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Research Article

Total Phenolic and Flavonoid Contents of Bintangur (*Calophyllum inophyllum* L.) Leaf Extract and Their Antioxidant and Antibacterial Activities

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A B S T R A C T

Acne is commonly caused by *Propionibacterium acnes*, a bacterium that reaches the skin surface through sebum flow. Excessive use of synthetic antibacterial agents can lead to bacterial resistance, while the accumulation of free radicals on the skin may trigger inflammation, highlighting the need for effective natural antioxidants. Antioxidant and antibacterial activities in plants are often associated with their total phenolic and flavonoid contents. Therefore, this study investigated the bioactive potential of Bintangur (*Calophyllum inophyllum* L.) leaf extract, which is known to contain various secondary metabolites. The aim of this study was to determine the total phenolic and flavonoid contents of the leaf extract and evaluate its antioxidant and antibacterial activities against *P. acnes*. The extraction was carried out using 96% ethanol by the maceration method. Total phenolic content was determined using gallic acid as the standard, while total flavonoid content was measured using quercetin. The extract contained 132.85 mg GAE/g and 26.095 mg QE/g of total phenolics and flavonoids, respectively. Antioxidant activity exhibited a strong effect with an IC_{50} value of 19.3 µg/mL. Antibacterial activity showed inhibition zone diameters of 4.89 mm at 0.18% (weak category), 8.89 mm at 0.40% (moderate), and 8.90 mm at 0.47% (moderate). These findings indicate that Bintangur leaf extract possesses promising antioxidant properties and moderate antibacterial activity against *P. acnes*.

Keywords: *Calophyllum inophyllum; phenolic; flavonoid; antioxidant; antibacterial*

1. Introduction

Infection was a major health concern that continued to increase and could be caused by bacteria, viruses, fungi, and protozoa (Gibson, 1996). Among these, bacterial infections often resulted in common skin disorders. Acne vulgaris was one of the most prevalent conditions, especially among individuals entering adolescence and adulthood (Indarto et al., 2019). In Indonesia, the incidence of acne was reported to rise from 60% to nearly 90% between 2006 and 2008 (Afriyanti, 2015). Acne typically appeared with comedones, which formed when follicles were blocked by excess sebum and dead skin cells (Knutsen-Larson, 2012).

Propionibacterium acnes was identified as the primary bacterium responsible for acne. It reached the skin surface through sebum flow and converted unsaturated fatty acids into saturated fatty acids. Increased triglyceride production in sebum promoted the growth of *P. acnes*, which triggered inflammation (Bramono et al., 2015). Treatment of acne commonly involved reducing sebum formation and decreasing bacterial colonies, often using antibiotics such as tetracycline, erythromycin, and clindamycin (Hafsari et al., 2015). However, excessive use of antibacterial agents resulted in bacterial resistance (Roslizawaty, 2013), defined as the ability of bacteria to withstand and reduce the effectiveness of antimicrobial compounds (Depkes, 2011). Therefore, natural antibacterial agents were needed to help overcome resistance issues (Hafsari et al., 2015).

Excess sebum on the skin also contributed to inflammation caused by free radicals, making antioxidants necessary to address this problem (Sari et al., 2014). Free radicals were unstable molecules containing unpaired electrons. When present in high amounts, they oxidized nucleic acids, proteins, lipids, and DNA, leading to chronic conditions such as cancer, premature aging, cataracts, and other degenerative diseases (Ariavianti, 2016). Antioxidants were required to inhibit oxidation processes (Fajriah et al., 2012). Synthetic antioxidants were known to have adverse effects, so natural antioxidants from plant extracts were considered safer and more effective (Inggrid et al., 2014).

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Antioxidant activity was strongly associated with total phenolic and flavonoid contents, as these compounds were capable of scavenging free radicals. Higher phenolic and flavonoid levels generally resulted in stronger antioxidant effects (Nur et al., 2019). Previous studies showed that greater phenolic and flavonoid content also contributed to stronger antibacterial activity (Wahyuni et al., 2019). Therefore, natural antioxidants with high bioactive compound levels were required to provide safer alternatives for preventing oxidative damage and bacterial infections.

One plant species from the Clusiaceae family with notable bioactivity was *Calophyllum inophyllum* L., which had been reported to exhibit antibacterial and antioxidant properties (Violet, 2018). Phytochemical studies indicated that its leaves contained flavonoids, tannins, and steroids (Liska et al., 2021). Flavonoids, in particular, were known to possess antioxidant, antibacterial, antiviral, antitumor, and anti-inflammatory activities (Parubak, 2013). *Calophyllum inophyllum* L. was widely distributed in the Bangka Belitung region but had not been extensively utilized by the local community. It was a medium-sized plant (10–25 m) commonly found in coastal areas, lowlands, and sandy soils. Traditionally, *Calophyllum inophyllum* L. had been used to treat eye irritation, skin infections, wounds, and abscesses (Chrystomo et al., 2016). Based on previous findings regarding its secondary metabolites, further evaluation of its bioactive properties was considered necessary.

2. Methods

2.1 Materials

The materials used in this study were *Calophyllum inophyllum* L. leaves, 96% ethanol, gallic acid, Folin–Ciocalteu reagent (Merck), 7% sodium carbonate (Na_2CO_3), quercetin, 10% aluminum chloride ($AlCl_3$), 1 M sodium acetate (CH_3COONa), nutrient broth (NB, Merck), DPPH (1,1-diphenyl-2-picrylhydrazyl; Sigma Aldrich), methanol (Merck), agar powder (Swallow), distilled water, dimethyl sulfoxide (DMSO), amoxicillin, and *Propionibacterium acnes*.

2.2 Equipment

The equipment used included a Philips HR2115 blender, Pyrex and Iwaki beakers (50, 100, and 150 mL), Pyrex and Iwaki measuring cylinders (5, 10, 20, and 100 mL), dropper pipettes, Iwaki test tubes, Bunsen burner, tripod stand, inoculating loop, test tube racks (Pyrex and Iwaki), paper disks, petri dishes, volumetric pipettes (1 and 25 mL), glass stir rods, vortex mixer, Pioneer™ analytical balance, incubator (Memmert, Germany), digital electric autoclave (Hirayama), rotary evaporator (IKA), laminar flow cabinet, and a UV–Vis spectrophotometer (Shimadzu UV-1800).

2.3 Procedures

Preparation and Extraction of Calophyllum inophyllum L. Leaves

The *Calophyllum inophyllum* L. leaves were dried at room conditions for 5 days, pulverized using a blender, and sieved to obtain a fine powder. A total of 150 g of leaf powder was macerated with 750 mL of 96% ethanol (1:5 ratio) in a closed container for 48 hours at room temperature. The mixture was filtered, and the filtrate was concentrated using a rotary evaporator.

