



Test of Invitro and Invivo Ethanol Extract of Corillars (*Coriandrum Sativum, L*) as Antibacterials

¹Uly Chairunisa*, ²Dwi Dini Aulia B, ³Rina Desni Yetti, ⁴Aried Eriadi

^{1,2,3,4}Pharmacy, School of Pharmacy (STIFARM), Padang, Indonesia

*E-mail:ulychairunisa1234@gmail.com

ABSTRACT

Coriander (*Coriandrum sativum L.*) is a popular spice plant in Indonesia. The assay content of coriander is phenolic linalool. In this study, in vitro and in vivo ethanol extracts of coriander stem (*Coriandrum sativum L.*) were performed on several hematological parameters consisting of leukocytes, lymphocytes, monocytes and granulocytes. The study in vitro results, the highest inhibitory diameter at 5% with a diameter of inhibition of 21.83 mm. In vivo test of ethanol extract of coriander (*Coriandrum sativum L.*) was carried out on male white mice induced by *Staphylococcus aureus* on several hematological parameters, used in study leukocytes, lymphocytes, monocytes and granulocytes. The results obtained ethanol extract of coriander (*Coriandrum sativum L.*) the effect on leukocytes, lymphocytes, monocytes and granulocytes induced by *Staphylococcus aureus* (sig <0.05). Conclusion: coriander stem ethanol extract has antibacterial activity in vitro and in vivo.

Keywords: Corrilars, antibacteri, in vitro and in vivo

INTRODUCTION

Coriander is a type of spice plant that is well known in Indonesian society as a cooking spice (Elshabrina, 2013). Coriander seeds (*Coriandrum sativum L.*) have long been used and utilized by humans as medicine or to improve the taste of food (Purseglove et al., 1981). Coriander (*Coriandrum sativum L.*) is a spice plant that is popular in Indonesia. The benefits taken from coriander are the leaves, seeds and fruit. Of all the contents there are vitamins, minerals and iron in the leaves, while the seeds contain essential oils such as linalool 70% (Bhat et al., 2014). Based on research (Silva et al., 2011), essential oil from coriander seeds effectively inhibits the growth of gram-positive and gram-negative bacteria with a concentration series of 0.2-0.8% w/v.

One of the plants that produce essential oils is coriander seeds. camphene, mycrene, camphor, ester, geranyl acetate, ketone, Linalyl acetate, alcohol, coumarin or furanokoumarin, umberliferon and -terpiene (Burdock & Carabin, 2009). Coriander (*Coriandrum sativum L.*) has long been used by humans as medicine, formulations containing coriander seed extract have been used as a stimulant, carminative, antispasmodic, diuretic and antirheumatic (Singh et al., 1996). Coriander fruit can reduce blood sugar levels (Sogara et al., 2014) and also coriander has antibacterial activity, especially on *Propionibacterium acnes* bacteria (Hapsari et al., 2016). Many studies of coriander as an antibacterial have been carried out, especially those used are coriander fruit extracts on the bacteria *E.coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae* and *P.ericilium* (Cao et al., 2012). Coriander fruit is also efficacious for the treatment of diarrhea, abdominal pain due to bloating, pain during menstrual disorders, gastric disorders, and is known to have gram-positive and gram-negative antibacterial activity (Lalitha et al., 2011). Based on the description above, the authors are interested in adding to the study of the medicinal plant coriander (*Coriandrum sativum L.*). This research focuses on in vitro and in vivo tests of the ethanolic extract of coriander stems (*Coriandrum sativum L.*) as an antibacterial. From the chemical composition and pharmacology of coriander fruit extract showed antibacterial, antifungal, antioxidant, insecticide, hypertension, antidiabetic, diuretic, anti-inflammatory, and anticancer activity (Singh et al., 1996). Coriander fruit is also efficacious for the treatment of diarrhea, abdominal pain due to bloating, pain during menstrual disorders, gastric disorders, and is known to have gram-positive and gram-negative antibacterial activity (Lalitha et al., 2011). Secondary metabolite compounds are chemical compounds produced by plants that function as defenses consisting of alkaloids, terpenoids, phenolics, flavonoids, tannins, saponins and others.

RESEARCH METHODS

Tools and materials

The tools used in this study were micro (thermoscientific) pipettes, ose needles, test tubes, test tube racks, petri dishes (pyrex), volumetric pipettes, aseptic cabinets, analytical balances, cotton, gauze, spirit lamps, Erlenmeyer (pyrex), hotplate, beaker glass (Iwaki), measuring cup (Merck), rotary evaporator (Hahnvapors model HS-2361N5), laminar air flow (Innotech), autoclave (25x-2 model Wisconsin aluminum foundry co.,inc), vortex (vortex mixer model VM-1000), magnetic stirrer, animal scales Triple Beam Balance (Ohous), mouse cage, mortar and stanfer, tissue, dropper, drip plate, watch glass, filter paper, aluminum foil, scissors, spatel, spatula, sonde (Terumo), microhematocrit capillary tubes (Marienfeld), Hematology Analyzer and camera.

The materials used in this study were coriander stems obtained in the Simabur area, Tanah Datar Regency, West Sumatra, distilled water (PT Brataco), 70% ethanol (PT Brataco), 0.9% sodium chloride (Otsuka), Nutrient Agar (NA) (Merck), disc chloramphenicol (Becton, Dickinson and Company Sparks, MD 21152 USA), Na CMC (PT Brataco), toluene (PT Brataco), ethyl acetate (PT Brataco), hydrochloric acid (PT Brataco), sulfuric acid (PT Brataco), lead acetate (PT Brataco), Ferric Chloride (PT Brataco), Chloroform (PT Brataco), mouse food (PT Central Proteinaprima Tbk Medan), chloramphenicol (Indo Farma), *Staphylococcus aureus* test microbes, and hematology reagents.

Experimental animal

Experimental animals used in this study were male white mice aged 2-3 months weighing between 20-30 grams.

WORKING PROCEDURE

Sample Preparation

The ways of making simplicia according to Prasetyo, 2013 are as, wet sorting, washing materials, chopping, drying, refining / powder making. Identification of Plants

Preparation of Coriander Stem Ethanol Extract (*Coriandrum sativum* L.)

The extract was made by maceration. 900 g of dry simplicia was put into a macerator and added 900 mL of 70% LP ethanol solvent. Then soaked for the first 6 hours, stirring occasionally, then allowed to stand up to 18 hours. Maserat is separated by filtration using flannel cloth. The extraction process was repeated at least twice with the same type and amount of solvent. The liquid extract was put into a rotary evaporator at a temperature of 50°C and continued with drying using an oven at a temperature of 40°C to produce a thick extract (Sogara et al., 2014).

Extract Characterization

1. Non Specific Characterization

Extract characterization carried out includes: determination of drying shrinkage, determination of water content, determination of total ash content and acid insoluble ash content.

2. Specific Characterization

Specific characterization of extracts include: identity and organoleptic test.

