia black. Conidia: hyaline, ellipsoid to cylindrical.
	GNBP-5	Coelomycetes	Colonies: cottony, white creamish, produce yellow exudates. Mycelium: immersed, branched, septate, hyaline. Sclerotia: black, tuberculate.
	GNBP-6	Coelomycetes	Colonies: thick-cottony, grey brownish. Mycelium: immersed, branched, septate, hyaline. Sclerotia: flattened, black, tuberculate.
	GNBP-7	nd	Colonies: cottony, white-brown-green, produce brown exudates, sterile. Mycelium: immersed, branched, septate, hyaline.
	GNBP-8	Coelomycetes	Colonies: thick-cottony, white brownish. Mycelium: immersed, branched, septate, hyaline. Sclerotia: flattened, black, tuberculate.
	GNBP-9	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli: dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
	GNBP-10	*Diaporthe* sp.	Colonies: thick cottony white turn to dark yellow. Mycelium: immersed, branched, septate, hyaline. Sclerotia black, immersed. Ascoma a perithecium black, immersed in a stroma.
Leaf	GNDP-1	nd	Colonies: cottony, creamish-brown, produce brown exudates, sterile. Mycelium: immersed, branched, septate, hyaline.
	GNDP-2	*Fusarium* sp.	Colonies: cottony, white, formed pink concentric zone. Macroconidia: hyaline, several celled, curved or bent at pointed ends, with pedicellate. Microconidia hyaline, 1-celled, ovoid or oblong.
	GNDP-3	nd	Colonies: cottony, white-creamish, reverse colony brown, sterile. Mycelium: immersed, branched, septate, hyaline.
	GNDP-4	*Phoma* sp.	Colonies: cream grey with aerial mycelium, formed dakr and light grey zonation, produce orange exudates. Pycnidia black. Conidia: hyaline, ellipsoid to cylindrical.
	GNDP-5	*Aspergillus* sp.	Colonies: hairy-powdery, dark violet. Conidial: head globose or radiate, uniseriate. Conidiophore slightly granular, colourless. Conidia: ornamented, globose or subglobose
	GNDP-6	*Phoma* sp.	Colonies: cream grey with aerial mycelium, formed dakr and light grey zonation, produce orange exudates. Pycnidia black. Conidia: hyaline, ellipsoid to cylindrical.
	GNDP-7	*Phoma* sp.	Colonies: cream grey with aerial mycelium, formed dark and light grey zonation, produce orange exudates. Pycnidia black. Conidia: hyaline, ellipsoid to cylindrical.
	GNDP-8	*Pestalotiopsis* sp.	Mycelia: immersed, branched, septate, hyaline to pale brown. Acervuli dark, discoid or cushion-shaped, subepidermal. Conidia: dark, ellipsoid to fusifoid, 3-4 euseptate, with hyaline pointed end cells and 2-3 apical appendages.
Fruit	GNFP-1	nd	Colonies: cottony, white-creamish, sterile. Mycelium: immersed, branched, septate, hyaline
	GNFP-2	nd	Colonies: thick-cottony, white, sterile. Mycelium: immersed, branched, septate, hyaline
	GNFP-3	nd	Colonies: cottony, creamish-brown, produce brown exudates, sterile. Mycelium: immersed, branched, septate, hyaline.
	GNFP-4	nd	Colonies: cottony, white-creamish, reverse colony dark in center colony, sterile. Mycelium: immersed, branched, septate, hyaline.
	GNFP-5	nd	Colonies: cottony, white-grey-creamish, distinct zonation, sterile. Mycelium: immersed, branched, septate, hyaline

Note**:** nd = cannot identified due to lack of both asexual (anamorph phase) or sexual organelles (teleomorph phase) formation on PDA. (*) = The above identifications were conducted by incubation of endophytic fungi on PDA, at 26-28° C for 14-20 days.

