ปฏิกิริยาต่อต้านราสาเหตุโรคพืชในห้องปฏิบัติการของสารสกัดหยาบของไลเคนบางชนิดจากอุทยานแห่งชาติภูหินร่องกล้า

IN VITRO ANTIFUNGAL ACTIVITY AGAINST PLANT PATHOGENIC FUNGI OF CRUDE EXTRACTS OF SOME LICHENS FROM PHU HIN RONGKLA NATIONAL PARK.

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บทคัดย่อ: สารสกัดหยาบจากไลเคน 10 ชนิดที่ได้จากการสกัดด้วยวิธีเอทิลแอลกอฮอล์บริสุทธิ์ เมื่อนำไปทดสอบยับยั้งการเจริญเติบโตของเส้นใยรา *Colletotrichum gloeosporoides* สาเหตุโรคแอนแทรคโนสของมะม่วง, *C. piperatum* สาเหตุโรคแอนแทรคโนสบนพริก, *Curvularia eragostidis* สาเหตุโรคจุดสนิมบนดอกกล้วยไม้สกุลหวายตัดดอก, *Fusarium moniliforme* สาเหตุโรคหลาวของข้าว, *Phytophthora parasitica* สาเหตุโรคต้น และรากเน่าของส้ม *Pythium diliensis* โรครากเน่าของมะกอก และ *Sclerotium rolfsii* สาเหตุโรคโคนเน่าของถั่วเหลือง โดยใช้ 5 ความเข้มข้นของสารสกัดหยาบที่ 1,000 500 100 50 และ 10 ppm และคำนวณหาค่าประสิทธิภาพของความเข้มข้นระงับการเจริญเติบโตของเส้นใยราที่ความน่าจะเป็น 50 เปอร์เซ็นต์ (EC_{50}) ตามวิธีของ Finney (1) พบว่าสารสกัดหยาบจากไลเคน *Canoparmelia owariensis* ให้ผลการยับยั้ง (EC_{50}) ราสาเหตุโรคพืชดังกล่าวที่ 51.14, 68.91, 21.29, 66.03, 133.12, 193.05 และ 3432.45 ไมโครกรัม/มิลลิลิตร ตามลำดับ

Abstract: The crude extracts from absolute ethanol alcohol of 10 lichen species are tested *in vitro* for the mycelial growth inhibition of *Colletotrichum gloeosporoides*, causing mango anthracnose, *Colletotrichum piperatum* causing chili anthracnose, *Curvularia eragostidis* causing rusty spot disease of *Dendrobium* cutting flower, *Fusarium moniliforme* causing bakanea disease of rice, *Phytophthora parasitica*, causing stem and root rot disease of citrus, *Pythium diliensis*, causing root rot disease of hog plum and *Sclerotium rolfsii* causing stem rot disease of mung bean. The five concentrations, 1,000 500 100 50 and 10 ppm (part per million), of crude extract were tested. The median effective inhibitory concentration (EC_{50}) against plant pathogenic fungus was calculated using the linear relation between the inhibitory probability and concentration logarithm according to method out line by Finney (1). With highly distinguished to inhibit the mycelial growth of those plant pathogenic fungi, the crude extract of *Canoparmelia owariensis* resulted in the EC_{50} of the above listed pathogens as 51.14, 68.91, 21.29, 66.03, 133.12, 193.05 and 3432.45 µg/ml respectively.

Introduction: Phu Hin Rongkla National Park has an area of 307 square kilometers. It is one of the Phetchabun Mountain Range sharing the border with 3 provinces, Phetchabun, Phitsanulok and Loei. The communist party used as their base in the past. It covers with 5 forest types, dry dipterocarp forest, mixed deciduous forest, dry evergreen forest, lower montane rain forest, and lower montane shrub. It is rich

with various plant species, including mosses, ferns and especially lichens. There are many lichen species. Some of them are wide spread over the others. While other species grow very poor and have limited distribution. The dominant lichen species should produce effective secondary metabolite in order to compete other species. The purpose of this study is to find lichen crude extracts feasible to inhibit mycelial growth of crucial plant pathogenic fungi *in vitro*.

