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Mushroom and macrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caused by *Colletotrichum coffeanum*

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Luo Yaling, Wattanachai Pongnak and Soytong Kasem (2014) Mushroom and macrofungi collection for screening bioactivity of some species to inhibit coffee antharcnose caused by *Colletotrichum coffeanum.* Journal of Agricultural Technology 10(4):845-861.

The 60 collected specimens from different locations in Thailand were morphological identified into 7 orders (Agaricales, Auriculariales, Boletetales, Cantharellales, Polyporales, Russulales, Xylariales), 17 Families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae). Descriptions of *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27 were described. Crude extracts were yielded from *L. fragilissimus* PH06, *C. strictipes* PH07, *Clitocybe* spp AJ2-2, *B. affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27. Result showed that the highest obtained from crude MeOH of *Lactarius* sp CH3-27, up to 6.76 %. The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against coffee anthracnise caused by *Colletotrichum coffaenum.* Result showed that Methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm, crude methanol from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 89.08 % for spore production of *C. coffaenum* at concentration of 100 ppm. Crude ethyl acetate from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 33.53 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm. Crude methanol from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 76.69 % for the spore production of *C. coffaenum* at the concentration of 100 ppm. These investigations are also reported for the first that time that *L. fragilissimus*, *C. strictipes*, *Clitocybe*, *B. affinis* var. *maculosus* and *Lactarius* have shown some antimicrobial substances against coffee anthracnose canused by C. coffaenum. Further investigation would be studies on chemical elucidation of these antagonistic substances.

Keywords: Mushrooms, Agaricales, Crude extracts, Morphological identify

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Introduction

Basidiomycota are macrofungi characterized by a multi-layered cell walls, barrel-shaped structures or pulley wheel occlusions at the septa of hyphae (dolipore septa), an extended dikaryophase, clamp connections that often develop on septa, and the formation of basidia that produce basidiospores at the tips of sterigmata (Kendrick, 2000). Almost 30,000 species have been found and described (Kirk *et al*., 2001). Basidiomycetes are mostly being saprobes, symbionts and play ecologically important roles, such as oxygen, carbon and nitrogen cycling. Humans are first attracted to mushrooms since ancient times because of their edible or poisonous traits. Mushrooms are an important group in the biosphere and their significance in diversity and conservation issues have been recognized extensively (Kaul, 2001).

Agaricales comprises the so-called mushrooms and toadstools, and is the largest clade of mushroom-forming fungi. More than 9000 species in more than 300 genera, and 26 families had been described. Mostly they are terrestrial, lignicolous and saprobic, and many are mycorrhizal fungi (Kirk *et al*., 2001). It is a class of widely distributed around the world, camp life and play an important economic saprophytic fungi. Their morphological characteristics were investigated through the canopy fleshy, smooth or scaly. Spores are oval or elliptical, smooth, dark brown or purple-brown (Hui and Changbiao, 2005). Field classification features are gill free, and easy separation with stipe, first as a white to pink, light brow, brown or dark brown when mature; a ring, single or double and spore print. They can grow in forests, grasslands, fields, farm, roadsides, gardens and other places (Hui, 2006, Rui-Lin *et al*., 2008; 2012; 2013).

The majority of mushrooms are edible, medicinal or health care value, development value is high. For example, *Agaricus bisporus* (Jellange) Imbach, ocher scaly mushrooms *A. crocopeplus* Berk, woodland mushrooms *A. silvaticus* Schaeff, large purple mushroom *A. augustus* Fr, white mushrooms *A. bernardii* (Quél.) Sacc, big fat mushrooms *A. bitorquis* (Quél.) Sacc and the four spore mushrooms *A. campestris* L. They have long been carried out artificial cultivation in order to serve people to edible. *Agaricus subrufescens* Peck are reported to make a liquid fermentation and found the mycelia contains large amounts of polysaccharides and other biologically active substances for human body's immune system regulating function (Genpei and Jigui, 2008).

Moreover, the wild mushrooms *A. arvensis* Schaeff and Brazil mushrooms *A. blazei* Murr, etc. can affect in lowering blood sugar, improve arteriosclerosis and suppress cancer cell lines (Xiaoping and Junyan, 2007).

The objective was to collect and find out the metabolites from some mushrooms against coffee anthracnose caused by *Colletotrichum coffeanum*.

Materials and methods

Collection and identification

Mushroom samples were collected during the raining season from July, 2013 to October, 2013. Collection was made in the forests and grass areas in 5 provinces of Thailand, which are Chanthaburi, Chiangrai, Phetchabuti, Kanchanaburi and Bangkok Provinces. Each collection site was recorded the macroclimates, chemical test and photograph of fresh specimens. Spore prinit was done as necessary in the collection sites. The specimens were brought to laboratory for further works, imorphologically identification and isolation to pure cultures. The field trip was followed the instruction described by Largent (1986).

