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Antifungal activity of Plant Extracts against Colletotrichum capsici Causal Agent of Chili Anthracnose

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Abstract

Anthracnose disease destroys on chili the worldwide. The fungi of the Colletotrichum genus are the pathogens that cause of chili anthracnose disease. This study was searching for the efficiency of plant extracts antifungal properties against C. capsici. The antifungal activities of five plants Aegle marmelos L. Correa., Eupatorium odoratum L., Phyllanthus acidus L. Skeels, Houttuynia cordata Thunb., and Clausena excavata, were evaluated on the plant pathogenic. The plant extracts assay was carried out in vitro at 5, 10, and 20 mg/ml concentrations using the disk diffusion technique. The efficacies of plant extracts were against mycelia growth of C. capsici, their effect significantly for fungi toxicity. The crude extracts of E. odoratum L. indicated the highest effectiveness against (>90%) fungicide. The antifungal activities have a high inhibition percent (>85%), such as hexane extracts of E. odoratum L., and A. marmelos L.; including ethyl acetate extracts of C. excavate and methanol extract of E. odoratum. Furthermore, strong exhibited antifungal percent (>70%) as hexane extracts of H. cordata, P. acidus L., and ethyl acetate extract of A. marmelos L. The phytochemicals in the plant extracts were identified to be alkaloids, flavonoids, phenols, steroids, and terpenoids. The bioactive of alkaloid compounds naturally appear in plant extracts potential against mycelia growth of C. capsici; they could be a source for future time development of natural product fungicides.

Keywords: Antifungal, Colletotrichum capsici, Chili, Anthracnose, Plan extract

1. Introduction

Chili (Capsicum annuum L.) is one of the economic essential vegetable crops worldwide. It is used in many diets and indicates medicinal properties, reducing the risk of cancer [1]. Chili is thought of as the essential crop in the tropics. The regions cultivated with chili worldwide is about ≈3.8 million hectares, with a total production of 4.1 million tons [2]. While the harvest area in Thailand is approximately 54,970 hectares with a show of 283,515 tons, revenue is estimated to be US$ 426.67 million [3]. However, infections by Colletotrichum ssp. are the crucial malady problematical in chili production, which leading to anthracnose disease. Consequently, chili is infected by anthracnose, which shall cause product losses of most up to 50% worldwide. Losses due to both pre-and post-harvest are caused with this effect has been reported that in Vietnam, it causes product loss 20-80%, in Malaysia 50%, in India 10-54%, and as highest as 80% in Thailand [4]. Anthracnose of C. annuum L. has been reported to be

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motive by at least four species of Colletotrichum such as C. capsici, and C. gloeoporioides found in Thailand, Indonesia, India, and Korea; C. coccodes in New Zealand, C. acutatum in Australia and Indonesia [5]. Which pathogenesis disease was able to use control procedure by use chemicals, biological agents, or plant extracts. Fungicide chemical is a widely used control of anthracnose disease; the fungicide control was a quick response .However, for a longtime used of chemicals fungicide shall cause risk toxic effects to environmental concerns. Other choices, spontaneous plant products are an important source of fungicide and are nontoxic to mammals and the environment; they are more careful and environmentally friendly . Uses of plant extracts to control anthracnose diseases on chili have reported much research . For example, crude extracts of sweet flag, Palmorosa oil, and Neem oil showed effectiveness in stoppage the growth of Colletotrichum ssp. Plant extracts of Azadirachta indica, Swietenia mahagoni, and Allium sativum were the combination plant extracts that showed significant disease reduction of chili [5]. The objective focus of this study was to evaluate the efficacy of plant extracts against C. capsici of anthracnose disease on chili.

2. Materials and Methods

2.1 Plant extracts

Research plants were, for example, Aegle marmelos L .Correa., Eupatorium odoratum L., Phyllanthus acidus L .Skeels, Houttuynia cordata Thunb., and Clausena excavata,, they were compiled from Lampang province, Thailand. Plants samples were identified by the Biology Laboratory, Faculty of Science, Lampang Rajabhat University. Leaves were cleaned up the dirtiness; underneath, take the air at room temperature; after that, the entire powder was used to use millstone. Afterward, plant powder brings percolated in continuance with n-Hexane, Ethyl acetate, and Methanol (100 g × 0.5 L × 3 days × 3 times) underneath room temperature, respectively. Filtration used filter paper to have filtrates solution; these were evaporated underneath reduce pressure at 40°C; the plant crude extracts received brought for the test biological activity.

2.2 Phytochemical screening

The plant extracts screenings apply the procedure has been research reported of Trease and Evans; testing approaches show up the chemical component of plants including phenols, flavonoids, alkaloids, terpenoids and steroids, etc., [6, 22].

