BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 15, Number 1, April 2026 | Pages: 1655-1666 | DOI: 10.14421/biomedich.2026.151.1655-1666 ISSN 2540-9328 (online)

Fractionation and Antibacterial Activity of the Methanol Extract of White Champaka Flowers (*Magnolia alba* (DC.) Figlar)

Ulil Amna[1]*, Irma Setia Fitri[1], Halimatussakdiah Halimatussakdiah[1], Mastura Mastura[1], Syarifah Yanti Astryna[2]
[1]Department of Chemistry; Faculty of Science and Technology, Universitas Samudra, Jl. Prof. Dr. Syarief Thayeb, Langsa 24416, Indonesia.
[2]Department of Pharmacy, Faculty of Health Sciences, Universitas Ubudiyah Indonesia, 23114, Aceh, Indonesia.

Corresponding author*
ulil_amna@unsam.ac.id

Manuscript received: 17 June 2026. Revision accepted: 16 August 2026, Published: 22 August 2026.

Abstract

Research on fractionation and antibacterial activity test of methanol extract of *cempaka putih* (*Magnolia alba* (DC.) Figlar) flower was conducted. This study aimed to investigate the class of chemical compounds contained in the methanol extract of *cempaka putih* (*M. alba* (DC.) Figlar) flowers and to test the antibacterial activity of the methanol extract of *M. alba* (DC.) Figlar flowers against *Staphylococcus aureus* and *Escherichia coli* bacteria. Based on the results of phytochemical screening, these showed that the methanol extract and its fractions contained secondary metabolites of alkaloid, terpenoids, steroids, saponins, flavonoids, phenols and tannins. Testing for antibacterial activity was carried out by the Kirby-Bauer method (agar diffusion) against *S. aureus* and *E. coli* bacteria, at concentrations of 5, 10, 20 and 40 ppm. The antibacterial activity test showed that the methanol extract gave moderate activity against *E. coli* and fraction A gave moderate activity against *S. aureus.* While other fractions showed weak activities against both bacteria.

Keywords: Antibacterial; White Champaka; Extraction; Fractionation; *Magnolia alba*.

INTRODUCTION

Indonesian society consists of diverse ethnic groups and cultures, each possessing unique traditional medicinal practices derived from the rich biodiversity of flora and fauna. The diversity of plant species, both cultivated and wild, represents an important source of medicinal agents. The use of plants in traditional medicine has long been recognized and passed down from one generation to the next. However, many medicinal plants commonly utilized by local communities are not adequately conserved, resulting in their decreasing availability in natural habitats (Nursanty, 2017). In general, almost all parts of a plant can be used for traditional medicinal purposes, including leaves, stems, roots, fruits, seeds, and flowers. One of the plants traditionally utilized for medicinal purposes is white champaka (Sukewijaya *et al*., 2016).

White champaka (*Magnolia alba* (DC.) Figlar) is one of the medicinal plants found in Indonesia. In several regions, this plant is used not only as a traditional remedy but also in religious offerings and worship rituals among Hindu communities (Sukewijaya *et al*., 2016). Traditionally, white champaka flowers have been used to treat various ailments, including as an expectorant, diuretic, and remedy for vertigo, flatulence, sinusitis, vaginal discharge, respiratory disorders, and coughs (Puspita and Poerbantanoe, 2019). In addition, white champaka has been reported to possess various pharmacological activities, including anticancer, antifungal, antioxidant, and antibacterial properties (Nasution *et al*., 2019; Sukewijaya *et al*., 2016).

Antibacterial agents inhibit bacterial growth or kill bacteria by disrupting the metabolism of harmful microorganisms (Novaryatiin *et al*., 2018). These compounds are commonly found as secondary metabolites in living organisms. In general, secondary metabolites that contribute to antibacterial activity include flavonoids, tannins, alkaloids, and saponins (Oroh *et al*., 2015; Pasaribu *et al*., 2013; Septiani *et al*., 2017), which are often present in polar plant extracts such as methanol extracts. The antibacterial mechanisms of these compounds generally involve damaging the bacterial cell wall, altering membrane permeability, disrupting protein synthesis, and inhibiting enzymatic activity (Septiani *et al*., 2017). Secondary metabolites found in plants are bioactive substances associated with various biological activities and therefore have potential applications as therapeutic agents for numerous diseases (Yuda *et al*., 2017). Although bacteria play beneficial roles in nature, some species are important human pathogens, including *Staphylococcus aureus* and *Escherichia coli*.

Staphylococcus aureus is considered a dangerous bacterium because it is commonly found in the air and surrounding environment (Trisia *et al*., 2018). One of the serious diseases associated with *S. aureus* infection is pneumonia (Parra *et al*., 2016; Trisia *et al*., 2018). Pneumonia is an acute lung infection that can interfere with the exchange of oxygen and carbon dioxide in the lungs (Trisia *et al*., 2018). *Escherichia coli* is one of the bacterial species capable of causing infections in humans (Dusturia *et al*., 2016). This bacterium can become pathogenic when its population increases excessively in the gastrointestinal tract, leading to urinary tract infections and digestive disorders such as diarrhea (Islam *et al*., 2016; Herdiyanti *et al*., 2019). Diarrhea is a condition characterized by excessive loss of fluids and electrolytes through feces (Mulyanto *et al*., 2018).

Based on the literature described above, research on the antibacterial activity of white champaka (*M. alba* (DC.) Figlar) is important. Therefore, this study aimed to evaluate the antibacterial activity of the methanol extract of white champaka flowers against *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Samples and Bioindicators

The sample used in this study was white champaka flowers (*M. alba* (DC.) Figlar) (Figure 1), which were collected from Kisaran, Asahan Regency, North Sumatra, Indonesia. The bacterial strains used as bioindicators were *Staphylococcus aureus* and *Escherichia coli*.

Figure 1. *Magnolia alba* (DC.) (Figlar).

Procedures

Extraction

The white champaka flowers were cleaned, separated from the stems, cut into small pieces, and air-dried. The air-dried sample (86 g) was macerated with methanol for 3 × 24 h. The resulting extract was then filtered, and the solvent was removed using a rotary evaporator to obtain the methanol extract, which was subsequently weighed.

Fractination

The methanol extract was fractionated by column chromatography using silica gel 40 (0.065–0.200 mm) as the stationary phase. The mobile phase consisted of *n*-hexane, ethyl acetate, and methanol, which were applied using a predetermined gradient elution system. The initial eluent composition was selected through a trial-and-error approach based on thin-layer chromatography (TLC) analysis. The fractions obtained were monitored by TLC, and fractions exhibiting similar spot patterns were combined into several fraction groups. The resulting fractions were concentrated by solvent evaporation and subsequently weighed.