Isolation of pathogen and pathogenicity test

Colletotrichum coffeanum causing anthracnose of coffee var arabica was isolated from leaf symptom by tissue transplanting techniques and performed pathologenicity test followed Koch's Postulate.

Extraction of biological active substances

The bioactive compounds were extracted from some selected species of Agaricales as crude extracts. The extraction was performed using the method of Kanomedhakul *et al*. (2006). Some species of Agraricaeae were cultured in potato dextrose broth (PDB) at room temperature (28-30 C) for 45 days. Fungal biomass were collected by moving from PDB, filtered through cheesecloth and air-dried overnigrht. Fresh and dried fungal biomass was recorded. Dried fungal biomss were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 24 hour at room temperature. The filtrate from ground biomass was separated by filtration through Whatman No.4 filter paper. The filtrate was evaporated in *vacuo* to yield crude extract. The marc was further extracted with ethyl acetace (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. Each crude extract was weighted, and then kept in refrigerator at 4 C until use.

Biological activity against coffee anthracnose caused by C. coffeanum

The crude extracts were tested for inhibition of the most aggressive isolate of *C. coffeanum*. The experiment was conducted by using 3 x 6 factorial in Completely Randomized Design (CRD) with four replications. Factor A

represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations 0, 10, 50, 100 and/or 500, and 1,000 μg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxiden (DMSO), then mixed into potato dextrose agar (PDA) before autoclaving at 121C, 15 1bs/inch^2 for 30 minutes. The tested pathogen were cultured on PDA and incubated at room temperature for 5 days, and then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room tmperature (28-30C) for 5 days. Data were collected as colony diameter and computed the percentage of inhibition. Data were statistically computed analysis of variance. Treatment means were compared with DMRT at P=0.05 and P=0.01.

Results

Collection and identification

57 specimens were collected from five provinces of six points in Thailand. These were morphologically identified into 7 orders (Agaricales, Auriculariales, Boletetales, Cantharellales, Polyporales, Russulales, Xylariales), 17 Families (Agaricaceae, Auriculariaceae, Boletaceae, Cantharellaceae, Clavariaceae, Exidiaceae, Hydnangiaceae, Inocybaceae, Lyophyllaceae, Marasmiaceae, Mycenaceae, Pleurotaceae, Polyporaceae, Russulaceae, Schizophyllaceae, Tricholomataceae, Xylariaceae). Thease were morphological identified into 57 species as follows:- *Agaricus macrosporus*, *Agaricus* spp., *Auricularia auricular*, *Boletus affinis var. maculosus*, *Boletus retisporus*, *Cantharellus cibarius*, *Clavulinopsis fusiformis*, *Clavulinopsis helvola*, *Clitocybula atrialba*, *Clitocybe* spp., *Collybia dryophila*, *Collybia iocephala*, *Collybia strictipes*, *Collybia* spp., *Coprinus* spp., *Inocybe fastigiata*, *Tricholoma* spp., *Lactarius controversus*, *Lactarius sanguifluus*, *Lactarius* spp., *Laccaria vinaceoavellanea*, *Laccaria* spp., *Leucocoprinus fragilissimus*, *Marasmiellus albuscorticis*, *Marasmiellus ramealis*, *Marasmius androsaceus*, *Marasmius foetidus*, *Marasmius purpureostriatus*, *Marasmius oreades*, *Marasmius plicatulus*, *Marasmius scorodonius*, *Marasmius* spp., *Mycena inclinata*, *Mycena rosella*, *Mycena subcaerulea*, *Mycena vulgaris*, *Mycena* spp., *Pleurocybella porrigens*, *Pluerotus giganteus*, *Resinomycena rhododendri*, *Russula crassotunicata*, *Russula* spp., *Schizophyllum commune*, *Termitomyces microcarpus*, *Trametesversicolor*, *Tremiscus* spp., *Termitomyces* spp., *Tricholoma* spp. and *Xylaria hypoxylon* as seen in Table 1.

