

Universities Research Journal 2008

Vol. 1, No. 1

Editorial Board

Editors in Chief

Prof. Dr. Kyaw Kyaw Khaung, Head of Department of Botany, University of Yangon

Prof. Dr. Aye Phay, Department of Botany, University of Yangon

Prof. Dr. Nu Nu Yi, Department of Botany, University of Mandalay

Editors

Prof. Maung Maung Myint, Head of Department of Botany, University of Mawlamyine

Prof. Aye Pwa, Head of Department of Botany, University of Patheingyi

Prof. Daw Sam Khing, Head of Department of Botany, University of Sittway

Prof. Dr. Than Than Htay, Head of Department of Botany, University of Taungtha

Prof. Khin Po Po, Head of Department of Botany, University of Pyaw Oo

Daw Mar Mar Aung, Head of Department of Botany, University of Dawei

Prof. Dr. Thandar Oo, Head of Department of Botany, University of West Yangon

Prof. Dr. San Aye, Head of Department of Botany, University of Hinthada

Prof. Daw Marlar, Head of Department of Botany, University of Myeik

Prof. Dr. Hla Hla Tin, Head of Department of Botany, University of Bago

Prof. Dr. Khin Thidar, Head of Department of Botany, University of Mandalay

Prof. Dr. Yee Yee Win, Head of Department of Botany, University of Taunggyi

Prof. May Than Su, Head of Department of Botany, University of Magway

Prof. Daw Thai Thai Aye, Head of Department of Botany, University of Yadanabon

Prof. Daw Tin Ye, Head of Department of Botany, University of Meiktila

Prof. Nay Win, Head of Department of Botany, University of Kyaukse

Prof. U Aung Myint Thein, Head of Department of Botany, University of Kalay

Prof. Swe Mar Tin, Head of Department of Botany, University of Lashio

Asso. Prof. Dr. San San Aye, Head of Department of Botany, University of Kyainge Tong

Contents

	Page
The Study of Fresh Water Algae from Kantharyar Lake, Pathein Township <i>Nyar Kyi, Mon Mon Lwin and Khin Min Min Phyo</i>	1
The Study of Selected Hydrophytes in Lake-pya-kan, Bago Township <i>Kyi Nyunt, Aye Mie Myat and San Nu</i>	25
Some Algae of Three Artesian Wells found in Ywathayar Village, Yinmarpin Township (Monywa District) <i>Theingi Htay</i>	37
Fresh Water Algae Found in Kalay University Campus and its Surrounding Areas <i>Moat War Dine Naw and Thein</i>	53
A Study on Usefulness of Some Woody Plants in Mon State <i>Eh Khu Hpaw, Win Win Nwe and Myo Hteik Aung</i>	63
A Study on Dyes Extracted from Natural Pigments of Some Resource Plants in Magway Township <i>May Than Su, Pa Pa Win, Kyaw Swe Lin and Thida Than</i>	85
Study on the Relationship of Plant Resin and Myanmar Society <i>Shwe Sin Ei</i>	101
Study on the Cultivated Legumes in Taungthaman Lake and Its Environ <i>Thai Thai Aye</i>	117
Effect of Direct-seeding and Transplanting Methods on Rice Cultivar Manawthukha in Meiktila Township <i>Nang Doi and Tun Chun</i>	133

