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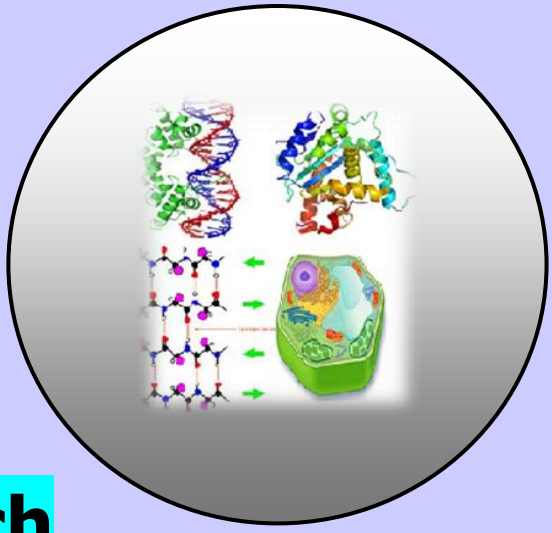
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

Index Copernicus International Value
IC Value of Journal 46.52 Poland, Europe (2015)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 34 (1) 2017 Pages No. 201-207



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 34, No. 1: 201-207, 2017
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 34/01/102/2017

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

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http:// www.sasjournals.com
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RESEARCH PAPER
Received: 31/03/2017 Revised: 17/04/2017 Accepted: 21/04/2017

Antifungal Activity of *Ficus septica* Leaf Extract against *Colletotrichum gloeosporioides* Cause Anthracnose Disease on Local Fruits in Bali

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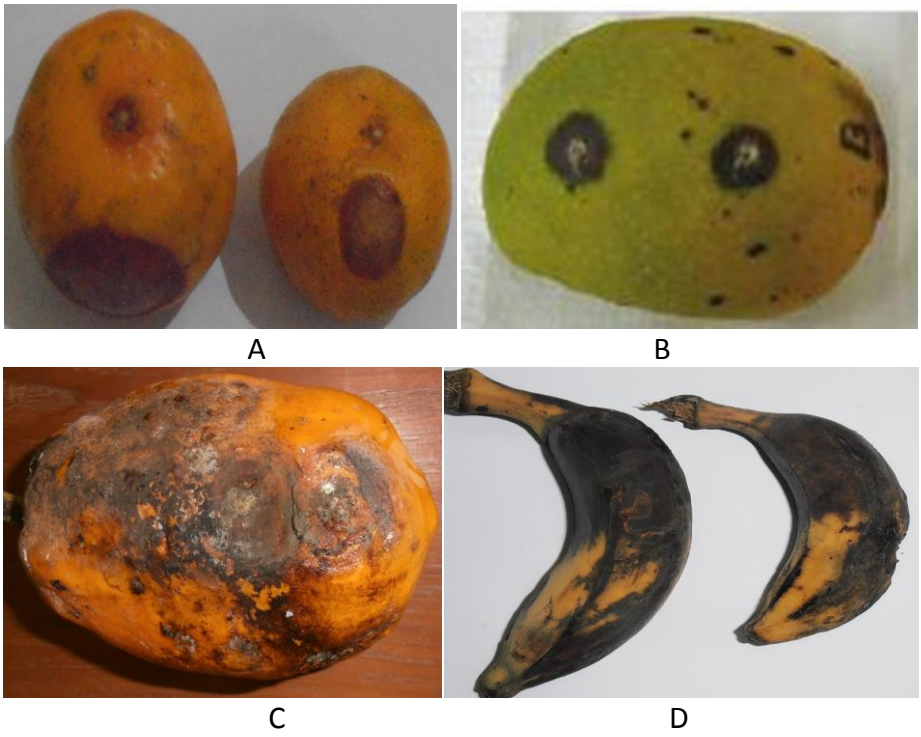
ABSTRACT

A research on antifungal activity assay leaf extract of awar-awar (Ficus septica Brum .f) against Colletotrichum gloeosporioides causes anthracnose disease on local fruits in Bali has been conducted. The study was conducted at the Laboratory of Biochemistry Department of Biology, Faculty of Mathematics and Natural Sciences Universitas Udayana Bukit Jimbaran with the extraction method and diffusion wells. The study was designed with a completely randomized design (CRD) comprised of 5 treatments with 5 replications. Qualitative data were analyzed by descriptive and quantitative data were analyzed by analysis of variance (ANOVA), when the data show significant differences continued with DMRT at 5% level. The results showed that the leaf extract of awar-awar can inhibit the growth of Colletotrichum gloeosporioides with the formation of inhibition zone around the well diffusion. Colony growth, formation of spore, germination of spores and fungal biomass C. gloeosporioides can be inhibited by a leaf extract of awar-awar significantly (P<0, 05) when compared with controls.
Keywords: Anthracnose, Antifungal, Ficus septic and Colletotrichum gloeosporioides.

