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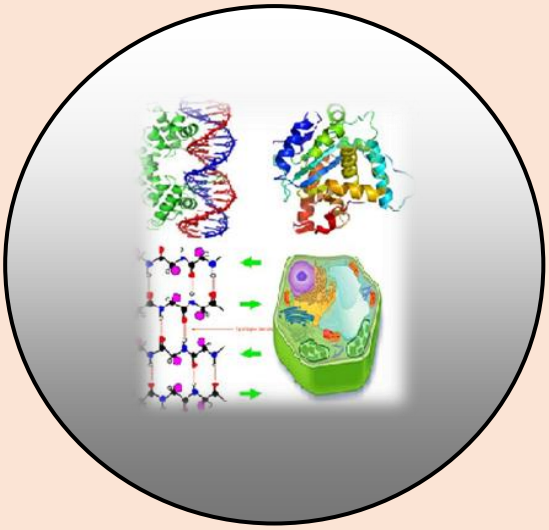

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RESEARCH PAPER

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Elucidation and Inhibition of Sembung Delan (*Sphaeranthus indicus* L.) Leaf Extract against BalineseLontar Destructive Fungi

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ABSTRACT

Lontar is a manuscript written on lontar leaves containing Balinese script that is passed down from generation to generation. The presence of fungal activity can damage the lontarbali in storage. Efforts to control fungus on lontarbali need to be done. One of them is by utilizing natural ingredients from plants. Preliminary research obtained 7 types of fungi isolated from 6 different locations in the province of Bali, namely Penicilliumrestricum, Aspergillus fumigatus, Mucorracemosus, Candida krusei, Aspergillus niger, Fusariumsp. and Rhodoterolamucilaginosa. This study aims to determine the class of compounds contained in the extract of SembungDelan leaf (Sphaeranthus indicus L.) and its inhibition on 7 types of fungi that have been isolated from lontar Bali. In vitro testing of the inhibition of the SembungDelanleaf extract on each fungal isolated on lontarbali was carried out by the diffusion well method. The compound group contained in the leaf extract of SembungDelan was known through phytochemical tests. The results showed that all types of fungi isolated in Balinese palm oil were able to be inhibited by crude extracts of Sembungdelan leaves with different barriers. Phytochemical tests are known that the sembungdelan leaves contain alkaloid, terpenoids, phenolic, saponin, flavonoid and tannin compounds.

Keywords: Lontarbali, Fungi, Sphaeranthus indicus and Extract.

INTRODUCTION

Lontar is one cultural heritage in form of manuscripts containing Balinese scripts which have been handed down from generation to generation. The manuscripts written on palm leaves are considered classic or ancient which tends to be sacred and religious (Geriani, 2010, Sedana et al., 2013).The environment conditions with high humidity might cause lontar to be easily damaged. In addition to insects, the existence of microorganisms such as fungi became another cause of the damage.Rontar leaves from rontar plants (*Borassus flabellifer*) as raw material for making lontar are mostly composed of cellulose components which are good substrates for fungal growth. According to Yosmar *et al.* (2015), cellulose is a carbohydrates making up the cell walls of plants that are easily damaged by cellulase enzyme.

Sancana (2014) stated that lontar manuscript as a cultural object does not have a strong resistance to environmental influences. Furthermore, there were reportedly several groups of fungi as a cause of damage to the Balinese lontar, namely: Aspergillus, Penicillium and Fusarium. In the initial research, there were 7 types of fungi found to be isolated from Balinese lonta in different locations, namely: 2 locations in Denpasar City (GriyaBalun and GriyaBatukandik); 2 locations in Tabanan Regency (Griya Kediri and GriyaSunantaya) and 2 locations in Gianyar Regency (GriyaUbud 1 and GriyaUbud 2). The types of fungi found were *Penicilliumrestricum*, *Aspergillus fumigatus*, *Mucorracemosus, Candida krusei, Aspergillus niger, Fusarium* sp. and *Rhodoterolamucilaginosa*. The growth of fungi in Balinese lontar needs to be managed so that the damage can be prevented. In this study, using natural materials as a source of raw materials in designing the formulations to inhibit the growth of fungi that could damage Balinese lontar.

SembungDelan leaves (*Sphaerantusindicus* L.) are plants which growagricultural land that have a potential to control the growth of fungi. A research from Darmayasa (2002) proved that the extract of this leaf was able to inhibit *Alternaria*sp., *Gleosporium*sp and *Phytophthora*sp. fungi in *in-vitro.* Furthermore, Darmayasa (2014)reported that the extract of SembungDelan was able to inhibit the growth of *Phytopthorainfestan*fungi which is the cause of leaf blight in potato plants. Based on those researches, a deeper research on the ability of SembungDelan leaves to inhibit the growth of fungi in lontar Bali needs to be conducted.