	Page
Study of <i>Glycine max</i> Merr. on its Productions and Uses in Lashio Township <i>Swe Mar Tin, Thida Aung, Kay Thi Aung and Nang Mya Oo</i>	145
Ethnomedicines used by Mro Tribes in Kyauk-Taw Township, Northern Rakhine State for Gastrointestinal Disorder <i>Khin Thet Kyaw</i>	157
Some Edible Wild and Cultivated Plants Used as Food for Palaung Tribe in Kyaukme Township <i>Nyo Nyo Tin</i>	167
Genetic Diversity and Relationships Among the Myanmar Banana Varieties Using PBA Molecular Markers <i>Saw Yee</i>	183
Noncoding Pastid tRNA-Leu (trnL) Intron Region Sequences Report for Genetic Separation of <i>Cinnamomum spp.</i> from China and Myanmar <i>Khin Thantsin</i>	197
Culture of <i>Musa chiliocarpa</i> Back. in Murashige and Skoog Liquid Medium For Shoot Proliferation and Cell Types <i>Cho Cho Nyunt and San San Aye</i>	209
Studies on the Antifungal Agent Isolated from <i>Solanum indicum</i> Linn. Applicable for the Specific Treatment for Mycosis <i>Moe Moe Aye and Nyunt Phay</i>	221
Production of Antibacterial Metabolite by <i>Lecanicillium waksmanii</i> MKN-09 <i>Moe Moe Aye, Khine Swe Nyunt and Nyunt Phay</i>	231
Antifungal Compound Isolated from Leaf of <i>Cassia fistula</i> L. (Ngu Shwe Wah) <i>Khine Swe Nyunt, Moe Moe Aye and Nyunt Phay</i>	239
Investigation on the Isolation of Soil Fungi from Different Soil in Dawei Township	247

Mar Lar Aung, Thi Thi Moore and Tin Tin Aye

Survey on Some Herbal Plants in Bago Yoma 257

Than Than Htay, Mar Mar Aye, Mar Mar cho and Yin Yin Waing

Morphology and Preliminary Phytochemical Studies on Some Medicinal Plants Found in Pyay Area 269

Thet Thet May

The Study of Some Medicinal Plants in Family Verbenaceae 283

Tin Thaw Oo

Study on Some Medicinal Plants Concerning with Six Major Diseases (Phase I) 295

Thandar Oo

Pharmacognostic Study on Fruits of *Terminalia catappa* L. (Banda fruit) 311

Shwe Shwe Hla

Studies on Pollen Morphology of Some Flowers 323

Tin Kyi Kyi

Preliminary Survey on Plant Species (Angiospermae) of Myeik Archipelago 337

Nwe' Nwe' Yi

Antifungal Compound Isolated from Leaf of *Cassia fistula* L.

(Ngu Shwe Wah)

Khine Swe Nyunt¹, Moe Moe Aye², Nyunt Phay³

Abstract

In the investigation of antifungal compound against rice plant pathogenic fungus *Magnaporthe grisea*, five kinds of plants grown in Patheingyi area were utilized. These plant parts such as leaves, barks and stems were extracted with ethanol, methanol, and acetone solvents and tested the antifungal activity on *M. grisea* by paper diffusion assay. In this study, methanol extract of leaf of *Cassia fistula* L. (Ngu Shwe Wah) showed highly activity on *Magnaporthe grisea* (24.1 mm inhibitory zone). Therefore, methanol extract of leaf *Cassia fistula* L. was selected for further investigations such as isolation, purification and biological properties. In the isolation and purification, silica gel column chromatography, C18 gel column chromatography, silica gel column rechromatography and Sephadex LH-20 gel chromatography were undertaken with using suitable ratios of organic solvents. MICs of this compound is 0.156 µg/ml on *M. grisea*, 0.3125 µg/ml on *Cladosporium* sp. and *Alternaria alternata*, and 0.625 µg/ml on *Aspergillus flavus*, *A. fumigatus*, *Curvularia oryzae*, and *Fusarium oxysporum*. This compound affects on the growth of *M. grisea* at a concentration of 0.156 µg/ml; any further increase in its concentration above 0.156 µg/ml resulted in a more suppressive effect on growth. At a concentration of 0.6.25 µg/ml, cell lysis was observed that indicating that this plant compound cut acts as a fungicidal agent.

