UNIVERSITAS AHMAD DAHLAN
JURNAL BIOEDUKATIKA
http://journal.uad.ac.id/index.php/BIOEDUKATIKA
2338-6630 (Print)|2541-5646 (Online)

The Effect of Methanol and Ethyl Acetate Extracts of Kirinyuh Stem (*Eupatorium odoratum* L.) on Antifungal Activity of *Fusarium oxysporum*

Fika Pita Loka [a, 1, *], Sabirin Matsjeh [a, 2], Etika Dyah Puspitasari [a, 3]

[a] Biology Education, Faculty of Teacher Training and Education, Universitas Ahmad Dahlan, Yogyakarta Indonesia
[1] fikapitaloka16@gmail.com *;[2] sabirin.matsjeh@pbio.uad.ac.id ;[3] etika.puspitasari@pbio.uad.ac.id
* Corresponding author

ARTICLE INFO

Article history
Submission February 23, 2023
Revision March 19, 2023
Accepted May 27, 2023

Keyword:
Fusarium oxysporum
Eupatorium odoratum L.
Metanol
Etil Asetat

ABSTRACT

Fusarium oxysporum is a fungus that has a negative role, namely causing moler disease or Fusarium wilt in shallot plants. The purpose of this study was to determine the effect of methanol and ethyl acetate extracts of kirinyuh stem (Eupatorium odoratum L.) on the antifungal activity of Fusarium oxysporum. This study used a complete randomized design (CRD) with 8 treatments, namely negative control, positive control, concentrations of 10%, 20%, 40%, 60%, 80%, 100% with 3 replicates. The activity test was conducted using the Kirby Bauer method. Data from the study were processed by analysis of variance (one way anova) and continued by Duncan's test. The results showed that methanol and ethyl acetate extracts of kirinyuh stems affect the antifungal activity of Fusarium oxysporum. Ethyl acetate extract is more active to inhibit Fusarium oxysporum fungi compared to methanol extract. The highest inhibition zone diameter in the methanol extract was at 100% concentration which was 7.5 mm and the lowest at 10% concentration which was 2.67 mm. The highest inhibition zone diameter in the ethyl acetate extract was at 100% concentration which was 12.33 mm and the lowest at 10% concentration which was 4.67 mm.

Introduction

Fusarium oxysporum is a fungus that has a negative or detrimental role, which can cause moler disease or Fusarium wilt disease in shallot plants. Fusarium oxysporum fungus attack is a major obstacle faced by shallot farmers (Juwanda et al., 2016). The growth of Fusarium oxysporum fungus is very fast and has dense colonies. Initially, the colony is white like cotton or accompanied by purple or red at the center of the colony (Suryani et al., 2020). Moler disease is characterized by plants withering quickly, roots becoming rotten, plants drooping as if they are about to collapse, yellowing and twisting withered leaves, and at the base of the tuber appear white fungal colonies (Juwanda et al., 2016).

Data from the Directorate of Horticultural Plant Protection noted that in 2003 the area of Fusarium fungus attack was only 48.2 hectares. In 2007 it expanded to 404.9 hectares and now moler disease is the main disease in shallot plants (Emeliawati et al., 2022). In addition, shallot production in Southeast Sulawesi has decreased. In 2015 it was 4.4 tons/hectare. In 2016, production decreased to 2.27 tons/hectare or decreased ± 51 percent from the previous year due to moler disease (Disbunhorti, 2015 in Asaad et al., 2020).

This can cause losses for shallot farmers, so a way to control the disease that attacks the shallot is needed.

Control of plant diseases caused by fungi is generally done using chemical pesticides because they are considered very effective in eradicating microorganisms and are widely sold in the market (Astuti & Widyastuti, 2016). The use of chemical pesticides is harmful to the environment, which can cause residues and pathogens will be increasingly resistant to pesticides, so environmentally friendly control is needed by using vegetable pesticides. Vegetable pesticides are natural products from various plant organs such as roots, stems, leaves, seeds, fruits, flowers, and skins that contain bioactive compounds or secondary metabolite compounds (Wulandari et al., 2019).

Control with vegetable pesticides can provide optimal results and is relatively safe for living things and the environment (Emeliawati et al., 2022). One example of a plant that can be used as a vegetable pesticide is kirinyuh (Nurhasbah et al., 2017). Kirinyuh has the scientific name Eupatorium odoratum L. with the synonym Chromolaena odorata (Backer, 1965). Kirinyuh is a woody shrub, easily found in various regions in Indonesia and can grow quickly (Thamrin et al., 2013). This plant has potential as a medicine, namely wound medicine for skin infections and inflammation. In addition, it contains compounds that can be used as vegetable pesticides to control plant diseases, especially those caused by fungi (Permatasari & Asri, 2021). However, kirinyuh plants, especially the stem part, are not optimally utilized by the community because they are considered weeds that are difficult to control (Sari et al., 2017). Many studies have proven the effectiveness of kirinyuh plants to control moler disease, especially the leaves. However, no research has been found that uses kirinyuh stems to control moler disease in shallots.