Phytochemical Screening

Phytochemical screening was performed on both the crude plant material and the extracts to identify the presence of alkaloids, terpenoids, steroids, saponins, flavonoids, phenolic compounds, and tannins (Halimatussakdiah *et al.*, 2019).

Alkaloids. Two gram of the plant materials was crushed and then 1 mL of ammonia was added. Furthermore, 10 mL of chloroform was added, and then the materials were crushed and filtered. 10 mL of sulfuric acid 2N was added to the filtrate, shaken vigorously, and left for a minute until the sulfuric acid solution and chloroform separated. The sulfuric acid layer was taken and divided into three test tubes and each test tube was tested by Meyer, Dragendorff, and Wagner reagents to determine the presence of alkaloids. The addition of Meyer reagent established white precipitate, Dragendorff' reagent caused reddish precipitate, and Wagner reagent raised yellow precipitate. These results indicate the presence of alkaloids.

Terpenoids, steroids, and saponins. Ten grams of the plant materials was finely ground, and then extracted with hot methanol. The obtained filtrate was concentrated with the rotary evaporator to yield methanol extract. The methanol extract was partitioned with hexane. The soluble extract in hexane was tested with the Liebermann–Burchard reagent. The blue or green color exhibits the presence of steroids and red color exhibits terpenoids. The insoluble residue in hexane was added with water and shaken vigorously. The presence of stable foam for 30 min indicates the existence of saponins; if positive for saponins, the solution was hydrolyzed with HCl and tested with the Liebermann–Burchard reagent. The green or blue color indicates the presence of steroidal saponins and the purple or red color shows the existence of terpenoid saponins.

Flavonoids. Plant materials (10 g) were extracted with methanol and concentrated. The concentrated methanol extract was partitioned with hexane. The residue was extracted with 10 mL of 80% ethanol, and 1 mg of magnesium and HCl 0.5 M were subsequently added. The pink or purple color shows the presence of flavonoids.

Phenolics. The extract was tested by ferric chloride. Three or four drops of $FeCl_3$ solution were to the extract, and the formation of bluish black color exhibits the phenol compound.

Tannins. One gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of $FeCl_3$ 0.1% were added. The formation of a brownish green or bluish black color indicates the presence of tannins.

Antibacterial Activities

The antibacterial activity assay was conducted on both the crude plant material and the extracts using the agar diffusion (Kirby–Bauer) method. Approximately 15 mL of nutrient agar (NA) was poured into sterile Petri dishes and allowed to solidify. The bacterial inoculum was then applied onto the surface of the NA medium using an inoculating loop. The inoculum was evenly spread across the agar surface by streaking at a 90° angle to ensure uniform bacterial growth. Subsequently, sterile paper discs (5 mm in diameter) impregnated with the test extract were placed on the agar surface.

For the positive control, paper discs containing chloramphenicol were placed on each Petri dish, while blank paper discs without extract served as the negative control. The plates were then incubated at 35–37°C for 24 h. The appearance of a clear zone surrounding the paper disc indicated positive antibacterial activity. The diameter of the inhibition zone was measured and compared with that produced by the standard antibiotic chloramphenicol (Pasaribu *et al*., 2013). The inhibition zones were interpreted in Table 1 (Kurniawan, *et al*., 2019).

Tabel 1. Categories of Antibacterial Inhibition Zones.

Diameter	Category
>12 mm	Strong (+++)
9< O ≤ 12 mm	Moderate (++)
Diameter 7< O ≤ 9 mm	Weak (+)
≤ 6 mm	Inactive

RESULTS AND DISCUSSION

Result

Extraction and Fractination

The sample preparation process included cleaning, stem separation, drying, and grinding. A total of 250 g of the collected sample was air-dried for 10 days to reduce its moisture content and prevent fungal growth. The dried sample was then ground into a fine powder to increase its surface area, thereby maximizing direct contact between the sample and the solvent during the extraction process. This procedure yielded 86 g of fine powdered material.

The sample was extracted using the maceration method with methanol as the solvent. The maceration process was carried out for 3 × 24 h and repeated four times until a clear filtrate was obtained. The resulting filtrate was light brown in color and was subsequently concentrated using a rotary evaporator to obtain a crude methanol extract with the yield extract obtain in Table 2.

Table 2. Yield Extract of *M. Alba* Flower.

Extract	Crude Extract Weight (g)	Yield Extract (g)	Crude Extract Color
Methanol	39.3	45.65	Blackish brown

Fractination

A total of 29 g of the *M. alba* methanol extract was dissolved in a small amount of methanol, loaded onto a chromatography column, and covered with a 1-cm layer of sand. The sample was then eluted successively with 100% *n*-hexane, mixtures of *n*-hexane and ethyl acetate in various proportions, 100% ethyl acetate, mixtures of ethyl acetate and methanol in various proportions, and finally 100% methanol until colorless fractions were obtained. The elution process resulted in the collection of 143 fractions. The fractions obtained were subsequently analyzed by thin-layer chromatography (TLC) to determine groups of fractions with similar spot patterns. The TLC monitoring results are shown in Figure 2. Fractions exhibiting similar TLC profiles were pooled, yielding five combined fraction groups, which are presented in Table 3.