Determination of Total Phenolic Content

A 1000 ppm gallic acid stock solution was prepared and diluted to 20, 50, 100, and 150 ppm. A total of 10 mg of extract was dissolved in 10 mL of methanol p.a. For each concentration, 0.5 mL of sample was transferred into a test tube and mixed with 0.5 mL of Folin–Ciocalteu reagent and 2.5 mL of 7% Na_2CO_3. The mixture was vortexed and the absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Mukhriani et al., 2019).

Determination of Total Flavonoid Content

A 1000 ppm quercetin stock solution was prepared and diluted to 10, 20, 50, 80, and 150 ppm. A total of 10 mg of extract was dissolved in 10 mL of methanol p.a. For each concentration, 0.5 mL of sample was added to a test tube followed by 0.1 mL of 10% $AlCl_3$, 1.5 mL methanol p.a., 0.1 mL of 1 M CH_3COONa, and 2.8 mL distilled water. The mixture was vortexed for 30 seconds and incubated at room temperature for 30 minutes. Absorbance was measured at 420 nm using a UV–Vis spectrophotometer (Mukhriani et al., 2019).

Antioxidant Activity Assay (DPPH Method)

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A 100 ppm extract solution was prepared by dissolving 0.005 g of extract in 50 mL of methanol. This solution was further diluted to obtain concentrations of 2, 5, 10, 30, and 50 ppm. A total of 1 mL of each concentration was placed into a test tube, followed by the addition of 2 mL methanol and 1 mL DPPH solution (0.5 g in 50 mL methanol). The blank solution contained 3 mL methanol and 1 mL DPPH. All tubes were vortexed for 30 seconds, incubated for 30 minutes at 37°C, and the absorbance was measured at 515 nm (Mahardika et al., 2018).

Antibacterial Activity Assay

Media Preparation and Sterilization

Nutrient broth (1.6 g) and agar (3 g) were mixed with 200 mL distilled water and heated until completely dissolved. Petri dishes were wrapped in paper, placed in plastic bags, and sterilized in an autoclave at 121°C for 15 minutes (Putra, 2015).

Rejuvenation of Propionibacterium acnes

Sterile media were poured into petri dishes and allowed to solidify. *P. acnes* was streaked onto the surface using an inoculating loop and incubated for 24 hours at 37°C (Putra, 2015).

Antibacterial Inhibition Zone Test

Extract solutions were prepared at concentrations of 0.18%, 0.40%, and 0.47% and mixed with 1 mL DMSO. Paper disks were soaked in the extract and placed onto the inoculated agar surface. Plates were incubated for 24 hours at 37°C. The diameter of the inhibition zones was measured using a digital caliper. Amoxicillin served as the positive control, and DMSO as the negative control (Putra, 2015).

3. Results and Discussion

The cleaned leaves were dried in an open room without direct exposure to sunlight for 5 days to prevent ultraviolet rays from damaging the chemical constituents in the sample (Winagsih, 2013). The dried leaves were then ground and sieved to obtain a fine powder for extraction. The maceration method was used because it is easy to perform, requires simple equipment, and does not involve heating, thereby preventing the degradation or loss of active compounds in the sample (Sa'adah, 2015). The principle of the maceration method is the distribution of active compounds in the solvent based on their polarity. Ethanol 96% was used because it can dissolve almost all types of organic compounds, is polar, easily evaporates (Arifiyana, 2018), and is non-toxic (Munawarah et al., 2010). Ethanol is able to dissolve phenolic and flavonoid compounds because these compounds are polar and require a polar solvent for extraction (Ismail et al., 2014). The sample was then filtered to obtain the filtrate, which was concentrated using a rotary evaporator. The purpose of concentration was to evaporate the solvent present in the extract. The final product obtained was a thick, dark brown viscous extract.

3.1 Total Phenolic Test

The determination of total phenolic content aimed to identify the amount of phenolic compounds contained in the bintangur leaf extract. Phenolic compounds have potential antioxidant activity (Abbas, 2017) as well as antibacterial activity (Jannah, 2020). This test used the Folin-Ciocalteu method. The principle of the Folin–Ciocalteu method is that phenolate ions reduce phosphomolybdic–phosphotungstic acid complexes in the Folin-Ciocalteu reagent under alkaline conditions during the oxidation of phenols, producing a blue-colored molybdenum–tungsten complex. Phenolate ions are formed through proton dissociation under alkaline conditions provided by the alkali compound. The alkali used in this study was sodium carbonate (Na_2CO_3) (Haci et al., 2009).

The Folin-Ciocalteu method is widely used because the procedure is simple. Gallic acid functions as a standard because it is a natural and stable phenolic compound. Gallic acid reacted with Folin-Ciocalteu reagent produces a yellow solution, indicating the presence of phenolic compounds. The addition of Na_2CO_3 acts to create an alkaline environment, enabling the reduction reaction of the Folin-Ciocalteu reagent. During the reaction, phenolic compounds react with the Folin-Ciocalteu reagent to form a blue molybdenum–tungsten complex (Figure 1) (Tahir et al., 2017). The reaction involves the reduction of molybdenum ions (Mo^{6+}) to (Mo^{5+}), resulting in a color change from yellow to blue (Prior, 2005). The more intense the blue color produced, the higher the phenolic content present.

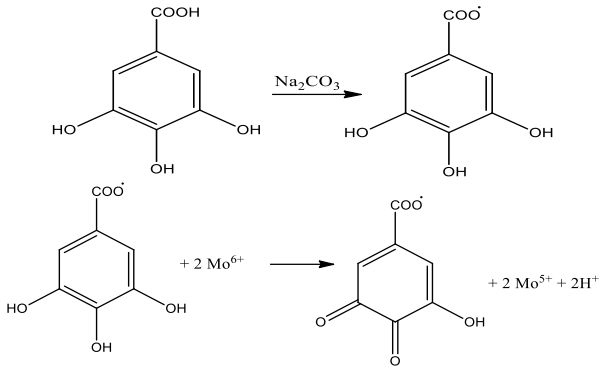


Figure 1. Reaction of gallic acid with the Folin–Ciocalteu reagent

The absorbance of the sample was measured using a UV–Vis spectrophotometer at a wavelength of 760 nm. Based on the results of this study, the total phenolic content of the *Calophyllum inophyllum* L. leaf extract was 132.85 mg GAE/g. This value was higher when compared to other species within the same genus, such as *Calophyllum pulcherrimum* (56.16 mg GAE/g), *Calophyllum soulattri* (100.66 mg GAE/g), and *Calophyllum teysmannii* (93.25 mg GAE/g). However, it was lower than the findings reported by Yohed (2017) for *Calophyllum inophyllum* L. extracted using methanol, which yielded 291.044 mg GAE/g extract. Thus, the *Calophyllum inophyllum* L. obtained using 96% ethanol exhibited higher phenolic content compared to other species within the same genus, but lower when compared to the same species extracted with a different solvent. The measurement results of the total phenolic content of *Calophyllum inophyllum* L. are presented in Table 1.