INTRODUCTION

Anthracnose is a major disease caused by the fungus *Colletotrichum* spp. that attack fruits in the tropics and subtropics when postharvest (Figure 1). In Indonesia, anthracnose pathogen most often found attacking local fruits is a fungus *Colletotrichum gloeosporioides* (Suryaningsih et al., 1996). Anthracnose besides resulting in yield loss can also damage the aesthetic value of the local fruits. The pathogen attacks can occur both

before and after harvest. Yield reduction due to anthracnose on local fruits can reach 50 percent or more (Semangun, 2007).



A B C D

Figure 1. There are some fruits local in Bali show symptoms anthracnose (A= orange, B= mango, C= papaya and D= banana).

Controls of anthracnose symptoms disease are still based on the use of synthetic fungicides. The use of synthetic fungicides continuous basis may result in pathogen resistance, pollute the environment and are harmful to consumers. Contamination by pesticides not only in agriculture but also the environment can harm human and animal life which pesticide residues accumulate in agricultural products and in waters (Sa'id, 1994). Based on this it is necessary to find out the alternative to control anthracnose on local fruits by utilizing the potential plant as a botanical fungicide that is not dangerous for the consumer and the environment.

According Sudirga et al. (2014) in the preliminary study as many as 20 species of plants have been tested in terms of antifungal activity against *Colletotrichum acutatum* the cause of anthracnose disease on chili pepper, and found six kinds of plants that can inhibit the growth of *C. acutatum*. These 6 kinds of plants are *Ficus septica*, *Albizia saman*, *Piper nigrum*, *Piper crocatum*, *Piper retrofectum* and *Thitonia difersifolia*. Among the six species, *F. septica* leaf extract has the highest inhibitory activity with inhibition zone of 30 mm.

MATERIAL AND METHODS

Place and Time Research

In vitro studies conducted at the Laboratory of Biochemistry Department of Biology, Faculty of Science, University of Udayana Bukit Jimbaran.

Sampling local fruits Bali (citrus, mango, papaya and banana) showing symptoms of anthracnose are taken from some of the traditional market in Denpasar. The study was conducted over five months from June to October 2016.

Extraction Method

Extraction leaf of *awar-awar* (*Ficus septica*) done by chopping the leaves, then dried at room temperature, and after dried leaves are made into a powder by means of a blender. *Awar awar* leaf powder (100 grams) and then macerated with 1000 ml of methanol PA (Pro Analysis) for 72 hours at room temperature, dark place. The filtrate obtained by filtering and dregs obtained were then macerated again with 1000 ml of methanol twice. To obtain crude extract leaves of *awar-awar* that will be used for further testing, the filtrate obtained from the maceration process is then evaporated with a vacuum rotary evaporator (Iwaki, Japan) at 40 °C.