Kirinyuh contains alkaloids, flavonoids, phenolics, saponins, tannins, terpenoids, and steroids (Gultom et al., 2020). As stated by Wulandari et al. (2018), that tannin, alkaloid, flavonoid, saponin, terpenoid and steroid compounds are secondary metabolite compounds that can control plant pathogens. The compounds contained in kirinyuh plants, especially flavonoids, can be taken through the extraction process (Verdiana et al., 2018). Proper solvent selection can improve the extraction process. Things that need to be considered in the selection of solvents include selectivity, polarity, toxicity, ease of evaporation, and solvent price. Extraction can be done using methanol and ethyl acetate solvents.

Methanol solvent is a universal solvent that can attract most polar and non-polar compounds. Methanol can attract flavonoid compounds, saponins, tannins and terpenoids in plants (Salamah & Widyasari, 2015). Meanwhile, ethyl acetate solvent is semi-polar which can bind compounds that provide antimicrobial activity, including phenols and flavonoids (Kurang & Penlaana, 2022). Based on the background description above, it is necessary to conduct this study to determine the effect of methanol and ethyl acetate extracts of kirinyuh stems on the antifungal activity of Fusarium oxysporum.

Method

Material

The materials used in this study include 70% alcohol, 80% methanol, 95% ethanol, ethyl acetate, 0.9% physiological NaCl, $BaCl_2$, H_2SO_4, kirinyuh stem powder, Fusarium oxysporum fungal isolates from shallots, PDA media, SDB media, distilled water, water, cotton, clear plastic, umbrella paper, filter paper, disc paper, aluminum foil, label paper, and tissue.

Tools

The tools used in this study include knives, cutting boards, bamboo baskets, blenders, filters, plastic jars, cake scales, analytical scales, funnels, stainless basins, round bottom flasks, separating funnels, thermometers, electric stoves, 500 mL erlenmeyers, 100 mL and 500 mL beakers, 100 mL and 1000 mL measuring cups, spoon, petri dish, round ose, glass spatula, stainless spatula, test tube, test tube rack, 10 mL propipette, 1 mL measuring pipette,

micro pipette, MC farland standard paper, cotton swab, vortex, refrigerator, autoclave, oven, tray, bunsen, hot plate, alcohol spray, tweezers, yellow tip, ruler, stationery, and SPSS software.

Research Stages

Sample Preparation

Kirinyuh stems are selected good stems. The kirinyuh stems were cut into small pieces of approximately 1 cm and washed with running water, then drained the water using bamboo baskets. Next, the kirinyuh stems were dried by airing them indoors. After drying, it is grounded until kirinyuh stem powder is obtained.

Maceration

Kirinyuh stem powder weighed as much as 100 g, put into a jar and soaked using 80% methanol as much as 700 mL until the surface of the kirinyuh stem powder sinks. Soaking of kirinyuh stem powder was carried out for 24 hours with one stirring. The powder that has been soaked is filtered using a sieve, so that the extract and pulp are obtained. Then, the pulp was soaked again like the maceration process done before. The extracts from the first and second maceration were combined (Herdiana & Aji, 2020).

Evaporation

Extracts obtained from maceration results are evaporated using a simple distillation device. The extract is put into a round bottom flask, then a condenser is installed and clamped with a stative. Next, it was heated on a water bath. The evaporation process is carried out for about 6 hours until the methanol does not drip anymore, so that the crude methanol extract of kirinyuh stem is obtained (Juliantari et al., 2018).

Fractination

A total of 100 mL of crude extract of kirinyuh stem was put into a separatory funnel, then added with 40 mL ethyl acetate. The separatory funnel containing the solution was digojog for 2-5 minutes with occasional release of gas contained in the solution, allowed to stand for several minutes until two layers were formed. The top layer (ethyl acetate layer) was removed, while the bottom layer (methanol layer) was added back 40 mL ethyl acetate as previously done up to three repetitions. Next, the methanol and ethyl acetate fractions were evaporated by standing in the open air. The ethyl acetate fraction was evaporated until 10 mL remained (Anjaswati et al., 2021).