Thin Layer Chromatography (TLC)

1. Vessel saturation

The filter paper is placed in the chromatography vessel. The height of the filter paper is 18 cm and the width is the same as the width of the vessel. A number of developer solutions are introduced into the chromatography vessel to a height of 0.5 to 1 cm from the bottom of the vessel. Then closed tightly and left until the filter paper should always be immersed in the developer solution at the bottom of the vessel.

2. TLC test solution

Approximately 10 mg of coriander (*Coriandrum sativum* L.) stem extract was weighed carefully and then soaked while shaking with 1 mL of ethanol for 10 minutes.

3. Mobile phase

Toluene : ethyl acetate (93:7) (Ministry of Health of the Republic of Indonesia, 2010)

4. Silent phase

Silica Gel 60 F254

5. TLC procedure

The test solution was spotted at a distance of 1.5 to 2 cm from the bottom edge of the plate, and allowed to dry. The plate on the rack is placed into the chromatographic vessel. The developer solution in the vessel must reach the bottom edge of the absorbent layer, the spots should not be submerged. The lid of the vessel is placed in place and the system is left until the mobile phase propagates up to the creepage limit at the creepage limit. The plate is removed and dried in air, and observing the spots with visible light, short-wave ultraviolet 254 nm. Then with long-wave ultraviolet 366 nm. The distance of each spot was measured and recorded from the spotting point and recorded the wavelength for each spot observed to determine the Rf value.

The distance of development of compounds on the chromatogram is usually expressed by the Rf number: $R_f = \frac{\text{Distance from the center point of the spot from the starting point}}{\text{Distance of the front line of the mobile phase from the starting point}}$. The value of Rf can be influenced by the humidity of the air or the absorbent is slightly distorted (Stalh, 1985).

Secondary Metabolite Test

Ethanol Extract of Coriander Stem (*Coriandrum sativum* L.)

1. Alkaloid test

The extract sample was dissolved in 2 mL of hydrochloric acid, heated for 5 minutes and filtered. The filtrate obtained was added 2-3 drops of Dragendorff reagent. The presence of alkaloid compounds was indicated by an orange precipitate. Identification of alkaloids with Meyer's reagent, the presence of alkaloids was indicated by the formation of a yellow precipitate (Harbone, 1987).

2. Phenolic test

A total of 2 mL of the extract was dissolved in 10 mL of distilled water, part of the solution was taken and transferred with a pipette into a test tube. Then add 2 drops of FeCl₃ solution to form a blue or purple color which indicates the presence of phenolics (Harbone, 1987).

3. Test for steroids and terpenoids.

A total of 2 mL of extract was added with 1 mL of Liebermen-Buchard reagent. The presence of steroid and terpenoid compounds was indicated by the formation of a dark blue or blackish green color (Harbone, 1987).

4. Tannin test

The addition of 3 drops of FeCl₃ reagent to 10 mg of extract produces a blue-black color (Hanani, 2017).

5. Saponin test

Weigh 20 mg of the extract, shaken with 2 mL of water, if foam appears for ten minutes, it indicates the presence of saponins (Tiwari et al, 2011).

In Vitro Test

1. Preparation of Test Microbial Suspension

The tested microbial colonies were taken from agar sloping 1-2 oses and then suspended in 0.9% Physiological Sodium Chloride as much as 10 mL in a sterile test tube and then homogenized by centrifugation and then the transmittance was measured using a spectrophotometer. Then the turbidity was compared using the Mc Farland standard (Hasmila et al., 2015).

2. Sterilization

Preparation Tool sterilization. The tools to be used are washed, drained, sprayed with 70% ethanol and then dried in an oven. Wrap it using double brown paper. For the test tube and Erlenmeyer, the mouth of the test tube is first plugged, then wrapped in double brown paper. The instrument is sterilized using an autoclave at 1210C for 15 minutes. The sterilized equipment is used for antibacterial testing.

3. Making Bacterial Media

Preparation of Nutrient Agar (NA) media was carried out by weighing 5 g of nutrient agar (NA) powder into an erlenmeyer, dissolved with 250 mL of aqua distillate. Heat over a water bath while stirring until the solution is clear. Then sterilized by using an autoclave at a temperature of 1210C at a pressure of 2 atm for 15 minutes.

4. Testing Coriander Ethanol Extract As Antibacterial

Concentration of ethanol extract of coriander stems was made. The concentration of ethanol extract of coriander stem used was 5%, 3% and 1%. Antibacterial test. Bacterial suspension was planted on solid NA media. The outside of the petri dish was marked with a sample disc dripped with ethanol extract of coriander stems with a concentration of 5%, 3% and 1%.

The positive control used was chloramphenicol disk, and the negative control used was DMSO. Incubated at 37°C for 2x24 o'clock. The growth that occurred was observed and the diameter of the clear zone formed was observed using a caliper (Bani et al., 2016).

In Vivo Test

1. Preparation of Experimental Animals

The experimental animals used were healthy male white mice weighing 20-30 grams, approximately 2-3 months old, as many as 30 mice. Before the study was conducted, the mice were acclimatized for 7 days. Animals are declared healthy if the deviation of body weight before and after adaptation is not more than 10% and visually shows normal behavior (Vogel, 2002).

2. Dosage Planning

a. Coriander (*Coriandrum sativum* L.)

Ethanol Extract Dosage The dose of coriander ethanol extract given to white male mice at a dose of 100 mg/kgBW, 300 mg/kgBW, and 500 mg/kgBW was administered orally.

b. *Staphylococcus aureus* Induction Dose

The inducer given to mice was *Staphylococcus aureus* with a population of 108 cfu/mL with a volume of 0.2 mL per day for seven days to see hematological parameters. c. Comparative Dose The comparison used was chloramphenicol. The human dose of 250mg/BW was converted to 0.0026 mice. The dose of chloramphenicol used was 0.65 mg/20gram.

3. Preparation of Test Preparation

a. Preparation of Coriander Ethanol Extract Suspension

Na CMC powder weighed 50 mg. Sprinkle over hot water 20 times in a hot mortar and leave for 15 minutes. then grind until homogeneous, add coriander ethanol extract that has been weighed according to the planned dose, grind homogeneously, then add distilled water to a volume of 10 mL.

b. Production of *Staphylococcus aureus* Bacteria

Suspension The tested microbial colonies were taken from agar slanted 1-2 oses and then suspended in 10 mL of 0.9% physiologic sodium chloride in a test tube. then homogenized by centrifugation and the transmittance was measured using a spectrophotometer. Then the turbidity was compared with Mc Farland's solution.

c. Manufacture of Comparative Suspension

The dose of chloramphenicol in 1 capsule is 250 mg. the dose for mice was 20g/kg BW converted = $0.0026 \times 250 = 0.65$ mg. the 10-day dose for 5 mice is $0.65 \text{ mg} \times 10 \times 5 = 32.5$ mg Chloramphenicol capsules are opened and the shells are finely ground, weighed carefully. homogenized hot water.