MATERIAL AND METHODS

Time and Place of the Research

This research is conducted from May to September, 2019 in the Microbiology Laboratory , Faculty of Mathematics and Natural Sciences, Udayana University and a joint Laboratory of the Mathematics and Natural Sciences of Udayana University.

Method of Extraction

The SembungDelan leaves are washed clean and air dried. Then, blended until become flour consistency. Next, measured to 100grammacerated with 1 liter of methanol at room temperature for 72 hours. The maceration results were then filtered. The filtrate obtained was evaporated with a vacuum rotary evaporator at 40^{o}C to separate the solvent. The crude extract obtained was used for further analysis (Harborne, 1996).

The Inhibitory Test of SembungDelan (S. *indicus* L.) Crude Extract on Balinese Lontar Destructive Fungi

The inhibitory test of SembungDelan (*S. indicus*L.) on Balinese lontar destructive fungi used the diffusion well method (Hewitt and Vincent, 1989). First, prepare each fungal suspension that had been isolated from the Balinese lontar by media *Potato Dextrosa Agar*, poured as much as 15 mL into a sterile petri dish. Next, implant the fungal colonies taken from culture stock of the sloping media right in the middle of the petri dish. After incubation time of 4 days at room temperature, the growing fungi were then rinsed using 5 mL of sterile water in order to obtain a suspension of mold spores. The density of mold spores was calculated using*NouemHaemocytometer*.

Each suspension of mold spores was taken as much as 200 μL. Next, put into a sterile Petri dish then poured PDA media and homogenized in order to obtain an even growth of fungi. After the mixture of PDA media and isolate was frozen up, several wells with a diameter of 0,5mm were made on that media. As much as 40 μL crude extract of SembungDelan leaves was put into the well. However, for the negative control on the other wells, only methanol was being deposit. All of those treatments were repeated 3 times. The inhibitory of the crude extract of SembungDelan leaves on the tested fungi was determined by measuring the diameter of the clear zone around the well. The measurements were conducted using calipers for 4 times and then being averaged. The crude extract of SembungDelan leaves which has shown a potential as a vegetative fungicideis given a concentration of 5% (b/v) by dilution using the following formula:

$$V_1 \cdot M_1 = V_2 \cdot M_2$$

Keterangan: Note:

V_1: initial volume V_2: expected volume M_1: initial concentration M_2: expected concentration

The Phytochemical Test of the crude extract of Sembungdelan leaves (*Sphaeranthusindicus*L.)

The Phytochemical test of the crude extract of SembungDelan leaves being conducted were the test for alkaloids, flavonoids, phenolics, steroids, terpenoids, and saponins.

Test for Alkaloids

The sample of Sembungdelan leaf extract was dissolved into 10 ml of ammonia chloroform, then added 0.5 mL of H_2SO_4 and homogenized, after that placed until two layers were formed which were pulp in the lower layer and clear extract in the upper layer. The top layer was taken, then dripped withmeyer reagent as much as 1 drop. If the extract turn out to be positive to contain alkaloids, it would form intosediment. (Harborne, 1996).

Test for Flavanoids and Phelonics

Test for flavonoids and phelonics in Sembungdelan extract was conducted by dissolving the extract in to 70% of ethanol and being heated up. Then, the extract was filtered to obtain the filtrate, and the result of the filtrate would be placed onto a drip plate and added with Mg and HCL to test for flavonoids, and added with $FeCL_3$ to test for the phenolics. The change of color on the extract would be red to show that it contained flavonoids, while the formation of a green to purplish green ring on the extract would signify the phenolic extract (Harborne, 1996).

Test for Steroids and Terpenoids

Test for steroids and terpenoids in Sembungdelan extract was conducted by adding a sample of Sembungdelan leaf extract into chloroform and heated up for 10 minutes. After being heated up, the sample was placed on a drip plate and then added with Lb reagent (concentrated antridic acetic acid + concentrated H_2SO_4). If the extract turned out to be positive to containterpenoids, the color would changeinto red, pink or violet in the SembungDelan extract, and if the sample turned out to be positive to contain steroids, the color would changeinto green or purple in the sample (Harborne, 1996).

Test for Saponins

Test for saponins in the sembungdelan extract was conducted by dissolving as much as 0,1 g Sembungdelan leaf extract into 5 mL of hot aquadest and shaked for 10 seconds. If the extract turned into a stable froth or foam for 10 minutes, the extract must be positive to contain saponins (Harborne, 1996).