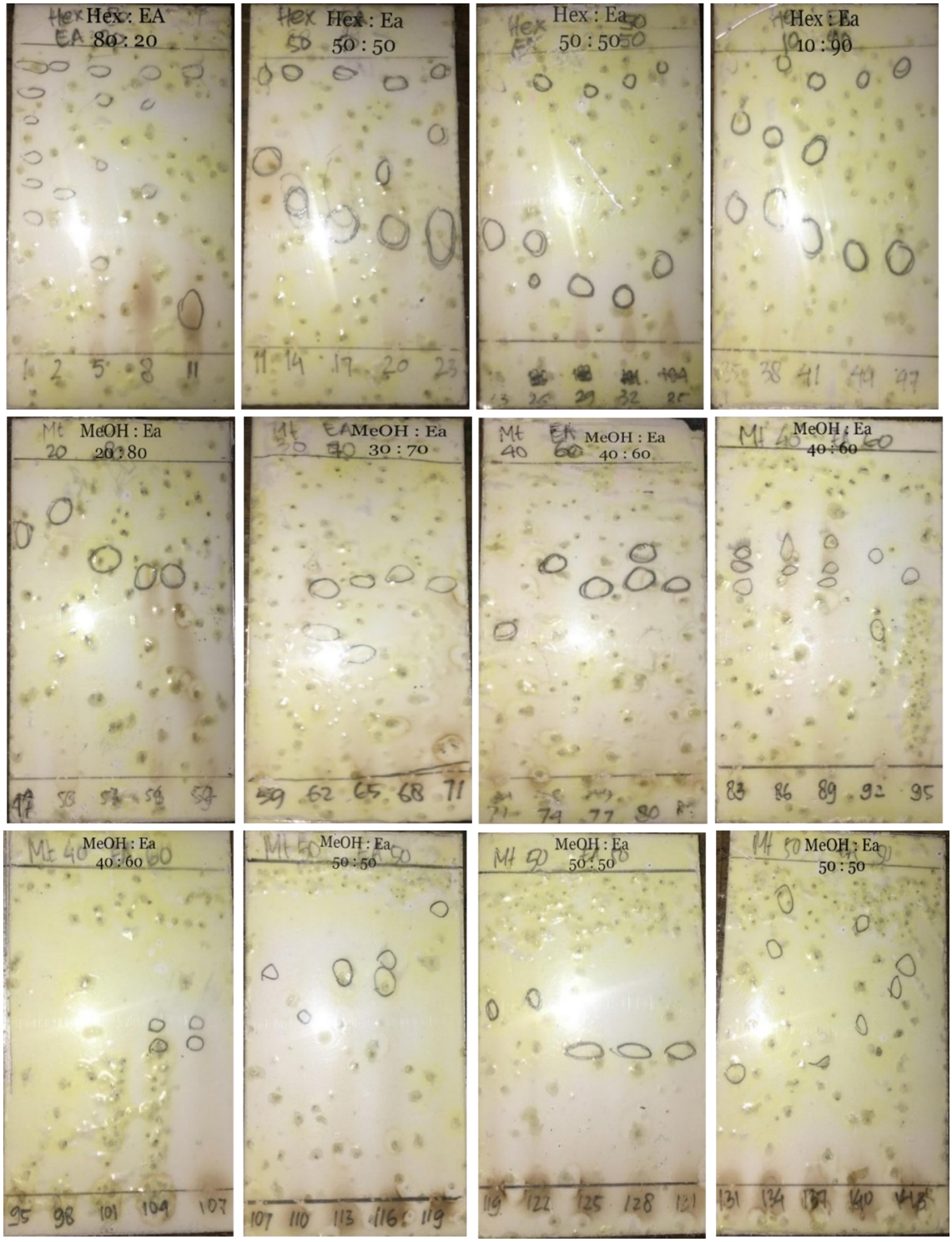


Figure 2. TLC Profiles of the Methanol Fractions of *M. alba* Flowers.

Table 3. Classification of the Methanol Fraction Groups of *M. alba* Flowers.

Fraction Group	Fraction
A	1 – 17
B	18 – 43
C	44 – 91
D	92 – 122
E	123 – 143

Phytochemical Screening

Phytochemical screening was performed to identify the classes of compounds present in *M. alba* flowers. The phytochemical tests were based on precipitation reactions and colour changes produced by specific reagents, and the results were used to classify the secondary metabolite groups present in the samples. Due to its high polarity, methanol is capable of penetrating the cell walls of the

plant material, allowing both polar and non-polar compounds to be efficiently extracted. Consequently, the phytochemical screening results of the methanol extract indicated the presence of almost all major classes of secondary metabolites in the sample. The phytochemical screening results of fresh white champaka flowers, the methanol extract, and its fractions are presented in Table 4.

Table 4. Phytochemical Screening Results.

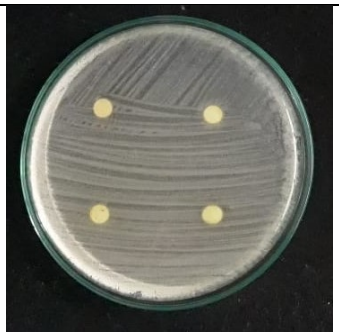
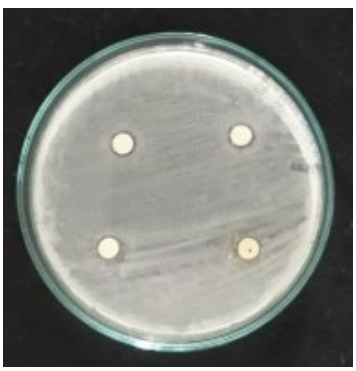
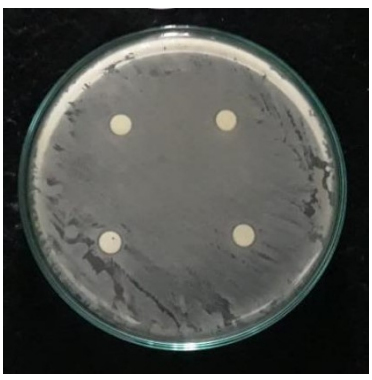
Sample	Fresh Flower	Methanol Extract	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
Alkaloid	+	+	+	+	+	+	+
Terpenoid	+	-	-	-	-	-	-
Steroid	-	+	+	+	+	-	-
Saponin	-	+	+	+	+	+	+
Flavonoid	+	-	-	-	-	-	-
Phenolic	+	++	-	-	-	-	-
Tanin	-	++	-	-	-	-	-