Table 1. Total phenolic content of *Calophyllum inophyllum* L leaf extract and related species and genera

Sample	Solvent	Total Phenolic (mg GAE/g extract)	Reference
C. inophyllum L.	Ethanol 96%	132.85	This study
C. inophyllum L.	Methanol	291.044	Yohed, 2017
C. pulcherrimum	Ethanol 96%	56.16	Septiana et al, 2018
C. soulattri	Ethanol 96%	100.66	Septiana et al, 2018
C. teysmannii	Ethanol 96%	93.25	Septiana et al, 2018

Differences in total phenolic content were influenced by the type of solvent used. Methanol has a higher dielectric constant than ethanol. The dielectric constant of methanol is 33.640, whereas ethanol has a dielectric constant of 25.16 (Mohsen et al., 2010). A higher dielectric constant indicates a more polar solvent; therefore, methanol is more polar than ethanol and is able to extract a greater amount of phenolic compounds compared to ethanol, in accordance with the principle of "like dissolves like" (Ramayani et al., 2021).

3.2 Total Flavonoid Assay

The determination of total flavonoid content aimed to measure the amount of flavonoids present in the bintangur leaf extract. The principle of this assay is based on a colorimetric method using $AlCl_3$, which forms an aluminum–flavonoid complex that produces a yellow color that can be measured with a UV–Vis spectrophotometer (Kusuma et al., 2010). This study used quercetin as the standard solution because quercetin is a flavonoid compound commonly found in almost all plant species (Kaur et al., 2001). The addition of $AlCl_3$ functioned to allow the reaction between $AlCl_3$ and flavonoid groups, forming a complex compound (Sari, 2017). Sodium acetate (CH_3COONa) was added to enhance the shift in absorbance intensity, producing a stronger peak (Kusuma et al., 2010). The incubation step prior to measurement ensured that the reaction proceeded completely, resulting in maximum color intensity (Azizah et al., 2014). The tested samples produced a yellow color, indicating the presence of flavonoid compounds due to the formation of a flavonoid–$AlCl_3$ complex (Figure 2) (Harborne, 1987).

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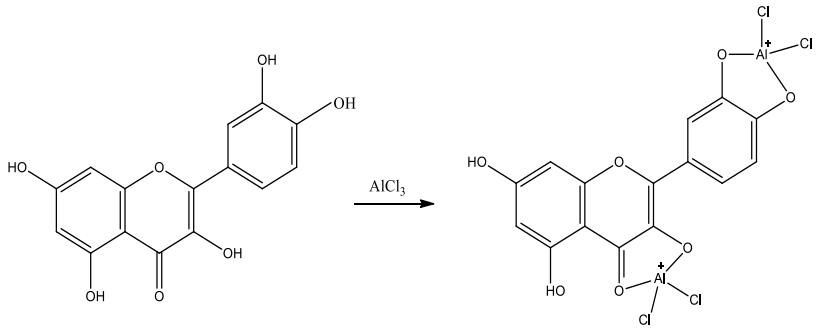


Figure 2. Reaction of flavonoids with $AlCl_3$.

The absorbance of the samples was measured using a UV–Vis spectrophotometer at a wavelength of 420 nm. Based on the results of this study, the total flavonoid content of the bintangur leaf extract (Calophyllum inophyllum L.) was 26.095 mg QE/g extract. The total flavonoid content obtained was expressed in mg QE (Quercetin Equivalent) per gram of extract. The measured total flavonoid content was higher than that reported for *Calophyllum pulcherrimum* (9.31 mg QE/g), *Calophyllum soulattri* (8.06 mg QE/g), and *Calophyllum teysmannii* (6.56 mg QE/g) (Septiana et al., 2018). However, the value was lower than that reported for *Calophyllum inophyllum* L. extracted using methanol in the study by Yohed (2017), which was 1289 mg QE/g. Thus, the flavonoid content of *Calophyllum inophyllum* L. extracted with 96% ethanol was higher compared to other species within the same genus, but lower than that of the same species extracted using methanol.

Differences in total flavonoid content were influenced by the type of solvent used. Methanol has a higher dielectric constant than ethanol. The dielectric constant of methanol is 33.640, whereas ethanol has a dielectric constant of 25.16 (Mohsen-Nia et al., 2010). A higher dielectric constant indicates greater solvent polarity, enabling methanol to extract more flavonoid compounds than ethanol, in accordance with the principle of "like dissolves like" (Ramayani et al., 2021). The results of the total flavonoid analysis of *Calophyllum inophyllum* L. are presented in Table 2.

Table 2. Total flavonoid content of *Calophyllum inophyllum* L leaf extract and related species and genera

Sample	Solvent	Total Flavonoid (mg QE/g extract)	Reference
C. inophyllum L.	Ethanol 96%	26.095	This study
C. inophyllum L.	Methanol	1289	Yohed, 2017
C. pulcherrimum	Ethanol 96%	9.31	Septiana et al, 2018
C. soulattri	Ethanol 96%	8.06	Septiana et al., 2018
C. teysmannii	Ethanol 96%	6.56	Septiana et al, 2018

The differences in total flavonoid content were also influenced by the various types of flavonoid compounds present in plants, as these compounds generally differ in their physical, chemical, and physiological properties (Kumar et al., 2013). However, the polarity of flavonoid compounds in *C. pulcherrimum*, *C. soulattri*, and *C. teysmannii* remains unknown due to the lack of supporting data. Flavonoids are part of the phenolic compound group; therefore, when analyzing polyphenolic content, the results of the total phenolic test were higher than those of the total flavonoid test (Yohed, 2017).

3.3 Antioxidant Assay

The antioxidant activity test was performed using the DPPH method. DPPH acted as a free radical that reacted with antioxidant compounds. The solution turned purple due to the presence of unpaired electrons. It was then incubated for 30 minutes at 37 °C to optimize the interaction between antioxidants and free radicals, enabling the antioxidants to capture the unpaired electrons (Chotimah, 2019). The antioxidant activity was subsequently measured using a UV–Vis spectrophotometer at a wavelength of 515 nm. The mechanism of DPPH action involved the transfer of a hydrogen atom from antioxidant compounds to the free radical. The

DPPH radical accepted the hydrogen atom, allowing it to pair its unpaired electron, and was reduced from 1,1-diphenyl-2-picrylhydrazyl to 1,1-diphenyl-2-picrylhydrazine (DPPH) (Antolovich et al., 2001). The reaction mechanism between DPPH radicals and antioxidant compounds, according to Eggiwanto (2021) in Nurohma (2021), is shown figure 3.

