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Article

Phyrochemical Screening and Potential Inhibition of Pacing Extract *(Costus speciousus, J.Sm)* Against *Mycobacterium Tuberculosis* H37Rv

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Abstract. Tuberculosis (TB) is an infectious disease caused by the bacterium Mycobacterium tuberculosis (Mtb) and can attack various organs of the body. Transmission occurs through sputum splashes when coughing, sneezing or talking. TB treatment using antituberculosis drugs (OAT) can cause side effects that affect health. Therefore, alternative therapies from natural ingredients are needed. This study aims to explore secondary metabolites in extracts and plant fractions of Pacing *(Costus speciousus, J.Sm)* and determine their effectiveness against Mycobacterium tuberculosis H37Rv. The extraction method used is remaceration with methanol solvent. The concentrations of the samples tested varied with isoniazid positive controls. The results showed that Pacing plant extracts and fractions contained alkaloids, flavonoids, saponins, and tannins. Terpenoid compounds were not detected in the samples tested. The antituberculosis test showed that the n-hexane fraction had the best anti-TB activity, although the IC50 value of the extract and fraction was still lower than isoniazid as a positive control. However, Pacing extract and fraction still have potential as very strong anti-TB agents because their IC50 values are below 50 ppm.

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis* (Mtb). This disease can be transmitted through sputum splashes containing the bacteria. Although the lungs are the organs most often attacked by this bacteria, tuberculosis can also attack other organs such as the brain, intestines, kidneys or spine [1-3]. The incidence of TB in this country continues to increase from year to year. In 2020, there were 393,323 cases of TB found, while in 2021 the number of cases increased to 443,235 cases that were successfully treated [4-5].

Tuberculosis (TB), a curable and preventable disease. Treatment for active TB involves the use of four standard antimicrobial drugs, namely isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z), over a 6-month period according to WHO guidelines 2020 [6]. The use of antituberculosis drugs (OAT) can cause side effects, ranging from mild to severe. Some of the side effects that may arise from using OAT include headaches, itching, loss of appetite, nausea, vomiting, joint pain, tingling, hearing loss, and vision problems. One of the most serious side effects is hepatotoxic, i.e. liver damage [7-8]. Lack of understanding of the side effects of OAT can lead to non-adherence to therapy, and can even lead to discontinuation of treatment. Stopping TB treatment can cause bacterial resistance which exacerbates the disease and threatens the patient's health [9-10].

Phytochemical screening is a method used to identify the content of secondary metabolites in plant extracts, such as roots, stems, leaves, flowers, and so on. The secondary metabolites that are generally sought are alkaloids, flavonoids, terpenoids, saponins, and tannins. The purpose of this screening is to look for active ingredients that have medicinal potential. The use of natural resources derived from plants as an alternative to anti-tuberculosis treatment is increasingly attracting attention [11-13].

Research has been conducted on the Pacing plant to evaluate its potential for various biological activities. The methanol extract of Pacing leaves has potential as an antidiabetic and has inhibitory activity against *α-Glucosidase* [14]. Pacing also exhibits antifungal activity against several types of fungi such as *Trichophyton mentagrophytes* and *Trichophyton rubrum*, as well as antibacterial activity against various types of bacteria such *as Shigella spp., Staphylococcus aureus*, and *Escherichia coli* [15]. In addition, other studies [16] showed that Pacing leaves have in vitro antituberculosis activity with a *Minimum Inhibition Concentration* (MIC) of 100-200 µg/mL and a *Minimum Bactericidal Concentration* (MBC) of 200 µg/mL against *Mycobacterium tuberculosis* H37Rv. Based on these studies, researchers wanted to further investigate the in vitro antituberculosis content and activity of extracts and fractions of all parts of the Pacing plant against *M. tuberculosis* H37Rv using a combination of resazurin and colorimetry methods.

The extraction method to be used is maceration with methanol solvent. The extraction results will be concentrated and fractionated using the liquid-liquid partition method using n-hexane, dichloromethane (DCM), and ethyl acetate as solvents in the fractionation [17-18]. In addition, researchers will also conduct a phytochemical screening to determine the secondary metabolites contained in the Pacing extract and evaluate the inhibition percentage of the methanol extract, n-hexane fraction, DCM fraction, and ethyl acetate fraction from the Pacing plant against *Mycobacterium tuberculosis* H37Rv. This study aims to identify the class of secondary metabolites contained in the methanol extract, n-hexane fraction, DCM fraction, and ethyl acetate fraction from the Pacing plant *(Costus speciousus, J.Sm)* and determine the IC50 value of the methanol extract, n-hexane fraction, DCM fraction, and ethyl acetate fraction from Pacing plant (*Costus speciousus, J.Sm)* against *Mycobacterium tuberculosis* H37Rv. The results of this study can provide further understanding of the potential of Pacing as an alternative tuberculosis treatment and contribution to the development of effective and sustainable antituberculosis therapy.

2. Experimental Section

2.1. Types of Research

This type of research is true experimental (research conducted in laboratories) to find out what classes of secondary metabolite compounds are contained in methanol extract, n-hexane fraction, DCM, ethyl acetate and the inhibition percentage of methanol extract, n-hexane fraction, DCM and ethyl acetate of Pacing against *Mycobacterium tuberculosis* H37Rv.

