Antifungal Potential of Leaf Extract of Teak (*Tectona grandis* L.f) Against *Acremonium butyri* (v.Beyma) W.Gams

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ABSTRACT

The objective of this research was to investigate potential of teak (*Tectona grandis* Lf) leaf extract in inhibiting the growth of *Acremonium butyri* (v.Beyma) W.Gams , the cause of wood damage. The purpose of this study was to test the potential of leaf extracts of teak as biofungicide. Ectraction was done based on maceration method using methanol as solvent. Antifungal activity of teak leaf extract was done based on well diffusion method on Potato Dextrose Agar (PDA). Five concentrations of leaf extracts, i.e. 0 % (control), 0.5 %, 1 %, 2 % and 4 % were tested in this study.The result of this study showed that the teak leaf extract significantly suppressed the growth of *Acremonium butyri* (v.Beyma) W.Gams with Minimum Inhibitory Concentration (MIC) 0.1 %. The extract inhibited fungal radial growth, total biomassa dry weight and spores formation.

Key words : *Tectona grandis* Lf, *Acremonium butyri* (v.Beyma) W.Gams , *maceration method, well diffusion method*

INTRODUCTION

Acremonium butyri (v.Beyma) W.Gams is one of important fungi that can cause the wood spoilage. The wood spoilage can reduce the durability and quality of wood (Novianto, 2009). To control the fungus, the people mainly rely on the use of synthetic chemical fungicides as wood preservatives. Along with the intensive use of synthetic chemical food preservatives, there is an increase in the awareness of the people on the negative impacts of these chemicals particularly on human health and environment. Many chemical wood preservatives have been prohibited for use as wood preservative (Priadi, 2005).

Higher plants of tropical origin can produce a diverse of anti-microbe or anti-insect substances (Downum *et al*., 1993; Lis-Balchin *et al*., 1996; Nakamura *et al*., 1996). Substances such as flavonoids, alkaloids, terpenoids etc. are the secondary metabolites produced by the plants as chemical defense from pests and diseases attacks. It is estimated, from total amount of plants species of the world, only 10% of them have been investigated for their pesticidal activity.

Manoharachary and Gourinath (1988) have determined the efficacy of some tropical plant extracts against four pathogenic fungi, i.e. *Curvularia lunata*, *Cylindrocarpon lichenicola*, *Fusarium solani* and *Myrothecium leuchotrichum*. The plants tested were *Calatropis*, *Datura*, *Ocimum*, *Ricinus* and *Thidax*. Among the plant parts tested, extracts of roots and flowers were

found to be effective to inhibit the sporulation and the growth of fungi. Bandara and Wijayagunasekeya (1988) evaluated three rhizomatous herbs, i.e. *Acorus calamus* (Araceae), *Zingiber zerumbet* and *Curcuma longa* (Zingiberaceae) for their antifungal activity to *Cladosporium* sp., *Btryodiplodia theobromae*, *Fusarium solani*, *Phytophthora infestans*, *Phytium* sp., and *Pyricularia oryzae*. Their results revealed that extract of *A. calamus* and *Z. zerumbet* had profound effect on growth of all fungi tested.

Fifteen plant species of different families were evaluated for antifungal acitivity by Suprapta *et al*. (2001) to control *Ceratocystis* fruit rot on Snake fruit (*Salacca edulis*). Their finding revealed that root extract of *Alpinia galanga* and the leaf extract of *Carica papaya* significantly inhibited the growth of *Ceratocystis* sp. both on PDA medium and on Snake fruit. Leaf extract of *Pometia pinnata* was found to posses antifungal activity against *Phytophthora infestans*, the causal agent of late blight disease on potato (Suprapta *et al*., 2002). Application of leaf extracts of *Piper betle* and root extract of *Alpinia galanga* on banana plant in the field significantly controlled the wilt disease of banana caused by *Fusarium oxysporum* and *Pseudomonas solanacearum* (Arya *et al*., 2002). Astiti (1998) found that the water extract of teak leaves obviously inhibited the growth of the fungus *Monilia* sp., the cause of wood spoilage.

Appropriate technological improvement, which result in more effective use of natural resources is required to preserve the wood particularly against the attack of fungi. This study was done to evaluate the antifungal potential of teak leaf extracts particularly against *Acremonium butyri* (v.Beyma) W.Gams .