Table 1. Collection of specimens

Species	Locations	Family/Order	Specimen No.
Agaricus macrosporus	Chanthaburi province, Amphoe Khao Khichakut	Agaricaceae, Agaricales	CH02
Agaricus spp	Chanthaburi province, Amphoe Khao Khichakut	Agaricaceae, Agaricales	CH3-25
Auricularia auricula	Phetchabuti Privince, Ampkoe Khao Khichakut	Auriculariaceae, Auricuriales	PH15
Boletus affinis var. *maculosus*	Kanchanaburi Province, AmphoeMueangKanchanaburi	Boletaceae, Boletale	AJ2-3
Boletus retisporus	Chiangrai Province, Chiang Kong	Boletaceae, Boletale	AJ07
Cantharellus cibarius	Chiangrai Province, Chiang Kong	Cantharellaceae, Cantharellales	AJ03
Clavulinopsis fusiformis	Chanthaburi province, Amphoe Khao Khichakut	Clavariaceae, Agaricales	CH3-15a
Clavulinopsis helvola	Chanthaburi province, Amphoe Khao Khichakut	Clavariaceae, Agaricales	CH3-15b
Clitocybula atrialba	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-08
Clitocybe spp	Kanchanaburi Province, AmphoeMueangKanchanaburi	Tricholomataceae Agaricale	AJ2-2
Clitocybe spp	Kanchanaburi Province, AmphoeMueangKanchanaburi	Tricholomataceae Agaricale	AJ2-5
Collybia dryopjila	Chanthaburi province, Amphoe Khao Khichakut	Tricholomataceae Agaricales	CH3-26
Collybia iocephala	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae Agaricales	PH11
Collybia strictipes	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae Agaricales	PH07
Collybia spp	Bangkok Province, Khet Lat Krabang(KMITL)	Tricholomataceae Agaricales	LB2
Coprinus spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Agaricaceae, Agaricales	PH09
Inocybe fastigiata	Kanchanaburi Province, AmphoeMueangKanchanaburi	Inocybaceae, Agaricales	AJ2-4
Lactarius controversus	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-20
Lactarius sanguifluus	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-06
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-01
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-24
Lactarius spp.	Chanthaburi province, Amphoe Khao Khichakut	Russulaceae, Russulales	CH3-27
Laccaria	Kanchanaburi Province,	Hydnangiaceae,	AJ2-1

vinaceoavellanea	AmphoeMueangKanchanaburi	Agaricales	
Laccaria spp	Chanthaburi province, Amphoe Khao Khichakut	Hydnangiaceae, Agaricales	CH3-13
Leucocoprinus fragilissimus	Phetchabuti Privince, Ampkoe Khao Khichakut	Agaricaceae, Agaricales	PH06
Marasmiellus albuscorticis	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-12
Marasmiellus ramealis	Kanchanaburi Province, Amphoe Sai Yok	Agaricaceae, Agaricales	SY09
Marasmius androsaceus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-04
Marasmius foetidus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-17
Marasmius purpureostriatus	Kanchanaburi Province, Amphoe Sai Yok	Marasmiaceae, Agaricales	SY16
Marasmius oreades	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-22
Marasmius plicatulus	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-18
Marasmius scorodonius	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-21
Marasmius spp.	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-02
Marasmius spp.	Chanthaburi province, Amphoe Khao Khichakut	Marasmiaceae, Agaricales	CH3-23
Marasmius spp.	Phetchabuti Privince, Ampkoe Khao Khichakut	Marasmiaceae, Agaricales	PH08
Marasmius spp.	Kanchanaburi Province, Amphoe Sai Yok	Marasmiaceae, Agaricales	SY02
Mycena inclinata	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-11
Mycena rosella	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-03
Mycena subcaerulea	Chanthaburi province, Amphoe Khao Khichakut	Mycenaceae, Agaricales	CH3-07
Mycena vulgaris	Kanchanaburi Province, AmphoeMueangKanchanaburi	Mycenaceae, Agaricales	AJ2-06
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY01
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY03
Mycena spp	Kanchanaburi Province, Amphoe Sai Yok	Mycenaceae, Agaricales	SY05
Pleurocybella porrigens	Phetchabuti Privince, Ampkoe Khao Khichakut	Marasmiaceae, Agaricales	PH13
Pluerotus giganteus	Phetchabuti Privince, Ampkoe Khao Khichakut	Pleurotaceae, Agaricales	PH05
Resinomycena	Chanthaburi province,	Mycenaceae,	CH3-16

rhododendri	Amphoe Khao Khichakut	Agaricales	
Russula crassotunicata	Chiangrai Province, Chiang Kong	Russulaceae, Russulales	AJ06
Russula spp	Chiangrai Province, Chiang Kong	Russulaceae, Russulales	AJ01
Schizophyllum commune	Kanchanaburi Province, Amphoe Sai Yok	Schizophyllaceae, Agaricales	SY13
Termitomyces microcarpus	Chanthaburi province, Amphoe Khao Khichakut	Tricholomataceae, Agaricales	CH3-14
Trametesversicolor spp	Chanthaburi province, Amphoe Khao Khichakut	Polyporaceae , Trametes	CH3-05
Tremiscus spp	Chanthaburi province, Amphoe Khao Khichakut	Exidiaceae, Auriculariales	CH3-09
Termitomyces spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Lyophyllaceae, Agaricales	PH03
Tricholoma spp.	Chanthaburi province, Amphoe Khao Khichakut	Tricholomatacea Agaricales	CH2-09
Tricholoma spp	Phetchabuti Privince, Ampkoe Khao Khichakut	Tricholomataceae, Agaricales	PH02
Xylaria hypoxylon	Chanthaburi province, Amphoe Khao Khichakut	Xylariaceae, Xylariales	CH3-19