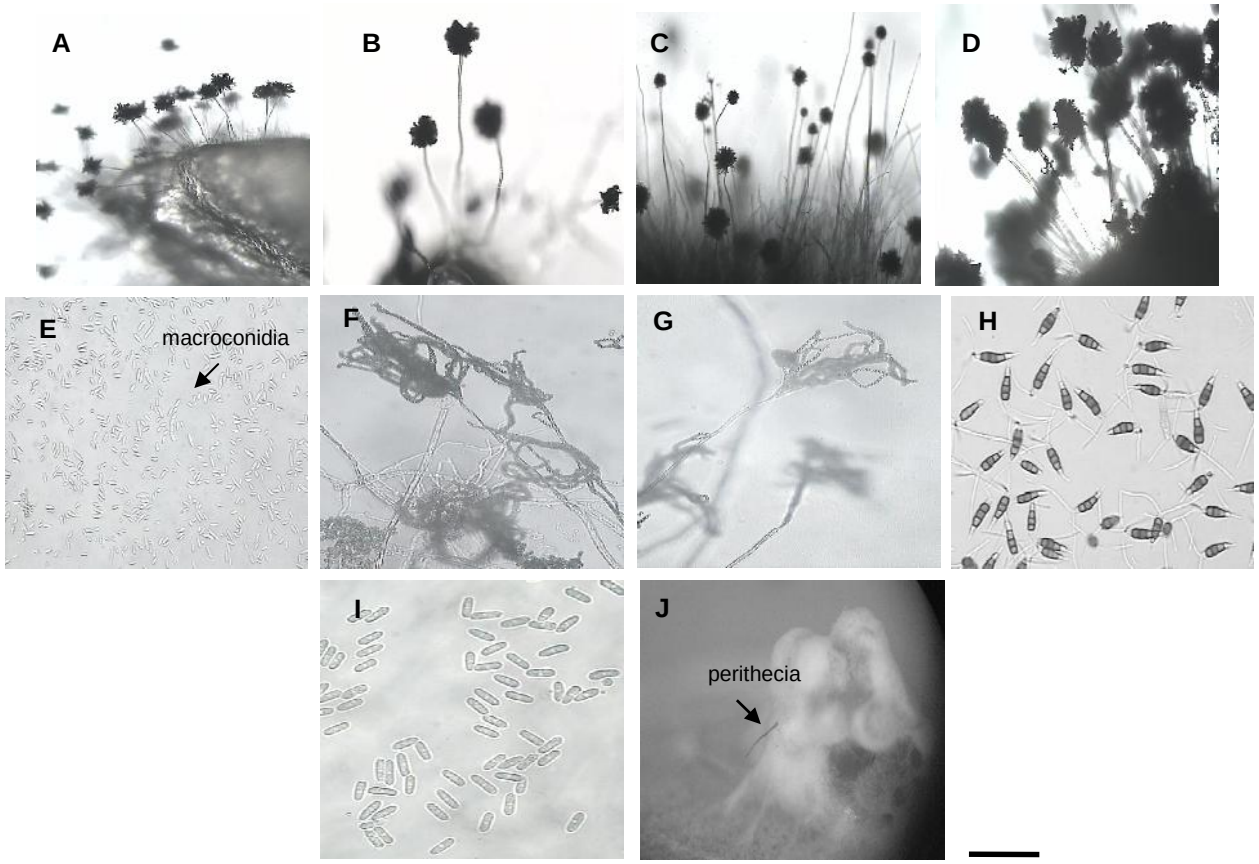

Figure 1. Microscopic structures of reproductive stages of endophytic fungi isolated from *U. gambier*. **A**. *Aspergillus* sp. GNAP-2; **B**. *Aspergillus* sp. GNDP-5; **C**. *Aspergillus* sp. GUBP-15; **D**. *Aspergillus niger*; **E**. *Fusarium* sp. GNDP-2); **F**. *Penicillium* sp. GNAP-3; **G**. *Penicillium* sp. GNAP-6; **H**. *Pestalotiopsis* sp.; **I**. *Phoma* sp.; **J**. *Diaporthe* sp. GNBP-10. Bar: A, B, C, D = 40 um, E, H, I = 10 um, F, G = 20 um, J = 50 um.

observed 13 isolates member of Coelomycetes produce asexual spores and identified into genera level, and they could be divided into 3 genera include *Pestalotiopsis* (GUBP-3, GUDP-7, GNBP-1, GNBP-3, GNBP-9, GNDP-8), *Phoma* (GUDP-4, GUBP-13, GNBP-4, GNDP-4, GNDP-6, GNDP-7) and *Phomopsis* (GUDP-1).