MATERIALS AND METHOD

Sample Collection and Extraction

Mature leaves of *Tectonia grandis* L.f. were collected from Bukit, Jimbaran Denpasar Bali. The leaves were washed in tap water, and chopped off into small pieces and air dried for three days under room temperature. The leaves were then powdered using blender. Extraction was done using methanol (PA grade) by soaking the powdered leaves for 48 h in the dark under room temperature. The filtrate was obtained through sieving with two layers cheese cloth and followed by filtration using Whatman No.1 filter paper. The filtrates were then evaporated in rotary evaporator (Iwaki, Tokyo Japan) to separate the methanol and the crude extract. This crude extract was used for antifungal bioassay to determine the minimum inhibitory concentration (MIC) against *Acremonium butyri* (v.Beyma) W.Gams .

Determination of Minimum Inhibitory Concentration (MIC)

The fungus *Acremonium butyri* (v.Beyma) W.Gams was isolated from spoilage wood and maintained in the Laboratory of Microbiology, Faculty of Science Udayana University. The fungus was re-cultured on PDA medium to allow it to produce spores and mycelia. The propagules (spores and mycelia) were harvested in sterile distilled water. Propagule's suspension (200 □ l) were put on a Petri dish and added with 10 ml melted PDA medium in a laminar flow. The Petri dish was shaken gently to allow the propagules distributed evenly on PDA. After the medium become solid, a diffusion well was made in the center of PDA using cork borer (5 mm diam.). Into the well, 20 □ l crude extract of teak leaf was put using micro pipette at

concentrations 0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 1%, 2% and 4%. Five Petri dishes were prepared for each concentration. The cultures were then incubated for 48 h in the dark under room temperature. The formation of inhibition zone around the diffusion well was observed to determine the antifungal activity. The lowest concentration in which the leaf extract of teak leaf produced inhibition zone is known as minimum inhibitory concentration (MIC).

Effect of Extract to the Radial Growth of *A. butyri*

The teak leaf extract at various concentrations (0%, 0.5%, 1%, 2% and 4%) were put on Petri dishes and then added with 10 ml melted PDA medium. The Petri dishes were shaken gently to allow the extract distributed evenly. After the medium become solid, a mycelial plug of *A. butyri* (v.Beyma) W.Gams (5 mm diam.) taken from the edge of a 3-day old culture was put in the center of PDA. Five Petri dishes were prepared for each concentration. The cultures were incubated for 7 days in the dark under room temperature. The diameter of fungal colony was measured daily. The percent inhibition of mycelia growth (PIMG) was determined according to the following formula (Pinto et al., 1998):

$$\text{PIMG (\%)} = \frac{dc - dt}{dc} \times 100$$

where

PIMG = Inhibitory activity (%)
dc = average increase in mycelia growth in control plates
dt = average increase in mycelia growth in treated plates.

Effect of Extract to the Spore Formation

Spores were harvested in sterile distilled water from a culture maintained in slant PDA. The suspension was passed through a filter paper (Whatman No.2) to separate the spore and mycelia or hypae. A 200 □ l spore's suspension (2 x 10^5 spores/ml) was added into 10 ml potato dextrose broth in a test tube containing various concentrations of teak leaf extract, i.e. 0%, 0.5%, 1%, 2% and 4% (w/v). Five test tubes were prepared for each concentration. The cultures were incubated in the dark under room temperature for five days. The number of spores were determined under microscope using haemocytometer. The inhibitory activity to the spore's formation (I) was calculated according to the following formula :

$$\text{I (\%)} = \frac{DC - DT}{DC} \times 100$$

where

I = Inhibitory activity (%)
DC = spore's density on control (without extract treatment)
DT = spore's density with extract treatment.

Effect of Extract on Fungal Biomass

Determination of the effect of teak leaf extract to the fungal biomass was done in 100 ml PDB medium that was placed in a 200-ml Erlenmeyer flask. The teak leaf extract was added into the flask at concentration varied from 0%, 0.5%, 1%, 2% and 4%. The medium was then inoculated with 1 ml of spore's suspension (the spore's density was 2 X 10^5 spores/ml). The final volume of the culture was 100 ml. Five flasks were prepared for each concentration. The cultures were incubated in the dark for 8 days under room temperature. The biomass was harvested through centrifugation at 5000 rpm for 5 minutes. The pellet (biomass) was taken and dried up in an oven at 60^{o}C until constant weight.

The inhibitory activity to the fungal biomass (I) was calculated according to the following formula :

$$I\ (\%) = \frac{WC - WT}{WC} \times 100$$

where

I = Inhibitory activity (%)
WC = weight of biomass on control (without extract treatment)
WT = weight of biomass with extract treatment.