Test for phenols: Samples 0.10 g was dissolved by ethanol 1.0 ml, poured down 1.0 ml of aqua in a test tube following adds two drops of $FeCl_3$ solution, appear blue, green, red or purple colorable are a positive test.

Test for flavonoids: Samples 0.10 g was dissolved with ethanol 5.0 ml, drops 1-5 of conc. HCl and add magnesium turnings 0.50 g. appear pink or red color when found flavonoids.

Test for alkaloids: Uses 2.0 ml add a test tube of samples 0.10 g following 2N HCl 0.20 ml, and 1.0 ml of Meyer’s reagent. These indicating was yellowish coloration when showed alkaloids.

Test for terpenoids: Samples 0.10 g adds a test tube with 2.0 ml of $CHCl_3$; finally drops 3 ml of conc. H_2SO_4. A positive test formed a layer, reddish-brown color of the interface.

Test for steroids: Use samples, 0.10 g was dissolved with 3.0 ml acetic anhydride, following with drops of conc. H_2SO_4. Positive tests were appearances of bluish-green color.

2.3 C. capsici material

Samples infect anthracnose disease with C. capsici on chili fruit brings from regions Lampang province. Chili fresh fruit disease was sliced into diminutive pieces and sterilized

with ethyl alcohol next 2% sodium hypochlorite. After that, these pieces were cleansing by sterilized water and placed onto a potato dextrose agar (PDA) plate incubating. Afterward, after 24 hours of incubating, conidiospores from the surface of the slit were spread into solution agar and incubation overnight. Germinated conidiospores were isolated and transferred onto PDA. The subculture cell pathogen to procure pure culture was retained for further testing [7].

2.4 Molecular variability

The identifications of C. capsici were based on molecular analysis of DNA. They were extracted from each pure culture using modified the cetyltrimethyl ammonium bromide method of Doyle and Doyle [8]. The ITS of rDNA was amplified by ITS3 (5´GCATCGATGAAGAACGCAGC3´) and ITS4 (5´TCCTCC GCTTATTGATATGC3´) under the following thermal conditions: 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 60 ºC on 30 s, and 72 ºC on 1 min. Gel/PCR DNA Fragments Extraction Kit (Geneaid Taiwan) was used during PCR purification. The sequences were determined in a genetic analyzer at Macrogen, Inc. Sequences were compared with other sequences found in the GenBank database via Basic Local Alignment Search Tool (BLAST) [9].

2.5 Evaluation of plant extracts inhibition C. capsici in vitro

Procedure evaluation of the efficacy of plant extracts inhibition C. capsici by poisoned food diffusion technique. Four plant crude extracts were dissolved with PDA thoroughly mixed by lukewarm PDA to successfully produce an aftermost concentration of 5, 10, and 20 mg/ml. Completely Randomized Designs were triplicate replicate per treatment adoption, and 20 ml of media was splashed over in each sterilized petri plate. Later solidification, fungi discs were punctured from the 7-day-old culture of C. capsici (1×10^8 spores/ml) at the middle of the petri plate. The plates were incubated at room temperature internal the Biological Oxygen Demand incubator and observe radial growth every 24 hours after inoculation; finally, a whole surface is fraught with mycelia growth in petri plates of the controller. The controller has maintained the pathogen on plates contains PDA without crude extracts. The mycelia growth interior petri plates increasingly with different concentrations and crude extracts. They were measure and compared with the standard controlled. Fungi toxicity was reported in the percentage of inhibition and calculated according to the equation [7].

$$\text{PIA} = \frac{\text{Ro} - \text{Rt}}{\text{Ro}} \times 100$$

PIA =Percentage of inhibitory activity against radial growth
Ro =Radial growth of the fungal in the control
Rt =Radial growth of the fungal in the treatment

3. Results

Phytochemical screenings were indicated such as phenols, flavonoids, alkaloids, terpenoids, and steroids .The phytochemical tests of different plants extracts using various solvents like n-hexane, ethyl acetate, and methanol were presented in Table 1. In this study, five plants were amassed from native plants. These plants were reported to treat severe diseases in traditional medication and antimicrobial agets. Qualitative analyses of phytochemicals were found of steroids, alkaloids, and terpenoids entire in the plant extracts. The plant extracts, namely E. odoratum L. cannot find flavonoids. In addition, A. marmelos L. Correa.; was not found phenol compounds.

Table 1: Phytochemical screening of plant extracts.