Descriptions of *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01and *Lactarius sp* CH3-27 are described as follows:-

***Leucocoprinus fragilissimus* PH06**

A small, white or nearly transoarent, easy to crack mushroom. Cap 2.4 cm in diameter, flat with a distinct yellow umbo, sometimes broadly bel-shaped, white, nearly transprrent, margin clearly lined, thick, small yellow scales. Gill free, white, unequal length. Stem 3.5 x 0.1 cm, very slim, white, ring small, easily detachable in the lower part of the stem; Habitat grows in grassland or tea garden (Fig.1).

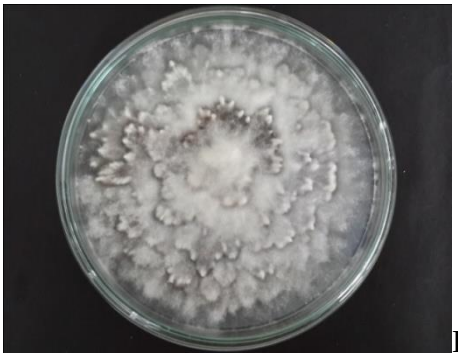
 A  B

Fig .1. *Leucocoprinus fragilissimus;* A: Fruiting bodies in the field B: Pure culture

Collybia strictipes PH07

A white, brittle mushroom. Cap 4.5 cm in diameter, bell-shaped with margin remaining inrolled and clearly lined, smooth. Gill free, pink, broad, unequel lenghth. Stem 4.5 x0.5 cm, white, fresh, smooth, peanut smell. Habitat scattered in grassland (Fig.2).

 A  B

Fig .2. *Collybia strictipes;* A: Fruiting body in the field B: Pure culture

Clitocybe spp AJ2-2

Cap 0.5-7 cm across, purperish to pink to pale brown, horn with strongly depress in the center and inrolled margin becoming wavy. Gills decurrent, white to olive-yellow. Stem *3.5*-9 cm, cylindrical, smooth, pink to dark brown. Habitat grows in clusters (Fig. 3).

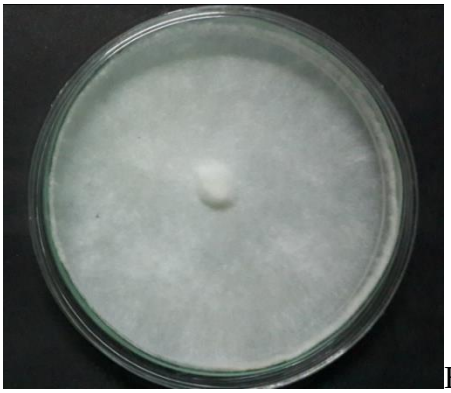

A

B

Fig. 3. *Clitocybe* spp; A: Fruiting bodies in the field*;* B:Pure culture

Boletus affinis var. *maculosus* AJ2-3

Cap 1-3.5 cm across, velvety redish-brown, dry shin, having a membraneous vein on the top part which promptly turns to tobacco color due to the falling spores. Gills adnate, white. Stem *6*-9 cm long, cylindrical, silky membranous, smooth. Habitat grows in clusters (Fig. 4).

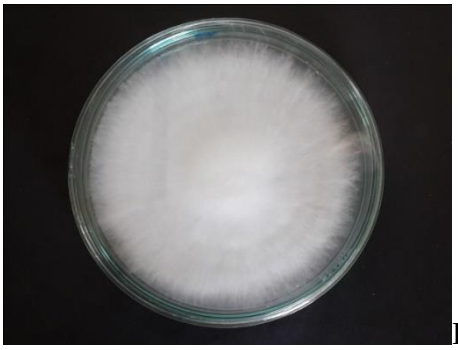

A

B

Fig. 4. *Lactarius* sp CH3-01; A: Fruiting bodies in the field B: Pure culture

Lactarius sp CH3-01

A flesh mushroom, fruit body makes people think pf milk. *Cap* 0.5-4 cm in diameter, convex, smooth, cream yellow with white, slight incurrent margin with not clearly lined, Color changes to buff when dry; Gill, free, close, cream yellow to pink; Flesh white, Stem 0.5-6 X 0.1-0.5 cm, white then becoming buff, smooth, having rooting base, spore print brown. Habitat scatter in sandy soild (Fig. 5).