RESULTS AND DISCUSSION

The teak leaf extract significantly suppressed the growth of *Acremonium butyri* (v.Beyma) W.Gams with minimum inhibitory concentration (MIC) 0.1%. This extract significantly ($P<0.05$) inhibited the radial growth of *A. butyric* on PDA medium. Treatment with 0.5% teak extract resulted in 50.45% inhibitory activity toward fungal radial growth. Results of this study showed that the higher the teak extract concentration, the higher the inhibitory activity. No fungal growth was observed on plates treated with teak leaf extract at concentration 4% (w/v) (Table 1; Figure 1).

Table 1. Inhibitory activity of teak leaf extract against the radial growth of *Acremonium butyri* (v.Beyma) W.Gams

No.	Extract concentration (%, w/v)	Diameter of fungal colony (mm)	Percent of inhibitory activity
1	0	89.2a*	-
2	0.5	44.2b	50.45
3	1	24.0c	73.09
4	2	14.0d	84.30
5	4	0e	100

*) Values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at 5%.

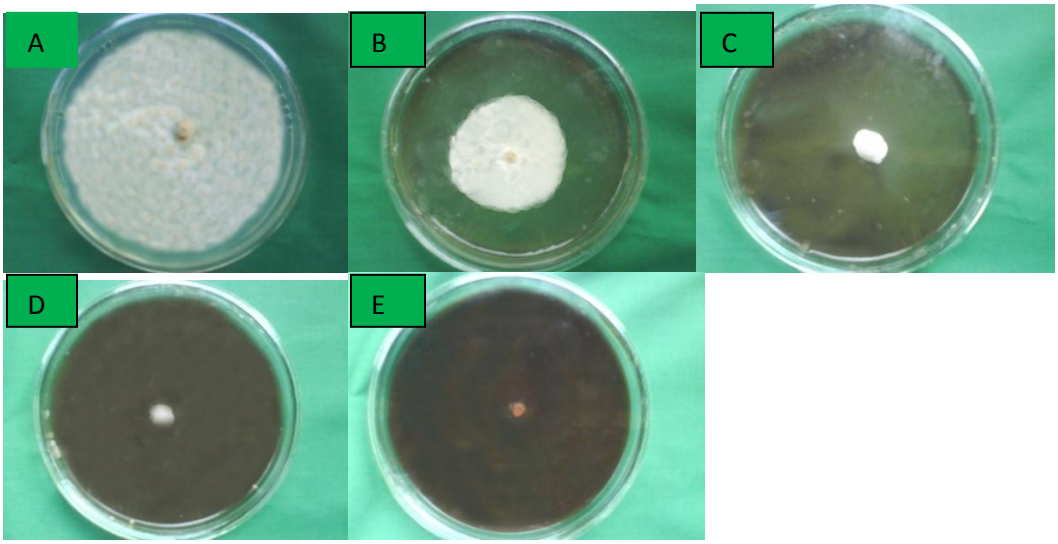

Figure 1. Effect of crude extracts against mycelium of *Acremonium butyri* in PDA medium after 7 days incubation.Tested concentration A . 0 % (control); B. 0.5 %; C. 1 % ; D.2 % ; E. 4 %.

The treatment with teak leaf extract as much as 2% (w/v) significantly ($P<0.05$) suppressed the spore's formation of *A. butyri* (v.Beyma) W.Gams on PDB medium. However,

there was no significant different (P>0.05) of spore's formation between control and treatment with 0.5% and 1% of teak leaf extract. The highest inhibitory activity was shown by treatment with 4% teak leaf extract (Table 2). Likewise, the treatment with teak leaf extract significantly (P<0.05) inhibited the biomass formation of *A. butyri* (v.Beyma) W.Gams on PDB medium. At extract concentration as low as 1% (w/v) the biomass formation of *A. butyri* (v.Beyma) W.Gams was suppressed by 58.05% (Table 3).

Table 2. Inhibitory activity of teak leaf extract against the spore's formation of *Acremonium butyri* (v.Beyma) W.Gams

No.	Extract concentration (%, w/v)	Spore's density/ml (x 10^5 spores)	Percent of inhibitory activity
1	0	134.5a*	-
2	0.5	124.5a	7.43
3	1	110.4a	17.92
4	2	77.5b	46.09
5	4	13.5c	89.97

*) Values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at 5%.