Concentration Preparation of Test Materials

The variation in the concentration of methanol extract of kirinyuh stem is made by dilution using distilled water, while the variation in the concentration of ethyl acetate extract is made by dilution using 95% ethanol. The concentration variations of methanol and ethyl acetate extracts of kirinyuh stems are 10%, 20%, 40%, 60%, 80%, and 100%. Concentration of 10% (methanol/ethyl acetate extract 0.2 mL + 1.8 mL distilled water/ethanol 95%), 20% (methanol/ethyl acetate extract 0.4 mL + 1.6 mL distilled water/ethanol 95%), 40% (methanol/ethyl acetate extract 0.8 mL + 1, 2 mL of distilled water/ethanol 95%), 60% (methanol/ethyl acetate extract 1.2 mL + 0.8 mL distilled water/ethanol 95%), 80% (methanol/ethyl acetate extract 1.6 mL + 0.4 mL distilled water/ethanol 95%), and 100% (methanol/ethyl acetate extract 2 mL).

Media creation

The medium used for the growth of Fusarium oxysporum is potato dextrose agar (PDA). PDA media powder was weighed as much as 15.8 g. PDA powder was dissolved in 500 mL of distilled water in a 500 mL beaker, then brought to a boil while occasionally stirring. The media that has been made, then poured into 500 mL erlenmeyer, tightly closed using cotton that has been wrapped with umbrella paper. Next, it was sterilized in an autoclave for 15 minutes at 121 ℃ with a pressure of 1.5 atm (Tusa'diah & Chatri, 2021).

Fusarium oxysporum Fungus Rejuvenation

PDA media that has been sterilized, poured into test tubes aseptically. The test tube is placed in an inclined position and

left until the PDA media solidifies. After the PDA media has solidified, pure culture of Fusarium oxysporum is scratched zig zag on the surface of the PDA media using a sterile ose, then incubated at 37℃ for 7 days (Putri et al., 2019).

Preparation of Mc Farland Standards (Scale 0.5)

A total of 0.05 mL of 1% $BaCl_2$ was put into a test tube and 9.95 mL of 1% H_2SO_4 was added. Then, the test tube was covered with aluminum foil, homogenized using a vortex and stored in the refrigerator (Aviany & Pujiyanto, 2020).

Preparation of Fungal Suspension

Fusarium oxysporum fungal culture was taken as much as 1 ose added to a test tube containing 1 mL SDB (sabouraud dextrose broth) liquid media and incubated for 24 hours at 37 ℃. Then, the fungal culture was taken as much as 0.1 mL, added 10 mL of 0.9% physiological NaCl until the turbidity was the same as the Mc Farland standard (on a scale of 0.5 and a fungal concentration of 1.5 x 10^8 CFU/mL). After obtaining the desired turbidity, then the fungal suspension is taken 0.1 mL and put into 9 mL SDB media and digojog until homogeneous (concentration of 10^6 CFU/mL) (Wahyuni & Karim, 2020).

Fusarium oxysporum Antifungal Activity Test

Determination of antifungal activity using the disc paper method (Kirby-Baeur) or disc paper. Paper discs were soaked in methanol and ethyl acetate extracts with various concentrations, namely 10%, 20%, 40%, 60%, 80%, and 100% as well as negative control using distilled water and positive control using dithane M-45 for 15 minutes, then placed on the surface of PDA media that had been inoculated with Fusarium oxysporum. Measurement of the inhibition zone is seen from the clear zone formed around the disc paper on PDA media after incubation for 24 hours at 37 ℃ (Kandoli et al., 2016). The diameter of the inhibition zone of Fusarium oxysporum antifungal activity was calculated by the formula:

$$\frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Dv = Diameter vertikal ;
Dh = Diameter horizontal ;
Dc = Diameter cakram (Warbung et al., 2013)

Experiment Design

The research design used was a complete randomized design (CRD). This study uses two variables, namely the independent variable (X) and the dependent variable (Y). The independent variable (X) in this study is methanol and ethyl acetate extracts of kirinyuh stems with various concentrations of 10%, 20%, 40%, 60%, 80%, and 100%, where each was done three times. As a negative control using distilled water and positive control using dithane M-45. The dependent variable (Y) in this study is the diameter of the inhibition zone which is seen from the formation of a clear zone around the disc paper. The data obtained were analyzed by one way analysis of variances (ANOVA) method. If the analysis shows a significance value of less than 0.05 so that H0 is rejected, then the data is significantly different which is continued with the Duncan test.

