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The Terpenoid Activity of Ethanol extracted from Purple Yam Sap to Inhibit the Growth of *R. oligosporus* and *S. cerevisiae*

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***Abstract*—Plants that contain secondary metabolite components can be used as an anti-microbial. Terpenoids are part of secondary metabolic, which is naturally in the plants. This study aims to investigate the terpenoid activity of purple yam sap as an anti-microbe to prevent the growth of *R. Oligosporus'*mold and *S.Cerevicea's yeast.* The terpenoid property within the purple *yam sap* was identified using the thin-layer chromatography (TLC) eluent toluene of *Etil Asetat* (3: 7). The anti-microbial activity was tested using the agar diffusion method, and the cell damage analysis was carried out using SEM. This study showed that the anti-microbial activities of the terpenoid to inhibit the growth of *R. Oligosporus* mold were as follow: the 96% ethanol extract had the inhibition zone of 8.5 mm, the 80% ethanol extract had the inhibition zone of 9.5 mm, and 65% ethanol extract had the inhibition zone of 10.03 mm, whereas the 50% ethanol extract had the highest inhibition zone by 10.93 mm. Meanwhile, 96% ethanol extract had the most robust ability to inhibit the growth of *S.crevicea* yeast by 11.07 mm, and 80% of the ethanol extract had the weakest ability to inhibit the growth of this yeast only 9.23 mm diameter of inhibition zone. The terpenoids substance with the minimum concentrate (0.01%). Extract causes the cell of the *R.oligosporus* fungus and cells the *S. Cereviceae's* yeast to leak; thus, the cell ruptured and died. On the one hand, the *S.cereviceae* cell changes the shapes and experiences cell damage.**

***Keywords*— Antimicrobial; *terpenoid*; purple yam sap; extract.**

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I. INTRODUCTION

Molds and yeasts can grow and multiply rapidly in humid areas. Molds and yeasts are microorganisms that can cause disease and bring damage to plants, animals, and human beings, as well as food products. Anti-microbial agents, both natural and synthetic, aim at inhibiting the growth of the microbes. Plants that contain secondary metabolite components, like *phenols, alkaloids, tannins, flavonoids, steroids, terpenoids, saponins* etc., can be used as an anti-microbial. Terpenoids, polyphenols, thiols prevent the growth of pathogenic microorganisms [1]

Utilization of active agents from plants, as either anti-fungi, traditional medicine, or natural preservation, is widely explored, i.e., extraction of essential oil from lime leaves effectively prevents the growth of *Aspergillus*sp fungus that causes aflatoxins in food [2]. The extract of *wualaerhizome* can inhibit the growth of *C. Albicans* fungus [3]. The onion skin extract has a strong property that inhibits Trichophyton mentagrophytes fungus's growth [4]. Besides, the Nano-emulsion cinnamon wood extract has a strong ability to inhibit the growth of several types of fungus, such as *Rhizopus* [5]. The use of Jatropha leaf extract can inhibit the growth of the fungus *Candida albicans* [6].

The Uwi or purple yam has a sap that is yet to be explored mainly as an anti-microbial agent. In the previous study, Uwi sap has been detected to contain a secondary metabolic compound of terpenoid with the anti-microbial property. Terpenoid has the molecular structure of isoprene carbon unit (2-*methylbuta*- 1, 3-diene). *Terpenoid* is classified as a secondary metabolite, an ingredient in an essential oil [7]. *Terpenoid* has the potential to act as anti-fungus, anti-bacterial, or anti-viral [8]. The anti-fungus property of terpenoid is(R)-6-[(Z)-1-heptenil]-5,6-*dihydro*-2H-*piran*-2-one, which is isolated from Hyptisovalifolia Benth [9]. By in vitro, terpenoid shows anti-fungus activity toward the *Microsporumcanis, Microsporumgypseum, Trichophyton mentagrophytes,* and *Trichophyton rubrum*.

Based on these, a text needs to be carried out to explore the anti-microbial terpenoid activity of the Uwi/sweet yam sap, hypothesized to inhibit the growth of fungus yeast.

II. Material and Methods

A. Samples

The samples in this study were the purple yam obtained from Enrekang Regency of South Sulawesi Province, Indonesia.