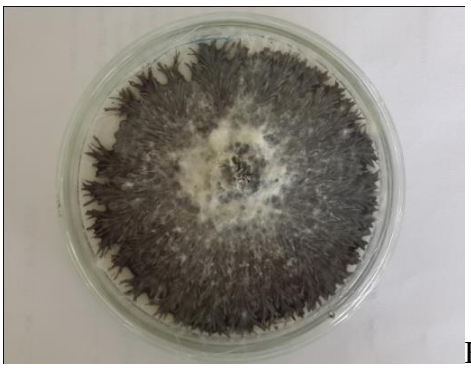
 A  B

Fig. 5. *Lactarius* sp; A: Fruiting bodies in the field B: Pure culture

Lactarius sp CH3-27

Cap 10 cm in diameter, flat with a white strongly depress in the center, reddish brown with lined, dark scales including the wavy margin. Gills decurrent, pink, close, equel. Stem 7x0.7cm, dark brown, cylindrical, downy the part attach gills are red. Habitat grows singly in soil (Fig. 6).

 A  B

Fig.6. *Lactarius* sp. A: Fruiting body in the field B: Pure culture

Extraction of biological active substances

Pure cultures of *L. fragilissimus* PH06, *C. strictipes* PH07, *Clitocybe* spp AJ2-2, *B. affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01, *Lactarius* sp CH3-27 (Fig. 1-6) were isolated from fruting bodies and were separately cultured in PDB for 45 days. Each fungal biosmass was separately extracted to get crude hexane, crude ethyly acetate and crude methanol. With this, the crude

hexane, crude ethyly acetate and crude methanol from *L. fragilissimus* PH06 yielded 0.12, 1.12 and 4.06 %, respectively. The crude hexane, crude ethyl acetate and crude methanol from *C. strictipes* PH07 yielded 0.36, 0.36 and 0.40 %, respectively. The crude hexane, crude ethyly acetate and crude methanol from *Clitocybe* spp AJ2-2 yielded 5.92, 5.48 and 5.99%, respectively. The crude hexane, crude ethyly acetate and crude methanol from *B. affinis* var. *maculosus* AJ2-3 yielded 0.43, 0.47 and 5.32 %, respectively. The crude hexane, crude ethyl acetate and crude methanol from *Lactarius* sp CH3-01 yielded 0.54, 2.12 and 5.03 %, respectively. The crude hexane, crude ethyly acetate and crude methanol from *Lactarius sp* CH3-27 yielded 3.88, 5.49 and 6.76 %, respectively (Table 2).

Table 2. Extraction of biological active substances from biomass culture for 45 days

Specimens	Fresh weight (g)	Fresh weight (g)	Yield[1], %	Crude Hexane(g)	Crude EtOAc(g)	Crude MeOH(g)
PH06 *L. fragilissimus*	3927	124.65	3.17	0.15 (0.12%)	1.39 (1.12%)	5.06 (4.06%)
PH07 *Collybia strictipes*	2010	55.00	2.73	0.2 (0.36%)	0.2 (0.36%)	0.22 (0.40%)
AJ2-2 *Clitocybe* spp	2500	72.10	2.88	4.27 (5.92%)	3.95 (5.48%)	4.32 (5.99%)
AJ2-3 *Boletus affinis var. maculosus*	5230	91.56	1.75	0.39 (0.43%)	0.43 (0.47%)	4.87 (5.32%)
CH3-01 *Lactarius* spp	1920	79.10	4.12	0.43 (0.54%)	1.68 (2.12%)	3.98 (5.03%)
CH3-27 *Lactarius* spp	4200	140.00	3.33	5.43 (3.88%)	7.69 (5.49%)	9.46 (6.76%)

[1](%)Yield = Weight after drying/ Weight before drying x 100%

Biological activity against coffee anthracnose caused by C. coffeanum

The crude extarcts from *Clitocybe* sp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 were selected for bioactivity test against coffee anthracnose caused by *C. coffaenum*. Result showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm when compared to the control (Table 3). Crude methanol from *Clitocybe* sp AJ2-2 gave significantly highest inhibited the spore production of *C. coffaenum* as 89.08 % and followed by crude ethyl acetate inhibited 86.48 % and crude hexane 70.45 % (Tables 4). The ethyl acetate crude extract from *B. affinis* var. *maculosus* AJ2-3 gave significantly highest inhibition of 33.53 % for the colony growth of *C.*

coffaenum at the concentration of 1,000 ppm when compared to the control (Table 5). Crude methanol and ethyl acetate from B. affinis var. maculosus AJ2-3 gave significantly highest inhibited the spore production of C. coffaenum as 67.86 % and followed by crude hexane inhibited 55.95 % (Tables 6).