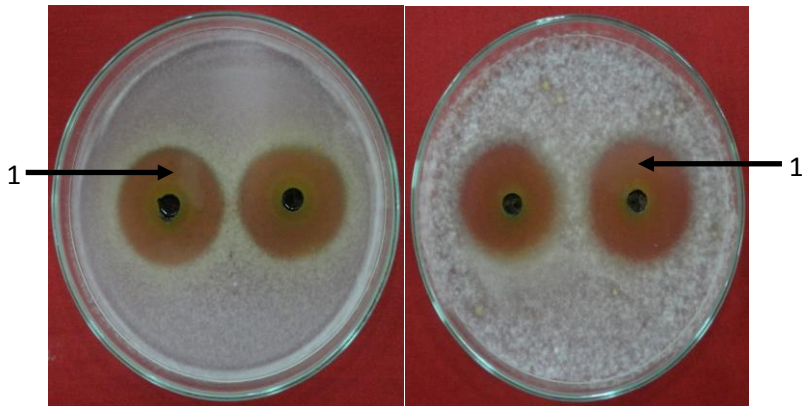
Antifungal Activity Test

Test the antifungal activity of crude extract of *awar-awar* leaves against *Colletotrichum gloeosporioides* done well diffusion method to measure the inhibition zone formed around the well diffusion. If the inhibition zone formed ≥ 20 mm means the barriers are very strong; if the inhibition zone formed between 10-20 mm means strong inhibition; if the inhibition zone formed between 5-10 mm mean inhibition moderat; whereas if the inhibition zone formed ≤ 5 mm means less or weaker inhibition (Ardiansyah, 2005). Some of the tests performed in this study as the effect of the extract on the growth of fungal colonies on PDA; the effects of extracts on spore density; spore germination; and fungal biomass in PDB media.

RESULTS AND DISCUSSION

Results

After the isolation and identification of fungi that cause anthracnose disease on local fruits in Bali, its fungus identified the *Colletotrichum gloeosporioides*. Then its are used as the test material for further research that is testing the antifungal activity of leaf extract of *awar-awar* with the well diffusion method. The parameter means like this: diameter of the colony, spore density, spore germination and fungal biomass. The test results as presented in Figure 2 and Table 1.



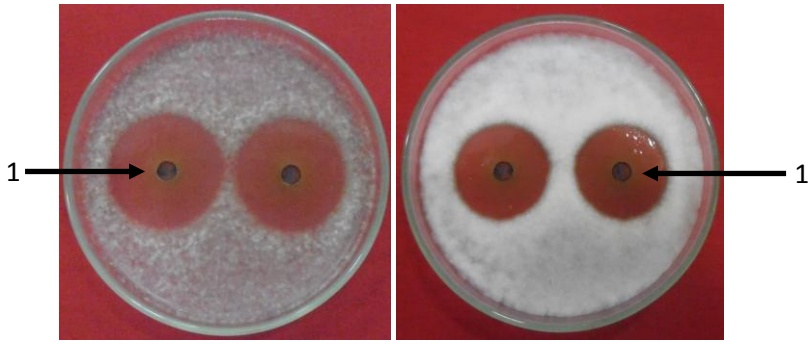


Figure 2. The result of the antifungal activity of leaf extract awar-awar against the *Colletotrichum gloesporioides* with the well diffusion method (A = bananas; B = papaya; C = orange; D = mango and 1 = diameter zone of inhibition).

Table 1. Inhibition of leaf extract of awar-awar on the growth of *Colletotrichum gloeosporioides* isolated from local fruits in Bali.

Colletotrichum gloeosporioides on isolated	Diameter of colony (mm)	Spore density (spore/ml x 10^5)	Spore germination (spore/ml x 10^5)	Biomass of fungi (g/100 ml)
Control	90,00 ± 0,21^a	11,38 ± 0,08^a	6,76 ± 0,10^a	0,86 ± 0,09^a
Orange	63,25 ± 0,10^b	5,19 ± 0,21^b	2,59 ± 0,12^b	0,52 ± 0,08^b
Papaya	58,00 ± 0,31^b	4,77 ± 0,08^b	1,96 ± 0,01^b	0,51 ± 0,21^b
Banana	57,75 ± 0,50^b	4,21 ± 0,10^b	1,56 ± 0,14^b	0,50 ± 0,13^b
Mango	58,50 ± 0,09^b	5,33 ± 0,20^b	2,09 ± 0,18^b	0,52 ± 0,09^b

** = Values followed by different letters in the same column means significantly different (P <0.05) based DMRT at the level of 5%.*

DISCUSSION

Based on Figure 2, the test results of the activity of the leaf extract awar-awar against the *Colletotrichum gloeosporioides* causes anthracnose on local fruit Bali such as fruit papaya, oranges, banana and mango with methods well diffusion, showed that the leaf extract awar-awar is able to inhibit the growth of fungi Colletotrichum gloeosporioides with the formation of inhibition zone on the media with inhibition zone diameter of 30 mm to 35 mm. According Ardiansyah (2005) when the inhibition zone formed above 20 mm it can be concluded that the extract is very strong to inhibit the growth of mold tests.