3. Animal Grouping

After acclimatization for 7 days, the experimental animals were divided into six treatment groups, each consisting of 5 animals. The negative control was mice that were not induced by *Staphylococcus aureus* and were not given coriander ethanol extract. Positive controls were mice induced with *Staphylococcus aureus* 108 cfu/mL. The comparison control was given *Staphylococcus aureus* 108 cfu/mL and chloramphenicol. Group I, Group II, and Group III were mice induced with *Staphylococcus aureus* 108 cfu/mL and given coriander ethanol extract orally at a dose of 100mg/kgBW, 300mg/kgBW, and 500 mg/kgBW for 7 consecutive days. come along.

Group	Treatment
Negative control	No treatment
Positive control	0.2 mL 108 cfu/mL <i>Staphylococcus aureus</i>
Comparative control	0.2 mL 108 cfu/mL <i>Staphylococcus aureus</i> + 0.65 mg/kgBW chloramphenicol
Group I	0.2 mL 108 cfu/mL <i>Staphylococcus aureus</i> + coriander ethanol extract 100 mg/kgBB
Group II	0.2 mL 108 cfu/mL <i>Staphylococcus aureus</i> + coriander ethanol extract 300 mg/kgBB
Group III	0.2 mL 108 cfu/mL <i>Staphylococcus aureus</i> + coriander ethanol extract 500 mg/kgBB

Table 1. Grouping of male white mice based on the treatment given.

How does hematological testing work on male white mice:

1. On day 1 to 7

Positive group, comparison group, group I, II and III were induced first with 0.2 mL of *Staphylococcus aureus* 108 per day for 7 days, then continued with coriander ethanol extract with each predetermined dose.

2. On the 8th to 14th day

Group II was given an inducer of *Staphylococcus aureus* as much as 0.2 mL once a day. The comparison group was given chloramphenicol at a predetermined dose. Groups I, II and III were given a suspension of coriander ethanol extract according to a predetermined dose.

3. Hematology measurements were carried out on the 15th day where the blood of mice would be taken from the eyes using micro capillaries, then inserted into the EDTA tube, then inserted into the Hematology Analyzer tool automatically the samples would be analyzed.

Hematological Parameter Check

The blood of the experimental animals was taken through the eyes using a capillary tube, then inserted into the EDTA tube, then inserted into the Hematology Analyzer.

Data analysis

The data obtained were analyzed statistically using the SPSS 25 computer application program

Results and discussion

The coriander plant was taken from the Simabur area, Tanah Datar Regency, identified at the Andalas University Herbarium (ANDA) -UNAND (FMIPA-BIOLOGI), the identification of the species *Coriandrum sativum* L. from the Apiaceae family. Extraction of coriander stems (*Coriandrum sativum* L.) was macerated with 70% ethanol, obtained a thick extract weighing 143.4 g and the yield of 15.93%. According to the Pharmacopoeia, the yield for coriander is not less than 13.2%. Non-specific characteristics consisting of simplicia drying shrinkage obtained drying shrinkage content of $1.0413 \pm 0.0058\%$, moisture content: $7.0968 \pm 0.001\%$, total ash content: $2.39 \pm 0.04\%$, acid insoluble ash content: $0.37 \pm 0.24\%$, these results are in accordance with Supplement 1, Indonesian Herbal Pharmacopoeia. In the Thin Layer Chromatography (TLC) test the objective is to see the separation of the compounds contained in the ethanol extract of coriander stems. The separation of compounds can be seen by the formation of stains on the TLC plate. The principle of separation using TLC is the separation of compounds based on their level of polarity. In this study, researchers used TLC silica gel F254, with a comparison of toluene solvent: ethyl acetate (93: 7) (Ministry of Health of the Republic of Indonesia, 2010).

From the results of TLC examination, it is known that the ethanol extract of coriander stems (*Coriandrum sativum* L.) was separated using toluene: ethyl acetate with Rf values at a wavelength of 366 nm: 0.53 cm and 0.26 cm (Appendix 1, Table IX). This is far from what is determined by Supplement 1, the Indonesian Herbal Pharmacopoeia, which is 0.12 cm for coriander and 0.31 cm for the comparison of linalool. The different Rf values obtained may be due to the different content of compounds in the extract, so that produce different separation distance.

The ethanol extract of coriander stems (*Coriandrum sativum* L.) for testing its antibacterial activity was made in various concentrations, namely 5%, 3%, and 1% using dimethylsulfoxide (DMSO) as the solvent. In this study, a positive control was used as a comparison, namely disc chloramphenicol. Chloramphenicol is an antibiotic that has broad and effective action against gram-positive and gram-negative (Gunawan, 2007). As a negative control, DMSO was used. interfere with the observation of antibacterial activity testing with the agar diffusion method, besides that DMSO is a solvent that can dissolve all polar, semi-polar and non-polar compounds (Maryati & Sutrisna, 2007).

The antibacterial activity test method against the ethanolic extract of coriander stems (*Coriandrum sativum* L.) using the diffusion method was chosen because this method is relatively simple and the results obtained are good enough to determine the presence of antibacterial activity.

The results of the antibacterial activity test showed that the thick extract of coriander stems had an inhibitory diameter at a concentration of 5%, an average inhibitory diameter of 21.83 mm, at a concentration of 3% the average inhibitory diameter was 16.83 mm and at a concentration of 1% the average the inhibition diameter was 15.33 m (Appendix 1, Table II).

From these results the concentration of 5% can be classified as having a strong diameter of inhibition, while the concentrations of 3% and 1% can be classified as having moderate inhibitory power. However, if you look at the best results, it was formed from the comparison antibiotic chloramphenicol which was used as a positive control, showing an inhibitory diameter of 22.17 mm. The most potent was the thick extract of coriander stems (*Coriandrum sativum* L.) at a concentration of 5%.

According to Mulyani et al., (2013) the classification of the inhibitory response to bacterial growth is that the diameter of the inhibition zone of more than 20 mm is classified as strong, 16-19 mm is classified as moderate, 10-15 mm is classified as weak and less than 10 mm does not have an inhibitory response. Based on the results of the study (Appendix 1, Table II) showed that the thick extract of coriander stems (*Coriandrum sativum* L.) which produced the largest average diameter of inhibition to inhibit the growth of *Staphylococcus aureus* bacteria was 21.83 ± 0.76 mm, it can be categorized as activity strong inhibition.

Appendix 1, Table II).