Introduction

Rice is an Asian countries' most economically important product. However, decreasing yield continues to be a problem because of phytopathogenic fungus such as *Magnaporthe grisea* which cause a rice blast disease. The only form of control available at present is chemical treatment which resulted in accumulation of harmful chemicals in the environment. Alternative biological treatment is thus more environmental friendly and less dangerous to farmers and customers (Chutrakul, 2005). Fungal belonging to the genus *Magnaporthe* is either as plant pathogens, or saprophytic association with plant tissues. Rice blast disease, caused by

1. Biological Resources and Biotechnology Development Centre, Patheingyi University

2. Assistant Lecturer, Botany Department, Patheingyi University

3. Pro-Rector, University of Patheingyi and Head of BR-BDC

Magnaporthe is also the most wide-spread and destructive fungal disease of rice (Oliver and Lionel, 2002). The discovery of antimicrobial agents from natural sources (plants) has been a goal of mankind since prehistoric times, and through previous efforts, plants have become a viable source of clinically useful antimicrobial agents (Richard, 1998). Plants have also afforded leads for synthetic modification and have served as tool for mechanistic studies (Colegate, and Molyneus, 1993).

The higher plants are logical; choices, chiefly because of their seemingly infinite variety of novel organic molecules, which are often referred to as secondary metabolites (Simon, and Alexander, 1991). *Cassia fistula* L. of the Caesalpinaceae family belongs to a group of plants through Myanmar and are popular medicinal and ornamental plant.

Materials and Methods

Sample preparation

Five kinds of plants such as *Cassia fistula* L., *Oldenlandia diffusa* (Wild.) Roxb., *Apium graveolens* Linn., *Alostonia schlaris* R.Br., and *Mimosa pudica* Linn. were collected at Pathein Township. Initially the leaves of these plants were dried at 17°C and dried leaves (40 g) was extracted with organic solvents (each 100 ml, methanol, ethanol, and acetone) according to the method of Lammens, 1979 and Hostettmann, and Marston, 1994. The organic extracts were evaluated for their abilities to inhibit the *in vitro* growth of phytopathogenic fungi by paper disc diffusion assay method.

Investigation of antimicrobial activity

The sample (20 μ l) was put onto the paper disc. The assay medium (Glucose 1%, Polypepton 0.3%, KNO₃ 0.1%, Agar 51.8%, Distilled water 100ml, pH 7.0) was used for the antimicrobial activity test. One percent (1.5 x 10⁸/ml CFU) of test organism was added to assay medium, then poured into plates. The plates were incubated for 24-36 hours at 28 to 30°C. Clear zones (inhibitory zones) surrounding the agar well indicates the presence of bioactive metabolites which inhibit the growth of test organisms.

Test organisms utilized in this study were phytopathogenic fungi, *Magnaporthe grisea*, *Cladosporium* sp., *Alternaria alterna*, *Aspergillus flavus*, *A. fumigatus*, *Curvularia oryzae*, and *Fusarium oxysporum*. Since it is intended to get the highly selective antifungal agent (Table 1 and 2),

based on antifungal activities, methanolic extract of *C. fistula* was selected to proceed the further isolation and purification.

Extraction, Isolation and Purification of antifungal agent

The methanolic extract of *C. fistula* leaves was concentrated *in vacuo* and the residue was dissolved in water (1:20, v/v) and was investigated the suitable pH for re-extraction with EtOAc. According to the results of pHs effects (Table 3), it was found that pH 4.0 was suitable for re-extraction with EtOAc. Based on the result, the methanolic extract (100ml) of *C. fistula* leaves was concentrated *in vacuo* (5 ml) and dissolved in water (1:20, v/v). This sample (105 ml) was adjusted at pH 4.0 and ethyl acetate (105 ml) was added for the re-extraction of antifungal agent. Ethyl acetate layer (105 ml) was concentrated *in vacuo*.

It is necessary to separate the bioactive compound by rechromatography, TLC was developed by the solvent such as hexane and hexane-acetone mixture. R_f values reported were acquired on Merck Kieselgel 60F₂₅₄ pre-coated TLC plates, which were utilized for analytical preparative purpose. The obtained EtOAc extracted samples (20 μ l) were applied on the TLC plate and allowed to dry. The TLC plates were developed in the solvent of hexane and hexane-acetone (9:1, 8:2, 7:3) mixture. Then, bioautography was done to check the antifungal activity of each. In this case also, the inhibitory zone was measured yielding an R_f value for the corresponding antifungal metabolites.

