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Antifungal of Fruit and Shoot Extracts of *Orania palindan* on *Coriolus versicolor* Growth

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ABSTRACT

Orania palindan is a species of areca nut from Family Arecaceae that produces toxins, especially in its fruit and shoot. This research aimed to test the antifungal property of *O. palindan* fruit and shoot using hot water extraction on the growth of *Coriolus versicolor*. Complete randomized design was used with four extract concentrations (0 %, 2 %, 4 %, and 6 %), each with three replications. Data were analyzed using Analysis of Variance (ANOVA) using SPSS. Results indicated that *O. palindan* fruit and shoot extracts significantly inhibited *C. versicolor* growth at 2%, 4%, and 6%. Duncan post-hoc test indicated that shoot extract at 6% concentration was the most effective at inhibiting *C. versicolor* growth.

Keyword: *Orania palindan*, Antifungal, *Coriolus versicolor*

1. INTRODUCTION

Papua Island is one of the areas in Indonesia with tremendous biodiversity. Plants in Papua island are estimated to reach 20,000 to 25,000 species, so Papua island is known as a megadiversity. One of the flora in Papua is the Areca palm from the family of Palmae or Arecaceae. About 2,300 palm species globally, 1,000 species are in Malesiana, with 270 species found in Papua and Papua New Guinea [1,10].

Areca plant, one of the Arecaceae species, is commonly found in India, Malaysia, Taiwan, Indonesia, and other Asian countries [4,6]. One of the areca palm species that the Papuan community is not exploited is *Orania* because its shoot contains toxin [1]. A scientific investigation is needed to test the toxin of the *Orania* plant, learn about the antifungal property of its fruit and shoot with hot water solvent (100°C) on the growth of white root fungus (*Coriolus versicolor*).

2. MATERIAL AND METHODS

2.1. Sample Preparation

Fruit and shoot samples of *O. palindan* were taken from Demini village, Momiwaren District, South

Manokwari Regency, Papua Barat province. Samples were taken from two trees. The fruits and shoots were cut up to ± 0.5 cm x 0.5 cm x 0.5 cm and dried in an air-conditioned room at 16-18°C until air-dry. Air-dry samples were grounded using a hammermill.

2.2. Sample Extraction

Five hundred grams of powder resulting from grinding using the hammermill was macerated using hot water (100°C) with 1:5 powder to solvent ratio and was let cool for 24 hours. Filtrate extracted was then evaporated in an air-conditioned room at 16-18°C aided by a fan to get a solid extract.

2.3. Fungi Preparation

Antifungal testing on wood-weathering fungi was done on white root fungus, *C. versicolor*. *C. versicolor* isolate was obtained from Tropical Silviculture and Forest Ecology Busgenweg Gottingen Germany in the form of culture in a petri dish. Before testing it in vitro, the fungus was first rejuvenated in a petri dish using PDA media.

2.4. Statistical Analysis

A complete randomized design was used in this experiment with four treatments (0%, 2%, 4%, and 6%), each with three replications. The response variable measured in this study was *C. versicolor* growth and the Average Activity of Anti Fungi (AAF) treated with *O. palindan* fruit and shoot extracts with concentrations of 2%, 4% and 6%. The *C. versicolor* growth treated with *O. palindan* fruit and shoot extracts with concentrations of 2%, 4% and 6%, which refers to the American Wood Preserve Association (Standard No. M10-63). The growth of *C. versicolor* was calculated using the following equation:

$$Pc(\%) = \frac{LPc}{Lc} \times 100$$

Where:

Pc = Percentage of petri dish area covered with hypha (%)

LPc = Total area covered by fungi (cm²)

Lc = Total area of the petri dish (cm²)

The total area covered by fungi (LPc) on a petri dish with a 9 cm diameter was calculated using the following equation:

$$LPc = \frac{1}{4} \pi D^2$$

LPc = Total area covered by fungi (cm²)

π = 3.14

D = Diameter of the distribution of hypha

The Average Activity of Anti Fungi (AFA), which refers to [3,4], calculated using the following equation:

$$I = \frac{(C-T)}{C} \times 100$$

Where:

I = inhibition (%)

C = colony diameter of mycelium from control Petri dish (mm)

T = colony diameter of mycelium from the Petri dish containing the solutions (mm)

Analysis of chemical component of extraction using LCMS was conducted at Indonesian Police Headquarters Forensic Laboratory Centre (Puslabfor), Jakarta.