Descriptive								
diameter daya hambat								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
konsentrasi 5 %	3	21.8333	0,76376	0,44096	19.9360	23.7306	21.00	22.50
konsentrasi 3 %	3	16.8333	1.04083	0,60093	14.2478	19.4189	16.00	18.00
konsentrasi 1 %	3	15.3333	1.75594	1.01379	10.9713	19.6953	13.50	17.00
Kloramfenikol	3	22.1667	1.12731	0,65085	19.3663	24.9671	21.00	23.25
Total	12	19.0417	3.31120	0,95586	16.9378	21.1455	13.50	23.25

Tests of Normality				
	perlakuan	Shapiro-Wilk		
		Statistic	df	Sig.
diameter daya hambat	konsentrasi 5 %	0,964	3	0,637
	konsentrasi 3 %	0,923	3	0,463
	konsentrasi 1 %	0,993	3	0,843
	kloramfenikol	0,996	3	0,878

In this study, the ethanolic extract of coriander (*Coriandrum sativum* L.) had an effect on monocyte parameters. This is indicated by the results of the ANOVA calculation where the value ($P < 0.005$). In Duncan's follow-up test, the results showed that group I (dose of 100 mg/kg BW) was the best dose group, while Group II (dose of 300 mg/kg BW) and group III (dose of 500 mg/kg BW) were the same dose as the comparison control.

The results of the effect of ethanol extract of coriander stems (*Coriandrum sativum* L.) on granulocyte parameters in male white mice induced by *Staphylococcus aureus* obtained the number of granulocytes in the negative group with an average of 3 ± 0.7 cells/mm³, the positive group with an average of 0.47 ± 0.06 cells/mm³, the comparison group with an average of 3.1 ± 0.95 cells/mm³, group I (dose of 100 mg/kg BW) with an average of 4.77 ± 0.35 cells/mm³, group II (dose of 300 mg/kg BW) with an average of 1.8 ± 1.51 cells/mm³ and group III (dose of 500 mg/kg BW) with an average of 2.2 ± 0.35 cells/mm³. The normal value for granulocytes is 0.4-2.0 cells/mm³.

In this study, the ethanolic extract of coriander (*Coriandrum sativum* L.) had an effect on granulocyte parameters. This is indicated by the results of the ANOVA calculation where the value ($P < 0.05$). In Duncan's follow-up test, the results showed that group I (dose of 100 mg/kg BW) was the best dose, while for group II (dose of 300 mg/kg BW) and group III (dose of 500 mg/kg BW) the hematocrit was returned to its normal state. normal well.

Bibliography

- Atmadja, A. P., Kusuma, R., Dinata, F. (2016).Pemeriksaan laboratorium untuk membedakan infeksi bakteri dan infeksi virus.CDK, 6(43), 457-461.
- Balai Penelitian Tanaman Rempah dan Obat. 2004. *Tanaman Obat : Ketumbar (Coriandrum sativum L.)*. Jakarta (ID) : Departemen Kesehatan Republik Indonesia.
- Bani,F., Serang,Y., Safitri, (2016). Kajian efektivitas filtrat perasan.minyak atsiri dan ekstrak etanol daun ketumbar (*Coriandrum sativum L.*). *Jurnal Farmasi dan Sains Indonesia*, 1(1).39-46.
- Bhat,S.,Kaushal,P.,Kaur,M and Sharma, H. K, (2014). Coriander (*Coriandrum sativum L.*): Processing, nutritional and functional aspects. *African Journal of Plant Science*, 8(1),25-33.
- Bhuiyan,M.N.I.,Begum,J.,Sultana,M. (2009).Chemical composition of leaf and seed essential oil of *Coriandrum sativum L.* from Bangladesh. *Bangladesh.J Pharmacol*, 3(4). 150-153.
- Brooks,G.F.,Butel,J.S.,Morse,S.A. (2005). *Mikrobiologi Kedokteran(Medical Microbiology)* Penerjemah, Bagian Mikrobiologi Fakultas Kedokteran Universitas Airlangga. Jakarta: Salemba Medika.
- Burdock, G.A. dan I. G. Carabin. (2009). Safety assessment of coriander (*Coriandrum sativum L.*)essential oil as a food ingredient. *Food and Chemical Toxicology*. 47(1):22-34.
- Cao, X. Z., You, J. M., Li, S. X.,& Zhang, Y. L.(2012). Antimicrobial activity of the extract from *Coriandrum sativum*.*International Journal of Food Nutrition and Safety*, 1(2).54-59.
- Departemen Kesehatan Republik Indonesia, (1980).Materia Medika Indonesia jilid IV. Jakarta: Departemen Kesehatan Republik Indonesia.
- Departemen Kesehatan Republik Indonesia.(1989). *Hematologi*. Jakarta:Departemen Kesehatan Republik Indonesia.
- Departemen Kesehatan Republik Indonesia.(2000). *Parameter standar umum ekstrak tumbuhan obat*.Jakarta : Direktorat Jendral Pengawasan Obat dan Makanan, Direktorat Pengawasan Obat Tradisional.
- Departemen Kesehatan Republik Indonesia.(2010). *Suplemen 1, Farmakope Herbal Indonesia*.Jakarta : Departemen Kesehatan Republik Indonesia.
- Elshabrina.(2013). *Dasyatnya Daun Obat Sepanjang Masa*.Yogyakarta : Cemerlang Publishing.
- Gandasoebrata, R. (2010). *Penuntun Laboratorium Klinik*. Jakarta Timur : Dian Rakyat.
- Gould,D.,& Brooker,C. (2003).*Mikrobiologi Terapan Untuk Perawat.Penerjemah*, Pendiit,B.U. Jakarta: EGC.
- Gunawan, S.G., Setiabudy, R., Nafrialdi,R.S., & Elysabeth. (2007). *Farmakologi dan Terapi* (Edisi 5). Jakarta : Departemen Farmakologi dan Terapeutik, Fakultas Kedokteran Universitas Indonesia.