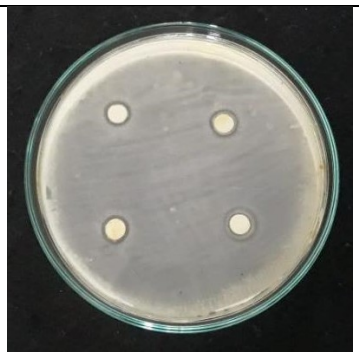
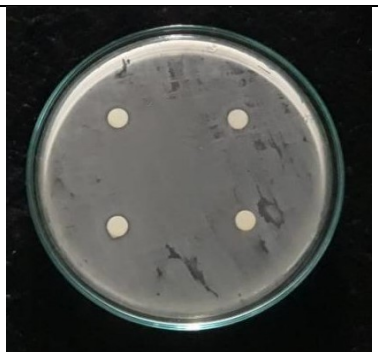
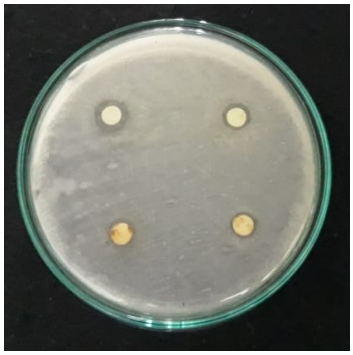
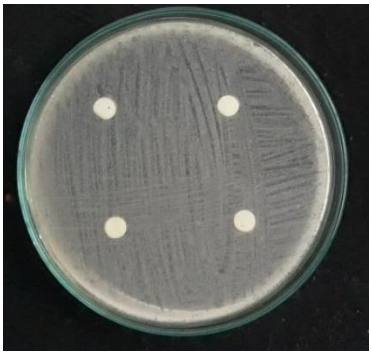
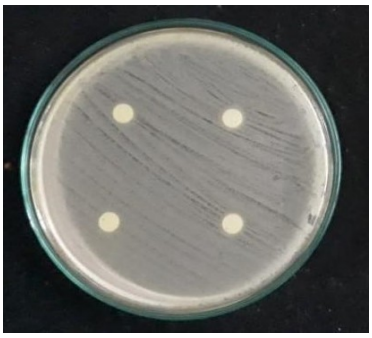
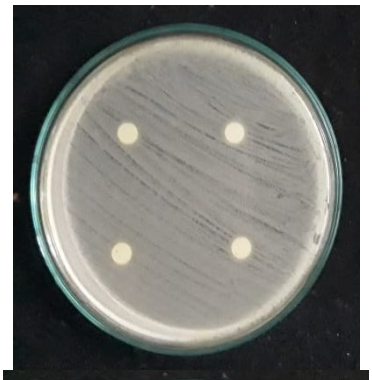
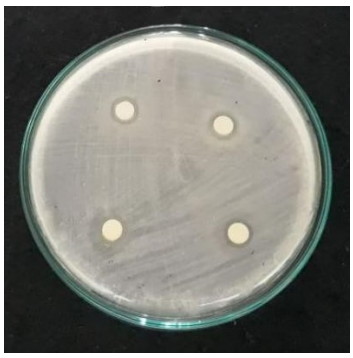
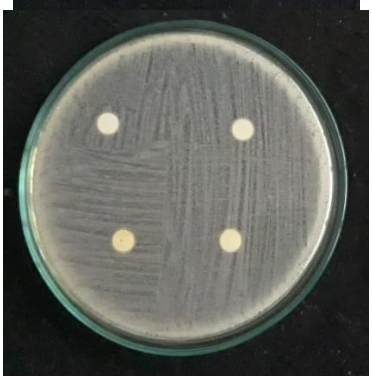
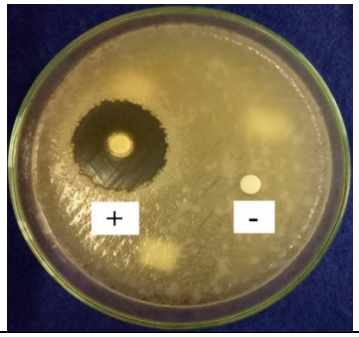
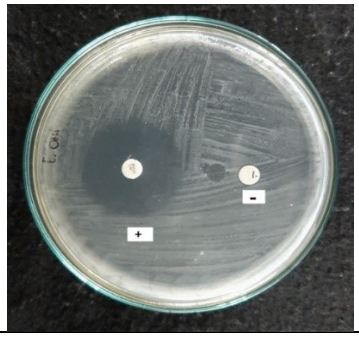
Remarks : (+) Secondary metabolites detected. (-) Secondary metabolites detected not detected.

Antibacterial Activities

Antibacterial agents are compounds used to inhibit bacterial growth, and their effectiveness is highly dependent on concentration. Although bacterial cells contain numerous potential targets for antimicrobial compounds, some bacteria are capable of undergoing cellular adaptations that enable them to develop resistance to certain antibacterial agents or antibiotics. The antibacterial activity assay of the methanol extract of white champaka flowers (*M. alba* (DC.) Figlar) and its fractions demonstrated inhibitory effects against the growth of *S. aureus* and *E. coli*. The assay was performed using the agar diffusion method at concentrations of 5, 10, 20, and 40 ppm, as indicated by the formation of inhibition zones around the test samples. The inhibition zone diameters of the methanol extract and its fractions from *M. alba* (DC.) Figlar flowers are shown in Tabel 5 and Table 6.

Table 5. Appearance of Inhibition Zone of Methanol Extract and Its Fractions against *S. aureus* and *E. coli.*

Sample	*S. aureus*	*E. coli*
Methanol Extract		
Fraction A		

Sample	*S. aureus*	*E. coli*
Fraction B		
Fraction C		
Fraction D		
Fraction E		
Control (+) and (-)		

Sample	*S. aureus*	*E. coli*

Table 6. Diameter of the Inhibition Zones.

Name of Sample	Sample concentration (ppm)	Mean Inhibition Zone Diameter (mm)	
		S. aureus	*E. coli*
Methanol Extract	5	8.53	8.03
	10	8.80	9.93
	20	9.20	10.27
	40	9.60	10.47
Fraction A	5	9.80	8.23
	10	10.17	8.53
	20	10.47	8.97
	40	10.80	9.40
Fraction B	5	8.90	8.03
	10	9.23	8.43
	20	9.50	8.93
	40	9.63	9.20
Fraction C	5	8.47	7.17
	10	8.90	7.43
	20	9.23	7.77
	40	9.43	8.10
Fraction D	5	8.03	7.23
	10	8.47	7.47
	20	8.77	7.77
	40	9.03	8.17
Fraction E	5	7.47	6.07
	10	7.80	6.37
	20	8.13	6.57
	40	8.40	6.87
Chloramphenicol (Positive Control)		25.50	25.50
Blank Disc (Negative Control)		0.00	0.00

Discussion

Extraction and Fractination

Extraction is a separation method used to isolate one or more components from a mixture by dissolving the sample in a suitable solvent. The basic principle of this separation method is the solubility of the sample in a particular solvent. The sample was extracted using the maceration method with methanol as the extraction solvent. The high polarity of methanol allows the extraction process to proceed efficiently. The maceration method was selected because it does not require heating, thereby minimizing the degradation of bioactive compounds present in the sample. In addition, this method is suitable for both small and large quantities of samples. The maceration process was carried out for 3 × 24 h and repeated four times until a clear filtrate was obtained. This was intended to maximize the extraction yield, as a longer contact time between the sample and solvent generally results in a more efficient extraction process. The resulting filtrate was light brown and transparent, and was subsequently concentrated using a *rotary evaporator* to obtain a concentrated methanolic extract, which shown in Table 2.