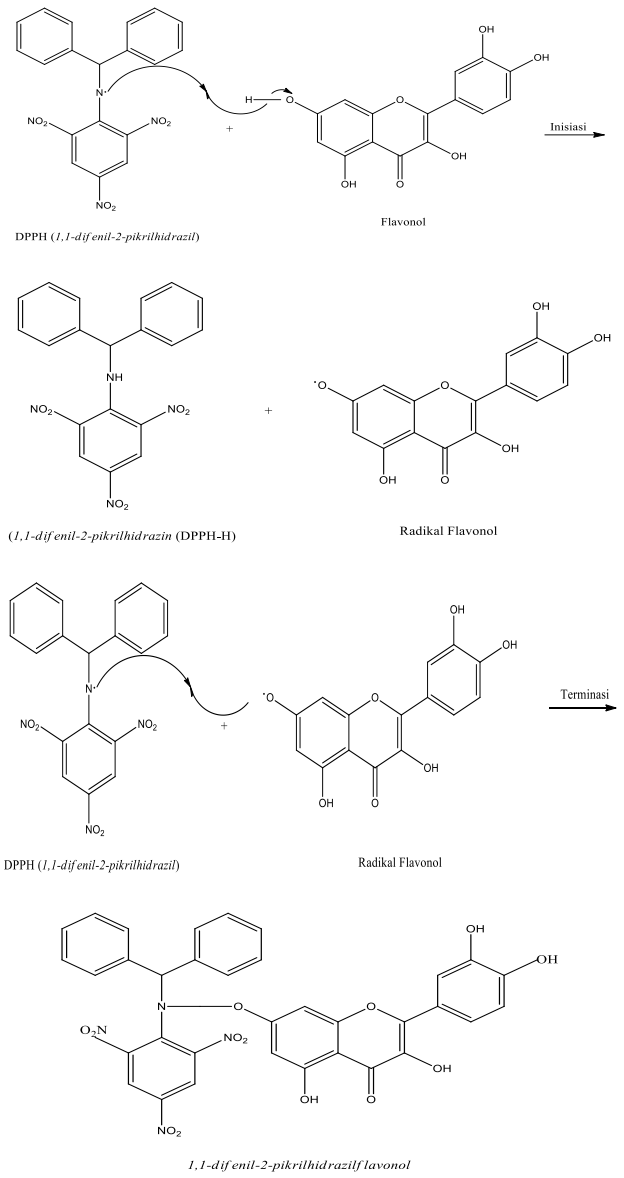


Figure 3. Mechanism of DPPH with antioxidant compounds according to Eggiwanto (2021) in Nurohma (2021)

The reaction between DPPH radicals and antioxidant compounds proceeded through two stages, namely initiation and termination. In the initiation stage, the radical compound accepted a hydrogen atom from the OH group of the flavonol compound, forming a flavonol radical and the stable DPPH-H (1,1-diphenyl-2-picrylhydrazine). In the subsequent termination stage, the oxygen radical of the flavonol formed a bond with the DPPH radical, producing 1,1-diphenyl-2-picrylhydrazyl-flavonol (Nurohma, 2021). The pairing of free electrons in the DPPH radical caused the sample color to change from purple to yellow, indicating that the free radical characteristics of DPPH decreased or were eliminated (Wijayanti, 2016). The parameter used to determine antioxidant activity was the IC_{50} value. IC_{50} referred to the concentration of an antioxidant compound required to reduce DPPH radicals by 50%, obtained from the linear regression equation describing the relationship between the extract concentration of *Calophyllum inophyllum* leaves and the % inhibition (Kate, 2014).

Based on the calculations, the IC_{50} value of the *Calophyllum inophyllum* leaf extract was 19.3 µg/mL, which was categorized as very strong. This IC_{50} value was lower than that of *Garcinia dulcis* (Roxb.) Kurz from

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the same family, which had an IC_{50} of 47.45 µg/mL. According to Yohed (2017), the IC_{50} value was higher than that of *Calophyllum inophyllum* L. from the same species, which exhibited an IC50 of 14.16 µg/mL. Thus, the IC_{50} value of the *Calophyllum inophyllum* leaf extract was higher compared to other species within the same family but lower within the same species. Antioxidant activity increased as the IC_{50} value decreased. This antioxidant capacity was attributed to the presence of secondary metabolites contained in the *Calophyllum inophyllum* leaf extract. Antioxidant activity was closely related to the total phenolic and flavonoid content in plants, which act as radical scavengers (Nur et al., 2019). The antioxidant assay results of *Calophyllum inophyllum* L. are presented in Table 3.

Table 3. Antioxidant activity of *Calophyllum inophyllum* L. leaf extract and comparison with one species and one family

Sample	Solvent	IC_{50} (µg/mL)	Reference
C. inophyllum L.	Ethanol 96%	19.3	Penelitian ini
C. inophyllum L.	Methanol	14.16	Yohed, 2017
G. dulcis (Roxb.)	Ethanol 96%	47.45	Widodo et al, 2021

Based on the study by Yohed (2017), which reported a total phenolic content of 291.044 mg GAE/g and a total flavonoid content of 1289 mg QE/g, the IC_{50} value obtained for *C. inophyllum* L. was 14.16 µg/mL. This indicated that the relationship between total phenolic and flavonoid contents and antioxidant activity followed the principle that higher phenolic and flavonoid levels resulted in stronger antioxidant activity. In the present measurement of *Calophyllum inophyllum* leaf extract, the total phenolic content was 132.85 mg GAE/g and the total flavonoid content was 26.095 mg QE/g, with an IC_{50} value of 19.3 µg/mL. The mechanism of phenolic compounds as antioxidants involved the ability of phenolic groups to scavenge free radicals by donating hydrogen atoms through an electron transfer process, forming phenoxyl radicals (Janeiro et al., 2004). The phenoxyl radicals produced as a result of phenol–radical interactions subsequently stabilized themselves through resonance. Therefore, phenolic derivatives, as effective hydrogen donors, were capable of inhibiting radical-induced reactions, and thus phenols were referred to as radical inhibitors (Togo, 2004). The antioxidant mechanism of flavonoid compounds occurred through radical scavenging via electron transfer from hydrogen atoms, which indirectly neutralized the toxic effects of free radicals (Akhlaghi et al., 2009).