- Hanani, E. (2017). *Analisis Fitokimia*. Jakarta :Penerbit Buku Kedokteran EGC.
- Handayani, D., & Aminah, I. (2017). Antibacterial and cytotoxic activities of ethyl acetat extract of symbiotic fungi from West Sumatera marine sponge *Acanthoryglophora ingens*. *Journal applied Pharmaceutical Science*, 7(02). 237-240.
- Hapsari,R.A.,Suwendar.,Hazar,S. (2016). Potensi aktivitas antibakteri ekstrak etanol buah ketumbar (*Coriandrum sativum* L.) terhadap *Propionibacterium Acnes* .*ProsidingFarmasi*, 2(2).788-793.
- Harbone, J.B. (1987). *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan* (2nd ed). Bandung: ITB.
- Hasmillah,I., Amaliah.,&Daniel, M. (2005). Efektivitas salep ekstrak-ekstrak daun sirsak (*Annona muricata* L.) pada mencit yang terinfeksi bakteri *Staphylococcus aureus* . *ProsidingSeminar Nasional Mikrobiologi Kesehatan Lingkungan*, 54-62.
- Irianto, K. (2017). *Biologi Molekuler Teori – Pratikum – Glosarium*. Jakarta : Alfabeta.
- Kiswari,R.(2014). *Hematologi dan Transfusi*.Jakarta :Erlangga.
- Lalitha,V., Kiran,B., Raveesha,B. . (2011). Antifungal and antibacterial potentiality of six essential oils extracted from plant source. *International Journal of Engineering Science and Technology* (IJEST), 3(4).
- Maryati.,& Sutrisna, E. M. (2007). Potensi sitotoksik tanaman ciplukan (*Physalis Angulata* L) terhadap sel hela cytotoxic effect of physalis angulata plant on hela cell line. *Pharmacon*, 8(1), 1-6.
- Mulyani,M., Arifin, B., & Nurdin, H. (2013). Uji antioksidan dan isolasi senyawa metabolit sekunder dari daun Srikaya (*Annona squamosa* L.)
- Neal, M.J. (2005). *At a glance farmakologi medis* (5thed). Jakarta : Erlangga.
- Nimish,P., Sanjay,K.B., Nayna,M., Jaimik,R.D. (2011). Phytopharmacology properties of *Coriander sativum* as a potential medicinal tree : an overview. *Journal of Applied Pharmaceutical Science*, 1(4).20-25.
- Nugraha, G. (2015). *Panduan Pemeriksaan Hematologi Dasar*. Jakarta: TIM.
- Prasetyo,M.S dan Entang Inorih,S. (2013). Pengelolaan budidaya tanaman obat-obatan (simplisia).Bengkulu : Badan Penerbitan Fakultas Pertanian UNIB.
- Purseglove, J.W., Brown, e.g., Green, C.L.&Robbin, S.R.J. (1981). *Coriander in Spices* vol. 2, New York : Tropical Agricultur Series.
- Radji, M. (2010). *Buku Ajar Mikrobiologi*. Jakarta: EGC.
- Rani, P.S., Nagasowjanya, G., Ajitha, A., & Maheswarrao, V.U. (2015). Aquametry – The Moisture Content Determination, *World JournalOf Pharmacy And Pharmaceutical Science*, 4(8), 566-580.
- Silva, F., Ferreira, S., Queiros, J.A., Domingues, F.C. (2011). Coriander (*Coriandrum sativum* L.) essential oil : its antimicrobial activity and mode of action evaluated by flow cytometry. *Journal of Medical Microbiology*.60.1479-1486.
- Singh, B., Chaurasia, O.P., Jadhav K.L. 1996. An ethnobotanical study 2 of Indus valley (Ladakh). *J Econ Tax Bot Adtl Ser*; 12 :92-101
- Soekardjo, B. & Siswando, (2000).*Kimia Medisinal* (2nded). Surabaya : Airlangga University Press.
- Sogara, U. P. P., Fatmawati., Bodhi, W. (2014). Pengaruh ekstrak etanol buah ketumbar (*Coriandrum sativum* L.) terhadap penurunan kadar gula darah tikus putih yang diinduksi aloksan. *Pharmacon*, 3(3).196-203.
- Stahl, E. (1985). Analisis Obat Secara Kromatografi dan Mikroskopi. Bandung: ITB.
- Tiwari, P., Kumar,B., Kaur,M., Kaur,G., & Kaur,H. (2011).Phytochemical screening and extraction.*Internationale Pharmaceutica Scienta*, 1(1). 98-106.
- Tjay,T.H.,& Rahardja,K. (2007).*Obat-obat penting*.(Edisi 6). Jakarta:Gramedia.
- Vogel, H.G. (2002). *Drug Discovery and Evaluation : Pharmalogycal Assays*, New York: Springer-Verley, Deidelbarg

RESULTS AND DISCUSSIONS

Hasil

Setelah dilakukan penelitian tentang uji *in vitro* dan *in vivo* ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) sebagai antibakteri, diperoleh hasil sebagai berikut :

1. Hasil identifikasi sampel tanaman ketumbar dari spesies *Coriandrum sativum* L. dari famili *Apiaceae* yang diidentifikasi oleh Herbarium Universitas Andalas (ANDA), Jurusan Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam (FMIPA) Universitas Andalas Padang, Sumatera Barat (Lampiran 1, Gambar 2).
2. Surat keterangan nama bakteri *Staphylococcus aureus* yang dikeluarkan oleh Pusat Diagnostik & Riset Mikrobiologi, Bagian Mikrobiologi

Fakultas Kedokteran Universitas Andalas Padang, Sumatera Barat (Lampiran 1, Gambar 3).