In this study, compound separation was carried out using the column chromatography method, which aims to purify, isolate, and separate compounds from a mixture. The separation process was performed to obtain fractions of chemical compounds from the methanolic extract of *M. alba* flowers that potentially possess antibacterial activity. The principle of column chromatography is the separation of compounds between two phases, namely the stationary phase and the mobile phase. The stationary phase retains the components of the mixture, while the mobile phase dissolves and transports the compounds according to their polarity. Prior to sample elution, sample profiling was conducted to determine the most suitable solvent system to be used as the eluent. Based on Figure 2, the TLC results showed that several fractions exhibited similar spot patterns. Therefore, these fractions were combined, resulting in five pooled fraction groups, as presented in Table 3. The pooled fractions were subsequently subjected to phytochemical screening and antibacterial activity test.

Phytochemical Screening

Based on Table 4, the fresh flower of *M. alba* tested positive for the presence of alkaloids, terpenoids, flavonoids, and tannin. Methanol extracts positive for the presence of alkaloids, saponins, steroids, phenolics, and tannins. Fraction A, B, and C positive for the presence of

alkaloids, saponins, and steroids. Fraction D, and E positive for the presence of alkaloids and saponins.

In the alkaloid screening test, the sample was treated with several reagents, resulting in precipitate formation. The addition of Mayer's reagent produced a white precipitate, Dragendorff's reagent yielded a brick-red precipitate, and Wagner's reagent formed a brown precipitate. The formation of precipitates with each reagent indicates a positive result for alkaloids in the sample. The white precipitate formed upon the addition of Mayer's reagent is attributed to the interaction between the nitrogen atom of the alkaloid and K^+ ions from potassium tetraiodomercurate(II), leading to the formation of an insoluble potassium–alkaloid complex. The reaction mechanism involved in the Mayer test is illustrated in Figure 3.

$$HgCl_2 + 2KI \rightarrow HgI_2 + 2KCI$$
$$HgI_2 + 2KI \rightarrow K_2 + [HgI_4]$$

Potassium tetraiodomercurate (II)

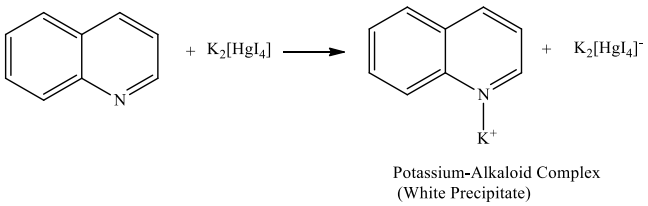


Figure 3. Mechanism of Potassium–Alkaloid Complex Formation in the Mayer Test. (Ergina *et al*., 2014)

In the alkaloid test using Dragendorff's reagent, a brick-red precipitate was formed. This precipitate is a potassium–alkaloid complex. During the preparation of Dragendorff's reagent, bismuth nitrate is dissolved in hydrochloric acid (HCl) to prevent hydrolysis, as bismuth salts are readily hydrolyzed to form bismuthyl ions (BiO^+). The reaction mechanism involved in this process is illustrated in Figure 4.

$$Bi(NO_3)_3 + 3KI \rightarrow BiI_3 + 3KNO_3$$
Brown
$$BiI_3 + KI \rightarrow K\,[BiI_4]$$
Potassium tetraiodobismuthate

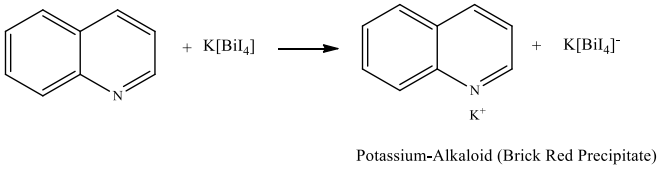


Figure 4. Mechanism of Potassium–Alkaloid Complex Formation in the Dragendorff Test (Rahmayani *et al*., 2013)

A positive result for alkaloids in the Wagner reagent test was indicated by the formation of a brown precipitate, which is presumed to be a potassium–alkaloid complex. During the preparation of Wagner's reagent, iodine reacts with I^- ions from potassium iodide to form triiodide ions (I_3^-), which impart a brown color to the solution. In the Wagner test, K^+ ions form coordinate covalent bonds with the nitrogen atom of the alkaloid, resulting in the formation of an insoluble potassium–alkaloid complex. The reaction mechanism involved in the Wagner reagent test is shown in Figure 5.

$$I_2 + I^- \rightarrow I_3^-$$
Brown

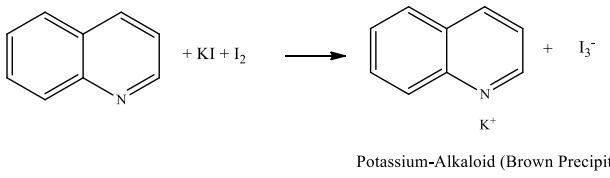


Figure 5. Mechanism of Potassium–Alkaloid Complex Formation in the Wagner Test (Rahmayani *et al*., 2013)

In the steroid and terpenoid screening using the Liebermann–Burchard method, the extract was dissolved in chloroform and subsequently treated with Liebermann–Burchard reagent (acetic anhydride). The appearance of a red color indicated a positive result for terpenoids, whereas the formation of a green color indicated the presence of steroids. This color development is attributed to the ability of terpenoid and steroid compounds to react with acetic anhydride, producing characteristic color changes. The difference in the colors produced by terpenoids and steroids is associated with differences in the functional groups attached to their carbon atoms. The chemical reaction mechanism involved in this test is illustrated in Figure 6.

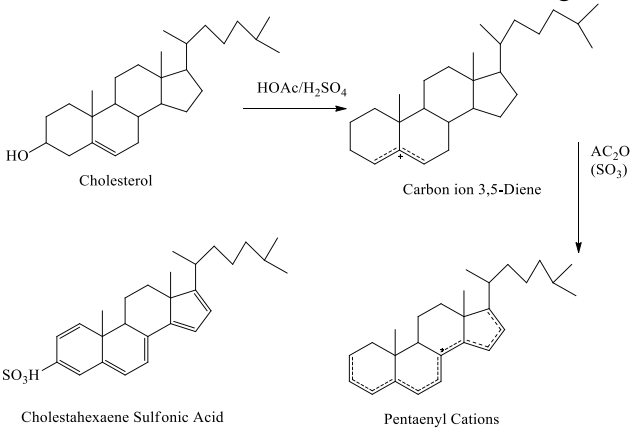


Figure 6. Mechanism of the Reaction with Liebermann–Burchard Reagent (Habibi *et al*., 2018).