2.5. Data Analysis

Data was analyzed by variance analysis (ANOVA). If the result was significant, Duncan posthoc test was conducted. Data analysis was performed using SPSS 25 for windows application.

3. RESULT AND DISCUSSION

3.1. Test of Antifungal Activity of Fruit and Shoot Extract of *Orania palindan*

Antifungal is a compound used to treat infectious diseases caused by fungi. Antifungal testing showed that fruit and shoot extract of *O. palindan* at concentration 2%, 4% and 6% were able to inhibit the growth of *C. versicolor* fungi (Figure 1 and 2).

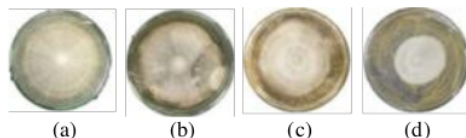


Figure 1 Inhibition activity of *Orania palindan* fruit extract on growth of *Coriolus versicolor* on day 7: (a) Control, (b) 2%, (c) 4 %, and (d) 6 % concentrations

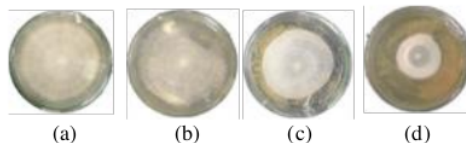


Figure 2 Inhibition activity of *Orania palindan* shoot extract on growth of *Coriolus versicolor* on day 7: (a) Control, (b) 2%, (c) 4 %, and (d) 6 % concentrations

As many researchers have found, *C. versicolor* is more resistant to antifungal treatment than other fungi. *C. versicolor* was more able to maintain its growth compared to *Schizophyllum commune* when treated clove-leaf extracts [2]. *C. versicolor* could support mycelium growth by up to 40%, while *Schizophyllum commune* only 30% [12]. The percentage of wood weight reduction due to *C. versicolor* attack was more significant than other fungi, which reached 15.23% [13]. *C. versicolor* was chosen in this study because of its known resistance.

The average percentage of *C. versicolor* growth of control was 100%, reached on the 7th day. The average percentages of *C. versicolor* growth treated with 2%, 4%, and 6% concentrations of *O. palindan* fruit extracts were 60.49%, 50.5%, and 31.73%, respectively. The average percentages of *C. versicolor* growth were 70.00%, 54.92%, and 24.67% when treated at 2%, 4%, and 6% concentrations with the shoot extract. Overall, the lowest average growth of *C. versicolor* was obtained in the treatment using shoot extract at a 6% concentration (Figure 3).

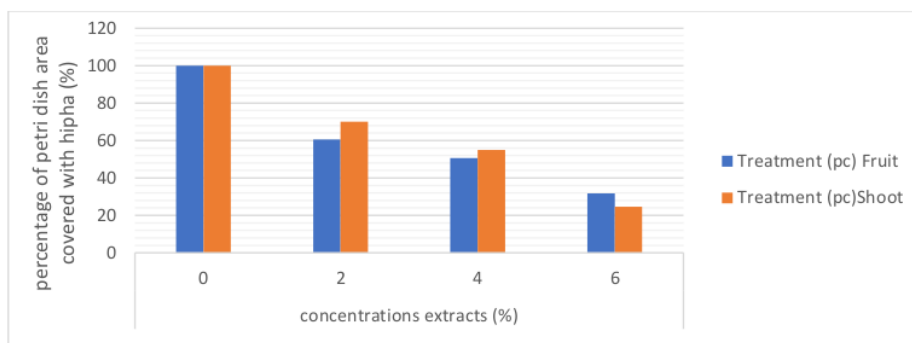


Figure 3 The average percentages of petri dish area covered by *C. versicolor* hypha (Pc) on different treatments

ANOVA test at 95% confidence level ($\alpha = 0,05$) indicated that 2%, 4%, and 6% of *O. palindan* fruit extract significantly affected the growth of *C. versicolor*. At 95% confidence level, Duncan test on the effect of *O. palindan* fruit extracts indicated that the 2%, 4%, and 6% treatments did not differ significantly, but all were very different from control (0%; Table 1).

Table 1. Duncan test on the effect of *Orania palindan* fruit extract treatments on the growth of *Coriolus versicolor* (N refers to the number of replicates)

Treatment (%)	N	Subset ($\alpha = 0,05$)	
		1	2
6	3	32.92	
4	3	51.26	
2	3	61.77	
0	3		100.00
Signification		0.054	1.000

At 95% confidence level, Duncan test on the effect of *O. palindan* shoot extracts indicated the 2% treatment was different from 0% and 6% treatments but not separate from the 4% treatment. It also showed that the 0% treatment differ from the 2%, 4% and 6% treatments and that the 6% treatment had the lowest mycelium growth (Table 2).