3. Hasil rendemen ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) sebesar 15,93 % (Lampiran 1, Tabel II).
4. Hasil karakterisasi non spesifik adalah :
 - a. Hasil susut pengeringan ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) yaitu $1,0413 \pm 0,0058$ % b/b (Lampiran 1, Tabel III).
 - b. Hasil kadar air ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) yaitu $7,0968 \pm 0,001$ % b/b (Lampiran 1, Tabel IV).
 - c. Hasil kadar abu total ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) yaitu $2,39 \pm 0,04$ % (Lampiran 1, Tabel V).
 - d. Hasil kadar abu tidak larut asam ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) yaitu $0,37 \pm 0,24$ % (Lampiran 1, Tabel VI).
5. Hasil karakterisasi spesifik adalah :
 - a. Hasil identitas ekstrak etanol batang ketumbar (Lampiran 1, Tabel VII).
 - b. Hasil organoleptis ekstrak etanol batang ketumbar yaitu berbentuk kental (semipadat), warna hijau kehitaman, rasa pahit dan bau aroma ekstrak menyengat (Lampiran 1, Tabel VIII)
6. Hasil pemeriksaan KLT, diketahui ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) dengan menggunakan toluen : etil asetat (93:7) sebagai fase geraknya dengan nilai R_f 0,53 cm dan 0,26 cm (Lampiran 1, Tabel IX).
7. Hasil uji aktivitas antimikroba ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) terhadap bakteri *Staphylococcus aureus* secara *in vitro*:
 - a. Ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) konsentrasi 5 % didapatkan rata-rata diameter hambat 21,83 mm (Lampiran 1, Tabel XI).
 - b. Ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) konsentrasi 3 % didapatkan rata-rata diameter hambat 16,83 mm (Lampiran 1, Tabel XI).
 - c. Ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) konsentrasi 1 % didapatkan rata-rata diameter hambat 15,33 mm (Lampiran 1, Tabel XI).
8. Hasil uji pengaruh ekstrak ketumbar terhadap hematologi mencit putih jantan yang diinduksi *Staphylococcus aureus* sebagai berikut :
 - a. Hasil uji pengaruh ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) terhadap parameter leukosit pada mencit putih jantan yang diinduksi *Staphylococcus aureus* diperoleh hasil rata-rata jumlah leukosit kelompok negatif yaitu $3,4 \pm 0,3$ sel/mm³, kelompok positif yaitu $6,8 \pm 0,0$ sel/mm³, kelompok pembanding yaitu $8,5 \pm 0,82$ sel/mm³, kelompok I (dosis 100 mg/kgBB) yaitu $14,07 \pm 1,85$ sel/mm³, kelompok II (dosis 300 mg/kgBB) yaitu $9,8 \pm 1,0$ sel/mm³ dan kelompok III (dosis 500 mg/kgBB) yaitu $7,0 \pm 0,72$ sel/mm³ (Lampiran 1, Tabel XIII).
 - b. Hasil uji pengaruh ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) terhadap parameter limfosit pada mencit putih jantan yang diinduksi *Staphylococcus aureus* diperoleh hasil rata-rata jumlah limfosit kelompok negatif yaitu $2,87 \pm 0,68$ sel/mm³, kelompok positif yaitu $0,5 \pm 0,1$ sel/mm³, kelompok pembanding yaitu $4,5 \pm 0,6$ sel/mm³, kelompok I (dosis 100 mg/kg BB) yaitu $7,6 \pm 2,88$ sel/mm³, kelompok II (dosis 300 mg/kg BB) yaitu $3,76 \pm 2,37$ sel/mm³ dan kelompok III (dosis 500 mg/kg BB) yaitu $4,13 \pm 0,71$ sel/mm³ (lampiran 1, Tabel XIII).
 - c. Hasil uji pengaruh ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) terhadap parameter monosit pada mencit putih jantan yang diinduksi *Staphylococcus aureus* diperoleh hasil rata-rata jumlah monosit kelompok negatif yaitu $0,63 \pm 0,21$ sel/mm³, kelompok positif yaitu $0,2 \pm 0,1$ sel/mm³, kelompok pembanding yaitu $0,77 \pm 0,15$ sel/mm³, kelompok I (dosis 100 mg/kg BB) yaitu $1,23 \pm 0,06$ sel/mm³, kelompok II (dosis 300 mg/kg BB) yaitu $0,67 \pm 0,5$ sel/mm³ dan kelompok III (dosis 500 mg/kg BB) yaitu $0,67 \pm 0,06$ sel/mm³ (lampiran 1, Tabel XIII).
 - d. Hasil uji pengaruh ekstrak etanol batang ketumbar (*Coriandrum sativum* L.) terhadap parameter granulosit pada mencit putih jantan yang diinduksi *Staphylococcus aureus* diperoleh hasil rata-rata jumlah granulosit kelompok negatif yaitu $3 \pm 0,7$ sel/mm³, kelompok positif yaitu $0,47 \pm 0,06$ sel/mm³, kelompok pembanding yaitu $3,1 \pm 0,95$ sel/mm³, kelompok I (dosis 100 mg/kg BB) yaitu $4,77 \pm 0,35$ sel/mm³, kelompok II (dosis 300 mg/kg BB) yaitu $1,8 \pm 1,51$ sel/mm³ dan kelompok III (dosis 500 mg/kg BB) yaitu $2,2 \pm 0,35$ sel/mm³ (lampiran 1, Tabel XIII).

The manufacture of seed extract is done by the maceration method, this method was chosen because it is relatively simple and fast, but it can already provide the active substance simplisia with the maximum. The main advantage of this method is that it is not done by heating so that it can prevent damage or loss of active substances that want to be abstracted in compounds that are not heat resistant (Sa'adah & Nurhasnawati, 2015). Antioxidant content is the content of compounds that are not heat resistant so that the maceration method which is cold extraction will be more optimal in extracting antioxidant compounds (Sie, 2013).

In this study, the manufacture of extracts by maceration method was carried out with 3 solvents based on its polarity, namely ethanol, ethyl acetate and n-Hexane. The purpose of using three solvents with different polarities is to know yield, get active compounds and know the antioxidant activity of kebiul seed based on their level of polarity. The percentage of yield obtained from ethanol extract 96% is 12.5868%, the percentage of yield obtained from ethyl acetate extract is 11.5264%, the percentage of yield obtained from n-Hexan extract is 9.0704%.

After getting a thick extract, then the extract is characterized with the aim of seeing the quality of the extract obtained. Determination of organoleptis obtained ethanol extract 96% with brownish yellow color, bitter taste, bad smell with viscous extract, ethyl acetate extract with yellow color, bitter taste, bad smell with thick extract and extract n- Heksan with yellow color, bitter taste, bad smell with thick extract. The determination of organoleptis includes

one of the specific parameters specified by the five senses and aims for simple and subjective early recognition (Ministry of Health of the Republic of Indonesia, 2000).

Then determine the phytochemical content in each extract. Qualitative phytochemical analysis is a method of initial analysis to examine the content of chemical compounds in the sample. Results obtained in ethanol extract 96% of seedlings showed positive results in alkaloids, flavonoids, saponins, tannins, phenols and terpenoids. Phytochemical screening results from ethyl acetate extract of castor seeds (*Caesalpinia bonduc*(L) Roxb.) showed positive results in alkaloids, saponins and steroids. Phytochemical screening results from n-Hexan extract of kebiul seed showed positive results in alkaloids, saponins and steroids.

These flavonoid compounds are powerful antioxidants that can prevent the formation of free radicals (Sakihama *et al.*, 2002). Flavonoids activity as antioxidants because they have clusters Hydroxyl can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen compounds (ROS) and has a hydroxyl ketone group that can act as a metallated catalyst that becomes a lipid peroxidation catalyst (Rezaeizadeh, 2011).

The determination of the antioxidant activity test of the seed extract was conducted using the DPPH (*2,2-diphenyl-1-picrylhydrazil*) method analyzed with UV-Vis spectrophotometry. DPPH is a commonly used method as a radical to test antioxidant activity because of its stable nature in the form of free radicals and is a simple, fast, and inexpensive method (Bozin *et al.*, 2008). The antioxidant activity of the sample was measured through measurements of the absorption intensity of each sample after adding DPPH using a UV-Vis spectrophotometer at a given wavelength. Based on this, before measuring antioxidant activity, first determine the maximum wavelength of solution *1,1-diphenyl-2-picrylhydrazil*.

The results of the experiment showed a maximum uptake of *1,1-diphenyl-2-picrylhydrazil* located at a wavelength of 515.50 nm with an absorbant of 0.656 nm. This means that the absorption measurement of the all extracts against DPPH free radicals is performed at a wavelength of 515.50 nm.

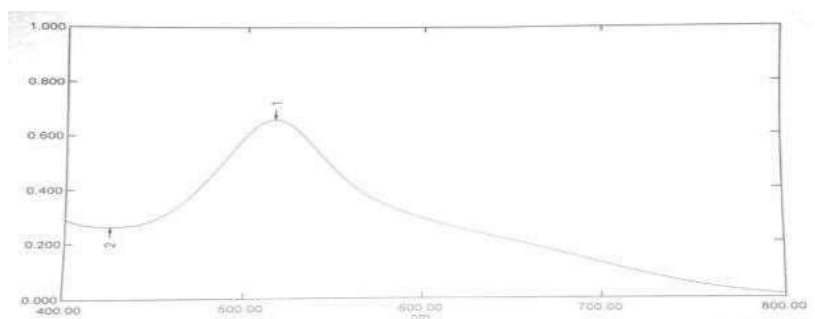


Figure 1. Maximum wavelength spectrum of DPPH solution 30 µg/mL

The size parameter used to indicate antioxidant activity is the value of inhibition concentration (IC₅₀) which is the concentration of an antioxidant substance that can cause 50% DPPH (*1,1-diphenyl-2-picrylhydrazil*) to lose radical character or concentration of an antioxidant substance that provides a percentage (%) inhibition of 50%. Substances that have high antioxidant activity, will have a low IC₅₀ value (Prasanto *et al.*, 2017).