Saponins are glycosidic compounds and therefore exhibit polar characteristics. In the saponin test, the formation of stable foam that persisted for approximately 30 minutes indicated a positive result for the presence of saponins in the sample. The foam formation is attributed to the presence of glycosides that are capable of producing froth in water and can be hydrolyzed into glucose and other compounds. Due to their polar nature, saponins tend to be extracted by semi-polar and polar solvents such as methanol. After the sample was

confirmed to contain saponins, it was hydrolyzed using HCl and subsequently tested with Liebermann–Burchard reagent. The development of a red color indicated a positive result for saponin compounds. The reaction mechanism involved in the saponin test is presented in Figure 7.

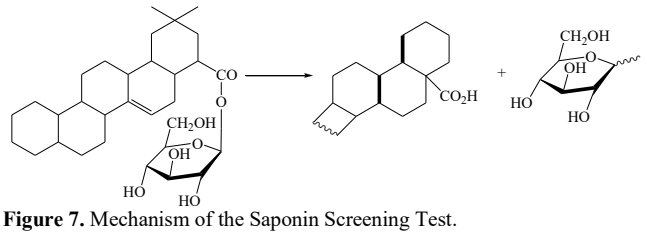


Figure 7. Mechanism of the Saponin Screening Test.

The flavonoid test yielded a positive result, as indicated by the development of a pink color in the extract solution. The addition of magnesium metal and hydrochloric acid (HCl) reduced the benzopyrone nucleus present in the flavonoid structure, leading to the formation of flavilium salts that exhibit a pink to purple coloration. This result may be attributed to the use of methanol as the extraction solvent, as methanol is capable of dissolving a wide range of compounds, including non-polar and semi-polar constituents (Rahmayani *et al*., 2013). The reaction mechanism involved in the flavonoid test is illustrated in Figure 8.

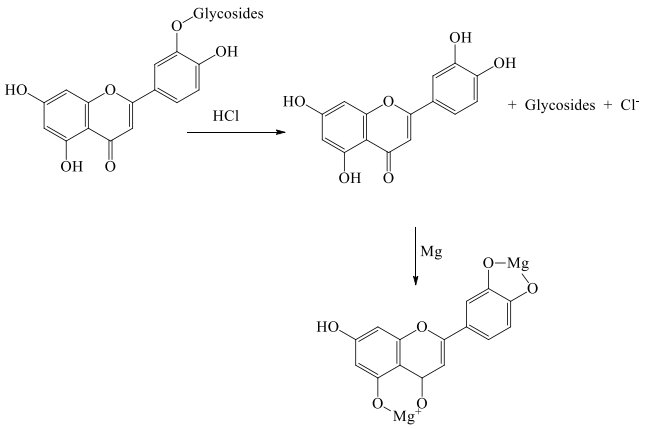


Figure 8. Mekanisme Reaksi Uji Kandungan Flavonoid (Iskandar, 2020).

Phenolic compounds are relatively soluble in water due to their association with sugars and glycosides. In the phenolic screening test, the addition of $FeCl_3$ to the sample resulted in the formation of a bluish-black color, indicating a positive result for the presence of phenolic compounds. Phenolic compounds contain hydroxyl groups that can react with Fe^{3+} ions in the $FeCl_3$ solution to form colored complex compounds. The reaction mechanism involved in this process is illustrated in Figure 9.

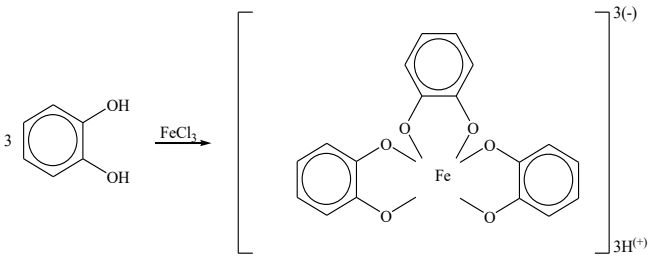


Figure 9. Reaction Mechanism for the Detection of Phenolic Compounds (Ergina *et al*., 2014).

In the tannin test, the sample was boiled in water, and the resulting filtrate was treated with $FeCl_3$ solution. The appearance of a greenish-brown color indicated the presence of tannins in the sample. The identification of tannins using $FeCl_3$ is based on the formation of a complex compound resulting from the reaction between tannin molecules and Fe^{3+} ions. The reaction mechanism involved in the tannin test is illustrated in Figure 10.

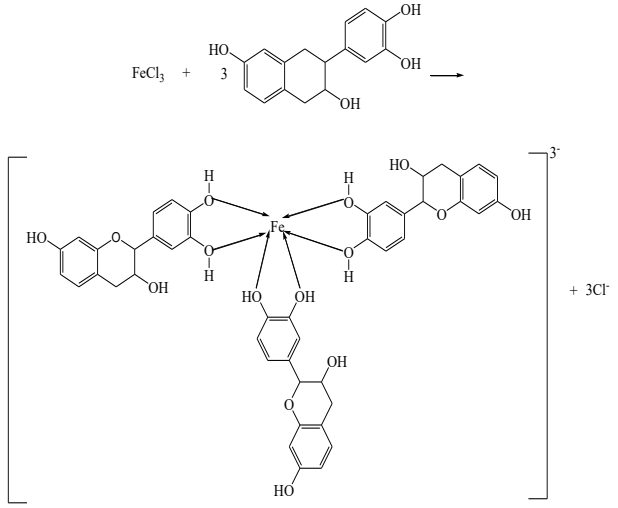


Figure 10. Reaction Mechanism of the Ferric Chloride Test for Tannins (Ergina *et al*., 2014).

Antibacterial Activity

Antibacterial agents are compounds used to inhibit bacterial growth, and their effectiveness is highly dependent on concentration. Most bacterial cells contain numerous potential targets for antimicrobial compounds; however, some bacteria are capable of undergoing cellular adaptations that enable them to develop resistance to certain antimicrobial agents or antibiotics. The antibacterial activity of the methanolic extract of white champaca flowers (*M. alba* (DC.) Figlar) and its fractions was evaluated against *S. aureus* and *E. coli* using the agar diffusion method at concentrations of 5, 10, 20, and 40 ppm. The principle of the agar diffusion assay is based on measuring the inhibition zone formed around the sample, which reflects its ability to suppress bacterial growth. In this study, chloramphenicol was used as the positive control to serve as a reference standard for evaluating the antibacterial activity of the extracts based on the diameter of the inhibition zone. Meanwhile, a blank paper disc without extract was used as the negative control to confirm that the disc itself did not exhibit antibacterial activity against the test organisms (Devi and Mulyani, 2017).