2.2. Research Variable

This study had several variables that were controlled and measured, namely the independent variables, the concentration of the methanol extract sample, the n-hexane fraction, the DCM fraction, and the ethyl acetate fraction from the Pacing plant (*Costus speciosus*, J.Sm). Concentrations used include 25, 100, 500, and 1000 ppm. Dependent Variable, measured using the IC_{50} value, which is the concentration of the sample required to inhibit the growth of 50% of M. tuberculosis H37Rv and Controlled Variable, using the extraction method, fractionation method, extraction time, type of solvent, and antituberculosis testing method.

2.3. Tool

The tools used are ELISA reader, 96 wellplates, shaker incubator, 37oC incubator, auto clave, rotary evaporator, BSL 2, Buchner funnel, ependorf, TLC chamber, micropipette, UV lamp, separating funnel, glassware, analytical balance, oven fume hood, spatula, tweezers, and dropper.

2.4. Material

The materials used in this study consisted of Pacing Plant, methanol, n-hexane fraction, DCM fraction and ethyl acetate fraction, distilled water, chloroform, acetic acid, butanol, caffeine, gallic acid, quercetin, TLC plates, can phosphate pH 6.9 , DMSO, filter paper, aluminum foil, dragendorff, FeCl3, anisaldehyde sulfate, Liberman-Burchard, Resazurin reagent, MT.H37Rv and modified 7H9 liquid medium.

2.5. Research Procedure

2.5.1. Plant Determination

This step was taken to ensure the identity of the plant used, namely Pacing *(Costus speciosus, J.Sm*). Plant identification is carried out at the Jember State Polytechnic to ensure the correct identity of plants and avoid mistakes in collecting the main components to be studied.

2.5.2. Making Simplici

In this step, Pacing roots, stems, leaves, and flowers are collected. The sample is then cleaned of dirt and washed with clean water. After that, the samples were dried by exposing them to the sun. After drying, the samples were ground into a homogeneous powder using a knife or scissors.

2.5.3. Sample Extraction

Pacing powder as much as 500 grams was weighed and extracted using the remaceration method. The remaceration process was carried out 3 times using 2.5 liters of methanol in each maceration process. After the maceration process, the filtered filtrate was collected and concentrated using a rotary evaporator with a temperature of 40°C and a speed of 100 rpm. Then, the extract is dried in an oven at 45-50°C until it reaches a constant yield weight.

2.5.4. Fractionation

Fractionation was carried out using the liquid-liquid partition method with n-hexane, DCM, and ethyl acetate as solvents. 2 grams of methanol extract was dissolved in 100 ml of methanol, and then added 100 ml of n-hexane in a ratio of 1:1. The mixture was shaken and allowed to stand until a layer of methanol (bottom) and n-hexane layer (top) formed. The n-hexane fraction was collected and concentrated, while the methanol fraction became the residue to be used for the next fractionation. This process was repeated with DCM and ethyl acetate.

2.5.5. Phytochemical Screening

Phytochemical screening was carried out using the thin layer chromatography (TLC) test method with several modifications, namely: Identification of Alkaloids, Identification of Flavonoids, Identification of Tannins, Identification of Saponins, and Identification of Terpenoids.

2.5.6. *In vitro* Antituberculosis Test

Antituberculosis test in vitro is a test method carried out in the laboratory to evaluate the effect of a substance or compound on the growth and activity of the bacterium *Mycobacterium tuberculosis*, which is the cause of tuberculosis [19-20]. Following are the general steps carried out in the in vitro antituberculosis test, namely the media used is media containing 10% *oleic acid-albumin-dextrose-catalase* (OADC), 0.1% calistone, and 0.5% glycerol. The isolate used was *Mycobacterium tuberculosis* H37Rv which had been enriched in the media. Isoniazid was used as a positive control. Isoniazid is an antituberculosis drug that has been proven effective.

Resazurin is used as an indicator of bacterial growth. Resazurin is a substance that changes color from blue to pink when there is bacterial growth [21-22]. Pacing extract, which is the test sample, was put into the microplate wells which already contained *M.tuberculosis* H37Rv and liquid media. Bacteria in liquid media were incubated in a shaking incubator at 37°C for 5-10 days. The bacterial suspension was then diluted in a microplate to a concentration of 5x107 CFU/ml, then continued incubation for 4 weeks. The microplate containing the test sample, bacteria, and liquid media was incubated at 37°C for 5 days. After 5 days of incubation, 20 µL of resazurin was added to each well of the microplate. The microplate was again incubated for 24 hours. If there is a blue color change, this indicates that the bacteria are not growing. If there is a pink color change, this indicates bacterial growth.

2.6. Data Analysis

Qualitative research data were obtained from the results of the sample phytochemical screening using the TLC method. The quantitative research data were obtained from the results of the in vitro antituberculosis test to determine the IC_{50} value based on the percent viability of mycobacteria. The IC_{50} value is the intersection of the line between the ln test concentration and the percent viability of mycobacteria contained in the equation $Y = bX+a$ with a value of $Y = 50$ and the anti ln(x) value is the IC_{50} value. The IC_{50} value of each test sample will be compared to determine which extract or fraction has the best inhibition against M. tuberculosis H37Rv [23].

3. Results and Discussion

3.1. Plant Determination

The Pacing plant (*Costus speciousus*, J.Sm) used in this study was obtained from the Tanggul area of Jember district, East Java, Indonesia in September 2022. Then a determination was made at the Faculty of Agriculture, Jember State Polytechnic to get a clear identity of the part of the plant used and avoid error in research. Based on the results of the determination, it was known that the plants used in this study were Pacing plants (*Costus speciousus*, J.Sm) from the Zingiberaceae tribe.