3.4 Antibacterial Assay

The antibacterial activity test was conducted to determine the ability of *Calophyllum inophyllum* leaf extract to inhibit the growth of *Propionibacterium acnes*. *P. acnes* is a bacterium commonly responsible for acne development (Bramono et al., 2015). Antibacterial agents are substances capable of inhibiting or even killing infection-causing bacteria. The assay was performed using the disc diffusion method. Sterilization of equipment and materials was carried out prior to the antibacterial test to prevent contamination by other microorganisms. This was followed by the rejuvenation of *P. acnes* cultures to obtain young, uncontaminated bacterial cells (Narulita, 2018). The clear zones formed around the paper discs were measured using a caliper. The inhibition zones indicated antibacterial activity of the *Calophyllum inophyllum* leaf extract. The diameter of the inhibition zone was calculated by subtracting the disc diameter from the total clear zone diameter. The disc diffusion method also had limitations, as the inhibition zone could be affected by incubation conditions, media thickness, inoculum density, prediffusion, and preincubation (Oktovia, 2017). The inhibition zone measurements against *P. acnes* are presented in Table 4.

Table 4. Antibacterial activity of *Calophyllum inophyllum* L. leaf extract

Concentration (%)	Inhibition Zone (mm)	Category
0.18	4.89	Weak
0.40	8.89	Moderate
0.47	8.90	Moderate
Positive control	13.37	Strong
Negative control	0	No Inhibition

Based on Table 4, at a concentration of 0.18%, an inhibition zone of 4.89 mm was obtained, which was categorized as weak; at a concentration of 0.40%, an inhibition zone of 8.89 mm was observed, categorized as moderate; and at a concentration of 0.47%, the inhibition zone measured 8.90 mm, also categorized as

moderate. These results indicated that concentration influenced the antibacterial activity, as higher concentrations of *Calophyllum inophyllum* leaf extract produced larger inhibition zones against *P. acnes*. The ability of the extract to inhibit the growth of *P. acnes* was attributed to its phenolic and flavonoid contents (Fadhilah, 2015). According to the study by Wahyuni et al. (2019), an extract of *M. paradisiaca* L. containing 150 mg GAE/g of total phenolics and 60 mg QE/g of total flavonoids exhibited antibacterial activity against *E. coli*, producing an inhibition zone of 14.75 mm at a concentration of 8.0%. The antibacterial activity of *C. inophyllum* leaf extract against *P. acnes* is presented in Figure 4.

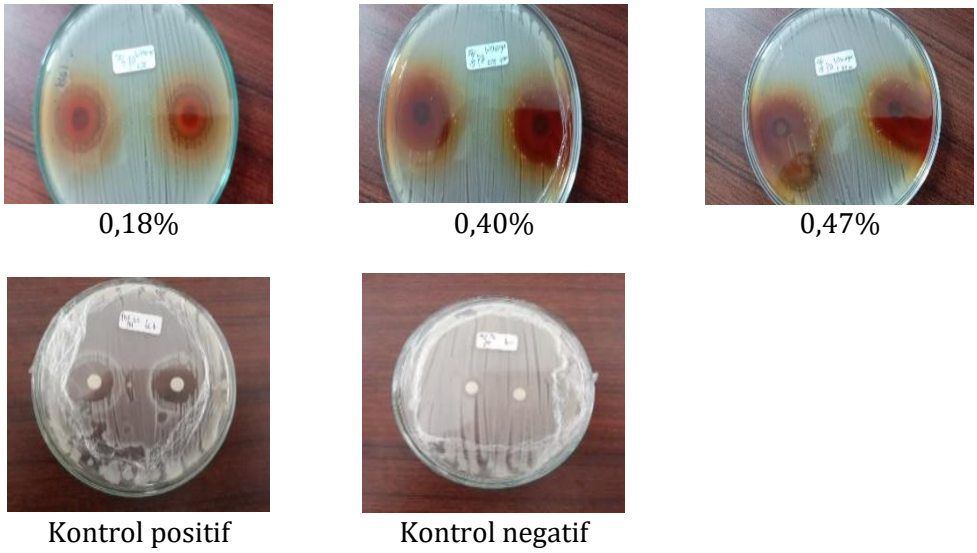


Figure 4. Antibacterial activity results against *Propionibacterium acnes*

Phenolic compounds acted by damaging the bacterial cell wall and disrupting bacterial enzymes (Narulita, 2018). Flavonoids functioned as antibacterial agents through several mechanisms, including the inhibition of nucleic acid synthesis, interference with bacterial energy metabolism, and disruption of cytoplasmic membrane function (Manik et al., 2014). The inhibition zone diameter obtained at the highest concentration in this study was greater than that reported by Hasibuan et al. (2013), in which *C. inophyllum* seed oil extract produced an inhibition zone of 7 mm against *S. aureus* and exhibited no inhibitory activity against *E. coli*. According to Husni et al. (2020), the extract of *C. soulattri* Burm. F demonstrated inhibition zones of 6.23 mm against *S. aureus*, 6.44 mm against *P. aeruginosa*, and 8.61 mm against *E. coli*, all of which fell into the moderate category. Another study within the same family reported that the peel extract of *G. mangostana* L. produced an inhibition zone of 6.70 mm against *S. sanguinis*. Therefore, the inhibition zones obtained from *C. inophyllum* L. leaf extract in this study-despite differences in solvents and bacterial strains used-were greater compared to those reported in related species, genera, and families.

Amoxicillin functioned as the positive control, while DMSO (dimethyl sulfoxide) served as the negative control in this study. Amoxicillin was used as a reference to confirm that the method employed was appropriate according to the literature, as indicated by the formation of an inhibition zone (Fitriana, 2018). The inhibition zone diameter obtained for the positive control was 13.37 mm, which fell into the strong category. The negative control produced an inhibition zone diameter of 0 mm, categorized as weak. The value of 0 mm occurred because DMSO was used solely as a solvent and possessed no antibacterial activity; therefore, it had no effect on the antibacterial activity of the sample. DMSO is capable of dissolving a wide range of compounds, both polar and non-polar (Assidqi et al., 2012). At high concentrations, DMSO may cause alterations in membrane fluidity, changes in microtubules, metal complex formation, induction of cell differentiation, and reduced collagen mRNA expression. The exact mechanism of DMSO cytotoxicity has not yet been fully elucidated (Zeng et al., 2010).

3.5 FTIR Analysis

Identification using FTIR spectroscopy was performed to determine the functional groups present in the *Calophyllum inophyllum* L. leaf extract. The FTIR analysis produced characteristic absorption peaks expressed as wavenumbers. Based on the FTIR spectra of the *Calophyllum inophyllum* L. leaf extract, a broad peak was observed at 3386 cm^{-1}, indicating the presence of an O–H alcohol functional group. An aromatic C–

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H functional group appeared at 2964 cm^{-1}. The aliphatic C–H stretching was shown by the absorption band at 2930 cm^{-1}. An aliphatic C=C functional group was detected at the wavenumber 1610 cm^{-1}, while an aromatic C=C group was found at 1521 cm^{-1}. The C–O functional group was identified at 1105 cm^{-1}. These functional groups supported the identification of secondary metabolites present in the extract (Sastrohamidjojo, 1991; Lambert et al., 1976; Iskandari, 2010). The *Calophyllum inophyllum* L. leaf extract was positively identified to contain flavonoids, steroids, and tannins (Liska et al., 2021). Based on the study by Riwanti et al. (2019), the presence of flavonoids, steroids, and tannins was suggested by the absorption band at 1521 cm^{-1}, which corresponds to an aromatic C=C functional group (Figure 5).