This assay was conducted by measuring the inhibition zones formed on the test medium, and the mean inhibition zone diameters obtained were tabulated in Table 4.5. Based on the antibacterial activity data presented above, it can be observed that the methanolic extract and its fractions exhibited varying antibacterial activities against *S. aureus*, although the differences were not significant. According to Table 6, the fraction A demonstrated moderate antibacterial activity against *S. aureus*, while other fractions demonstrated as weak bacterial activities. Several factors may influence antibacterial activity, including the type and potency of the antibacterial compounds as well as the active constituents extracted from the plant material. In general, the inhibitory effect of the white champaca flower extract against *S. aureus* may be attributed to the presence of antibacterial compounds capable of damaging the bacterial cell wall. According to Mahvirah *et al.* (2019), the mechanism of action of antibacterial active compounds involves disruption of the protoplasm as well as damage to and penetration of the bacterial cell wall.

Fractions A exhibited the largest inhibition zone compared to the other fractions against *S. aureus*. Based on the phytochemical screening results, the presence of polar compounds such as alkaloids and saponins may contribute to the observed antibacterial activity. Gram-positive bacteria are generally more sensitive to polar antibacterial compounds because their cell walls contain a thick peptidoglycan layer, low lipid content, and teichoic acids within the cell wall structure. Teichoic acids are water-soluble polymers that function in the transport of positive ions. Their water-soluble nature indicates that the cell wall of Gram-positive bacteria is predominantly polar.

According to Silvia (2015), alkaloids exhibit antibacterial activity through mechanisms that interfere with the peptidoglycan components of the bacterial cell wall, thereby disrupting proper cell wall formation and ultimately causing bacterial cell death. Saponins exert antibacterial effects by damaging the cell wall and penetrating the bacterial cell membrane, leading to the leakage of essential intracellular components (Karlina, 2013). The disruption of the cell wall by saponins interferes with bacterial metabolism and eventually results in bacterial death.

In this study, the Gram-negative bacterium used was *Escherichia coli*, a pathogenic bacterium characterized by a complex cell envelope structure consisting of three polymeric layers located outside the peptidoglycan layer, namely lipoproteins, an outer phospholipid membrane, and lipopolysaccharides (Kandau and Pandiangan, 2018). The inhibition zone diameters produced by the methanolic extract and fractions of white champaca flowers (*M. alba* (DC.) Figlar) against *S. aureus* and *E. coli* are presented in Table 5 and Table 6. As shown in the result, higher sample concentrations resulted in larger inhibition zones, suggesting a positive relationship between concentration and antibacterial activity.

The antibacterial assay against *E. coli* revealed that the methanolic extract produced the largest inhibition zone diameter. This result may be attributed to the presence of various secondary metabolites in the methanolic extract that are capable of inhibiting bacterial growth, including alkaloids, saponins, tannins, phenolics, and steroids.

Alkaloids are known to inhibit bacterial growth by interacting with DNA and disrupting membrane permeability. Saponins exhibit antibacterial activity by reducing the efficiency of glucose utilization in microorganisms, affecting growth and proliferation, decreasing enzymatic activity, and suppressing protein synthesis, ultimately leading to bacterial cell death. Tannins also possess antibacterial properties; their

inhibitory activity is associated with their ability to bind to the bacterial cell wall, inhibit bacterial growth, and interfere with protease activity (Mawan *et al*., 2018).

Based on the data presented in Table 6, the methanolic extract and its fractions exhibited varying antibacterial activities against *S. aureus* and *E. coli*. The results indicate that the methanol extract gave moderate activity against *E. coli* and fraction A gave moderate activity against *S. aureus.* While other fractions showed weak activities against both bacteria.

CONCLUSIONS

The results of the phytochemical screening of white champaca flowers (*M. alba* (DC.) Figlar) showed that the fresh flower sample contained secondary metabolites belonging to the alkaloid, terpenoid, and flavonoid groups. The methanolic extract and fractions A, B, and C were found to contain alkaloids, steroids, and saponins, whereas fractions D and E contained alkaloids and saponins as their major secondary metabolite constituents. The results of the antibacterial activity assay of white champaca (*M. alba*) flowers demonstrated that the methanol extract gave moderate activity against *E. coli* and fraction A gave moderate activity against *S. aureus.* While other fractions showed weak activities against both bacteria.

Acknowledgements: The authors gratefully acknowledge Universitas Samudra for facilitating this research.

Authors' Contributions: Ulil Amna and Halimatussakdiah designed the study. Irma Setia Fitri carried out the laboratory work and analyzed the data. Ulil Amna and Syarifah Yanti Astryna wrote the manuscript. Mastura and halimatussakdiah review the manuscript. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there are no competing interests.

Funding: No funding

REFERENCES

Devi, S., & Mulyani, T. (2017). Antibacterial Activity of Ethanol Extract Pacar Kuku Leaf (*Lawsonia inermis Linn)* in *Pseudomonas aeruginosa. Journal of Current Pharmaccutical Scinces. 1*(1):30-35.

Dusturia, N., Hikamah, R. S., & Sudiarti, D. (2016). Efektivitas Antibakteri Bunga Kenanga (*Cananga odorata*) dengan Metode Konvensional terhadap Pertembuhan *Staphylococcus aureus. Bioshell*, *5*(1):324 – 332.

Ergina., Nuryanti, S., & Pursitasari, D, I. (2014). Uji Kualitatif Senyawa Metabolit Sekunder pada Daun Palado (Agave angustifolia) yang diekstraksi dengan Pelarut Air dan Etanol. Jurnal Akademika Kimia, *3*(3):165-172.

Habibi, I, A., Firmansyah, A, R., & Setyawati, M, S. (2018). Skrining Fitokimia Eksrak n-Heksa Korteks Batang Salam (*Syzygium Polyanthum*). Indonesian Journal of Chemical Sience. *7*(1):1-4.

Halimatussakdiah., Amna, U., & Wahyuningsih, P. (2018). Preliminary Phytochemical Analysis and Larvicidal Activity of Edible Fern (*Diplazium esculentum* (Retz.) Sw.) Extract Against Culex. *Jurnal Natural*, *18*(3):141-147.

Herdiyanti., Alimsardjono, L., & Indiastuti, N. D. (2019). Resistance Pola *Escherichia coli* dan *Klebsiella pneumoniae* Bakteri Terhadap Amikacin, Ceftazidime, Meropenem, Nitrofurantoin Antibiotik pada Pasien Lansia dengan ISK di Dr. Soetomo Rumah sakit Surabaya. *Journal of Aesculap Ilmu Kedokteran*, *10*(1):21-24.