Table 3. Crude extracts of *Clitocybe* sp AJ2-2 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

Crude extracts	**Concentration (ppm)**	**Colonydiameter (cm)[/1]**	**Growth inhibition(%)[/2]**
Crude Hexane	0	4.97^{a}	0.00^{g}
	10	4.92^{ab}	1.02^{fg}
	50	4.90^{ab}	1.53^{fg}
	100	4.82^{ab}	3.03^{efg}
	500	4.70^{bc}	5.54^{ef}
	1000	4.57^{cd}	8.04^{de}
Crude EtOAc	0	4.98^{a}	0.00^{g}
	10	4.87^{ab}	2.56^{fg}
	50	4.72^{bc}	4.27^{efg}
	100	4.70^{bc}	5.76^{ef}
	500	4.42^{d}	11.29^{d}
	1000	4.17^{e}	17.30^{c}
Crude MeOH	0	5.00^{a}	0.00^{g}
	10	4.77^{abc}	3.00^{efg}
	50	4.85^{ab}	4.75^{efg}
	100	4.45^{d}	12.50^{d}
	500	3.85^{f}	23.00^{b}
	1000	3.50^{g}	30.00^{a}
C.V.(%)		3.05	27.68

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 4. Spore production inhibition of crude extracts from *Clitocybe* sp AJ2-2 to *Colletotrichum coffaenum* at 30days

Crude extracts	Concentration (ppm)	Number of spores /1(10x6)	Inhibition(%)/2\
	0	7.38def	0.00bc
	10	3.69efg	54.74ab
Crude Hexane	50	3.06fg	63.81a
	100	2.63fg	70.45a
	0	7.38def	0.00bc
Crude EtOAc	10	2.56fg	65.55a
	50	1.75g	76.06a
	100	1.00g	86.48a
Crude	0	7.38def	0.00bc
MeOH	10	3.69efg	51.34ab
	50	1.56g	78.67a
	100	0.81g	89.08a
C.V.(%)		3.05	31.43

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Table 5. Crude extracts of *Boletus affinis* var. *maculosus* AJ2-3 testing for growth inhibition of *Colletotrichum coffaenum* at 5days

Crude extracts	Concentration (ppm)	Colonydiameter (cm)/1	Growth inhibition(%)/2
	0	5.00a	0.00h
	10	4.80bc	4.00fg
Crude Hex	50	4.72cd	5.50fg
	100	4.40e	12.00e
	500	4.15gh	17.00 cd
	1000	3.82i	23.50b
	0	4.92ab	0.00h
	10	4.20fg	14.72de
Crude EtOAc	50	4.17fgh	15.23cde
	100	4.05h	17.77cd
	500	3.70i	23.86b
	1000	3.27j	33.53a
	0	4.97a	0.00h
	10	4.80bc	3.53g
Crude	50	4.62d	7.03f
MeOH	100	4.30ef	12.06e
	500	4.30ef	12.06e
	1000	4.07gh	18.34c
C.V.(%)		2.17	13.87

[1]Average of four replications. Means followed by a common letter are not significantly differed by DMRT at P=0.01.

[2]Inhibition(%)=R1-R2/R1x100 where R1 was colony diameter of pathogen in control and R2 was colony diameter of pathogen in treated plates.

Table 6. Spore production inhibition of crude extracts from *Boletus affinis* var. *maculosus* AJ2-3 to *Colletotrichum coffaenum* at 30days

Crude extracts	Concentration (ppm)	Number of spores [1] (10^{x6})	Inhibition(%)[2]
Crude Hexane	0	1.56^{cde}	0.00^{b}
	10	1.13^{cde}	27.98^{ab}
	50	0.75^{de}	51.78^{ab}
	100	0.69^{de}	55.95^{ab}
Crude EtOAc	0	1.56^{cde}	0.00^{b}
	10	1.50^{cde}	3.57^{ab}
	50	1.25^{cde}	19.64^{ab}
	100	0.50^{e}	67.86^{a}
Crude MeOH	0	1.56^{cde}	0.00^{b}
	10	0.50^{e}	67.86^{a}
	50	0.50^{e}	67.86^{a}
	100	0.50^{e}	67.86^{a}
C.V.(%)		19.67	12.63

[1]Average of four replications, Means followed by a common letter are not significantly differed by DMRT at P=0.05.