3.2. Making Simplicia

The manufacture of simplicia involves a series of important processing steps to maintain the quality and content of active compounds in plant raw materials. In this study, the Pacing plant used was obtained in dry form because it is easily broken. The quality characteristics of simplicia which are easily broken are good for processing and safe storage [24]. Pacing plants were dried for 14 days using the air dry method in the shade. Drying is done to reduce the water content and prevent decay and hydrolysis of compounds by enzymes. Furthermore, particle size reduction was carried out on the stem bark using a grinder and blender. The aim is to facilitate penetration of the solvent into the cell and optimize the withdrawal of compounds during extraction. Simplisia that has been properly processed is ready for the extraction process [25].

3.3. Extraction

Pacing plant extraction was carried out using the maceration method. The maceration process was carried out by immersing the sample in an organic solvent for 3x24 hours at room temperature and protected from direct light. This method is used to extract the active compounds contained in the sample. The choice of the maceration method aims to ensure the solubility of the active compounds in organic solvents. In addition, the use of organic solvents such as methanol was chosen because it has the ability to dissolve organic compounds both polar and nonpolar [26].

Methanol is also volatile so it can be easily released from the extract. After the maceration process, the resulting macerate is then concentrated using a rotary evaporator [27]. This step was carried out to remove organic solvents and obtain a thick extract from the Pacing plant. The concentration results showed that 32.209 grams of viscous extract was obtained with a yield of 6.44%. The resulting extract has a blackish green color. By using maceration and concentration methods, it was successful to obtain a viscous extract from the Pacing plant containing the desired active compounds. The extract can be used for further analysis or testing of biological activity required in the research.

3.4. Fractionation

The fractionation of the Pacing plant methanol extract was carried out using a multilevel liquid-liquid fractionation method with the help of a separatory funnel. Fractionation was carried out using solvents with different polarities respectively, namely n-hexane, DCM, and ethyl acetate [28]. The yield percentage of the Pacing plant fraction resulting from the study can be seen in the research data in Table 1.

Table 1. Yield Percentage of Pacing Plant Fractionation Results

Sample	Yield Weight (g)	% Yield (%b/b)
n-hexane fraction	0.4026 ± 0.2507	20.1333 ± 12.5357
DCM faction	0.6123 ± 0.2223	30.6166 ± 11.5904
Ethyl acetate fraction	0.5313 ± 0.1097	26.5666 ± 5.4887

The results of the standard deviation of the large % yield in fractionation can be caused by several factors, namely the inhomogeneity of the sample. Sample inhomogeneity can occur in fractionation if the desired components in the sample are not evenly distributed or properly separated from other components. This can result in significant variations in fractionation results, including yield percentages [29].

3.5. Phytochemical Screening

Phytochemical screening was carried out with the aim of knowing the class of secondary metabolite compounds contained in a research sample. Phytochemical screening in this study was carried out qualitatively on Pacing plant extracts and fractions using the TLC method. The TLC method has several advantages, namely it is easy to work with, it is cheaper, the equipment is simple, and all components in the sample can be detected because this method allows simultaneous separation of samples. The group of compounds examined consisted of alkaloids, flavonoids, tannins, saponins and terpenoids. These five groups of compounds are known to have different mechanisms as antibacterial agents [30-31]. Phytochemical screening results of pacing plant extracts and fractions

Table 2. Results of Phytochemical Screening of Pacing Plant Extracts and Fractions

Sample	Compound class				
	Alkaloids	Flavonoids	Tanin	Saponin	Terpenoid
Methanol Extract	+	+	+	+	-
n-hexane fraction	+	+	+	+	-
DCM faction	+	+	+	+	-
Ethyl acetate fraction	+	+	+	+	-

Description : (+) Yes; (-) There isn't any

3.5.1. Phytochemical Screening of Alkaloid Compound Groups

In this study, phytochemical screening was carried out to detect the presence of alkaloid compounds in the samples. The standard used is caffeine, which serves as a comparison. The method used involves the use of Dragendorff spray reagent to see the color change and the distance traveled by the alkaloid stain on the plate. Samples containing alkaloid compounds will show an orange color after being sprayed with Dragendorff [32-33].

The results showed that all the samples studied contained alkaloid compounds. This is in accordance with previous studies which also found the presence of alkaloids in Pacing plant extracts and fractions. Observation under UV lamp with a wavelength of 254 and 365 nm also revealed the presence of stains in the sample parallel to the alkaloid standard.

Figure 1 in the study shows the results that support the presence of alkaloid compounds in the sample. The presence of orange stains on all sample lines after being sprayed with Dragendorff indicated the presence of alkaloids. In addition, observation under a UV lamp with a wavelength of 254 nm also confirmed the presence of stains parallel to the alkaloid standard. Phytochemical screening in this study showed that samples from the Pacing plant contained alkaloid compounds.