Iskandar, D. (2020). Aplikasi Uji Skrining Fitokimia terhadap Daun Uncaria Tomentosa sebagai bahan Utama dalam Pembuatan Teh. Jurnal Teknologi Technoscientia, *12*(2):153-158.

Islam, A. M., Kabir, L. M. S., & Seel, K. S. (2016). Molecular Detection and Characterization of *Escherichia Coli* Isolated From Raw Milk Sold in Different Markets of Bangladesh. *Bangl J Vet. Med*, *14*(2):271-275.

Kandou, F. E. F. & Pandiangan, D. (2018). Aktivitas Antibakteri Ekstrak Metanol Tumbuhan Paku Diantum *capillus-veneris* dan *Asplenium nidus* Terhadap Bakteri Gram Negatif *Escherichia coli* Dengan Metode Difusi Agar. Jurnal MIPA Unsrat Online, *7*(1):25 – 28.

Karlina, Y. C., Ibrahim, M., & Trimulyono, G. (2013). Aktivitas Antibakteri Ekstrak Herba Krokot (*Portulaca Oleracea I*) terhadap *Staphylococcus aureus dan Escherichia coli*. LenteraBio, *2*(1):87 – 93.

Kurniawan, E., Jekti, D.S.D., & Zulkifli, L. (2019). Aktivitas Antibakteri Ekstrak Metanol Batang Bidara Laut (*Strychnos ligustrina*) Terhadap Bakteri Patogen. *Jurnal Biologis Tropis*. *19*(1):61-69.

Magvirah, T., Marwati., & Ardhani, F., (2019). Uji Daya Hambat Bakteri *Staphylococcus aureus* Menggunakan Ekstrak Daun Tahongai (*Kleinhovia Hospital L*.). Jurnal Peternakan Lingkungan Tropis, *2*(2):41-50.

Mawan, R. A., Indriwati, E. S., & Suhadi. (2018). Aktivitas Antibakteri Ekstrak Metanol Buah *Syzygium polyanthum* terhadap Pertumbuhan Bakteri *Escherchia coli*. Bioeksperimen, *4*(1):64-68.

Mulyanto, A., Mujahid, I, & Khasanah, U. T. (2018). Potentiality of Young Coconut Water as Antimicrobial Against *Escherichia coli* Bacteria Causing Diarrhea. Bio-Site, *4*(1):18 – 24.

Nasution, R., Azwar, I. A., Helwati, H., & Marianne. (2019). Kegiatan antibakteri Perfume: Kombinasi Bunga *Magnolia alba*, Cananga Kegiatan antibakteri Perfume: Kombinasi Bunga *Magnolia alba*, *Cananga odorata* dan *Mimusops elengi* L, Tetap dengan *Pogostemon cablin* Minyak. *Indonesia Jurnal Farmasi dan Clinical Research (IDJPCR)*, *2*(1):13-23.

Novaryatiin, S., Handayani, R., & Chairunnisa, R. (2018). Uji Daya Hambat Ekstrak Etanol Umbi Hati Tanah (*Angiotepris* Sp.) Terhadap Bakteri *Staphylococcus aureus. Jurnal Surya Medika, 3*(2):23-31.

Nursanty, R. (2017). Aktivitas Antibakteri Ekstrak Metanol Tanaman *Lawsonia inermis* L. dan *Capsicum frutescens* L. terhadap Bakteri *Staphylococcus aureus* ATCC 29213. *Bioleuser*, *1*(3):98-103.

Oroh, B. S., Kandou, F. E. F., Pelealu, J., & Pandiangan, D. (2015). Uji Daya Hambat Ekstrak Metanol *Selaginella delicatula* dan *Diplazium dilatatum* terhadap Bakteri

Staphylococcus aureus dan *Escherichia coli. Jurnal Ilmiah Sains*, *15*(1):53-58.

Parra, M., Beatriz, v., Sarita, S., & Modak. (2016). aktivitas antibakteri komponen kutikula dari *Heliotropium* spesies , terhadap *Staphylococcus aureus* dan *Salmonella typhimurium*. *Buletin Amerika Latin dan Karibia Tanaman Obat dan Aromatik*, *15*(6):422-428.

Pasaribu, P. S., Wahidatul, N., & Erwin. (2013). Toxicity Testa and Antibacterial Activity on Variuos Fraction of Plumeria acuminate Alt. Leaves Extract. *Jurnal Kimia Mulawarman*, *10*(2):94-99.

Puspita, A & Poerbantanoe, B. (2019). Galeri Bunga Cempaka di Kota Semarang. *Jurnal ediminasi arsitektur*, *7*(1):529-536.

Rahmayani, U., Pringgenies, D., & Djunaedi, A. (2013). Uji Aktivitas Antioksidan Ekstrak Kasar Keong Bakau (*Telescopium telescopium*) dengan Pelarut yang berbeda terhadap Metode DPPH (*Diphenyl Picril Hidrazil*). *Journal Of Merine Research*, *2*(4):36-45.

Septiani., Dewi, N. E., & Wijayanti, I. (2017). Antibacterial Activities of Seagrass Extracts (*Cymodocea rotundata*) Against *Staphylococcus aureus* and *Escherichia coli. Saintek Perikanan, 13*(1):1-6.

Silvia., Arreneuz, S., & Wibowo, A, M. (2015). Aktivitas Antimikroba Ekstrak Daun Soma (*Plolarium alternofolium Meich*) Terhadap Jamur *Malassezia furtur* dan Bakteri *Staphylococcus aureus. JKK*, *4*(3):84-93.

Sukewijaya, M. I., Mahendra, S., Rai, N.I., & Temaja, M. R. G. (2016). Karakter Morfologi dan Genetika Variabilitas Champaca di Bali. *Jurnal Internasional Biosains dan Biotechology*, *4*(1):77-230.

Trisia, A., Philyria, R., & Toemon, N. A. (2018). Antibacterial Activity Test of Ethanol Extract from Kalanduyung Leaf (*Guazuma ulmifolia Lam*) on *Staphylococcus aureus* Growth with Difussion Method (*Kirby-Bauer*). *Anterior Jurnal*, *7*(2):136 – 143.

Yuda, K. S. E. P., Cahyaningsih, E., & Winariyanthi, Y. P. N. (2017). Phytochemical Screening and Thin Layer Chromatographic Studies of *Euphorbia hirta* L. Extrac. *Medicamento*, *3*(2):61-70.