The Analysis of the Data

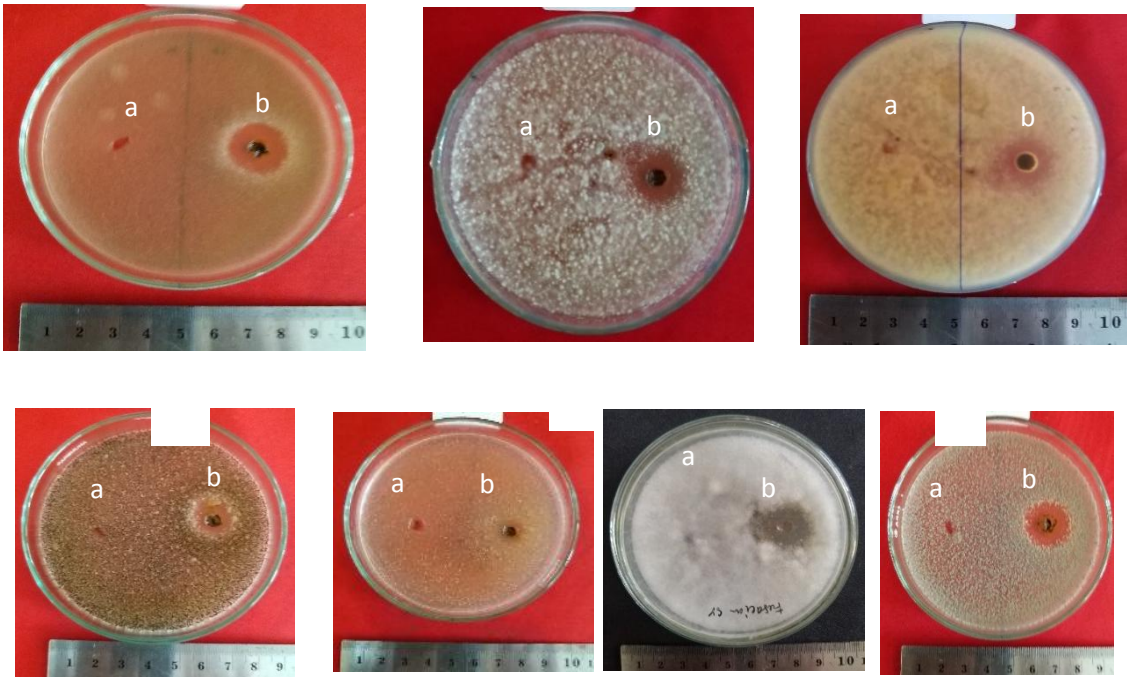
The obtained data in this research were in qualitative, which is showed in form of pictures or graphics, and in quantitative, which is analyzed using the Analysis of Variance (ANOVA). If the obtained data showed a significant difference at the test level of 5% ($P < 0.05$), it would be proceeded into Duncan test.

RESULT AND DISCUSSION

The crude extract of SembungDelan leaves at the concentration of 0,01g/20 µL in *in vitro* was able to inhibit the growth of fungi in Balinese lontar. This matter is shown by the presence of the diameter of the inhibitory zone that formed around the diffusion well. The diameter of the inhibitory zone of each fungus tested was different. The biggest resistance diameter occurred in the testing of SembungDelan crude extracts on the fungus *Aspergillus niger* was 21 mm. The smallest resistance obtained in the test of the fungus *Rhodoterulamucilaginosa* was with a diameter of the inhibitory zone of 10 mm, more explanation is presented in Table 2.

All types of fungi that have been isolated from Balinese lontar in different places in *in viro*were ableto be inhibited by the crude extract of SembungDelan leaves at the concentration of 0,01g/20 μL. In this study, the inhibitory had by the crude extract of SembungDelan leaves were varied. The difference of the inhibitory was thought to be closely related to the structure and characteristics of the isolated fungi. The biggest inhibition based on the measurement of the diameter of the inhibitory zone occured in the *Aspergillus niger* fungus, which was 21 mm. The smallest inhibition was found in the testing of *Rhodoterulamucilaginosa* fungus with a diameter of 10 mm in the inhibition zone. According to Nester *et al.* (2007), there appears to be a difference of inhibitory from one substance tested in *in vitro* is determined by the type, concentration of the test compound, and type and concentration of the microbes tested.

A similar research conducted by Darmayasa (2014) reported that Sembung Delan leaves were able to inhibit the growth of *Phytopthorain festan* fungi which is the cause of leaf blight in potato plants. Similarly to the research conducted by Mhetre*et al.*(2006) which showed that Sembung Delan plants were able to inhibit the growth of *Stappylococcus aureus, Eschericia coli, Fusarium* sp., and *Penicillium pinophilum*. The formation of the clear zone diameter around the diffusion well in this study proved that there was an active substance that functioned as an antifungal contained in the crude extract of Sembung Delan leaves.