Table 3. Inhibitory activity of teak leaf extract against the biomass of *Acremonium butyri* (v.Beyma) W.Gams

No.	Extract concentration (%, w/v)	Dry weight of biomass (mg)	Percent of inhibitory activity
1	0	94.4a*	-
2	0.5	78.2b	17.16
3	1	39.6c	58.05
4	2	7.6d	91.95
5	4	3.4e	96.43

*) Values followed by the same letter in the same column are not significantly different according to the Duncan's Multiple Range Test at 5%.

Several plant species have been studied for their antifungal activities against plant pathogenic fungi. Plant extracts from several plant species such as *Piper betle* (Family : Piperaceae), *Alpinia galanga* (Family Zingiberaceae), *Eugenia aromatica* (Family Myrtaceae), *Pometia pinnata* (Family Sapindaceae), *Sphaeranthus indicus* (Family Compositae) and *Carica papaya* (Family Caricaceae) were proven to posses antifungal activities on potato-dextrose agar (PDA) medium against several pathogenic fungi. Methanol extracts of *A. galanga* rhizome and *C. papaya* leaf obviously inhibited the radial growth of *Ceratocystis* sp., the causal agent of fruit rot disease on Salak fruit (*Sallaca edulis*) on PDA medium. Treatment with 0.5% (w/v) extracts of *A. galanga* or *C. papaya* inhibited the radial growth of *Ceratocystis* sp. by 92.5% and 73.3% respectively (Suprapta *et al*., 2001). The *P. betle* crude extract reduced significantly ($P<0.05$) the spore formation of *Fusarium oxysporum* f.sp. *vanillae* in potato dextrose (PD) broth medium. The spore formation was inhibited by the *P. betle* crude extracts as low as 0.1% (w/v) with inhibitory activity of 84.41%. Minimum inhibitory concentration (MIC) of this extract was 0.15% (w/v). The spore formation *F. oxysporum* f.sp. *vanillae* was completely inhibited when 0.3% to 0.5% *P. betle* crude extract was used (Suprapta *et al*., 2007). In addition to spore formation, the *P. betle* crude extract also affected the radial growth of *F. oxysporum* f.sp. *vanillae* on PDA medium. The radial growth of *F. oxysporum* f.sp. *vanillae* was inhibited significantly ($P<0.05$) by the *P. betle* crude extract at concentration as low as 0.15% with an inhibitory activity of 20.23%. The more the concentration of the extract increased, the more the inhibitory activity observed within the tested concentration (Suprapta *et al*., 2007).

Five plant species namely *E. aromatica*, *A. galanga*, *Pometia pinnata*, *Sphaeranthus indicus* and *P. betle* exhibited the antifungal activity against *Phytophthora palmivora*, the causal agent of cocoa black pod disease. The crude extract of these plant species showed inhibitory activity against the radial growth of *P. palmivora* of more than 50% at a concentration of 0.5% (w/v) on PDA medium (Suprapta *et al*., 2008). The leaf extract of *Pometia pinnata* exhibited the antifungal activity against *Phytophthora infestans*, the causal agent of potato late blight disease. Treatment with 0.5% (w/v) crude extract of *P. pinnata* on PDA medium inhibited 85% of the radial growth of *P. infestans* (Suprapta *et al*., 2002).

Bandara *et al*. (1989) tested the effect of the crude extract of rhizome of *Acorus calamus* (Araceae) and *Zingiber zerumbet* (Zingiberaceae) against the growth and spore's formation of several pathogenic fungi. Results showed that these plant extracts significantly inhibited the growth of *Cladosporium* sp., *Btryodiplodia theobromae*, *Fusarium solani*, *Phythophthora infestans*, *Phythium* sp., and *Pyricularia oryzae*. The inhibiting activity of the extract of *A. calamus* against the growth of *F. solani* was bigger that that of Benlate, a synthetic fungicide.

The present study revealed that the methanolic extract of the teak leaf obviously inhibited the growth, spore's formation and biomass formation of *A. butyri* (v.Beyma) W.Gams . These results suggested that the extract of teak leaf contains antifungal substances against *A. butyri* (v.Beyma) W.Gams , one of important fungi that cause wood spoilage.

CONCLUSION

The methanolic extract of teak leaf obviously inhibited the growth of *Acremonium butyri* (v.Beyma) W.Gams one of the important fungi that cause the wood spoilage. The teak leaf extract inhibited the growth of *A. butyri* (v.Beyma) W.Gams through the suppression of the radial growth, spore's formation and biomass formation. A further study is needed in order to isolate and identify the active substances that responsible for antifungal activity against *A. butyri* (v.Beyma) W.Gams

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