Based on the results of TLC, silica gel (Wako gel C-300, Japan) column chromatography was carried out with eluting solvent hexane and hexane-ethyl acetate mixture (9:1) and then tested the activity against *Magnaporthe grisea*. The column volume and flow rate were 5.25 cm x 20 cm and flow rate 2ml/min.

The active hexane-EtOAc fractions were combined and concentrated *in vacuo*. Silica gel column re-chromatography was then developed with dichloromethane-methanol (9:1) mixture. The column volume and flow rate were 5.25 cm x 20 cm and flow rate 1.5 ml/min.

Active fractions of silica gel column re-chromatography were combined and concentrated *in vacuo*. The residue was chromatographed (C 18 gel) by the eluting solvent acetonitrile-water (6:4) in the 4.5 x 18 cm column volume and 1.5 ml/min flow rate.

The active fractions from C 18 gel chromatography) were combined and concentrated. This residue was purified by Sephadex LH-20 with chloroform-methanol (7:3)(column volume 3.5 cm x 15 cm and flow rate 1.0 ml/min).

Determination of the minimum inhibitory concentration and Fungicidal effect on *Magnaporthe grisea* (Phay, 1999)

The minimum inhibitory concentrations (MICs) of compound were determined in GYA liquid medium (10 ml) inoculated with approximately 1.4×10^7 CFU of test organism *M. grisea* and were introduced with the treatment of compound (0.1562 µg/ml, 0.3125 µg/ml, 0.625 µg/ml, 1.25 µg/ml and 2.5 µg/ml) into 5 tubes. The control was also done. After incubation at 27°C for 24 hr, the MICs were determined by selecting the lowest concentration of compound which caused complete inhibition of fungicidal growth. Experiments were done in triplicate.

Test organism *M. grisea* (0.1 ml) was separately inoculated into 6 Conical flasks containing medium (100 ml, 1.0% glucose, 0.3% Yeast extract, 0.3% NZ amine type, pH 7.0) and incubated for 60 hours. At 24 hr incubation, it was introduced with the treatment of compound (0.3125 µg/ml 0.625 µg/ml 1.25 µg/ml, 2.5 µg/ml, and 5 µg/ml) into 5 flasks. PCV % was measured in 12 hr intervals. Control is one flask.

Results

Investigation of antimicrobial activity

In the study of antimicrobial agent isolated from the leaves of five kinds of plants, it was found that although all extract of *Cassia fistula* showed antifungal activity, methanolic extract *Cassia fistula* L. showed highly antifungal activity against *Magnaporthe grisea*, *Cladosporium* sp., *Alternaria alterna*, *Aspergillus flavus*, *A. fumigatus*, *Curvularia oryzae*, and *Fusarium oxysporum* (Table 1). Other plant extracts did not show the activity.

Table 1. Antifungal activity of extracts of organic solvents from *Cassia fistula* L

Extract	<i>M. grisea</i>	<i>Clado.</i>	<i>A. alterna</i>	<i>A. flavus</i>	<i>A. fumi</i>	<i>C. oryzae</i>	<i>F oxys</i>
MeOH	24.1 mm	16.2 mm	18.8 mm	18.6 mm	18.7 mm	17.4 mm	20.4 mm
EtOH	13.2 mm	-	-	13.6 mm	13.4 mm	-	16.5 mm
Acetone	20.6 mm	16.0 mm	17.6 mm	18.4 mm	16.8 mm	16.6 mm	20.8 mm

M. grisea = *Magnaporthe grisea*

Clado = *Cladosporium*

A. alterna = *Alternaria alterna*

A. flavus = *Aspergillus flavus*

A. fumigatus = *Aspergillus fumigatus*

C. oryzae = *Curvularia oryzae*

F. oxysporium = *Fusarium oxysporium*

Table 2. The effects of pHs for re-extraction of antifungal agent

pHs	Inhibitory zone (mm)
3	20.2
4	21.8
5	19.6
6	18.4

Extraction, Isolation and Purification of antifungal agent

According to the R_f value of paper chromatography bioassay, it was considered that the antifungal agent could be re-extracted by ethyl acetate solvent at pH 4.0.