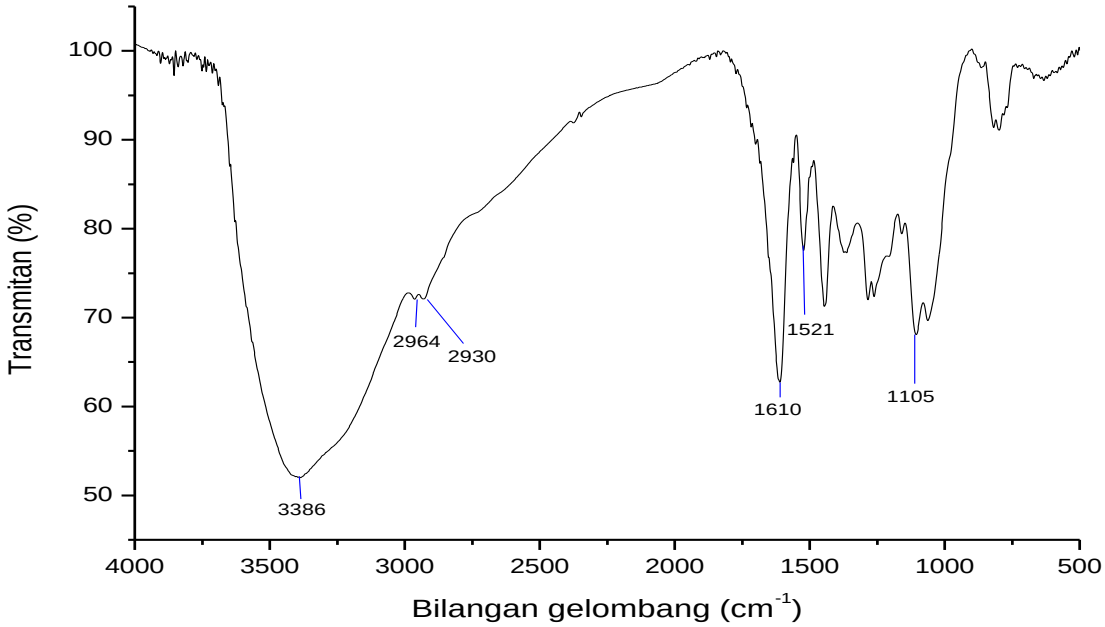


Figure 5. FTIR spectrum of *Calophyllum inophyllum* L. leaf extract

4. Conclusion

The total phenolic and flavonoid contents of the *Calophyllum inophyllum* L. leaf extract were 132.85 mg GAE/g extract and 26.095 mg QE/g extract, respectively. The antioxidant activity of the extract yielded an IC_{50} value of 19.3 µg/mL, which was classified as very strong. The antibacterial activity of the *Calophyllum inophyllum* L. leaf extract against *Propionibacterium acnes* showed inhibition zone diameters of 4.89 mm at a concentration of 0.18% (weak category), 8.89 mm at 0.40% (moderate category), and 8.90 mm at 0.47% (moderate category).

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References

Afriyanti, R. N. 2015. Akne Vulgaris pada Remaja. *J Majority*, 4(6): 102-109.

Akhlaghi, M., Bandy, B. 2009. Review Article : Mechanisms of Flavonoid Protection Against Myocardinal Ischemia-Reperfusion Injury. *Journal Molecullar and Cellular Cardiology*, 46(3): 309-317.

Antolovich, M., Prenzler, P. D., Patsalides, E., Mcdonald, S., & Robards, K. methods for Testing Antioxidant Activity. *Journal Homepage*, 127: 183-198.

Ariavianti, E. 2016. Uji Aktivitas Antibakteri dan Antioksidan dari Ekstrak Metanol, Fraksi N heksan dan Fraksi Etil Asetat Daun Vitex pubescens*. Skrpsi*. Universitas Jakarta, Jakarta.

Arifiyana, D & Dipahayu, D. 2018. Pengaruh Metode Pengeringan Terhadap Kadar Flavonoid Total Ekstrak Daun Ubi Jalar Ungu (*Ipomoea batatas* (L.) Lamk) Varietas Antin 3. *Prosiding SEMHAS PPM 2018*, 1(1): 583-591.

Assidqi, K., Tjahjaningsih, W & Sigit, S. 2012. Potensi Ekstrak Daun Patikan Kebo*(Euphorbia hirta)* Sebagai Antibakteri Terhadap *Aeromonas hydrophila* Secara In Vitro. *Journal of Marine and Coastal Science*, 1(2): 113-124.

Azizah, D. N., Endang, K., & Faramayuda, F. 2014. Penetapan Kadar Flavonoid Metode $AlCl_3$ pada Ekstrak Metanol Kulit Kakao (*Theobroma cacao* L.). *jurnal Ilmiah Farmasi*, 2(2): 45-49.

Bramono, S. L. S. M. K., & Indriatmi, W. 2015. *Ilmu Penyakit Kulit dan Kelamin*. Edisi ke-7. Jakarta : Fakultas Kedokteran Universitas Indonesia.

Chotimah, C. 2019. Uji Total Flavonoid dan Aktivitas Antioksidan Ekstrak Daun dan Kulit Batang Dadap Serep (*Erythrina subumbrans* (Hassk.) Merr.) Menggunakan Pelarut yang Bebeda. *Skripsi*. Universitas Islam Negeri Maulana Malik Ibrahim, Malang.

Chrystomo, L. Y., Karim, A. K., Antari, N. N., Dwa, S., Wona, Y., dan Pongtiku, A. 2016. *Tumbuhan Obat Tradisional Papua Berdasarkan Kearifan Lokal Masyarakat*. Edisi ke-1. Nulis Buku Jendela Dunia : Papua.

Eggiwanto, S. 2021. Penentuan Kadar Total Fenolik dan Flavonoid Fraksi Batang PucuK Idat (*Cratoxylum glaucum*) Sebagai Antioksidan dan Antidiabetes. *Skripsi*. Universitas Bangka Belitung, Bangka.

Fadhilah, L. N. 2015. Pengaruh Antibakteri Ekstrak Etanol Kulit Manggis (*Garcinia mangostana* Linn) Terhadap Zona Hambatan Bakteri *Streptococcus sanguinis* Dominan *Gingivitis* (Kajian In Vitro). *Skripsi.* Unversitas Muhammadiyah Surakarta, Surakarta.