Results and Discussion

The drying process of kirinyuh stems in this study was carried out by airing them indoors. During the drying process, the kirinyuh stems were turned over and over to dry evenly and prevent mold growth. According to Putri et al. (2022), the drying process by aerating indoors aims to keep the active compounds contained in the material from being damaged. The chemical content in the material can be damaged if the drying of the material is carried out in direct sunlight. During the drying process, the materials are arranged properly and should not be stacked and flipped so that the drying is evenly distributed and the drying takes place quickly. Drying aims to prevent the growth of microorganisms and so that the material can be stored for a relatively long time. Kirinyuh stems before maceration are mashed first until they become powder. According to Setyantoro et

al. (2019), the finer the powder size, the greater the surface area, so that more of the content contained in the kirinyuh stem can be drawn by the solvent.

Inhibition test of methanol and ethyl acetate extracts of kirinyuh stems against Fusarium oxysporum antifungal activity using the kirby bauer method using potato dextrose agar (PDA) media. The selection of the Kirby Bauer method is because this method has a smaller risk of failure compared to other methods. The media that has been inoculated with the fungus is placed upside down to prevent the water vapor droplets that arise from falling onto the media, where the droplets can affect the final results of the incubation (Putra, 2015). According to Intan et al. (2021), the advantages of the Kirby Bauer method are relatively low cost, fast testing process, easy to do, and does not require special skills. Selection of PDA media because PDA media is a suitable medium for fungal growth because it has a low pH of 4.5 - 5.6, thus avoiding bacterial contamination. Based on its components PDA is included in semi-synthetic media because it is made from potatoes (natural ingredients), dextrose and agar (synthetic ingredients). Potatoes are a source of carbohydrates, energy and vitamins, dextrose as an energy and sugar source, while the agar component serves to solidify the PDA medium. These components are needed for the growth and proliferation of fungi (Nurdin & Nurdin, 2020).

The diameter of the inhibition zone was determined based on the clear zone formed around the disc paper on the surface of PDA media that had previously been inoculated with Fusarium oxysporum and incubated for 48 hours at 37℃. Before the discs were placed on PDA media, the discs were soaked in negative control solution (distilled water), positive control (dithane M-45), methanol and ethyl acetate extracts (concentrations of 10%, 20%, 40%, 60%, 80% and 100%) for 15 minutes. Soaking the disc paper for 15 minutes aims to make the solution absorb perfectly into the disc paper (Intan et al., 2021). The positive control uses dithane M-45 because it contains the active ingredient menkozeb which can inhibit fungal growth, by damaging enzymes and fungal proteins (Sari et al., 2014).

Menkozeb is a fungicide from the dithiocarbamate group, which is in the form of maneb plus zinc ions. The addition of zinc can improve fungicidal properties and reduce membrane phytoxicity (Widiastuti et al., 2011). The negative control uses distilled water because there are no active compounds contained in distilled water or no antimicrobial effect on distilled water (Yanti et al., 2016). The use of 80% methanol solvent because 80% methanol is a universal solvent that has polar groups (-OH) and non-polar groups (-CH3), so it can attract polar and nonpolar compounds (Kurang & Penlaana, 2022). Methanol 80% can attract alkaloids, steroids, saponins, and flavonoids from plants (Salamah & Widyasari, 2015). The use of ethyl acetate solvent because ethyl acetate is semipolar, which can attract compounds that are polar and nonpolar (Harbone, 1998 in Warni et al., 2022). In addition, ethyl acetate can bind compounds that provide antimicrobial activity, such as phenols and flavonoids (Kurang & Penlaana, 2022). Ethyl acetate is a good solvent used for extraction because it is volatile, low toxicity and not hygroscopic (Putri et al., 2013). The results of measuring the diameter of the clear zone, ANOVA test was conducted which showed that methanol and ethyl acetate extracts had an effect on the growth of Fusarium oxysporum fungi, then continued with the Duncan test.

Table 1. Standard Deviation and Duncan Test Results of Methanol Extracts

Methanol Extract Concentration (%)	Inhibition Zone Diameter (mm) Repeat			Mean Zone of Inhibition Diameter (mm)±SD
	I	II	III	
Control (-)	0	0	0	0±0,00a
Control (+)	13,5	15	10	12,83±2,56f
10%	3	2	3	2,67±0,58b
20%	4	4	3	3,67±0,58bc
40%	5	5,5	4	4,83±0,76cd
60%	5	6	5	5,33±0,58cd
80%	6,5	6	6	6,17±0,29de
100%	8,5	8	6	7,5±1,32e

Notes: Notations (a, b, c, d, e, and f) are the results of Duncan's test with a 5% confidence level, if the same notation indicates not significantly different and if the notation is not the same, it indicates a significant difference.