B. The Extract of Uwi/Purple Yam

The purple yam sap was extracted with maceration [10] of 500 grams of purple yam for three days using the 96% single ethanol solvent, 80% ethanol, 65% ethanol, and 50% ethanol. The purple yam sap was filtered using the Whatman paper number 1 with a vacuum. The filtrate was then evaporated with a rotary evaporator at a temperature of 60 – 750 C for 30 minutes. The sap extract was then inserted into the desiccator.

C. Secondary Metabolite Activity

The analysis of the secondary metabolite activity of the uwi/purple yam sap was carried out using thin-layer chromatography (TLC) [10]. The thin coated 60 F_{254}silica gel plate of 6 x 7 cm and the purple yam sap sample was slabbed into the thin layer plate using the capillary pipet. The eluent/solvent used was the Toluene: (93:7). The plate was put into a chamber filled with eluent; thus, the plate was perfectly diluted. After the plate is dried, it was pulled and dried with a dried vacuum. The formed stain was observed using the UV 254 nm and UV 365 nm. Formed spot color (red-violet terpenoid) was observed using the sulfite acid vanillin solution.

D. Anti-microbial Activity

The anti-microbial activity was tested using the agar diffusion method [9]. The used bacterial isolate was *R.oligosporus* and *S.cerevisiae*. One inoculating loop with bacterial suspension was scratched into the sterile *saborauddextro agar* (SDA) media in a flowing laminar. The Paper disk was absorbed with 0.25 ml purple yam sap with a minimum concentration of (1%). Then the paper disc was put into a Petri dish that had previously spread with isolated bacteria. The positive control used was DMSO (dimethyl sulfoxide) and the negative control was tetracycline. The Petri dishes containing the extract of purple yam sap and each type of bacteria were incubated at 37^0C for 18 – 24 hours. After 24 hours, observation of the clear zone formed surrounding the media was carried out using the calipers.

III. Results and Discussion

A. Identified the Terpenoid

The terpenoid can be identified using the *Lieberman-Burchard method.* The result presented in (Table 1) for all types of ethanol extracted from the Uwi sap is detected to contain terpenoid substances positively. The observation was carried out under TLC of UV 25 purplish-red spot is observed with the Rf value of 0.14.

TABLE I
IDENTIFICATION OF TERPENOID SUBSTANCE OF ETHANOL EXTRACT EXTRACTED FROM THE PURPLE YAM/UWI SAP

No	Ethanol extract of uwi sap	Terpenoid	Retention time (Rf)
1	Ethanol extract 96%	Positive	0.14
2	Ethanol extract 80%	Positive	0.14
3	Ethanol extract 65%	Positive	0.14
4	Ethanol extract 50 %	Positive	0.14

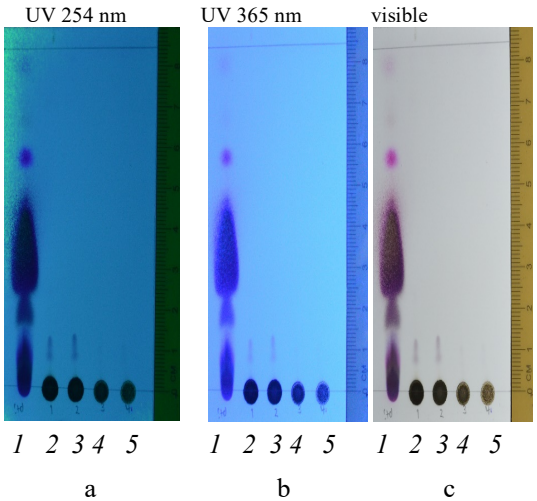

Fig. 1 Thin-layer chromatographic of terpenoid substance detection a) observation in UV 254 nm, b) UV 365 nm, c) without UV.

Notes:
1. Comparator terpenoid
2. 96% of ethanol extract
3. 80% ethanol extract
4. 65% of ethanol extract
5. 50% of ethanol extract.

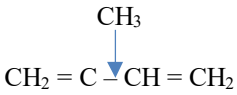

Fig. 2 The structure of terpenoid compounds [8].