[2]Inhibition (%) = R1-R2/R1x100 where R1was number of pathogen spores in control and R2 was number of pathogen spore in treated plate which number of spores are less than that in control .

Discussion

Thease were motphological identified into 49 species as follows:- *Agaricus macrosporus, Agaricus spp., Auricularia auricular, Boletus affinis var. maculosus, Boletus retisporus, Cantharellus cibarius, Clavulinopsis fusiformis, Clavulinopsis helvola, Clitocybula atrialba, Clitocybe spp., Collybia dryophila, Collybia iocephala, Collybia strictipes, Collybia spp., Coprinus spp., Inocybe fastigiata, Tricholoma spp., Lactarius controversus, Lactarius sanguifluus, Lactarius spp., Laccaria vinaceoavellanea, Laccaria spp., Leucocoprinus fragilissimus, Marasmiellus albuscorticis, Marasmiellus ramealis, Marasmius androsaceus, Marasmius foetidus, Marasmius purpureostriatus, Marasmius oreades, Marasmius plicatulus, Marasmius scorodonius, Marasmius spp., Mycena inclinata, Mycena rosella, Mycena subcaerulea, Mycena vulgaris, Mycena spp., Pleurocybella porrigens, Pluerotus giganteus, Resinomycena rhododendri, Russula crassotunicata, Russula spp., Schizophyllum commune, Termitomyces microcarpus, Trametesversicolor, Tremiscus spp., Termitomyces spp., Tricholoma spp. and Xylaria hypoxylon.* With this, there are some literature reviews found those species in Thailand (Akom, 1996; David and Brian, 1992; Gary, 1981; Soytong,

1994; Konemann, 1998; Smith, 2001; Roger, 1991; States, 2004; Susan and Van, 2000). *Leucocoprinus fragilissimus* PH06, *Collybia strictipes* PH07, *Clitocybe* spp AJ2-2, *Boletus affinis* var. *maculosus* AJ2-3, *Lactarius* sp CH3-01 and *Lactarius* sp CH3-27 were described which these species reported to be found in Thailand (Konemann, 1998; Roger, 1991; States, 2004; Susan and Van, 2000).

As result showed that methanol crude extract from *Clitocybe* sp AJ2-2 gave significantly highest inhibition of 30 % for the colony growth of *C. coffaenum* at the concentration of 1,000 ppm. Crude methanol from *Clitocybe* sp AJ2-2 inhibited the spore production of *C. coffaenum* as 89.08 % and followed by crude ethyl acetate inhibited 86.48 % and crude hexane 70.45 %. It was also found that crude methanol and ethyl acetate of *B. affinis* var. *maculosus* AJ2-3 inhibited spore production of *C. coffaenum* 67.86 % and followed by crude hexane inhibited 55.95 %. The research findings are reported for the first time that the metabolites from Clitocybe sp AJ2-2 and B. affinis var. maculosus could inhibit *C. coffaenum* causing coffee anthracnose. Similar report from Badalyan *et al.* (2002) stated that the antagonistic activity of 17 species of Basidiomycotina (*Coriolus versicolor, Flammulina velutipes, Ganoderma lucidum, Hypholoma fasciculare, H. sublateritium, Kühneromyces mutabilis, Lentinula edodes, Lentinus tigrinus, Pholiota alnicola, Ph. aurivella, Ph. destruens, Pleurotus ostreatus, P. cornucopiae, Polyporus squamosus, P. subarcularius, P. varius* and *Schizophyllum commune*) could inhibit plant pathogens, *Bipolaris sorokiniana, Fusarium culmorum, Gaeumannomyces graminis* var. *tritici* and *Rhizoctonia cerealis* that causing foot and root diseases of winter cereals.

The potential of fungal metabolites from fungi have been usually reported to produce antibiotic substances against human and plant pathogens. Kanokmedhakul *et al.* (2003) reported that a macrofungi, *Scleroderma citrinum* produces a bioactive triterpenoid and vulpinic acid derivatives that expressed against *Candida albicans*. Morober, Soytong *et al.* (2014) reported that the natural products were isolated from the fruiting bodies of *Scleroderma citrinum*. A new lanostane-type steroids were found namely 4,4'-Dimethoxymethyl vulpinate (DMV) and 4,4'-Dimethoxyvulpinic acid (DMVA). These compounds showed that 4,4'-Dimethoxyvulpinic acid inhibited *Colletotrichum gloeosporioides* than 4,4'-Dimethoxymethyl vulpinate at all tested concentrations. The effective dose (ED_{50}) of DMVA compound could inhibit the mycelium growth of *C. gloeosporioides* at the concentrations of 81 ppm, respectively. The ED_{50} of DMV compound for inhibition of mycelial growth was 2,114 and 5,231 ppm, respectively. The production of conidia of *C. gloeosporioides* was inhibited by both compounds which the ED_{50} of DMA and