Testing the antioxidant activity of gallic acid, obtained the result of the solution absorbant 0.496; 0.423; 0.339; 0.265; 0.196.

Viewed from the result of absorbant can be known that the greater the concentration of the sample, the smaller the absorbance value obtained, this is because the higher the antioxidant compounds that are able to reduce or ward off radicals in DPPH so that the percentage of inhibition will be greater (Bahriul *et al.*, 2014). IC₅₀ value or free radical antidote activity of 50% obtained from gallic acid of 12.8337 µg / mL. The results showed antioxidant activity with a very strong category due to IC₅₀ values < 50 µg / mL (Rosidah & Tjitraesmi, 2018).

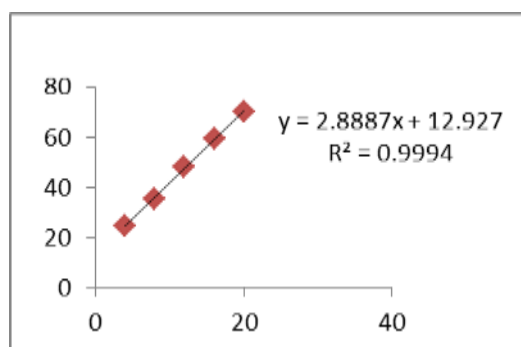


Figure 2. Galad acid extract calibration curve

In testing the antioxidant activity of ethanol extract 96% of kebiul seed, the result of absorbing a solution of 0.329; 0.311; 0.296; 0.280; 0.262. IC₅₀ value or free radical antidote activity of 50% was obtained at 40.3619 µg/mL. The results showed antioxidant activity with a very strong category due to IC₅₀ values < 50 µg / mL (Rosidah & Tjitraesmi, 2018).

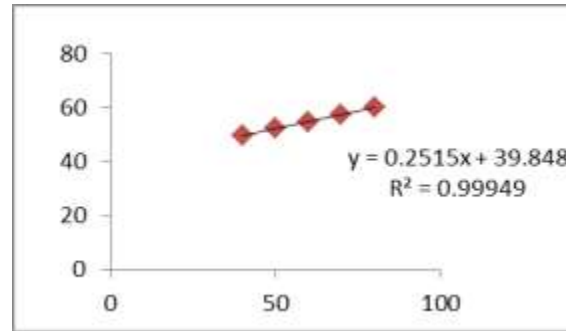


Figure 3. Calibration curve of ethanol 96% extract

In testing the antioxidant activity of n- Hexan extract from kebiul seed, a solution absorbant result of 0.655; 0,578; 0,490; 0,400; 0.309. IC50 value or free radical antidote activity of 50% was obtained at 198.2069 µg/mL. The results showed antioxidant activity in a moderate category due to IC50 values of 101-250 µg/mL (Rosidah & Tjitraresmi, 2018).

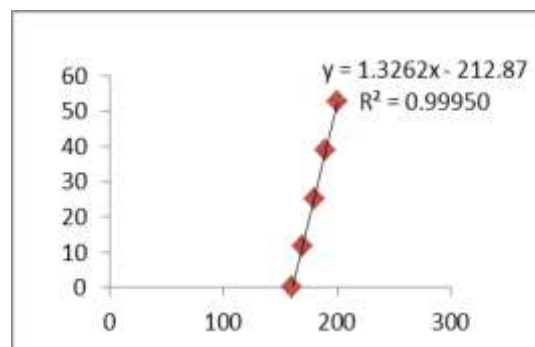


Figure 5. Calibration curve of n-Hexan extract

In testing the antioxidant activity of ethyl acetate extract from kebiul seed, a solution absorbant result of 0.599; 0,512; 0,437; 0,361; 0.292. IC50 value or free radical antidote activity of 50% was obtained at 184.6666 µg/mL. The results showed antioxidant activity in a moderate category due to IC50 values of 101-250 µg/mL (Rosidah & Tjitraresmi, 2018).

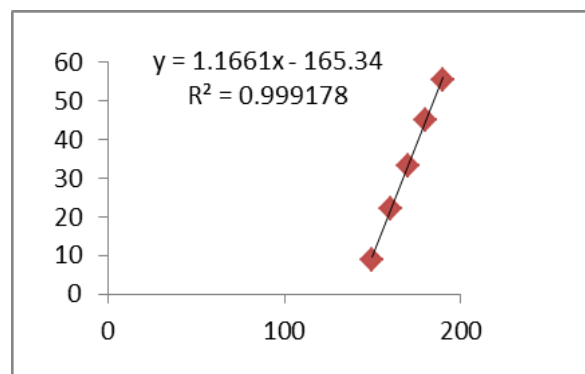


Figure 4. Calibration curve of ethyl acetate extract

From the value of IC50 can be seen the best antioxidant activity is ethanol extract 96% which is 40.3619 µg / mL and categorized very strongly in antioxidants. It is stated that very strong intensities have IC50 (<50 µg/mL), strong (50-100 µg/mL) values, moderate (101- 250 µg/mL), weak (250-500 µg/mL) (Rosidah & Tjitraresmi, 2018). The effect of solvent polarity can affect the antioxidant levels of kebiul seed as seen from the value of IC50, the polar a compound, the higher its antioxidant activity, because in polar compounds attract a lot of chemical content including chemical compounds that have strong antioxidant activity, namely flavonoids (Sakihama *et al.*, 2002).

CONCLUSIONS AND SUGGESTIONS

CONCLUSIONS

Based on research that has been done on phytochemical screening and the effect of solvent polarization on the antioxidant activity of castor seed extract (*Caesalpinia bonduc* (L) Roxb.) with the DPPH method, it can be concluded that:

1. Solvent polarization affects the chemical content of kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.), where ethanol extract 96% of kebiul seeds (*Caesalpinia bonduc* (L) Roxb.) attract the most chemical content compared to ethyl acetate extract and n- Hexan kebiul seeds (*Caesalpinia bonduc* (L) Roxb.).
2. Solvent polarization affects antioxidant activity in kebiul seed extract (*Caesalpinia bonduc* (L) Roxb.), where ethanol extract 96% of kebiul seeds (*Caesalpinia bonduc* (L) Roxb.) has the highest antioxidant activity compared to ethyl acetate extract and n- Hexan kebiul seeds (*Caesalpinia bonduc* (L) Roxb.).