Based on table 1, it shows that the methanol extract of kirinyuh stem can inhibit the growth of Fusarium oxysporum as indicated by the formation of a clear zone around the disc paper. The greater the concentration of kirinyuh stem methanol extract, the greater the average clear zone produced. At a concentration of 100%, the average clear zone produced was the largest compared to other concentrations, namely 7.5 ± 1.32 mm. The 80% concentration produced an average clear zone of 6.17 ± 0.29 mm. The 60% concentration produces an average clear zone of 5.33 ± 0.58 mm. The 40% concentration produced an average clear zone of 4.83±0.76 mm. The 20% concentration produced an average clear zone of 3.67±0.58 mm. The 10% concentration produced the smallest clear zone average of 2.67 ± 0.58 mm. The negative control in the form of distilled water, produces an average clear zone of 0 ± 0.00 mm, while the positive control in the form of dithane M-45 produces an average clear zone of 12.83 ± 2.56 mm.

Fungal growth inhibition response is classified based on the diameter of the inhibition zone including 0 mm (inactive), 20 mm (very strong) (Alioes et al., 2018). When viewed in the fungal growth inhibition response, the diameter of the inhibition zone in the negative control included an inactive response, the positive control included a moderate response, and concentrations of 10%, 20%, 40%, 60%, 80%, and 100% included a weak response in inhibiting the growth of Fusarium oxysporum fungi. The average value of the diameter of the inhibition zone of methanol extract increases with each addition of concentration. This is because the higher the concentration of methanol extract, the higher the antifungal activity produced. Research conducted by Azmi et al. (2021), stated that the higher the concentration given, the greater the percentage of inhibition of Fusarium oxysporum fungal growth. Further inhibition zone diameter testing uses ethyl acetate extract obtained using the fractionation method. The fractionation method is a separation process from methanol extracts using different solvents based on the level of polarity (Anjaswati et al., 2021).

Tabel 2. Standar Deviasi dan Hasil Uji Duncan Ekstrak Etil Asetat

Methanol Extract Concentration (%)	Inhibition Zone Diameter (mm) Repeat			Mean Zone of Inhibition Diameter (mm)±SD
	I	II	III	
Control (-)	0	0	0	0±0,00a
Control (+)	13,5	15	10	12,83±2,56e
10%	5	5	4	4,67±0,58b
20%	6	8	9	7,67±1,53c
40%	6	9	10,5	8,5±2,3c
60%	10	9	9	9,33±0,58cd
80%	11,5	11	11	11,17±0,29de
100%	13	12	12	12,33±0,58e

Notes: Notations (a, b, c, d, e, and f) are the results of Duncan's test with a 5% confidence level, if the same notation indicates not significantly different and if the notation is not the same, it indicates a significant difference.

Based on table 2, similar to the methanol extract of kirinyuh stem, the ethyl acetate extract of kirinyuh stem can also inhibit the growth of Fusarium oxysporum fungus, where the greater the concentration of ethyl acetate extract of kirinyuh stem, the greater the average clear zone produced. At a concentration of 100% concentration, the average clear zone produced was the largest compared to the other concentrations, namely 12.33 ± 0.58 mm. The 80% concentration produces an average clear zone of 11.17 ± 0.29 mm. The 60% concentration produces an average clear zone of 9.33 ± 0.58 mm. The 40% concentration produced an average clear zone of 8.5±2.3. The 20% concentration produced an average clear zone of 7.67±1.53 mm. The 10% concentration produced the smallest clear zone average of 4.67 ± 0.58 mm. The negative control in the form of distilled water produces an average clear zone of 0 ± 0.00 mm, while the positive control in the form of dithane M-45 produces an average clear zone of 12.83 ± 2.56 mm.

The inhibition response of fungal growth in the negative control is inactive, the inhibition response at concentrations of 10%, 20%, 40%, 60% is weak, the inhibition response at concentrations of 80%, 100% and positive control is moderate. The average value of the diameter of the inhibition zone of ethyl acetate extract increases with each additional concentration. According to Putri et al., (2019), the lower the concentration, the less the amount of active substances dissolved in the extract, so that the ability to inhibit fungal growth is lower. Conversely, the higher the concentration, the greater the amount of active substances that function as antifungals, so the ability to inhibit fungal growth is higher. Based on the results of the diameter of the inhibition zone of methanol and ethyl acetate extracts of kirinyuh stems, both extracts can inhibit the growth of Fusarium oxysporum fungi. This is because kirinyuh stems contain secondary metabolite compounds that play an important role in inhibiting fungal growth. The content of kirinyuh stem compounds includes alkaloids, phenolics, safonin, flavonoids, terpenoids, tannins and steroids (Gultom et al., 2020). These compounds have antifungal activity each with a different inhibitory mechanism.