A B C

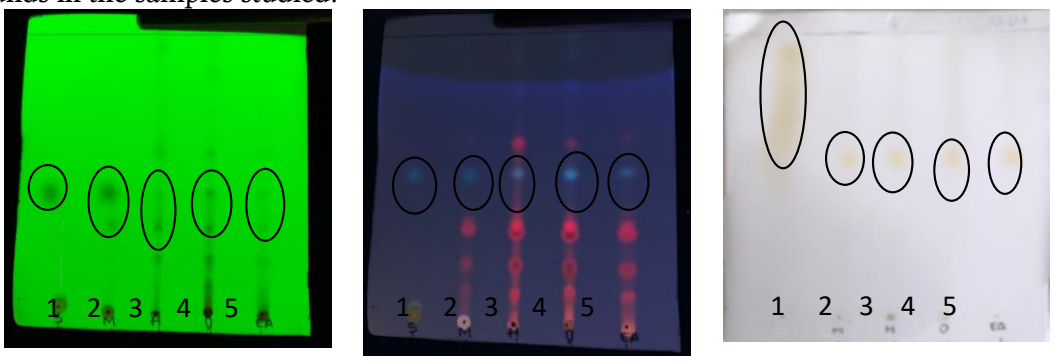
Figure 1. Phytochemical screening of alkaloid compounds using the TLC method with n-hexane and ethyl acetate as mobile phases (7:3)

Description: (1) caffeine standards; (2) methanol extract; (3) n-hexane fraction; (4) DCM fraction; (5) ethyl acetate fraction; (A) observed in a 254 nm UV lamp; (B) observed in 365 nm UV lamp; (C) after being sprayed with Dragendroff.

3.5.2. Phytochemical Screening of Flavonoid Compounds

Phytochemical screening to identify the presence of a class of flavonoid compounds in the sample. The standard used was quercetin, which is one of the flavonols and represents a group of flavonoids. Stain observation was carried out using a UV lamp with a wavelength of 254 nm and 365 nm, as well as ammonia vapor.

The results of the phytochemical screening showed that all samples contained a class of flavonoid compounds, as shown in Figure 2. Observation under UV light with a wavelength of 254 nm and 365 nm revealed stains in all samples that were similar to the stains on the standard flavonoid (quercetin). In addition, the use of ammonia vapor on the plate also produced a yellow-brown stain similar to the standard, indicating the presence of flavonoids in the sample. These results are consistent with previous studies which also found the presence of flavonoids in Pacing plant extracts and fractions. Thus, the phytochemical screening in this study verified the presence of a class of flavonoid compounds in the samples studied.



A B C

Figure 2. Phytochemical screening of flavonoid compounds using the TLC method with mobile phases of glacial acetic acid, butanol, and water (1:4:5)

Description: (1) standar kuersetin, (2) ekstrak metanol, (3) fraksi *n*-heksan; (4) fraksi DCM; (5) fraksi etil asetat; (A) diamati pada lampu UV 254 nm; (B) diamati pada lampu UV 365 nm; (C) setelah beri uap amonia

3.5.3. Phytochemical Screening of Tannin Compounds

Phytochemical screening was carried out to identify the presence of tannin compounds in the samples. Identification of tannins was carried out using methanol:water (6:4) and gallic acid as the tannin standard. Gallic acid was chosen as the standard because it has good stability, is relatively inexpensive, and has strong natural antioxidant activity. After the elution was complete, the plates used were observed using a UV lamp and sprayed with $FeCl_3$ reagent to observe stain formation. Samples are categorized as positive for containing a class of tannin compounds if black stains form after being sprayed with $_{FeCl3,}$ according to previous research [34-35].

The results of the phytochemical screening showed that all extract and fraction samples contained a group of tannin compounds, as shown in Figure 3 Observations using UV lamps with a wavelength of 254 nm and 365 nm revealed clear stains on all sample lines, as well as the formation of black spots after being sprayed with $FeCl_3$. This finding is consistent with previous studies which also stated that Pacing plant extracts and fractions contain a class of tannin compounds. Thus, the phytochemical screening in this study verified the presence of a class of tannin compounds in the samples studied. This information is important in understanding the chemical composition and potential biological activity of the Pacing plant.

A B C

Figure 3. Phytochemical screening of tannin compounds using the TLC method with methanol and water as mobile phases (6:4)

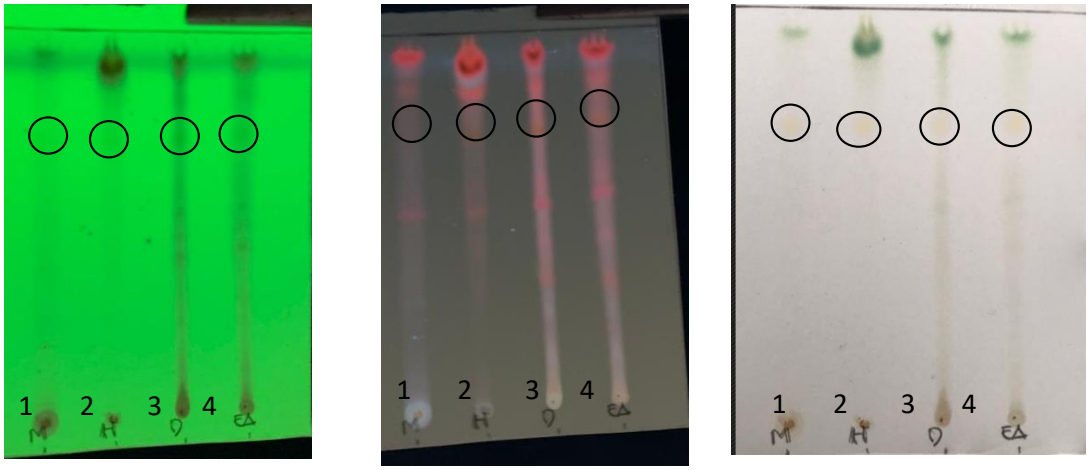
Description: (1) Gallic acid standard, (2) methanol extract, (3) n-hexane fraction; (4) DCM fraction; (5) ethyl acetate fraction; (A) observed in a 254 nm UV lamp; (B) observed in 365 nm UV lamp; (C) after being sprayed with $FeCl_3$