SUGGESTIONS

Researchers are further advised to test the antioxidant activity of kebiul seed ethanol extract (*Caesalpinia bonduc* (L) Roxb.) with the FRAP (Ferric Reducing Antioxidant Power) method.

References

- Andayani, R., Maimunah., & Lisawati, Y. (2008). Determination of Antioxidant Activity, Total Phenolic and Lycopene Levels in Tomatoes (*Solanum A lycopersicum* L.). *Journal of Pharmaceutical Science and Technology*, 13(1), 31-37.
- Badarinath, A. K., Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A Review on In – vitro Antioxidant Methods: Comparisons, Correlations and Considerations. *International Journal of PharmTech Research CODEN (USA): IJPRIF*, 2(2), 1276-1285.
- Ministry of Health of the Republic of Indonesia. (1977). *Medika Indonesia Material Volume I*. Jakarta: Ministry of Health of the Republic of Indonesia.
- Ministry of Health of the Republic of Indonesia. (2000). *General Standard Parameters of Medicinal Plant Extracts*. Jakarta: Directorate General of Drug and Food Control.
- Dwitasari, O., Seno, D. S. H., & Safitri, M. (2018). Identification of Bioactive Compounds and α -Glucosidase Inhibition Activity of *Caesalpinia bonduc* Seed Extract In vitro. *Current Biochemistry*, 5(2), 12-20.
- Gaur, R. L., Sahoo, M. K., Dixit, S., Fatma, N., Rastogi, S., Kulshreshtha, D. K., & Murthy, P. K. (2008). Antifilarial Activity of *Caesalpinia bonduc* Against Experimental Filariasis Infections. *Indian Journal of Medical Research*, 128(1), 65.
- Handayani, T. R., & Yuliani, S. (2016). Effect of Castration Fruit Ethanol Extract (*Caesalpinia bonduc* L. Roxb) on Sprague Dawley-Induced Rat Kidney Stones Ethylene Glycol 0.75% and Ammonium Chloride 2%. *Pharmaceutical Media: Journal of Pharmaceutical Sciences*, 13(2), 227-236.
- Kanerkar, U. R., Bhogankar, P. Y., & Indurwade, N. H. (2015). Antispermogenic Effect of *Caesalpinia bonduc* (L.) Roxb. Seeds. *Int. Res. J. of Science & Engineering*, 3(4), 173-178.
- Ministry of Health of the Republic of Indonesia. (2010). *Supplement I Pharmacopoeia Herbal Indonesi*. Jakarta: Ministry of Health of the Republic of Indonesia.
- Khan, H. U., Ali, I., Khan, A. U., Naz, R., & Gilani, A. H. (2011). Antibacterial, Antifungal, Antispasmodic and Ca^{++} Antagonist Effects of *Caesalpinia bonduc*. *Natural Product Research*, 25(4), 444-449.
- Maesaroh, K., Kurnia, D., & Anshori, J. A. (2018). Comparison of DPPH, FRAP and FIC Antioxidant Activity Test Methods Against Ascorbic Acid, Gallic Acid and Quercetin. *Chimica et Natura Acta*, 6(2), 93-100.
- Molyneux, P. (2004). The Use of the Stable A Free Radical Diphenylpicryl- Hydrazyl (DPPH) for Estimating Antioxidant Activity. *Songklanakar J. Sci. Technol*, 26(2), 211-219.
- Prasanto, D., Riyanti, E., & Gartika, M. (2017). Test the antioxidant activity of garlic extract (*Allium sativum*). *ODONTO: Dental Journal*, 4(2), 122-128.
- Reiza, I. A., Rijai, L., & Mahmudah, F. (2019). Phytochemical Screening Of Exochemicals Extracted Ethanol Skin Pineapple (*Ananas comosus* (L.) Merr). *In Proceeding of Mulawarman Pharmaceuticals Conferences*. 10(1), 104-108.
- Rezaeizadeh, A., Zuki, ABZ., Abdollahi, M., Goh, YM., Noordin, MM., Hamid, M., & Azmi, TI (2011). Determination of antioxidant activity in methanol extract and chloroformic *Momordica charantia*. *Journal of African Biotechnology*, 10(24), 4932- 4940.

- Rosidah., & Tjitraesmi, A. (2018). The potential of Melastomataceae plant as an antioxidant. *Farmaka*, 16(1), 24-35.
- Sa'adah, H., & Nurhasnawati, H. (2015). Comparison of Ethanol and Water Solvents in the Manufacture of Tiwai Onion Bulb Extract (*Eleutherine americana* Merr) using the Maceration Method. *Scientific journals of profit*, 1(2), 149-153.
- Sachan, N. K., Verma, S., Sachan, A. K., & Arshad, H. (2010). An Investigation to Antioxidant Activity of *Caesalpinia bonducella* seeds. *Annals of Pharmacy and Pharmaceutical Science*, 1(2), 88-91
- Sakihama, Y., Cohen, M. F., Grace, S. C., & Hamasaki, H. (2002). Plant Phenolic Antioxidant and Prooxidant Activities: Phenolics – Induced Oxidative Damage Mediated by Metals in Plants. *Toxicology*, 177(1), 67-68.
- Sayuti, K., & Yenrina, R. (2015). *Natural and Synthetic Antioxidants*. Field: Andalas University Press.
- Shukla, S., Mehta, A., John, J., Singh, S., Mehta, P., & Vyas, S.P. (2009). Antioxidant Activity and Total Phenolic Content of Ethanolic Extract of *Caesalpinia bonducella* Seeds *Journal of Food & Chemical Toxicology*, 47(8), 1848-1851.
- Shukla, S., Mehta, A., Mehta, P., Vyas, S. P., Shukla, S., & Bajpai, V. K. (2010). Studies on Anti-inflammatory, Antipyretic and Analgesic Properties of *Caesalpinia bonducella* F. Seed Oil in Experimental Animal Models. *Food and Chemical Toxicology*, 48(1), 61-64.
- Sie, J. O. (2013). Antioxidant Power of Mangosteen Peel Ethanol Extract (*Garcinia mangostana* Linn.) Stirring and reflux results. *Calyptra*, 2(1), 1- 10.
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical Screening and Extraction: A review. *Internationale Pharmaceutica Scientia*, 1(1), 98-106.
- Tukiran., Wardana, A. P., Nurlaila, E., Santi, A.M., & Hidayata, N. (2016). Initial Analysis of Phytochemicals on Methanol Extract of *Syzygium* Plant Stem Skin (Myrtaceae). *Prosiding National Seminar in Chemistry and Workshop*, 12(6), 38-43.
- Widodo, H., Rohman, A., & Sismindari. (2018). Utilization of Fabaceae Family Plant for the Treatment of Liver Disease by Traditional Ethnic Treatment in Indonesia. *R&D Media*, 29(1), 65 – 88.