The mechanism of action of flavonoids in inhibiting fungal growth is by inhibiting the work of fungal mitochondria, thus disrupting the process of food diffusion into the cell, resulting in cell death. In addition, the mechanism of action of flavonoids as antifungal is by forming complex bonds with proteins so that they can cause denaturation of proteins. This will result in disruption of cell permeability. The disruption of cell permeability causes damage to the plasma membrane so that the fungal cell membrane becomes lysed. The lysis of the fungal cell membrane will result in the death of the fungus (Jihad et al., 2020).

The mechanism of alkaloids in inhibiting fungal growth is by inserting between the cell wall and DNA and then preventing the replication of fungal DNA so that fungal growth will be disrupted (Komala et al., 2020). The mechanism of saponins in inhibiting fungal growth by causing fungal cells to lyse, which disrupts the stability of the cell membrane. Meanwhile, the mechanism of terpenoids in inhibiting fungal growth is by reducing the permeability of fungal cell membranes. Terpenoid compounds can bind to protein and lipid molecules so that they can affect the physiological functions of cell membrane proteins and enzyme proteins (Bayuaji et al., 2012).

Ethyl acetate extract has the most active antifungal ability by forming a larger clear zone compared to methanol extract. Research conducted by Rahmi et al. (2021) showed that semipolar ethyl acetate solvents can extract flavonoid compounds better than polar methanol solvents. According to Firdaus et al. (2015), the best antifungal ability is found in semi-polar extracts compared to polar and non-polar extracts, this is because semi-polar extracts with a level of polarity that is between polar and non-polar result in semi-polar extracts containing secondary metabolites that are more complex than polar and non-polar extracts. In addition to the nature of the solvent used, according to Ariyanti et al. (2012) factors that affect the difference in the diameter of the inhibition zone include the nature of the agar media, the concentration of chemicals, the speed of fungal growth, the speed of diffusion, the number of microorganisms inoculated and the conditions during incubation.

Conclusion

Methanol and ethyl acetate extracts of kirinyuh stems affect the antifungal activity of Fusarium oxysporum. Ethyl acetate extract of kirinyuh stem is more active to inhibit Fusarium oxysporum fungus compared to methanol extract of kirinyuh stem. The concentration of methanol and ethyl acetate extracts that have the most effect on Fusarium oxysporum is 100%. Based on the results of the study, further research needs to be done to determine the minimum inhibitory concentration (KHM) with a concentration of less than 10%.

Acknowledgment

This research was financially supported by Ahmad Dahlan University's Institute for Research and Community Service (LPPM). The authors would like to thank LPPM Ahmad Dahlan University for the funds that have been provided so that this research can be carried out properly and effectively.

References

Alioes, Y., Kartika, A., Zain, E. A., & Azzura, V. (2018). Uji Potensi Antijamur *Candida albicans* Ekstrak Daun Gelinggang (*Cassia alata* L.) Dibandingkan Dengan Sediaan Daun Sirih yang Beredar Di Pasaran Secara in Vitro. *Jurnal Kimia Riset*, *3*(2), 108–115. https://doi.org/10.20473/jkr.v3i2.12040

Anjaswati, D., Pratimasari, D., & Nirwana, A. P. (2021). Perbandingan Rendemen Ekstrak Etanol, Fraksi N-Heksana, Etil Asetat, dan Air Daun Bit (*Beta vulgaris* L.) Menggunakan Fraksinasi Bertingkat. *Stikes*, *1*(1), 1–6.

Ariyanti, N. K., Darmayasa, I. B. G., & Sudirga, S. K. (2012). Daya Hambat Ekstrak Kulit Daun Lidah Buaya (*Aloe barbadensis* Miller) terhadap Pertumbuhan Bakteri *Staphylococcus aureus* ATCC 25923 dan *Escherichia coli* ATCC 25922. *Jurnal Biologi*, *16*(1), 1–4. http://ojs.unud.ac.id/index.php/bio/article/download/5301/4057

Aviany, H. B., & Pujiyanto, S. (2020). Analisis Efektivitas Probiotik di dalam Produk Kecantikan sebagai Antibakteri terhadap Bakteri *Staphylococcus epidermidis*. *Jurnal Berkala Bioteknologi*, *3*(2), 24–31.

Azmi, F., Chatri, M., Advinda, L., & Irdawati. (2021). Effect of Rambutan Leaf Extract (*Nephelium lappaceum*

L.) on Colony Diameter and Percentage of Growth of Inhibition *Fusarium oxysporum*. *Serambi Biologi*, *6*(1), 7–11.