Fajriah, S., Darmawan, A., Sundowo, A., & Artanti, N. 2012. Isolasi Senyawa Antioksidan dari Ekstrak Etil Asetat Daun Benalu (*Dendrophthoe pentandra* L. Miq) yang Tumbuh pada Inang Lobi-lobi. *Jurnal Kimia Indonesia*, 2(1): 17-20.

Fitriana, G. A. V. 2018. Uji Efek Kombinasi Antibiotik Amoksisilin Dengan Ekstrak Metanol Daun Sirih (*Piper betle* L.) Terdapat Pertumbuhan *Staphylococcus aureus*. *Skripsi.*Univesitas Sanata Dharma, Yogyakarta.

Gibson, James, L. 1996. *Organisasi : Perilaku, Struktur dan Proses*. Edisi ke-8. Binarupa Aksara : Jakarta.

Haci, I. A., Didi, A., Bekkara, F. A., & Gherib, M. 2009. In Vitro Antioxidant Activity and Total Phenol Contents in Methanol Crude Extracts from The Algerian Medicinal Plant Limoniastrum Feei. *Sci Study Res*, 10(4): 329-336.

Hafsari, A. R., Cahyanto, T., Sujawo, T., & Lestari, R. I. 2015. Uji Aktivitas Antibakteri Ekstrak Daun Beluntas (*Pluchea indica* (L) Less.) Terhadap *Propionibacterium acnes* Penyebab Jerawat. *Research Gate*, 9(1): 141-161.

Hasibuan, S., Sahirman, S., & Yudawati, N. M. A. 2013. Karakteristik Fisikokimia dan Antibakteri Hasil Purifikasi Minyak Biji Nyamplung (*Calophyllum inophyllum* L). *Agritech,* 33(3): 311-319.

Husni, E., Dachriyanus., & Saputri, V. W. 2020. Penentuan Kadar Fenolat Total Uji Aktivitas Antioksidan dan Antibakteri dari Ekstrak dan Fraksi Kulit Batang Bintangor (*Calophyllum soulattri* Burm. F). *Jurnal Sains Farmasi dan Klinis*, 7(1): 92-98.

Indarto, I., Narulita, W., Anggoro, B. S., & Novitasari, A. 2019. Aktvitas Antibakteri Ekstrak Daun Binahong Terhadap *Propionibacterium acnes*. *Biosfer : Jurnal Tadris Biologi*, 10(1): 67- 78.

Inggrid, H. M., & Santoso, H. 2014. Ekstraksi Antioksidan dan Senyawa Aktif dari Buah Kiwi (*Actinidia deliciosa*). *Research Report Engeneering Science*, 2.

Iskandari, A. 2010. Isolasi dan Elusidasi Struktur Kuersetin dari Daun Nyamplung (*Calophyllum inophyllum* L.). *Skripsi*. Universitas Sebelas Maret, Surakarta.

Ismail, I., Handayany, G. N., Wahyuni, D & Juliandri. 2014. Formulasi dan Penentuan Nilai SPF *(Sun Protecting Factor)* Sedian Krim Tabir Surya Ekstrak Etanol Daun Kemangi *(Ocimum sanctum* L.). *Jurnal Farmasi UIN Alauddin Makassar*, 2(1): 6-11.

Janeiro., Patricia., Brett, O., & Maria, A. 2004. Catechin Electrochemical Oxidation Mechanisms. *Analytica Chimica Acta*, 1(1): 109-115.

Jannah, M. 2020. Uji Aktivitas Antibakteri Fraksi Eti Asetat dan Petroleum Eter Hasil Hidrolisis Ekstrak Matanol *Hydrilla verticillata* Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Skripsi*. Universitas Islam Negeri Maulana Malik Ibrahim, Malang.

Kaur, C & Kapoor, H. C. 2001. Antioxidants in Fruits and Vegetables The Millennium's Health. *International Journal of Food Science & Technology*, 36(7): 703-725.

Kate, D. I. 2014. Penetapan Kandungan Fenolik Total dan Uji Aktivitas Antioksidan Dengan Metode DPPH (*1,1-Diphenyl-2-Pikrilhydrazil)* Ekstrak Metanolik Umbi Bidara Upas (*Merremia mammosa* (Lour) Hallier f.). *Skripsi.* Universitas Sanata Dharma, Yogyakarta.

Khan, Z. Z., Assi, M. & Moore, T. A. 2009. Recurrent Epidural Abscess Caused by *Propionibacterium acnes. Kansas Journal of Medicane*, 2(4): 92-95.

Knutsen-Larson., Dawson, A. l., Dunnick, C. A., & Dellavalle, R. P. 2012. Acne Vulgaris : Pathogenesis, Treatment and Needs Assessment. *Dermatologic Clinics,* 30(1): 99-106.

Kumar, S., & Pandey, A. K. 2013. Chemistry and Biological Activities of Flavonoids : an Overview. *The Scientific World Journal*, 2013: 1-16.

 https://doi.org/10.68712/15db4k45

Kusuma, A. T., Adela, A., Abidin, Z., Najib, A. 2010. Penentuan Kadar Flavonoid Ekstrak Etil Asetat Daun Sukun (*Artocarpus artilis*). *Jurnal ad-Dawa'*, 1(1): 25-31.

Lambert, J. B., Shurvel, H. F., Verbit, L., Cooks, R. G., Stout, G.H. 1976. Organic Structural Analysis. *Mac Milan*, 55(5) 249.

Liska, Novianti, S., & Amanah, H. 2021. *Skrining Metabolit Sekunder Ekstrak Daun Bintangur* (*Calophyllum inophyllum L.*). Prosiding Seminar Nasional Penelitian dan Pengabdian Kepada Masyarakat Fakultas Teknik Universitas Bangka Belitung, 29-30 September 2021, 93-95.

Mahardika, R. G & Roanisca, O. 2018. Aktivitas Antioksidan dan Fitokimia dari Ekstrak Etil Asetat Pucuk Idat (*Cratoxylum glaucum*). *Jurnal Penelitian Kimia Indonesia*, 5(2): 69-74.

Manik, D. F & Hertiani, T. 2014. Analisis Korelasi Antara Kadar Flavonoid dengan Aktivitas Antibakteri Ekstrak Etanol dan Fraksi-fraksi Daun Kersen (*Muntingia calabura* L.) Terhadap *Staphylococcus aureus. Khanzanah : Jurnal Mahasiswa*. 6(2): 1-12.

Mohsen-Nia, M., Amiri, H., & Jazi, B. 2010. Dielectric Constants of Water, Methanol, Ethanol, Butanol and Acetone : Measurement and Computational Study. *Journal of Solution Chemistry*, 39(5): 701-708.

Mukhriani, M., Arsul, M. I., Sugiarna, R., & Farhan, N. 2019. Kadar Fenolik dan Flavonoid Total Ekstrak Etanol Daun Anggur (*Vitis vinifera* L.). *ad-Dawaa' Journal of Pharmaceutical Dciences*, 2(2): 95-102.