Terpenoid was identified (Figure 1) due to the changes of color from red to purple when reacted with *Liebermann-Burchard reactant* (*hydride acetate* acid and thick H_2SO_4) [4], [10], [11], [12], [13]. Terpenoid was identified with the addition of H_2SO_4reagent and the formation of red brick color [14]. The terpenoid compound has anti-bacterial property *Terpenoid* group compounds are antibacterial [12]. The terpenoid compound structure contains the aromatic C, H, and O. Terpenoid has a carbon structure that developed from two atoms or more of C_5 units, which is called as isoprene. Isoprene units are usually in orderly related.

B. The Activity Terpenoid to Inhibition Rhizopus oligosporus

The activity to inhibit *R. oligosporus,* which is formed in Table 2, shows that ethanol from Uwi/purple yam concentrate extract by the lowest of 0.01% produces various inhibition zone diameters**.**

TABLE II
THE MEASUREMENT OF INHIBITION ZONE DIAMETER OF RHIZOPUS *OLIGOSPORUS FUNGUS'* GROWTH

Type of Extracts	Minimum Extract	Positive control (ketocon	Negative Control (DMSO)	Zone Diameter of *Rhizopus oligosporus*			Average
				UI	U2	U3	
96%	0.01%	13.9	6.6	7.9	8.5	9.3	8.5
80%	0.01%	16.2	6.6	7.9	10.1	10.5	9.5
65%	0.01%	15	6.6	9.1	10.2	10.8	10.03
50%	0.01%	14.4	6.6	9.6	11.4	11.8	10.93

The 96% ethanol extract of Uwi sap produces an inhibition zone with a diameter of 8.5 mm, 80% ethanol extract of Uwi/purple yam sap produces an inhibition zone with a diameter of 9.55 mm. Moreover, 65% ethanol extract of Uwi sap produces an inhibition zone with a diameter of 10.03 mm, and 50% ethanol extract of Uwi sap produces an inhibition zone with a diameter of 10.93 mm. The smallest inhibition zone is formed by the 96% ethanol extract, which is only 8.5 mm, whereas the largest inhibition zone is produced by 50% ethanol extract, which produces 10.99 mm diameter of inhibition zone. Fifty percent ethanol extract has a different polarization level, meaning that it is more efficient in extracting the substance's secondary metabolic property. Thus, it results in the formation of a larger inhibition zone diameter toward the growth of *Rhizopus oligosporus.* The utilization of ethanol and water (50%) is more efficient in extracting a compound due to its different polarity levels. The most significant inhibition zone (10.93 mm) formed by 50% of ethanol extract is classified as a strong category. In determining bacterial growth's strength, inhibition is based on the formed inhibition zone [16]. A ≥ 20 mm diameter of inhibition zone is categorized as powerful inhibition ability, whereas 10- 20 mm diameter is categorized as solid ability, inhibition diameter of 5 – 10 mm is categorized as moderate, and 5 mm or less is categorized as weak inhibition ability.

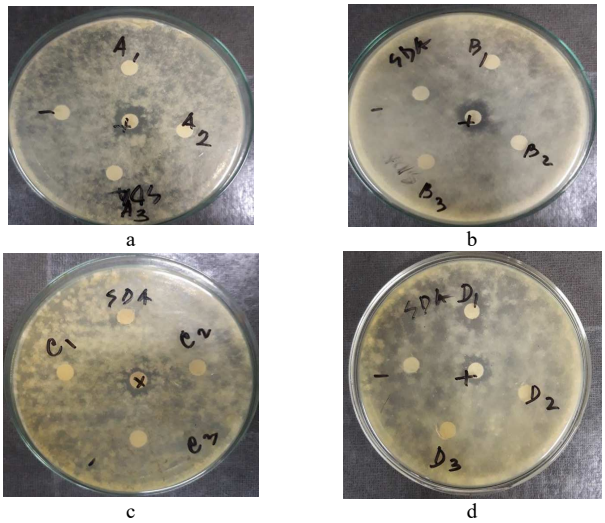

Fig. 3 The inhibition zone formed against *R. oligosporus* fungus (A) extracts ethanol 96% (B) extracts ethanol 80% (C) extracts ethanol 65% (D) extracts ethanol 50%