Methodology:
Growth of plant pathogenic mycelium, *Colletotrichum gloeosporoides*, causing mango anthracnose, *Colletotrichum piperatum* causing chili anthracnose, *Curvularia eragostidis* causing rusty spot disease of *Dendrobium* cutting flower, *Fusarium moniliforme,* causing bakanea disease of rice, *Phytophthora parasitica,* causing stem and root rot disease of citrus, *Pythium diliensis,* causing root rot disease of hog plum and *Sclerotium rolfsii,* causing stem rot disease of mung bean, was determined on PDA plates, containing 10, 50, 100, 500, or 1000 ppm of lichen crude extracts from *Canoparmelia ecaperata, C. owariensis*, *Heterodermia appendiculata, H. microphylla*, *H. lepidota, Parmotrema maclayanum, P. tinctorum, Pyxine coccifera, P. coralligera, Usnea experata*. (3),(4) Mycelium growth on PDA plates without crude extract, and PDA containing 0.2% DMSO (Dimethyl sulfoxide) were used as controls. Five replicates were performed for each experiment. Crude extract toxicity was recorded in term of percentage colony inhibition and calculates according to Pandey et al. (2). The mean effective inhibitory concentration (EC_{50}) against plant pathogenic fungus was calculated according to method out line by Finney (1).

Results, Discussion, and Conclusion: Almost of crude extracts from 10 dominant lichen species at Phu Hin Rongkla National Park area are capable to inhibit the mycerial growth of serious stem and root rot disease of citrus, *Phytophthora parasitica*. The most effective one comes from *Parmotrema tinctorum* (table. 1) while the EC_{50} of crude extracts from *Canoparmelia owariensis* and *C. ecaperata*, generally shown high inhibitory effect on 6 plant pathogenic fungi. The EC_{50} of crude extracts from *C. owariensis* against *Colletotrichum gloeosporoides, Colletotrichum piperatum, Curvularia eragostidis, Fusarium moniliforme, Phytophthora parasitica, Pythium diliensis* and *Sclerotium rolfsii* are equal to 51.14, 68.91, 21.29, 66.03, 133.12, 193.05 and 3432.45 µg/ml respectively. The crude extract from *Pyxine coocifera* and *Usnea exasperata* perform strong inhibition of *Sclerotium rolfsii*, the pathogen causing stem and root rot of mung bean (table 2). These results proof our hypothesis that lichens which are abundant in nature give high quantity of lichen substances that are efficient inhibitors of plant pathogenic fungi in laboratory culture.

Lichen crude cxtracts	**Toxicity regression equation (p=ax +b)**	**EC_{50} µg/ml**
Canoparmelia ecaperata	p= 0.40389X + (-2.02689)	151.18
Canoparmelia owariensis	p= 0.35503X + (-1.73656)	133.13
Heterodermia appendiculata	p= 0.33008X + (-2.27145)	974.04
Heterodermia microphylla	p= 0.42065X + (-2.35776)	271.81
Heterodermia lepidota	p= 0.39655X + (-2.61268)	730.06
Parmotrema maclayanum	p= 0.35125X + (-2.21925)	554.51
Parmotrema tinctorum	p= 0.45830X + (-2.06947)	91.43

Pyxine coccifera	p= 0.60313X + (-2.76762)	98.36

plant pathogenic fungi	EC_{50} (µg/ml) of lichen crude extracts								
	1*	2*	3*	4*	5*	6*	7*	8*	
Colletotrichum gloeosporoides	73.34	51.14	2287.15	2478.70	2486.67	2301.84	1234.51	1688.11	915
Colletotrichum piperratum	152.24	68.91	6139.75	4741.31	29558.90	645.55	2763.79	14725.41	229
Curvularia eragostidis	26.38	21.29	882.69	619.92	2042.89	613.44	590.95	796.71	117
Fusarium moniliforme	68.23	66.03	472.04	287.95	554.43	734.41	288.95	212.71	90
Phytophthora parasitica	151.18	133.13	974.04	271.81	730.06	554.51	91.43	98.36	5
Pythium diliensis	216.92	193.05	9846.19	1492.77	3466.91	4508.90	61.05	689.83	41
Sclerotium rofsii	1212.30	3432.50	4597.41	4884.07	18555.29	4768.50	1081.65	542.69	696

Pyxine coralligera	p= 0.33959X + (-2.14693)	556.69
Usnea experata	p= 0.32746X + (-2.48502)	1976.04

P = Probit, a = Regression, x = Log dose, b = Intercept

Table 2. Median effective inhibitory concentration (EC_{50}) of lichen crude extracts screened in vitro against 7 plant pathogenic fungi

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