Plants	Flavonoids	Phenolic	Alkaloids	Terpenoids	Steroids

	H	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**
A. marmelos (L.)	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+
E. odoratum L.	-	-	-	+	-	-	+	+	+	+	-	+	+	+	-
P. acidus (L.)	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+
H. cordata	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+
C. excavata	-	-	+	+	-	+	-	+	+	+	+	+	+	+	+

(+) present, (-) absent

H: Hexane extract, **E**: Ethyl acetate extract, **M**: Methanol extract

Table 2: Inhibition of Colletotrichum capsici with plant extracts.

Plants (Leaf)	% Inhibition of Colletotrichum capsici with plant extracts								
	H (mg/ml)			**E** (mg/ml)			**M** (mg/ml)		
	5	10	20	5	10	20	5	10	20
A. marmelos (L.)	44.68	77.82	85.08	58.41	63.53	72.22	9.24	34.48	56.04
E. odoratum L.	31.70	70.29	88.18	85.36	87.55	91.97	70.14	75.88	85.42
P. acidus (L.)	66.35	67.34	73.01	41.02	62.40	67.68	3.91	11.47	44.62
H. cordata	54.63	76.44	77.70	27.01	35.87	41.30	18.61	24.54	36.06
C. excavate	17.60	37.52	60.26	69.66	82.69	89.44	27.39	40.92	62.00

H: Hexane extract, **E**: Ethyl acetate extract, **M**: Methanol extract ($P<0.05$)

The plant extract evaluated efficaciously were against fungi C. capsici indicated a positive effect, and the percentage of inhibitions were showed in Table 2. The investigation of in vitro antifungal activity is the considerable goal of advancing potential new fungicides. The experiments were different solvents, and plant leaf extracts showed result significantly ($P<0.05$) figure 1 decrease mycelia growth. The restraining efficiently when use samples concentrations progressively increased at 5, 10, and 20 mg/ml, respectively (Figure 1). The experiments **H** extracts of E. odoratum and A. marmelos; these concentrations at 20 mg/ml showed significantly the highest percentage inhibitions of pathogen were 88.18 and 85.08, respectively. The testing **E** extracts of E. odoratum demonstrated the highest inhibition (91.97%) mycelia growth; while the high inhibition mycelia growth efficacy percentage 89.44, was found in the **E** extracts of C. excavate. The **M** plant extracts were showed efficacy inhibition growth on C. capsici; they were testing at concentrations 20 mg/ml good inhibited the mycelia growth percentage was 85.42 and 62.00 as follows E. odoratum and C. excavate, respectively.

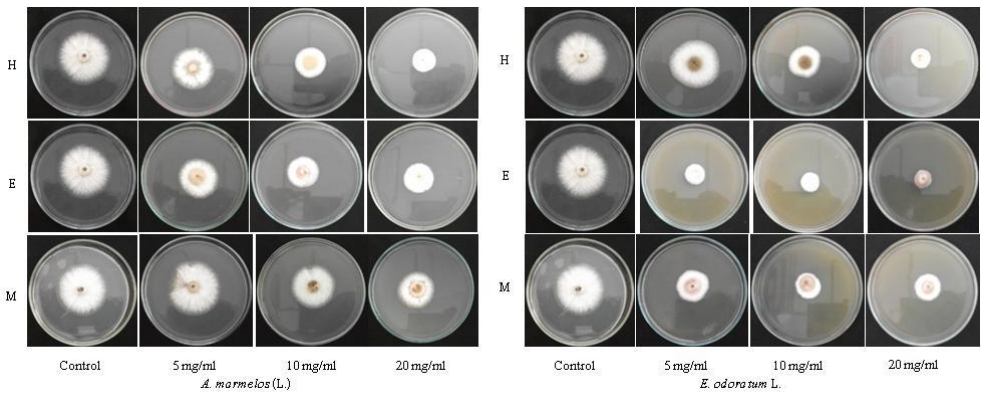

Figure 1: Inhibition of Colletotrichum capsici with plant extracts (in PDA).

4. Discussion and Conclusion

These studies revealed that plants extract of five sample plants be able to inhibit suitable fungal activities, all in against the mycelia growth of C. capsici. Compared with the positive control of C. capsici isolated showed the percentage of inhibition by crude plant extracts at 20 mg/ml concentration exhibited higher against previous studies report. Research reported of plant extracts were showed antifungal and antibacterial activity. Previously reported chemical constituents from plants were found several compounds such as flavonoids, phenols, alkaloids, terpenoid, and steroids. This will be discussed in detail as follows.

Aegle marmelos L. had the potential to treat the disease such as inflammatory, antioxidant, antibacterial, and antifungal. The extract of A. Marmelos (L.) indicates antimicrobial activity Staphylococcus aureus, S. epidermidis, and Proteus vulgaris; in addition, the plant was reported to efficacy antifungal activities inhibit the mycelia growth of Colletotrichum spp [10]. Likewise, the botanical extract was indicating the highest inhibition C. capsici 85%, water extract and 60.19%, ethanol extract respectively [11, 12]. Bael reported of phytochemicals showed as flavonoids, phenolic, terpenoids, coumarins, and alkaloids component; for that reason, plant extracts have efficacy against fungal [13].