Table 2. Results of Duncan test on the effect of *Orania palindan* hot extract treatments on the growth of *Coriolus versicolor* (N refers to the number of replicates)

Treatment (%)	N	Subset ($\alpha = 0,05$)		
		1	2	3
6	3	24.97		
4	3		55.41	
2	3		71.06	
0	3			100.00
Signification		1.000	0.174	1.000

Fruit and shoot extracts of *O. palindan* were able to inhibit the growth of *C. versicolor*. The greater the extract used, the smaller the percentage of petri dish area covered with hypha. As indicated by the Duncan test results, the 2% extract could inhibit *C. versicolor*, but the most effective at inhibiting growth was *O. palindan* shoot extract at 6% concentration.

Cinnamomum camphora extract can be developed as a natural wood preservative [7] So, the extract of the fruit and shoot extracts of *O. palindan* can also be developed as one.

3.2. Activity Result of Antifungal (AFA) Score Test

Result of the AFA calculation, shown in Table 3.

Table 3. Percentage Average score of (Activity of Anti Fungi=AFA)

Part of Section	Concentration (%)	AFA	Degree of Activities
Fruit	0	0.00	not active (-)
	2	39.51	moderate (++)
	4	49.43	moderate (++)
	6	68.27	strong (+++)
Shoot	0	0.00	not active (-)
	2	30.00	moderate (++)
	4	45.08	moderate (++)
	6	75.33	strong (+++)

Source: Primary data after processed

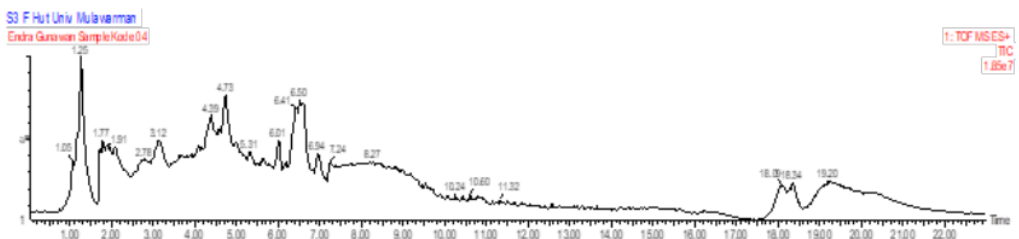


Figure 4 The Result of LCMS Analysis of *Orania palindan* Shoot's Extract using Hot Water Solvents

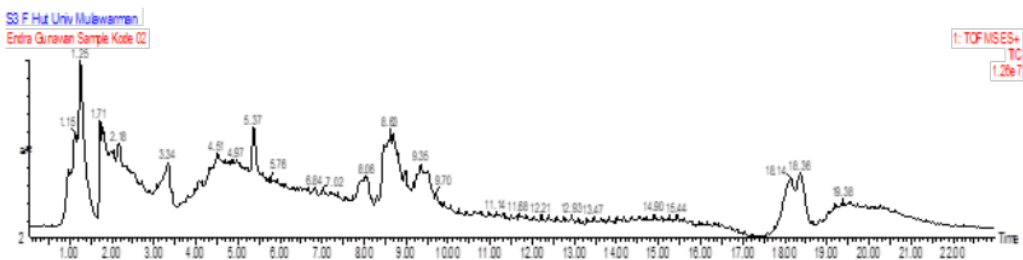


Figure 5 The Result of LCMS Analysis of *Orania palindan* Fruit's Extract using Hot Water Solvent

At the extraction of fruit and shoot of *O. palindan* using hot water, at concentration 2%, 4%, the resulting moderat and 6% resulted in a strong AFA score. *C. versicolor* is strong fungi but can be inhibited its mycelium growth by diluting the extract of fruit and shoot of *O. palindan* in hot water.

3.3. LCMS Analysis

The LCMS analysis of *O. palindan* shoot's and fruit extract extracted using hot water solvents shows in Figure 4 and 5. The LCMS analysis of *O. palindan* shoots, and fruit extract using hot water solvent resulted in 11 chemical compounds that can be antifungal. These compounds are listed in Table 4. At the same time, the fruit's extract contains six compounds, as the list in Table 5.