3.5.4. Phytochemical Screening of Saponin Compounds

In this study, phytochemical screening was carried out to identify the presence of saponin compounds in Pacing plant extract and fraction samples. Identification of saponins was carried out using the Thin Layer Chromatography (TLC) method using an eluent in the form of a mixture of chloroform and methanol in a ratio of 9:1. The elution plate was then observed using a UV lamp and sprayed with Lieberman-Burchard reagent. Samples containing saponins will form blue to blue-violet, dark blue, red, or yellow-brown stains after being sprayed, according to previous studies [36-37].

The results of the phytochemical screening showed that all the tested samples contained saponin compounds, as shown in Figure 4. Observation under UV light and after being sprayed with Lieberman-Burchard showed the formation of parallel stains in all samples, with yellow-brown stains. This finding is consistent with previous research which also stated that Pacing plant extracts and

fractions contain a class of saponin compounds. Thus, the phytochemical screening in this study verified the presence of a class of saponin compounds in the samples studied. This information is important in understanding the chemical composition and potential biological activity of the Pacing plant.



A B C

Figure 4. Phytochemical Screening of Saponin Compounds Using the TLC Method with the Mobile Phases of Chloroform and Methanol (9:1)

Description: (1) methanol extract, (2) n-hexane fraction; (3) DCM fraction; (4) ethyl acetate fraction; (A) observed in a 254 nm UV lamp; (B) observed in 365 nm UV lamp; (C) after being sprayed with Liberman-Burchard

3.5.5. Phytochemical Screening for Terpenoid Compounds

Phytochemical screening for the terpenoid compound group was identified using the Thin Layer Chromatography (TLC) method with n-hexane and ethyl acetate as eluents in a ratio of 7:3. The elution plate was observed using a UV lamp and sprayed with anisaldehyde sulfate reagent. The appearance of purplish-red stains after spraying indicates the presence of terpenoid compounds in the sample, according to previous research [38]. However, the results of the phytochemical screening in this study showed that there was no terpenoid content in all the samples tested. These results are in accordance with previous studies which stated that Pacing plant extracts and fractions contain groups of alkaloid compounds, flavonoids, tannins, and steroids, but do not contain terpenoids [32].

The results of the phytochemical screening can be seen in Figure 5. The sample did not show a purplish red stain after being sprayed with anisaldehyde sulfate. When observed using a UV lamp, the sample forms a stain similar to the stain formed during observation of the alkaloid compound group. This indicates that the stain formed is probably an alkaloid stain, because the mobile phases used in both observations are the same, namely n-hexane and ethyl acetate in a ratio of 7:3.

3.6. *In vitro* Antituberculosis Activity Test

In vitro antituberculosis activity test was carried out using the Resazurin Microtiter Assay (REMA) combined with colorimetry. The REMA method is a new method that provides fast and accurate antituberculosis drug sensitivity (OAT) test results with a simpler procedure. In the REMA method, readings are made by observing the color changes formed. This method uses Middlebrook 7H9 liquid medium and resazurin redox indicator. Resazurin initially has a non-fluorescent blue color and does not show growth. However, when reduced, resazurin changes to a fluorescent or resorphin pink color,

indicating the growth of Mycobacterium tuberculosis. This color change is used to detect the growth of M. tuberculosis. More detailed information about color changes can be seen in Figure 6.

Figure 6. In vitro Antituberculosis Test Results

Mycobacterial growth was calculated based on the percentage of mycobacterial viability. Furthermore, the percentage of inhibition of the test sample against M. tuberculosis H37Rv was calculated using the IC_{50} value. The IC_{50} value is the concentration of the test sample which can inhibit 50% of bacterial growth. The smaller the IC_{50} value, the greater the antibacterial activity of the test sample. The REMA method with colorimetry provides convenience and speed in testing antituberculosis activity and provides information regarding drug sensitivity and the effectiveness of inhibiting the growth of M. tuberculosis from the samples tested.

Antituberculosis activity test, the IC_{50} value is obtained by calculating using the equation Y = bX + a, where the Y value is 50 and the anti ln (X) value is the IC_{50} value [39-40]. The calculation of the IC_{50} value can be seen in Figure 6, while the IC_{50} value of each sample is listed in Table 3. The percentage of mycobacterial viability (viability) obtained can be seen in Appendix 4. The percentage of mycobacterial viability describes the survival rate or growth of mycobacteria after exposure to the sample or certain treatment.