DMVA compounds were 45 and 68 ppm, respectively. Rieger *et al* (2010) reported that pure culture of Basidiomycete, *Carpia montagnei* produced caripyrin as a new pyridylooxirane that inhibited *Magnaporthe oryzae* causing rice blast pathogen. These investigations were found biological active substances from *Clitocybe* spp AJ2-2 and *B. affinis* var. *maculosus* AJ2-3 to inhibit coffee anthracnose caused by *C. coffaenum.* The control mechanism would be involved in bioactive compound producing from these mushroom which possible be elucidated in further search finding.

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References

Akom Kongton (1996). Mushrooms and Toxic mushrooms in Thainland. Thainland, pp. 65.

David Pegler and Brian Spooner (1992). The Mushroom IDENTIFIE. New Burlington Books, pp. 39.

Gary, H. Lincoff (1981). The Audubon Society Field Guide to North American Mushrooms. Alfred A. Knop. US and simultaneously in Canada, pp. 299.

Genpei Yu and Jigui Bao (2008). Agaricus (Iwade 101) Applied Research in immune regulation . Shanghai Journal of Preventive Medicine 20(5):235–237.

Hui Zhang and Changbiao Li (2005). Agaricus antioxidant activity of polysaccharides. Chinese mushroom.

Hui Zhang (2006). Agaricus extracellular immunomodulatory activity of fermented AbEXP . Sheng yang institute of Chemical Technology 21(2):102-104.

Kanokmedhakul, S., Kanokmedhakul, K., Prajuabsuk, T., Soytong, K., Kongsaeree, P. and Suksamran (2003). A bioactive triterpenoid and vulpinic acid derivatives from *Scleroderma citrinum*. Planta Medica 69:566-568.

Soytong, K., Phoutthasone Sibounnavong, Kwanchai Kanokmedhakul and Somdej Kanokmedhakul (2014). Biological active compounds of Scleroderma citrinum to inhibit plant pathogenic fungi. Journal of Agricultural Technology 10(1):79-86.

Soytong, K. (1994). Mushrooms and macrofungi in Thailand. Alfred A. Siritham Offset in Thailand, pp. 106.

Kaul, T.N. (1997). Introduction to mushroom science (systematics). New Hampshire. Science Publishers Inc.

Kendrick, B. (2000). The fifth kingdom 3rd. City name The USA. Focus Publishing.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2001). Dictionary of the Fungi 9th ed. London. CAB International.

Konemann (1998). The Great Encyclopedia of Mushrooms. Neue Staling, Oldenburg. Germany. pp. 134.

Rieger Patrick H, Liermann Johannes C, Till Opatz, Heidrun Anke and Eckhard Thines (2010). Caripyrin, a new inhibitor of infection-related morphogensis in the rice blast fungus *Magnaporthe oryzae.* The Journal of Antibiotics 63:285-289.

Roger Phillips (1991). Mushroom in North America. Little Brown and Company (Canada) limited. pp. 62.

Rui-lin Zhao, Desjardin, D.E., Soytong, K. and Hyde, K.D. (2008). Advances in the Phylogenesis of Agaricales and its higher ranks and strategies for establishing phylogenetic hypotheses. Zhejiang Unversity Science B. 9(10):747-846.

Rui-Lin Zhao, Kevin, D. Hyde, Dennis, E. Desjardin, Olivier Raspe, Kasem Soytong, Jacques Guinberteau, Samantha C. Karunarathna and Philippe Callac. (2012). Agaricus flocculosipes sp. nov.,a new potentially cultivatable species from the palaeotropics. Mycoscience DOI 10.1007/s10267-011-0169-5, Published online 4 January.

Rui-Lin Zhao, Dennis, E., Desjardin, Philippe Callac, Luis A. Parra, Jacques Guinberteau, Kasem Soytong, Samantha Karunarathna, Ying Zhang and Kevin D. Hyde (2013). Two species of Agaricus sect. Xanthodermatei from Thailand. Mycotaxon, 122:187-195.

States J.S. (2004). Mushrooms and truffles of the southwest; Transition zone horticultural institute, Inc.pp. 71.

Smith, H. Alexander (1975). A Field Guide to Western Mushrooms. The University of Michigan Press pp. 153.

Susan Metzler and Van Metzler (2000). Texas Mushrooms. The university of Texas Press. Japan, pp. 95.

Xiaoping Luo and Tunyan Wang. (2007). Mushrooms of the genus pre; iminary study. Technoligy Imfomation (24):272.

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