Narulita, W. 2018. Uji Efektivitas Ekstrak Daun Binahong (*Anredera cordifolia*) Dalam Menghambat Pertumbuhan Bakteri *Propionibacterium acnes* Secara In Vitro. *Skripsi.* Universitas Islam Negeri Raden Intan, Lampung.

Nur, S., Sami, FJ, Awaluddin, A., & Afsari, Mia. 2019. Korelasi Antara Kadar Total Flavonoid dan Fenolik dari Ekstrak dan Fraksi Daun Jati Putih (*Gmelina arborea* Roxb.) Terhadap Aktivitas Antioksidan. *Jurnal Farmasi Galenika* (*Galenika Journal of Pharmacy*) (*e Journal*), 5(1): 33-42.

Nurohma, A. 2021. Kajian Fitokimia Ekstrak Etanol Daun Medang Sang (*Phoebe excelsa* Ness) Serta Bioaktivitasnya Sebagai Antioksidan dan Antifungi. *Doctoral Dissertation*. Universitas Bangka Belitung, Balunijuk.

Parubak, A. S. 2013. Senyawa Flavonoid yang Bersifat Antibakteri dari Akway (*Drimys becariana* Gibbs). *Chemistry Progress,* 6(1): 34-37.

Prior, R. L., Wu, x., & Schaich, K. 2005. Standarized Methods for Determination of Antioxidants Capacity and Phenolics in Foods and Dietary Suplements. *Journal of Agricultural Food Chemistry*, 52(10): 4290-4303.

Putra, I. M. A. S. 2015. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (*Annonae mucricata* L.) Dengan Metode Difusi Cakram Terhadap *Escherichia coli. Jurnal Ilmiah Medicamento*, 1(1): 15-19.

Ramayani, S. L., Octaviana, R. W., dan Asokawati, S. S. 2021. Pengaruh Perbedaan Pelarut Terhadap Kadar Total Fenolik dan Kadar Total Flavonoid Ekstrak Daun Kitolod (*Isotoma longiflora* (L.). *Jurnal Akademik Farmasi Prayoga*, 6(2): 1-10.

Riwanti, P & Izazih, F. 2019. Skrining Fitokimia Ekstrak Etanol 96% *Sargassum polycystum* Profile Dengan Spektrofotometri Infrared. *Acta Holistika Pharmaciana*, 1(2): 34-41.

Roslizawaty, Ramadhani, N. Y., Fakhrurrazi, & Herrialfian. 2013. Aktivitas Antibakterial Ekstrak Etanol dan Rebusan Sarang Semut (*Myrmecodia* sp.) Terhadap *Bakteri Escherichia coli. Jurnal Medika Veterinaria*, 7(2): 91-94.

Sari, N. R., & Setyowati, E. 2014. Pengaruh Masker Jagung dan Minyak Zaitun Trehadap Perawatan Kulit Wajah. *Beauty and Beauty Health Education*, 3(1): 1-7.

Sari, K.A. 2017. Penetapan Kadar Fenolik Total dan Flavonoid Total Ekstrak Beras Hitam (*Oryza sativa* L.) dari Kalimantan Selatan. *Jurnal Ilmiah Ibnu Sina*, 2(2): 327-335.

Sastrohamidjojo, H. 1991. *Spektroskopi*. Edisi ke-2. Liberty : Yogyakarta.

Sa'adah, H. H. Nurhasanawati. 2015. Perbandingan Pelarut Etanol dan Air Pada Pembuatan Ekstrak Umbi Bawang Tiwai (*Eleutherine smericana* Merr.) Menggunakan Metode Maserasi. *Jurnal Manuntung*, 1(2).

Septiana, E & Simanjuntak, P. 2018. Aktivitas Antioksidan Ekstrak Etanol Kulit Batang *Calophyllum pulcherrimum, C. soulattri, C. teysmanii*. *Buletin Penelitian Tanaman Rempah dan Obat*, 29(2): 59-68.

Syahputra, E., & Prijono, D. 2007. Sediaan Insektisida *Calophyllum soulattri* : Aktivitas Insektisida dan Residu Terhadap Larva *Crocidolomia pavonana* dan Keamanan pada Tanaman. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, 7(1): 21-29.

Tahir, M., Mauflihunna, A., & Syafrianti, S. 2017. Penentuan Kadar Fenolik Total Ekstrak Etanol Daun Nilam (*Pogostemon cablin* Benth) Dengan Metode Spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia*, 4(1): 215-218.

Togo, H. 2004. *Advanced Free Radical Reactions for Organic Synthesis*. Edisi ke-1. Elsevier : Belanda.

Violet, V. 2018. Identifikasi Pemanfaatan Tradisional dan Penapisan Senyawa Fitokimia Ekstrak Daun Bintangur (*Calophyllum soulattri* Burn F.). *EnviroScienteae* 14(1): 70-76.

Wahyuni, N. K. D. M. S., Rita, W. S., &Asih, I. A. R. A. 2019. Aktivitas Antibakteri Ekstrak Kulit Pisang Kepoh Kuning (*Musa paradisiaca* L.) *Escherichia coli* Serta Penentuan Total Flavonoid dan Fenol Dalam Fraksi Aktif. *Jurnal Kimia*, 13(1): 9-15.

Widodo, S., Yusa, A. M., &Ina, P. T. 2021. Pengaruh Waktu Maserasi Terhadap Aktivitas Antioksidan Ekstrak Daun Mundu (*Garcinia dulcis* (Roxb.) Kurz). *Jurnal Ilmu dan Teknologi Pangan*, 10(1): 14-23.

Wijayanti, D., Setiatin, E. T., & Kurnianto, E. 2016. Efek Ekstrak Daun Binahong (*Anredera cordifolia* (Ten) Steenis) Terhadap Profil Darah Merah pada Marmut (*Carvia cobaya*). *Jurnal Sain Veteriner*, 34(1): 75-83.

Yohed, I., & Kristianita, R. A. 2017. Pengaruh Jenis Pelarut dan Temperatur Terhadap Total Phenolic Conten, Total Flavonoid Content, dan Aktivitas Antioksidan di Ekstrak Daun Nyamplung (*Calophyllum inophyllum*). *Dissertation*. Institute Teknologi Sepuluh Nopember, Surabaya.

Zeng, X., Zhao, C., Wang, H., Li, S., Deng, Y., & Li, Z. 2010. Dimethyl Sulfoxide Decrease Type-I and Type-III Cpllagen Synthesis in Human Hepatic Stellate Cells and Human Foreskin Fibroblasts. *Advanced Science Letters*, 3(4): 496-499

 https://doi.org/10.68712/15db4k45