Pestalotiopsis is endophytic fungus which commonly isolated from higher plant than other member. Two isolates (GUDP-7, GUBP-3) isolated from var. udang, and 4 isolates (GNDP-8, GNBP-1, GNBP-3, GNBP9) from var. nasi are having identical morphological characters identified as *Pestalotiopsis*. Those isolates form spore as shown in Fig. 1H. Although they are morphologically identical each other, however TLC patterns of the ethyl acetate extract of culture medium are different. They form 4 different patterns employing that they are belong to 4 different species. The isolates of GNDP-8, GNBP-3 and GNBP-9 show their own specific TLC pattern, but GUDP-7, GUBP-3 and GNBP1 have identical profile and identified as *Pestalotiopsis* sp. GUDP.

From *U. gambier* var. udang obtained two isolates (GUDP-4, GUBP-13) which shows morphological characters similar to *Phoma*. The two fungus shows identical morphological characters similar to fungi namely GNDP-4, GNDP-6, GNDP-7, and GNBP-4 obtained from *U. gambier* var. nasi, thus the six fungi are identified as *Phoma* sp GUDP, and the six isolates shows similar TLC pattern (data not shown) which implies that those isolates produce similar secondary metabolites when grown in PDB and GYP. Anamorphic phase of *Phoma* is indicated by formation of conidia or phyliaspore inside pycnidia (Sutton, 1980). Grown on PDA, *Phoma* sp. GUDP produce a number phyliaspora (Fig. 1I).

One of the 14 isolated Coelomycetes (GNBP-10) produce black stick colored reproductive sexual (Fig. 1J), this is one character of *Diaporthe. Diaporthe* is endophytic fungi with wide range of host plant. Genera *Diaporthe* is a sexual stage (teleomorph), and mostly found in asexual stage (anamorphic stage) that belongs to genera of *Phomopsis* (Kobayashi, 1970). Morphologically *Diaporthe* is very difficult to be identified correctly since this genera is seldom to form perithecia grown in a synthetic medium. In a synthetic medium they mostly form pycnidia (Pioli et al., 2003).

Only two isolates (GNAP-3, GNAP-6) are isolated from root of var. nasi which identified as *Penicillium.* Species identification of *Penicillium* based on morphological observation is difficult (Samson et al., 1995). The two *Penicillium* isolated from var. nasi are also difficult to identify into species level. The two isolates have identical conidia (Fig. 1F and 1G), but

macroscopically the color of those isolates are different. The appearance of GNAP-3 colony is whitish blue, and GNAP-6 is deep blue. The TLC chromatogram patterns of fungi isolates (data not shown) are not identical implying that two isolates are different species. Generally *Penicillium* is saprophyte and some are parasitic to plant. *Penicillium* are endophytic in *P. janthinellum* and *Melia azedarach* (Marinho et al*.,* 2005), and *Taxus brevifolia* (Stierle et al., 1997).

We observed 15 sterile hypha, and they shows quite differs macroscopic view (Table 1 and Table 2). All of those isolates do not form sexual or asexual organelle grown in PDA till 60 days incubation. Identification for those isolates will be conducted in the future with molecular analyses of the ribosomal DNA.

CONCLUSIONS

We isolated 53 isolates of fungi from *U. gambier* var. nasi and var. udang. Based on morphological observation, those 53 isolates are belonged Coelomycetes and Hyphomycetes, 15 isolates are unidentified due to they form only sterile hypha. Identification at genera level, endophytic fungi obtained is *Aspergillus, Cladosporium, Diaporthe, Fusarium, Penicillium, Pestalotiopsis, Phoma,* and *Phomopsis*. Chemotaxonomy analyses based on TLC patterns of secondary metabolites extracted by ethyl acetate of cultures grown on PDB and GYP reinforce morphological identification, and grouping of endophytic fungi.

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