Bayuaji, T. S., Astuti, I. Y., & Dhiani, B. A. (2012). Aktivitas Antifungi Krim Daun Ketepeng Cina (*Senna alata* L. Roxb.) terhadap *Trichophyton mentagrophytes*. *Pharmacy*, *9*(3), 57–64.

Firdaus, R., Ardiningsih, P., & Arreneuz, S. (2015). Aktivitas Antijamur Ekstrak Teripang Butoh Keling (*Holothuria leucospilota*) Dari Pulau Lemukutan terhadap *Candida albicans*. *Jurnal Kimia Khatulistiwa*, *4*(4), 7–14.

Gultom, E. S., Sakinah, M., & Hasanah, U. (2020). Eksplorasi Senyawa Metabolit Sekunder Daun Kirinyuh (*Chromolaena odorata*) dengan GC-MS. *Jurnal Biosains*, *6*(1), 23–26.

Herdiana, I., & Aji, N. (2020). Fraksinasi Ekstrak Daun Sirih dan Ekstrak Gambir serta Uji Antibakteri *Streptococcus* mutans. *Jurnal Ilmiah Kesehatan*, *19*(3), 100–106. https://doi.org/10.33221/jikes.v19i03.580

Intan, K., Diani, A., & Nurul, A. S. R. (2021). Aktivitas Antibakteri Kayu Manis (*Cinnamomum burmanii*) terhadap Pertumbuhan *Staphylococcus aureus*. *Jurnal Kesehatan Perintis*, *8*(2), 121–127. https://doi.org/10.33653/jkp.v8i2.679

Jihad, A. F. A., Zulfa, F., & Bahar, M. (2020). Uji efektivitas ekstrak bawang bombai (*Allium cepa* L. Var. Cepa) terhadap pertumbuhan jamur *Mallasezia furfur* secara in vitro. *Seminar Nasional Riset Kedokteran*, 295–303.

Juliantari, N. P. D., Wrasiati, L. P., & Wartini, N. M. (2018). Karakteristik Ekstrak Ampas Kopi Bubuk Robusta (*Coffea canephora*) Pada Perlakuan Konsentrasi Pelarut Etanol dan Suhu Maserasi. *Jurnal Rekayasa Dan Manajemen Agroindustri*, *6*(3), 243–249. https://doi.org/10.24843/jrma.2018.v06.i03.p08

Juwanda, M., Khotimah, H., & Amin, M. (2016). Peningkatan Ketahanan Bawang Merah terhadap Penyakit Layu Fusarium Melalui Induksi Ketahanan Dengan Asam Salisilat Secara In Vitro. *Agrin*, *20*(1), 15–28.

Kandoli, F., Abijulu, J., & Leman, M. (2016). Uji Daya Hambat Ekstrak Daun Durian (*Durio zybethinus*) terhadap Pertumbuhan *Candida albicans* Secara In Vitro. *Jurnal Ilmiah Farmasi*, *5*(1), 46–52.

Komala, O., . Y., & Siwi, F. R. (2020). Aktivitas Antijamur Ekstrak Etanol 50% dan Etanol 96% Daun Pacar Kuku *Lawsonia inermis* L terhadap *Trichophyton mentagrophytes*. *Ekologia: Jurnal Ilmiah Ilmu Dasar Dan Lingkungan Hidup*, *19*(1), 12–19. https://doi.org/10.33751/ekol.v19i1.1657

Kurang, R. Y., & Penlaana, R. (2022). Daya Hambat Ekstrak Metanol dan Etil Asetat Daun Kirinyuh (*Chromolaena odorata* L.) terhadap Bakteri Escherichia Coli. *Jamb.J.Chem*, *4*(2), 22–29.

Nurdin, E., & Nurdin, G. M. (2020). Perbandingan Variasi Media Alternatif dengan Berbagai Sumber Karbohidrat Terhadap Pertumbuhan *Candida albicans*. *Bionature*, *21*(1), 1–5. https://doi.org/10.35580/bionature.v21i1.13920

Putra, I. M. A. S. (2015). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (*Annonae muricata* L.) dengan Metode Difusi Agar Cakram terhadap *Escherichia coli*. *Jurnal Ilmiah Medicamento*, *1*(1), 15–19. https://doi.org/10.36733/medicamento.v1i1.721

Putri, A., Nofita, N., & Ulfa, A. M. (2022). Perbandingan Aktivitas Antioksidan Ekstrak Daun Bidara (*Ziziphus spina-*

christi L.) dengan Teknik Ekstraksi Perkolasi dan Infusa. *Jurnal Ilmu Kedokteran dan Kesehatan*, *9*(4), 1178–1189. https://doi.org/10.33024/jikk.v9i4.5635

Putri, D. R., Asri, M. T., & Ratnasari, E. (2019). Aktivitas Antifungi Ekstrak Buah Pare (*Momordica charantia* L.) dalam Menghambat Pertumbuhan Jamur *Fusarium oxysporum*. *Lentera Bio*, *8*(2), 156–161. http://ejournal.unesa.ac.id/index.php/lenterabio

Putri, W. S., Warditiani, N. K., & Larasanty, L. P. F. (2013). Skrining Fitokimia Ekstrak Etil Asetat Kulit Buah Manggis (*Garcinia mangostana* L.). *Jurnal Farmasi Udayana*, *2*(4), 56–60.