Eupatorium odoratum L. was contributed to the investigation of antimicrobial medicinal plants, indicated concern with antifungal. Leaves extracts were inhibited fungal the in vitro growth of Cryptococcus neoformans, Microsporum gypseum, Trichophyton mentagrophytes, and Trichophyton rubrum [14]. Furthermore, the therapeutic plant's ethanol, cold-water, and warm water extracts against Phytophthora megakarya ethanol extract was most efficient [15]. Moreover, leaf extracts by methanol were inhibited Bacillus cereus, Bacillus subtilis, Staphylococcus aereus, Staphylococcus epidermidis, and Escherichia coli [16]. Phytochemicals of the extracts showed the biologically active constituents, for example, flavonoids, phenols, tannins, steroids, terpenoids, and alkaloids; consequently, E. odoratum L. extracts have efficacy against C. capsici [17].

Phyllanthus acidus L. Skeels has many pharmacological properties: antibacterial, antioxidant, antifungal, antiviral, and anti-parasitic, which, were potential protective properties [18]. A researcher reported leaves extracts with water and n-butanol were inhibited microbial growth as Staphylococcus aureus, Escherichia coli, and Aspergillus nigra [19]. Likewise, methanol extract of the fruit of P. acidus showed moderate to a good zone of inhibition against B. megaterium, B. subtilis, S. typhi, and S. dysenteriae. Furthermore, P. acidus showed moderate inhibition against pathogenic bacterial strains (Bacillus subtilis, Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus) [20]. Phytochemicals screening of P. acidus showed the constituents of alkaloids, terpenoids, tannin, flavonoids, saponins, phenols, glycoside, tannins, resins, emodols, and steroids [21]. The plant extracts have efficacy moderate to good antifungal activity in a dependent contain phytochemicals.

Hottuynia cordata has exhibited several pharmaceutical activities, including antifungal, antibacterial, anti-inflammatory, anticancer, antiviral, antioxidative, immunologic, and anti-mutagenic effects [22]. All parts of H. cordata exhibit bacteriostatic against active. Root extract has more potent inhibition on Staphylococcus aureus, and Escherichia coli. The MIC was 0.04, 0.06, and 0.06 g/mL, respectively; leaf extract has stronger inhibition on Bacillus dysenteriae, the MIC was 0.08 g/mL [23]. The H. cordata extract by 95% ethanol showed the highest mycelia inhibition at 50.2% for Fusarium oxysporum and 62.3% for Colletotrichum capsici when test with poisons food method. In addition, water extract showed antibacterial effects against murin salmonellosis as well [24]. Data research contains bioactive compounds, including flavonoids, phenolics, alkaloids, volatile oil, steroids, and terpenoids; these phytochemicals could reduce the intensity of pathogen on plants [25].

Clausena excavata have biological activities as antioxidant, antibacterial, anticancer, insecticidal, antinociceptive, anti-malarial, anti-HIV-1, and antifungal. The antifungal activity of phytochemicals from C. excavata, like 3-formylcarbazole, mukonal and 3-methoxycarbonylcarbazole

showed bioactivity with IC_{50} were 13.6, 29.3, 9.5, and 2.8 µg/mL, respectively, [26]. The leaves of C. excavate showed MIC was against 15 fungal strains pathogenic against plants as Rhizoctonia solani and Sclerotinia sclerotiorum. The human pathogen, Candida tropicalis, antifungal activities such as Aspergillus fumigatus, Mucor circinelloides, Colletotrichum gloeosporiodes, Lasiodiplodia theobromae, Fusarium oxysporum and Rhizopus stolonifer were high than that of the standard antimicrobials [27]. The MIC value of methanol extract from C. excavata showed active against Trichophyton mentagrophytes and Trichophyton rubrum were indicated at 62.5 and 62.5 µg/mL, respectively, [28]. Research reported chemicals constituents of C. excavata, found alkaloids, limonoids, terpenoids, carbazole, and steroids, which are valid for antifungal disease [29].

This research shows the crude extracts from the leaves of plants, the tested of bioactive metabolites extracted shows that they are effective against chili disease -causing fungi C. capsica. The crude plants extracted found the phytochemicals, for instance, alkaloids, flavonoids, phenols, steroids, and terpenoids. The conclusion demonstrated potential in using plants extract had alkaloid compounds as fungicides in organic farming, this research indicates bioactive metabolite from plants shows the highest possibilities as antifungals for chili agriculture in the future.

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