The observation on the minimum concentrate of 0.01% of ethanol extracted from the sap of Uwi/purple yam (Figure 3) shows that 96% of the ethanol extracted from the Uwi sap forms an inhibition zone of 8.5 mm diameter with moderate inhibition ability. Meanwhile, 80% of ethanol extract forms the inhibition zone of 9.5 mm, with also moderate inhibition ability. Further, 65% of ethanol extract forms an inhibition zone of 10.03 mm with strong inhibition ability. Lastly, the 50% ethanol extracted from this type of yam produces a 10.93 mm inhibition zone, which is categorized as strong inhibition ability. Based on the inhibition zone category, it is evident that 96% ethanol extract has a low inhibition property, whereas the 50% ethanol extract sample produces the highest inhibition property with a strong inhibition category. [16]categorizes the inhibition zone as follows: < 5 mm as weak category, 5-10 mm as moderate category, 10-20 mm as strong category, and > 20 mm as an excessive category. The ethanol extract extracted from the Uwi sap to inhibit the fungus' growth is due to the phytochemical property within the extract that serves as an anti-microbe. Secondary metabolic compounds can damage cell walls [17]. Anti-microbial compounds play a role in damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting the work of enzymes [18].

The terpenoid compound, which serves as the anti-microbial activity, is (R)-6-[(Z)-1-heptenil]-5,6-dihidro-2H-piran-2-one, which is isolated from the *Hyptisovalifolia Benth*. In vitro, this compound shows the anti-fungus activity against the *Microsporumcanis, Microsporumgypseum, Tricophytonmentagrophytes,* and *Tricophytonrubrum*. *Terpenoid* reacts with porin within the bacterial cell's outer membrane and forms strong polymeric bonds that cause damage to the porin. This leads to the bacterial cell being nutrition deficient; hence, the bacterial growth is inhibited or dies[9].

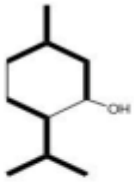

Fig. 4 The Core Structure of Terpenoid Compound (the menthol compound)[7].

Based on the highest inhibition zone formed by 50% ethanol extracted from the purple yam sap, further observation on the *Rhizopus oligoporus*cell damage due to the extract's addition with the concentrate of 0.01% was carried out.

R.oligosporus has the structural characteristics of hyphae, non-septate hyphae, fast growth, and cotton-like mycelium formation [19]. The appearance of the *R. oligosporus*cell in (Figure 5) shows that 50% ethanol extract from the Uwi/purple yam sap with 0.01% concentrate causes parts of the fungus to experience leakage. This is due to the secondary metabolic activity of the Uwi sap extract. The sap extract is detected to contain terpenoid based on the TLC test, where the terpenoid compound works to destroy the cell wall of the bacteria by disturbing the peptidoglycan

component of the bacteria; thus, the cell membrane is damaged, which causes the cell to experience lysis and the bacteria died [9].

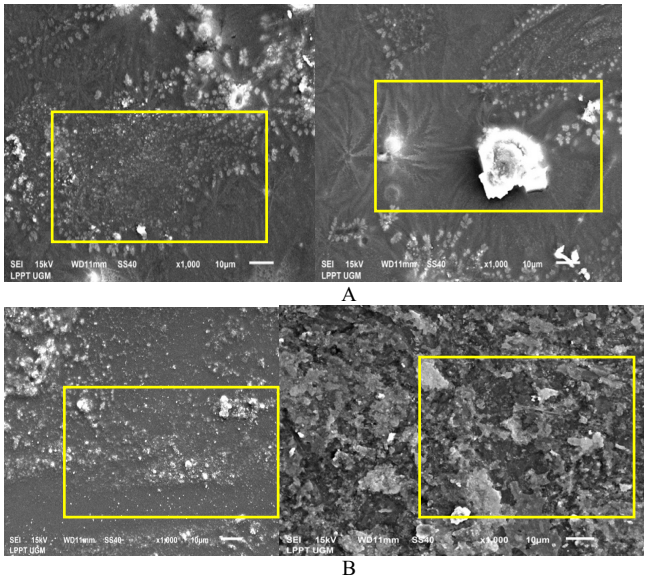

Fig. 5 The description of the damage of the R.oligosporus fungus cell using the microscopic electron scanning (SEM) after the addition of 50% ethanol extract from Uwi sap. A) The appearance of R.oligosporus mold cells changed shape after the addition of the sap extract. B) The mold cells leak due to exposure to the sap extract.