Based on the data presented in Table 1 shows that the awar-awar leaf extract can inhibit the growth of *Colletotrichum gloeosporioides* isolated from local fruits Bali. Measuring the diameter of the colony, spore density, spore germination and fungal biomass showed that among controls (media that is not mixed with leaf extract awar-awar) with treatments showed significant differences (P≤0,05), but among isolates that were tested did not showed difference real (P≥0,05). This is because among the isolates tested are the same type of fungi *C. gloeosporioides* thus providing a common response to the extracts which are used as the test material.

A similar study was reported by Gawade at al. (2014), that the leaf extracts *Aegle Marmelos* (L). extracted with methanol and ether in the ratio 1: 1 inhibits the growth of fungi *Colletotrichum acutatum* with inhibition zone diameter of 22 mm. Sayeed et al. (2012) reported that extracts of *Moringa oleifera* Lam. inhibit the growth of fungus *Colletotrichum* sp. with inhibition zone diameter of about 11 mm at the extract concentration of 100 ug / disc and 14 mm at a concentration of 200 ug / disc. According Nduagu et al. (2008), of 11 species of plants were tested against spore forming ability of the fungus *Colletotrichum capsici* after 7 days of incubation was found three types of plants are able to inhibit the formation of spores of *C. capsici* with barriers percentage above 90%. The third plant is *Cochlospermum planchonii* bark extract, bark extract and extract stem *Citrus limon, Tephrosia vogelii* with barriers percentage respectively 100%, 93.91% and 92.83%. Silva et al. (2008), reported that the *Origanum Majorana* L. leaf extract can inhibit the germination of spores of the fungus *Colletotrichum gloeosporioides* Penz to inhibition of 96%. Astiti and Suprapta (2012) reported that crude extract of leaves of teak at a concentration of 4% can inhibit the growth of fungal biomass *Nigrospora* sp., *Penicillium citrinium*, *Aspergillus flavus*, *Arthrinium phaeospermum* and *Acremonium butyri* with inhibition of respectively 100%, 96.43 %, 95%, 97.04% and 96.43%.

According to Baumgartner et al. (1990) the results of the methanol extract fractionation of *awar-awar* leaves contain active compounds in the form of 2 indolizidine alkaloid that is ficuseptine and antofine, the two compounds that have antifungal and antibacterial activity. Results of fractionation of ethanol and hexane leaf extract of *awar-awar* has potential as an anticancer compound, besides that of leaves, fruits and roots of *awar-awar* contain alkaloids, saponins and flavonoids that have the potential as antimicrobial compounds (Nugroho et al., 2013).The existence of barriers to growth of the fungus *Colletotrichum gloeosporioides* by crude extract of leaves awar-awar (*Ficus septica* Burm.f) may be due to the active compounds contained in the leaf-awar awar that are antifungal and antimicrobial. Castillo et al. (2012) reported that, awar-awar contain active compounds antofine and ficuseptine. Antofine compound has potential as an anticancer compound while the compounds ficuseptine potential as antibacterial and antifungal compounds. Lawal et al. (2012) reported methanol and ethyl acetate extracts of the root bark of *Ficus exasperata* Vahl. at a concentration of 200 mg / ml can inhibit the growth of *Colletotrichum gloeosporioides* with inhibition zone diameter each by 19 mm and 13 mm, and after phytochemical test, the extract containing saponins and glycosides. According Pelczar et al. (2003), mechanisms of antimicrobial substances in killing or inhibiting the growth of microbes are to: (1) damage the microbial cell wall, causing lysis or inhibit the formation of a cell wall in the growing, (2) change the permeability of the cell membrane, causing leakage of the cytoplasm and nutrients contained therein, (3) causes the denaturation of cells, and (4) inhibits the action of enzymes in the cell.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Crude extract of leaves awar-awar (*Ficus septica* Brum.f.) can inhibit the growth of fungi *Colletotrichum gloeosporioides* causes anthracnose on local fruits in Bali.

Recommendations

Further research is needed to determine the effective concentration of the crude extract of leaves awar-awar in inhibiting the growth of *Colletotrichum gloeosporioides*. As well as necessary to extract formulation and in vivo tests in the greenhouse before applied in the field.

ACKNOWLEDGEMENTS

The authors would like to express gratitude to the Rector of Udayana University for the research grants (Hibah Unggulan Udayana) and we would like to express to Laboratory of Biochemistry, Biology Departments, Faculty of Science, Udayana University for providing laboratory's facilities that made this study happened.

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