Note:

1 sigr control (b) Diameter hibitory zone of Sembungdelar eaf extract against*Candida krusei*

2 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Aspergillus fumigatus*

3 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Mucorracemosus*

4 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Aspergillus niger*

5 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Rhodoterulamucilaginosa*

6 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Fusarium*sp

7 sign (a) control (b) Diameter of inhibitory zone of Sembungdelan leaf extract against *Penicilliumrestricum*

The phytochemical test of Sembungdelan leaf extract, it turned out to be positive to contain alkaloids, terpenoids, phenolics, saponins, flavonoids and tannins. However, the test for steroids showed a negative result. Qualitativelu, the most abundant compound in SembungDelan leaf extract was terpenoids, proven from the test results giving a prominent reddish color. The least amount of compounds in SembungDelan leaf extract was alkaloids and tannins. More details are presented in Table 3.

Table 2. The diameter of the inhibitory zone of SembungDelan leaves (*S. indicus*L) crude extract on the fungi in Balinese lontar.

No	Type of Fungus	Inhibitory of SembungDelan(*Sphaerantus indicus* L.)crude extract (Ø mm)
1	*Penicillium restricum*	18±0,24
2	*Aspergillus fumigatus*	18±0,25
3	*Mucor racemosus*	20±0,15
4	*Candida krusei*	19±0,24
5	*Aspergillus niger*	21±0,15
6	*Fusarium*sp	19±0,24
7	*Rhodoterulamucilaginosa*	10±0,00

Table 3. Compound groups of SembungDelan leaf extract based on the Phytochemical test.

No	Compound Group	result	Unit
1	Alkaloids	+	white sediment
2	Steroids	-	greenish color
3	Terpenoids	+ + +	reddish color
4	Phenolics	+ +	greenish color
5	Saponins	+ +	foam
6	Flavonoids	+ +	reddish color
7	Tannins	+	bluish color

Note:
+++ :give sediment/a lot of color/prominent
++ : give sediment/medium color
+ :give sediment/enough color
- : no sediment/color

Based on the phytochemical test, SembungDelan plants contained several compound groups namely alkaloids, terpenoids, phenolics, saponins, flavonoids and tannins. The presence of the compound groups has been reported by Tiwari and Khosa (2009) in SembungDelan plants in the mainland of India, which contained *flavonoid glikosida, flavonoid C-glikosida, isoflavon, sterol, alkaloid, asam amino, 2-hydroxycostic acid, asamilicic*, essential oil which has methyl chavicol, d-cadinene, α-ionone, p-methoxycinnamaldehyde, eugenol, taninns and steroids.In this study, test for steroids has turned out to be negative. The difference in geographical condition was assumed to be the cause of the absence of steroid compounds in SembungDelan plants in mainland of Indonesia. The essential oil that is contained in SembungDelan leaves has the characteristic of being an antifungal against *Trichodermaviride*, *Rhizopusnodosus*, *Aspergillus niger, Trichophytonrubrum* and *Curvularia parasadii* (Mane and Badole, 2013). Emani*et al.* (2017) successfully isolated the compound *5a-hydroperoxy-7a-hydroxy-isosphaerantholide* and (*11a,13-dihydro- 7a-hydroxyfrullanolide-13-yl*)-adenine (**2**) from its flowers. However, it is still cannot be proven whether the isolated compound could be used as antifungal. It is in contrast with Bhuwan*et al.* (2016) which stated that the extract of SembungDelan plants (*S. indicus*L.) had a very broad biological activity. Selvanayagam*et al*. (2005) said that *S. indicus*L. were indigenous plants which grew on agricultural land, and is widely used as a medicine for dysentery, diseases of the uterus and vagina, urinary tract infections as well as a control of worms and pests. More over, stated in their research that *S. indicus*L. contained crystal with a structure of 3a-Hydroxy-3,5a,9-trimethyl 1,3,3a,4,5,5a,6,7,8, 9b, decahydro-2naphtha [1,2c]-imidazole-2-one Monohydrat. Reported by Sangeetha *et al.* (2010) that the extract of *S. indicus* L. using ethanol solvent, chloroform and petroleum were able to inhibit microbes that are the causes of several infection diseases in humans, such as *Candida albicans*, *Cryptococcus neoformans*, *Salmonella typhy*, *Klebsiella*sp., *Pseudomonas aeruginosa*, and *E. coli*.

CONCLUSION

The Extract of SembungDelan leaves (*S. indicus*L.) with methanol solvent was able to inhibit the growth of *Penicilliumrestricum*, *Aspergillus fumigatus, Mucorracemosus, Candida krusei, Aspergillus niger, Fusarium*sp. and *Rhodotorulamucilaginosa*fungi which were isolated from Balinese lontar. The compound groups contained in the SembungDelan leaf extract in this study were alkaloids, terpenoids, phenolics, saponins, flavonoids and tannins.

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