Table 4. List of The Chemical Compounds of *Orania palindan* Shoot's Extract extracted Using Hot Water Solvents

No.	Name of compound
1.	5-Aminotetrazolo[1,5-a]pyrazine-6,8-dicarbonitrile
2.	1-Acetoxy-5-fluoro-3-(2-oxiranylmethyl)-2,4(1H,3H)-pyrimidinedione;
3.	5-Chloro-4-(chloromethyl)-1-(4-fluorobenzyl)-3-isopropyl-1H-pyrazole
4.	3-(5-Bromo-2-thienyl)-6-methyl [1,2,4]triazolo[3,4-b][1,3,2]thiadiazole
5.	1-((2S,4S)-4-Fluoro-1-[(5-methyl-1,2,4-oxadiazol-3-yl)methyl]-2-pyrrolidinyl)-N-methylmethanamine dihydrochloride
6.	N-(2-[(5-Bromo-1,3,4-thiadiazol-2-yl)amino]ethyl) methanesulfonamide
7.	3,3'-(1-Methyl-1H-1,2,4-triazole-3,5-diyl)bis(4-azido-1,2,5-oxadiazole)
8.	3-(2,6-Dichlorophenyl)-N-(4-fluoro-3-nitrophenyl)-5-methyl-1,2-oxazole-4-carboxamide;
9.	2-Oxo-2H-imidazole-4,5-dicarbonitrile
10.	3,6-Dihydro-1H-[1,2,5]oxadiazolo[3,4-b][1,2,3]triazolo[4,5-e]pyrazine;
11.	2,2-Dihydroxy-1,3,5,7-tetraoxa-2-silaspiro [3.3]heptan-6-one ,

Table 5. The Chemical Compounds of *Orania palindan* Fruit's Extract extracted Using Hot Water Solvents

No.	Name of compound
1.	3-(4-(bromomethyl) phenyl)-5-methyl-1,2,4-oxadiazole
2.	N-(5-Nitro-3,8-dioxido[1,2,5]oxadiazolo[3,4-e][2,1,3]benzoxadiazol-4-yl)
3.	Ammonium 2-(dinitromethyl)-5-nitro-2,5-dihydro-1H-tetrazole-5-carboxylate
4.	2-[3-(Trimethylammonio)propyl]-1,2,3,4-tetrahydro isoquinolinium dibromide
5.	Dipropyl [5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl] phosphoramidate
6.	[(4,6-Dichloro-1,3,5-triazin-2-yl)(4-methoxyphenyl) carbamoyl] sulfamic acid.

The result of LCMS analysis shows that from the extract of fruit and shoot of *O. palindan* extracted using hot water, the compounds that become anti fungi are azole and pirimidin groups. Azole group in the extract of fruit and shoot of *O. palindan* is consist of tetrazole, pyrazole, thiadiazole, oxazole, and imidiazole. Tetrazole is a compound that acts as an anti-inflammatory, anti-bacteria, antivirus, anti-tuberculosis, anti-fungi, analgesic, and antitumor [3]. Derivation of triazole and thiadiazole are compounds that can be used as anti-bacteria and anti-fungi [9]. Imidazole compound contains purine, histamine, histidine, dan nucleic acid. Moreover, it is stated that the imidazole compound acts as anti-bacterial, anti-cancer, anti-tuberculosis, anti-fungi, analgesic, and anti-HIV [14]. Derivations of pyrimidine, such as furo [2,3-d] pyrimidine, triazolo [1,5-a] pyrimidine, and tetrazole [1,5] pyrimidine, are compounds that act as anti-cancer, antivirus, anti-hypertension, analgesic, antipyretic, anti-inflammatory, anti-fungi, anti-bacteria, and anti-psoriasis [11].

4. CONCLUSIONS

Fruit and shoot extract of *O. palindan* can inhibit the growth of *C. versicolor* fungus. The most effective treatment to inhibit the growth of *C. versicolor* fungus is fruit and shoot extracts at 6% concentration. The part of *O. palindan* plant that was most effective at inhibiting the growth of *C. versicolor* fungus was the shoot. ANOVA results showed that the fruit and shoot extracts of *O. palindan* had a significant effect on the growth of *C. versicolor* fungi. Duncan test results showed that the 4% concentration of fruits and shoot extract inhibited the growth of *C. versicolor*, but the most effective concentration was the shoot extract at 6% concentration. The anti-fungi compounds from the extract of fruit and shoot of *O. palindan* using hot water Solvent are azole and pyrimidine. Azole group consist of tetrazole, pyrazole, thiadiazole, oxazole, and imidiazole.

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