C. The Activity Terpenoid to Inhibition Saccharomyces Cerevisiae

The inhibition power that showed in (Table 3) toward the growth of *S. cerevisiae yeast* indicates that the formed inhibition zone in the concentrate of 0.01% for the 96%, 65%, and 50% ethanol extracts are categorized as strong. In contrast, 80% ethanol extract is in the moderate category, forming a low inhibition zone by only 9.23 mm.

TABLE III
THE MEASUREMENT OF INHIBITION ZONE DIAMETER OF SACCHAROMYCES *CEREVISIAE YEAST'* GROWTH

Type of extracts	Minimum extract	Positive control (ketoconazol	Negative control (dmso)	Zone diameter of saccharomyces cerevisiae			Average
				UI	U2	U3	
96%	0.01	19.4	6.6	10.5	10.9	11.8	11.07
80%	0.01	21.6	6.6	8.2	9.4	10.1	9.23
65%	0.01	21.6	6.6	9.1	10.1	10.9	10.03
50%	0.01	22.8	6.6	9.1	10.2	11.1	10.13

The 96% ethanol extract produces the highest inhibition zone by forming an inhibition zone of 11.07 mm. This is due to 96% ethanol extract's ability to extract a more dominant metabolic compound, as it has a similar polarity level to the extracted substance. The functional groups within the ethanol have polar and non-polar properties, where the (-OH) group has polar property due to its higher than oxygen electronegativity [18]. At the same time, the non-polar (C_2H_5-) is capable of dissolving non-polar compounds.

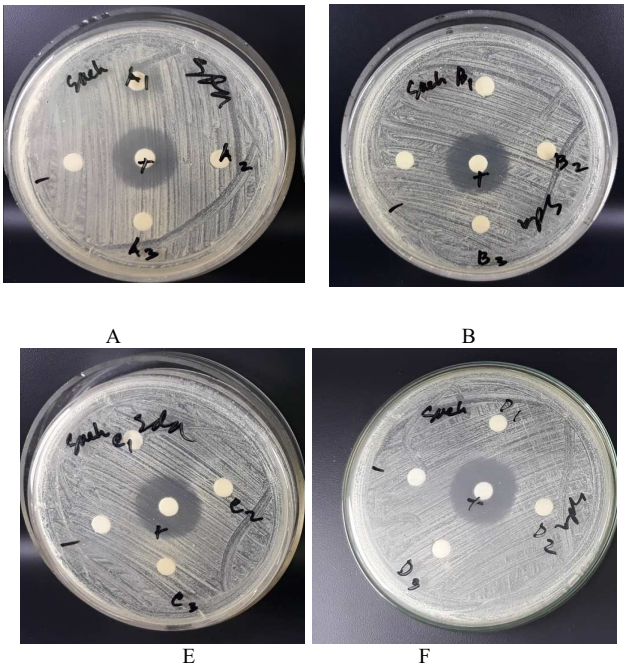

Fig. 6 The inhibition zone formed against S. *cerevisiae yeast*. (C) extracts ethanol 96% (D) extracts ethanol 80% (E) extracts ethanol 65% (F) extracts ethanol 50%

The active ethanol extract agent from the Uwi/purple yam sap in Figure 6 produces various inhibition zone toward the *S. cerevisiae yeast*. The 96% extract produces the highest inhibition zone by 11.07 mm, and the lowest inhibition zone is produced by the 80% extract by 9.23 mm. For the *R.oligosporus* fungus, the highest inhibition zone is produced by the 50% ethanol extract (10.93 mm), and the 96% ethanol extract produces the lowest inhibition zone. The diameter difference of the inhibition zone can be influenced by the cell structure owned by the microorganism. *R. oligosporus* can produce anti-bacterial agents [19], resulting in lower zone diameters compared to *S. cereviceae* yeasts. Yeast has microstructures consisting of the capsule, cell wall, cytoplasmic membrane, nucleus, vacuole, mitochondria, polyphosphate, and cytoplasm, whereas fungal cells contain *phospholipid* compounds, *sterols, nucleus proteins, mitochondria, endoplasmic reticulum, ribosomes, Golgic apparatus, peroxisomes, glyoxisomes, hydrogenesomes, lysosomes,* and *liposomes* [19]. Besides that, the fungus spore wall is thicker than the hyphae wall and has a cell membrane that protects cell contents [20].