Table 3. IC_{50} Value of Positive Test and Control Samples

Sampel	Nilai IC_{50} (ppm)
Ekstrak metanol	13.2394
Fraksi *n*-heksan	5.4151
Fraksi DCM	27.8156
Fraksi etil asetat	6.5659
Isoniazid	1.7831

Based on the research results, IC_{50} values were obtained for Pacing *(Costus speciosus J.sm)* methanol extract of 13.2394 ppm, n-hexane fraction of 5.4151 ppm, DCM fraction of 27.8156 ppm, and ethyl acetate fraction of 6.5659 ppm. Meanwhile, the IC_{50} value of isoniazid as a positive control was 1.7831 ppm. The smaller the IC_{50} value, the better the activity as an antituberculosis (Anti-TB) agent. Anti-TB activity was classified as very strong if the IC_{50} value was less than 50 ppm, strong if the IC_{50} value was between 50-100 ppm, moderate if the IC50 value was between 101-150 ppm, and weak if the IC_{50}

value was between 151-200 ppm [41-42]. Samples that have an IC_{50} value of > 200 ppm are considered to have very weak activity as an anti-bacterial. Of the four extracts and fractions of Pacing *(Costus speciosus J.sm),* the n-hexane fraction showed the best anti-TB activity. Although the anti-TB activity of the four extracts and the Pacing fraction was still lower than that of the isoniazid positive control, it still has the potential to be a very strong anti-TB candidate because it has an IC_{50} value <50 ppm [33].

The results of phytochemical screening on the n-hexane fraction, ethyl acetate fraction, methanol extract, and DCM fraction showed the presence of alkaloids, flavonoids, tannins, and saponins. These four groups of compounds have an important role as antibacterial agents. Alkaloids work as antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells, causing damage to the cell wall lining and bacterial cell death. In addition, alkaloids can also inhibit topoisomerase enzymes in bacterial cells, which are necessary for cell division [43].

Flavonoids work as antibacterials through three mechanisms. First, they can inhibit nucleic acid synthesis, which causes damage to the bacterial cell wall. Second, flavonoids can interfere with the function of cell membranes by forming complex compounds with extracellular proteins, thereby damaging the bacterial cell membrane. Third, flavonoids can inhibit bacterial energy metabolism, which results in inhibition of bacterial macromolecular biosynthesis [30].

Tannins work as an antibacterial by causing *Mycobacterium tuberculosis* cell lysis. Tannins affect the polypeptide wall of the bacterial cell, interfere with the formation of a perfect cell wall, and eventually cause bacterial cell death. Tannins can also inactivate bacterial enzymes and interfere with the passage of proteins in cells [16]. The antibacterial effect of tannins can also be strengthened through the formation of tannin complexes with metal ions, increasing the toxicity of tannins to bacteria. In addition, tannins can cause wrinkling of cell walls and membranes, disrupt cell permeability, and inhibit bacterial growth [44].

Saponins act as antibacterial by causing leakage of proteins and enzymes from inside the cell. The mechanism of action of saponins involves a decrease in the surface tension of the bacterial cell wall and damage to the permeability of the cell membrane. Saponins have surface properties similar to detergents, so they can bind to the cytoplasmic membrane, damage the stability of the cell membrane, and cause cytoplasmic leakage out of the bacterial cell [45-46]. The results of the phytochemical screening provide information about the groups of compounds contained in plant extracts or fractions, to identify specific compounds that act as anti-TB agents, the next step is to isolate these compounds. This isolation aims to separate and concentrate these potential compounds. By carrying out further isolation and testing, researchers can identify specific compounds responsible for the anti-TB activity in plant extracts or fractions. This information is important for understanding the mechanism of action of these compounds and for developing their potential as anti-TB agents.

4. Conclusion

Methanol extract, n-hexane fraction, DCM and ethyl acetate of the Pacing (Costus speciousus J.sm) plant contains alkaloids, flavonoids, tannins and saponins. The four samples did not contain terpenoid compounds. In vitro antituberculosis test results against M. tuberculosis H37Rv using the resazurin colorinetry method showed the IC50 value of the Pacing (Costus specious J.sm) methanol extract sample was 13.2394 ppm, the n-hexane fraction was 5.4151 ppm, DCM was 27.8156 ppm , and ethyl acetate 6.5659 ppm while the IC_{50} value of isoniazid as a positive control was 1.7831 ppm. Of the four extracts and fractions of Pacing (*Costus specious* J.sm) it was shown that the n-hexane fraction had the best anti-TB activity. However, the anti-TB activity of the four extracts and the Pacing fraction was still lower when compared to the isoniazid positive control but still had the potential to be a very strong anti-TB category because it had an IC_{50} value of <50 ppm.

References

[1] Miggiano, R., Rizzi, M., & Ferraris, D. M. (2020). Mycobacterium tuberculosis pathogenesis, infection prevention and treatment. *Pathogens*, *9*(5), 385.

[2] Ri, K. (2020). Pedoman Nasional Pelayanan Kedokteran: Tatalaksana Tuberkulosis. *Jakarta: Kementerian Kesehatan Republik Indonesia*.

[3] Wei, M., Zhao, Y., Qian, Z., Yang, B., Xi, J., Wei, J., & Tang, B. (2020). Pneumonia caused by Mycobacterium tuberculosis. *Microbes and infection*, *22*(6-7), 278-284.

[4] Lestari, T. (2023). Strengthening health systems to improve tuberculosis (TB) contact investigation and preventive treatment in Mimika, Indonesia.

[5] Kamila, D. A., Wardani, H. E., Tama, T. D., Hapsari, A., & Gayatri, R. W. (2023). Determinant of Successful Tuberculosis Treatment in Puskesmas Singosari in 2020-2022. *Keluwih: Jurnal Kesehatan dan Kedokteran*, *5*(1), 7-19.

[6] World Health Organization. (2022). *Global tuberculosis report 2021: supplementary material*. World Health Organization.