Re-extraction was undertaken by EtOAc at pH 4.0. The purification was developed with silica gel column chromatography (hexane-acetone), silica gel column chromatography (dichloromethane-methanol), C18 gel

column chromatography (acetonitrile-water) and Sephadex LH-20 (chloroform-methanol) to afford pure compound (27.8 mg).

Determination of the minimum inhibitory concentration and Fungicidal effect on *M. grisea* (Phay, 1999)

It was observed that MIC of compound is 0.156 $\mu\text{g/ml}$ on *M. grisea*. This compound affects on the growth of *M. grisea* at a concentration of 0.156 $\mu\text{g/ml}$; any further increase in its concentration above 0.156 $\mu\text{g/ml}$ resulted in a more suppressive effect on growth. At a concentration of 0.625 $\mu\text{g/ml}$, cell lysis was observed that indicating that compound acts as a fungicidal compound.

Conclusion

In the studies on the antifungal agent for the control the growth of *Magnaporthe grisea*, antifungal compound was isolated from the leaves of five kinds of plants using three solvents. Among them, the methanolic extract *Cassia fistula* Linn. showed highly antifungal activity. The methanolic extract was purified by silica gel column chromatography (hexane-acetone), silica gel column chromatography (dichloromethane-methanol), C18 gel column chromatography (acetonitrile-water) and Sephadex LH-20 (chloroform-methanol) to afford pure compound (27.8 mg) from 40 g of dried leaves. This antifungal compound potently inhibited the growth of *Magnaporthe grisea* with MIC of 0.625 $\mu\text{g/ml}$, comparable to that of griseofulvin.

YMICs of compound is 0.156 $\mu\text{g/ml}$ on *M. grisea*. This compound affects on the growth of *M. grisea* at a concentration of 0.156 $\mu\text{g/ml}$; any further increase in its concentration above 0.156 $\mu\text{g/ml}$ resulted in a more suppressive effect on growth means at a concentration of 0.625 $\mu\text{g/ml}$, cell lysis was observed that indicating that compound acts as a fungicidal compound.

From the traditional medicine knowledge, it was possible to select a plant species used to control the growth of fungi. The pharmacological value of this plant was demonstrated and was able to purify and isolate and antifungal agent, which represents a new molecular weight. Therefore, it is necessary to elucidate the compound structure.

Acknowledgement

We would like to express our gratitude to Pro-Rector U Hla Tint, University of Patheingyi for his allowing us to do this research. We are also grateful to the Minister and the Deputy Minister of Ministry of Education and the Director General, Department of Higher Education (Lower Myanmar) for their support to work the research. We thank Ma Nant Khin Cho Aye and Nant San Gi Bwar, Biological Resources and Biotechnology Development Center for their kind help in this research.

References

- Chuttrakul, (2005): Developing and screening for antifungal agents, *Biothainand*, 79
- Colegate, S.M and Molyneus, R.J. Bioactive natural products, 34-67, 1993
- Hostettmann, K. and Marston, A.: Search for new compounds from higher plants, *Pure Appl. Chem.* 66, 2231-2234, 1994
- Leven, M., and Lammens, E.: Screening of higher plants for biological activity, *Planta Medica* 36, 311-321, 1979
- Oliver, P. and Lionel, G. (2002): Population structure of *Xanthomonas*, *Magnaporthe* and *Curvularia* with endophytic and epiphytic phases, 209-221
- Phay, N. (1999): Fistulosin, antifungal compound from Welsh onion, *J. Phytochemistry*, 52, 271-274
- Richard J.P. Cannell. *Natural Products Isolation*; 1-53. 1998
- Simon, G., and Alexander, I. G.; *Natural products from plants*, 56-84, 1991
- Sion, G., and A. I. Gray.: Isolation by planar chromatography, *In methods in Biotechnology*, Vol.4, 209-245, 1998
- Williams, S.: (2003): Secondary metabolites in plants: 435-439