Rahmi, N., Salim, R., Miyono, & Rizki, M. I. (2021). Pengaruh Jenis Pelarut dan Metode Ekstraksi terhadap Aktivitas Antibakteri dan Penghambatan Radikal Bebas Ekstrak Kulit Kayu Bangkal (*Nauclea subdita*). *Jurnal Penelitian Hasil Hutan*, *39*(1), 13–26. https://doi.org/10.20886/jphh.2021.39.1.13-26

Salamah, N., & Widyasari, E. (2015). Aktivitas Antioksidan Ekstrak Metanol Daun Kelengkeng (*Euphoria longan* (L) Steud.) dengan Metode Penangkapan Radikal 2,2'-Difenil-1-Pikrilhidrazil. *Pharmaciana*, *5*(1), 25–34. https://doi.org/10.12928/pharmaciana.v5i1.2283

Sari, E. M., Suwirmen, & Noli, Z. A. (2014). Pengaruh Penggunaan Fungisida (Dithane M-45) terhadap Pertumbuhan Tanaman Jagung (*Zea mays* L.) dan Kepadatan Spora Fungi Mikoriza Arbuskula (FMA). *Jurnal Biologi Universitas Andalas (J. Bio. UA.)*, *3*(3), 188–194.

Setyantoro, M. E., Haslina, H., & Wahjuningsih, S. B. (2019). Pengaruh Waktu Ekstraksi dengan Metode Ultrasonik terhadap Kandungan Vitamin C, Protein, dan Fitokimia Ekstrak Rambut Jagung (*Zea mays* L.). *Jurnal Teknologi Pangan dan Hasil Pertanian*, *14*(2), 53–67. https://doi.org/10.26623/jtphp.v14i2.2445

Suryani, Y., Taupiqurrahman, O., & Kulsum, Y. (2020). *Mikologi*. PT. Freeline Cipta Granesia.

Tusa'diah, H., & Chatri, M. (2021). Uji Efektivitas Ekstrak Daun Sukun (*Artocarpus altilis* Park.) terhadap Koloni Jamur *Fusarium oxysporum* Secara In-Vitro. *Prosiding Semnas Bio*, 1679–1685. https://semnas.biologi.fmipa.unp.ac.id/index.php/prosiding/article/view/215/147

Wahyuni, & Karim, S. F. (2020). Uji Aktivitas Antibakteri Ekstrak Etanol Daun Kacapiring (*Gardenia jasminoides* Ellis) terhadap Bakteri *Streptococcus mutans*. *Jurnal Sains Dan Kesehatan*, *2*(4), 399–404.

Warbung, Y. Y., Wowor, V. N. S., & Posangi, J. (2013). Daya Hambat Ekstrak Spons Laut *Callyspongia* sp terhadap Pertubuhan Bakteri *Staphylococcus aureus*. *E-Gigi*, *1*(2), 1–12. https://doi.org/10.35790/eg.1.2.2013.3151

Warni, J., Marliah, A., & Erida, G. (2022). Uji Aktivitas Bioherbisida Ekstrak Metanol Teki (*Cyperus rotundus* L.) terhadap Pertumbuhan Bayam Duri (*Amaranthus spinosus* L.). *Jurnal Ilmiah Mahasiswa Pertanian*, *7*(2), 47–54. https://doi.org/10.17969/jimfp.v7i4.22071

Widiastuti, A., Agustina, W., Wibowo, A., & Sumardiyono, C. (2011). Uji Efektivitas Pestisida terhadap Beberapa Patogen Penyebab Penyakit Penting pada Buah Naga (*Hylocereus* sp.) secara In Vitro. *Jurnal Perlindungan Tanaman Indonesia*, *17*(2), 73–76.

Yanti, N., Samingan, & Mudatsir. (2016).

Uji Aktivitas Antifungi Ekstrak Etanol Gel Manjakani (*Quercus infectoria*) terhadap *Candida albicans*. *Jurnal IImiah Mahasiswa Pendidikan Biologi*, *1*(1), 1–9.