[7] Nisrina, H., & Hilmi, I. L. (2022). Description of Side Effects of Using Antituberculosis Drug in Pulmonary Tuberculosis Patients: Literature Review. *Jurnal eduhealth*, *13*(02), 684-688.

[8] Putra, I. W. A., Lonovi, K., Damayanti, L., & Kamajaya, T. (2021). Characteristic of SGOT and SGPT Elevation in Patient with Lung Tuberculosis within OAT Therapy. In *12th Annual Scientific Meeting, Medical Faculty, Universitas Jenderal Achmad Yani, International Symposium on" Emergency Preparedness and Disaster Response during COVID 19 Pandemic"(ASMC 2021))* (pp. 92-98). Atlantis Press.

[9] Pramono, J. S., Sudding, R., & Tini, T. (2023). Relationship between Side Effects of Anti-tuberculosis Drugs (ATDs) and Adherence to Medication in Elderly Patients with Tuberculosis. *Poltekita: Jurnal Ilmu Kesehatan*, *17*(2), 326-333.

[10] Hayati, S. M., Purba, J. M., & Siregar, C. T. (2022). Analysis of Factors Affecting Acceptance and Commitment Therapy (ACT) in Pulmonary TB Patients. *Indonesian Journal of Global Health Research*, *4*(3), 573-580.

[11] Barbouchi, M., Benzidia, B., Elamrani, K., Sabiri, M., & El Idrissi, M. (2024). Phytochemical screening, quantitative analysis and antioxidant properties of crude extracts from stems, leaves, and flowers of three Ruta species. *Kuwait Journal of Science*, *51*(4), 100287.

[12] Moteriya, P., Rinkal, S., & Chanda, S. (2015). Screening of phytochemical constituents in some ornamental flowers of Saurashtra region. *Journal of Pharmacognosy and Phytochemistry*, *3*(5), 112-120.

[13] Thirumurugan, D., Cholarajan, A., Raja, S. S., & Vijayakumar, R. (2018). An introductory chapter: secondary metabolites. *Secondary metabolites-sources and applications*, *1*, 13.

[14] Hasan, T., Islam, A., ara khanom Riva, R., Rahman, M. N., Ahmed, S., Islam, M. A., & Daula, A. S. U. (2023). Phytochemicals from Zingiber capitatum rhizome as potential α-glucosidase, α-amylase, and glycogen phosphorylase inhibitors for the management of Type-II diabetes mellitus: Inferences from in vitro, in vivo and in-silico investigations. *Arabian Journal of Chemistry*, *16*(10), 105128.

[15] Jayaprakash, S. N. (2021). *Antimicrobial and Anti-inflammatory Efficacy of Essential oils Loaded Ethosomes* (Doctoral dissertation, Indian Veterinary Research Institute).

[16] Mohamad, S., Ismail, N. N., Parumasivam, T., Ibrahim, P., Osman, H., & A. Wahab, H. (2018). Antituberculosis activity, phytochemical identification of Costus speciosus (J. Koenig) Sm., Cymbopogon citratus (DC. Ex Nees) Stapf., and Tabernaemontana coronaria (L.) Willd. and their effects on the growth kinetics and cellular integrity of Mycobacterium tuberculosis H37Rv. *BMC complementary and alternative medicine*, *18*, 1-14.