The largest diameter of the inhibition zone produced by the 96% and 50% extracts may be due to the extent of the anti-microbial agent within the substance. The phytochemical component (such as *terpenoid*) within the extract is a determining factor that causes damage to the fungus and yeast cell. The 96% ethanol extract sample, which has the highest inhibition ability, is further observed using the SEM method to investigate the types of damage in the yeast cell due to the addition of 0.01% concentrate extract.

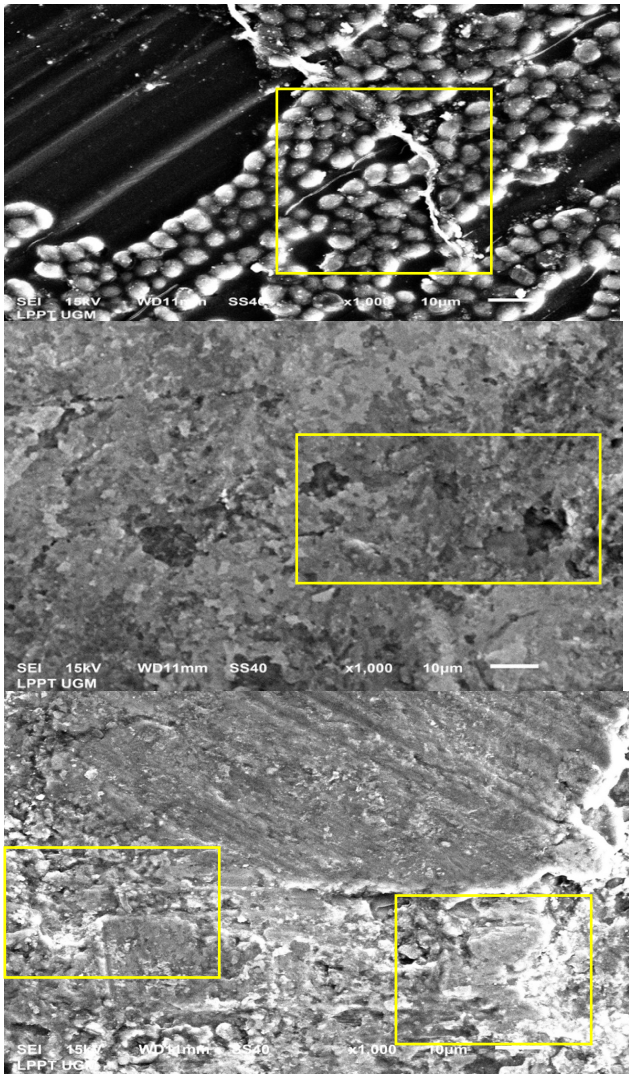

Fig. 7 The description of the damage of the *S. cerevisiae yeast* cell using the microscopic electron scanning (SEM) after the addition of 96% ethanol extract from Uwi sap. A) The appearance of S. *cerevisiae* cell morphology. B) *S. cerevisiae* bacterial cells undergo irregular shape changes.

S. cerevisiae can form a round shape, oval, even bar cells. The addition of 0.01% concentrate of Uwi sap extract (Figure7) causes the yeast cells' shape to change shape into irregular shapes and destroy the cells. The terpenoid compound causes the rupture of the cell membranes by the lipoic components, interferes with the fungus cell membrane's permeability, and further causes the destruction to the crista. Thus, the produced energy for the cell's growth and development is reduced, and the yeast growth is inhibited [9].

IV. Conclusion

The Uwi/purple yam sap extract contains secondary metabolic activity such as terpenoid substances that are effective as anti-microbes in inhibiting the growth of *R.oligosporus* fungus and *S. cerevisiae* yeast. In minimum concentrate extract, the terpenoid substance causes the cell of the *R.oligosporus* fungus to leak; thus, the cell ruptured and died. On the one hand, the *S. cerevisiae* cell changes the shapes and experiences cell damage.

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