[17] Das, D., & Shafi, S. (2023). Bioactivity-Guided Fractionation and Identification of Bioactive Molecules: A Basic Method in Drug Discovery. In *Drugs and a Methodological Compendium: From bench to bedside* (pp. 41-78). Singapore: Springer Nature Singapore.
[18] Lopes, A. G., & das Neves Costaa, F. (2021). Selection of three-phase solvent system for countercurrent chromatography–a practical guide using Syzygium malaccense leaves extract as an example. *Rev. Virtual Quim*, *13*, 1-10.
[19] Franzblau, S. G., DeGroote, M. A., Cho, S. H., Andries, K., Nuermberger, E., Orme, I. M., ... & Lenaerts, A. J. (2012). Comprehensive analysis of methods used for the evaluation of compounds against Mycobacterium tuberculosis. *Tuberculosis*, *92*(6), 453-488.
[20] Imperiale, B. R., Cataldi, Á. A., & Morcillo, N. S. (2017). In vitro anti-tuberculosis activity of azole drugs against Mycobacterium tuberculosis clinical isolates. *Revista Argentina de microbiologia*, *49*(4), 332-338.
[21] Chakansin, C., Yostaworakul, J., Warin, C., Kulthong, K., & Boonrungsiman, S. (2022). Resazurin rapid screening for antibacterial activities of organic and inorganic nanoparticles: Potential, limitations and precautions. *Analytical Biochemistry*, *637*, 114449.
[22] Sychev, A. V., Lavrova, A. I., Dogonadze, M. Z., & Postnikov, E. B. (2023). Establishing compliance between spectral, colourimetric and photometric indicators in Resazurin reduction test. *Bioengineering*, *10*(8), 962.
[23] Koleangan, H. S., Runtuwene, M. R., & Kamu, V. S. (2014). Penentuan Aktivitas Antioksidan Berdasarkan Nilai IC50 Ekstrak Metanol dan Fraksi Hasil Partisinya pada Kulit Biji Pinang Yaki (Areca vestiaria Giseke). *Jurnal MIPA*, *3*(2), 149-154.
[24] Taurina, W., & Andrie, M. (2022). Standardization of simplicia Clove Leaf (Syzygium aromaticum) from Lemukutan Island, West Kalimantan as Raw Material for Wound Healing Ointment. *International Journal of Pharmaceutical Research (09752366)*, *14*(3).
[25] Audah, K. A. (2018). Methods Development for Mangrove Extraction towards Standardization of Indonesian Extract Library. *Methods Development for Mangrove Extraction towards Standardization of Indonesian Extract Library*.
[26] Rahmawati, I. S., Widyanto, R. M., Maulidiana, A. R., Madani, M. S., & Riski, C. N. (2022). Aktivitas antioksidan dan antibakteri ekstrak etanol buah ihau (Dimocarpus longan var. malesianus Leenh) terhadap bakteri gram positif (Staphylococcus aureus). *Jurnal al-azhar indonesia seri sains dan teknologi*, *7*(2), 138.
[27] Mohamad, R. H., El-Bastawesy, A. M., Abdel-Monem, M. G., Noor, A. M., Al-Mehdar, H. A. R., Sharawy, S. M., & El-Merzabani, M. M. (2011). Antioxidant and anticarcinogenic effects of methanolic extract and volatile oil of fennel seeds (Foeniculum vulgare). *Journal of medicinal food*, *14*(9), 986-1001.
[28] Green, R. J. (2004). Antioxidant activity of peanut plant tissues.
[29] Tamrin, M. (2022). Studi literatur Penetapan Rendemen Ekstrak Etanol Myrtaceae Menggunakan Metode Maserasi.
[30] Aprillia, E., & Tjitraresmi, A. (2018). Uji Aktivitas Tumbuhan Sebagai Anti-Tuberkulosis. *Farmaka*, *16*(2).
[31] Kumar, M., Singh, S. K., Singh, P. P., Singh, V. K., Rai, A. C., Srivastava, A. K., ... & Kumar, A. (2021). Potential anti-mycobacterium tuberculosis activity of plant secondary metabolites: insight with molecular docking interactions. *Antioxidants*, *10*(12), 1990.
[32] Aristina, R. F., Astuti, W., & Pratiwi, D. R. (2019). Screening and phytochemicals test of extract endhophytes bacteria from stem of pacing (Costus sp.). *Jurnal Atomik*, *4*(1), 21-24.
[33] Situmeang, B., Ilham, I., Ibrahim, A. M., Amin, F., Mahardika, M., Bialangi, N., & Musa, W. J. A. (2022). Aktivitas antioksidan dan antibakteri dari fraksi ekstrak metanol kulit batang kesambi (Shleichera Oleosa). *Jurnal Kimia*, *16*(1).

[34] Dhull, S. B., Kaur, P., & Purewal, S. S. (2016). Phytochemical analysis, phenolic compounds, condensed tannin content and antioxidant potential in Marwa (Origanum majorana) seed extracts. *Resource-Efficient Technologies*, *2*(4), 168-174.
[35] Kamarudin, N. A., Muhamad, N., Salleh, N. N. H. N., & Tan, S. C. (2021). Impact of solvent selection on phytochemical content, recovery of tannin and antioxidant activity of quercus infectoria galls. *Pharmacognosy Journal*, *13*(5).
[36] Raj, D., & Kielisz, P. (2019). High-performance thin-layer chromatographic—densitometric determination of the major triterpene saponins and their aglycones from Centella asiatica. *JPC–Journal of Planar Chromatography–Modern TLC*, *32*, 25-30.
[37] Pothier, J. (1996). Thin-layer chromatography in plant sciences. *CRC Press: Boca Raton*.
[38] Arundina, I., Luthfi, M., & Indrawati, R. (2015). Identifikasi kromatografi lapis tipis sudamala (Artemisia vulgaris L.). *Majalah Kedokteran Gigi Indonesia*, *1*(2), 167-171.
[39] Hassan, N. W., Saudi, M. N., Abdel-Ghany, Y. S., Ismail, A., Elzahhar, P. A., Sriram, D., ... & El-Hawash, S. A. (2020). Novel pyrazine based anti-tubercular agents: Design, synthesis, biological evaluation and in silico studies. *Bioorganic chemistry*, *96*, 103610.
[40] Sturdy, M. (2010). *Natural Product Drug Discovery Regarding Mycobacterium tuberculosis and Quinone Reductase 2*. University of Illinois at Chicago, Health Sciences Center.
[41] Santoso, K. T. (2019). *The Synthesis and Evaluation of Heterocycles as Anti-Tuberculosis Agents* (Doctoral dissertation, Open Access Te Herenga Waka-Victoria University of Wellington).
[42] Tshinavhe, R. (2021). *Design, Synthesis and Anti-Tb Evaluation of 6 Dialkylaminopyrimidine Carboxamides*. University of Johannesburg (South Africa).
[43] Borges, A., J Saavedra, M., & Simoes, M. (2015). Insights on antimicrobial resistance, biofilms and the use of phytochemicals as new antimicrobial agents. *Current medicinal chemistry*, *22*(21), 2590-2614.
[44] Anggoro, A. (2015). Potensi Daun Pepaya (Carica papaya Sp.) sebagai Obat Anti Tuberkulosis. *Jurnal Kesehatan dan Agromedicine*, *2*(2), 86-89.
[45] Oakenfull, D., & Sidhu, G. S. (2023). Saponins. *Toxicants of plant origin*, 97-142.
[46] Fink, R., & Filip, S. (2023). Surface-active natural saponins. Properties, safety, and efficacy. *International journal of environmental health research*, *33*(7), 639-648.