Jurnal Akta Kimia Indonesia
INDONESIA CHIMICA ACTA

PAPER

View Article Online
View Journal View Issue

Cite this: *Indo. Chim. Acta.*, 2022, **15**, 1.

Analysis of Total Phenolic Content of Siam Weed Leaf Extract (*Chromolaena odorata* L.) and Antibacterial Activity Test Against *Escherichia coli* and *Staphylococcus aureus*

Yusriadi[1]*, Mega Fia Lestari[1], Hasri[2], Maryono[2], Netti Herawati[2]

Received Date:
27th May 2022
Accepted Date:
26th September 2022

Keywords:
Antibacterial;
siam weed leaves;
total phenolic

DOI: http://dx.doi.org/
10.20956/ica.v15i1.21074

Abstract. This study aimed to determine the total phenolic content of extracts of water, methanol, and ethyl acetate of Siame weed leaves and to test their bioactivity against *E. coli* and *S. aureus*. This research includes testing of active compound groups and determination of total phenolic and bioactivity tests. The total phenolic content was determined by the Folin-Ciocalteu method using a UV-Vis spectrophotometer. The results of the analysis showed that the extracts of water, methanol, and ethyl acetate were 54.0647; 44.9289; and 9.2005 mg GAE/g extract. The results of the bioactivity test of aqueous extracts were greater than that of methanol and ethyl acetate extracts where the minimum inhibitory concentration (MIC) of aqueous extracts against *E. coli* bacteria was at a concentration of 0.1%, while against *S. aureus* bacteria the bioactivity of methanol extracts was greater than that of aqueous extracts and ethyl acetate concentrations. minimum inhibitory (MIC) 0.5%. These results prove that Siam weed leaf extract can be used as an antibacterial agent.

Introduction

Indonesia is a mega-biodiversity country that is rich in medicinal plants and has the potential to be developed, but it has not been managed optimally. The natural wealth of plants in Indonesia includes 30,000 plant species out of a total of 40,000 plant species in the world, 940 species of which are medicinal plants (this number is 90% of the total medicinal plants in Asia) (Ministry of Forestry, 2010).

One of the plants that have potential as medicine is the Siam weed (*C. odorata* L.). This weed originally came from South and Central America, spread to tropical areas of Asia, Africa, and the Pacific, classified as an invasive weed. This plant has a characteristic triangular-shaped leaf that has three clearly visible leaf bones and when squeezed feels a very pungent odor with white compound flowers. (Prawidariputra. 2007). Siam weed leaves' benefits are treating leech bites, soft tissue wounds, burns, and skin infections. Siam weed leaves are traditionally used as medicine in wound healing, mouthwash for the treatment of sore throat, cough medicine, malaria medicine, antibacterial, headache, antidiarrheal, antihypertensive, astringent, antispasmodic, antioxidant anti-inflammatory, treating diabetes, anticholesterol, anticancer, and diuretic (Ikewachi and Ikewachi, 2011). The properties possessed by this plant due to the presence of secondary metabolite compounds. Siam weed leaves contain active compounds including alkaloids, flavonoids, saponins, and tannins, phenolic compounds such as protocatechuic, p-coumarin, ferulic, p-hydroxybenzoate, vanillic acid (Omokhua, 2015), and L-asparaginase enzyme (Yusriadi et al.). Phenolic compounds are reported to inhibit the growth and metabolism of a microbe (Hermawan and Laksono. 2013). However, no studies report the total phenolic content in Siam weeds.

Phenolic compounds have a solubility between polar and semipolar (Weecharangsan et al. 2006). Therefore, in this study, methanol (polar) and ethyl acetate (semipolar) and

[1]Departement of Chemical Analysis, Manufacturing Industry Community Academy of Bantaeng, 92461, Bantaeng, Indonesia; ***Email: yusri@akom-Bantaeng.co.id***
[2]Chemistry Study Program, Faculty of Mathematics and Natural Sciences, State University of Makassar, 90221, Makassar, Indonesia.

water were used as a solvent because people used to use these plants by dripping it into and then squeezing it remove the extract or boiling it.

One of the bacteria that spread disease to the community is *S. aureus* which is the main cause of skin disease, and *E. coli* which is the bacteria that causes digestive disease (Wertheim et al. 2005). To overcome the spread of disease from bacteria, an antibacteria is needed. However, recently it has been found that many types of bacteria are resistant to synthetic antibacterial medicine. With the emergence of antibiotic resistance, the need to look for alternative antibiotics has increased, including antibiotics derived from plants.

Based on the description above, we report the determination of the total phenolic content of siam weed leaves extracts and their bioactivity against *S. aureus* and *E. coli*.

Experimental

Materials and Method

Materials

The materials used in this study were Siam weed leaves, filter paper, methanol, ethyl acetate, Liebermann-Burchard, HCl 1%, Mg powder, Mayer reagent, Wagner reagent, FeCl 1%, gallic acid, Na_2CO_3 20%, Folin-Ciocalteu reagent, synthetic nutrient agar (NA) medium, *E. coli*, *S. aureus*, 0.9% NaCl solution, paper disc, and antalgic.

Method

Sample preparation and extraction

Siam weed leaves were cleaned and then dried at room temperature and then crushed. Then 700 g of Siam weed leaf powder were macerated with methanol ethyl acetate and water for 3 x 24 hours each, then filtered. Each macerate was evaporated until a thick extract is obtained and then evaporated to form a powder.

Phytochemical Test

Alkaloids. A few drops of extract were added with Mayer and Wagner reagents. The positive result indicated by yellowish-white precipitate (Mayer reagent) and brown precipitate (Wagner reagent).

Flavonoids. A few drops of the extract solution were added to a few drops of concentrated HCl and Mg powder. A positive test was marked red solution.

Terpenoids and steroids. A few drops of the extract solution were added with a few drops of Liebermann-Burchard reagent. A positive test is the presence of terpenoids (red-orange or purple) while the presence of steroids (green to blue).

Saponins. 0.5 g of extract was added with 0.5 mL of hot water and shaken for 1 minute. The solution was observed if it foamed, then 1% HCl was added and waited for 10 minutes, if the foam persisted then the extract was positive for saponins.

Tannins. 0.5 g extract of the sample from the extraction was added with 1-2 mL of water and 2 drops of 1% FeCl. The test is positive, the colour is bluish-green.

Determination of total phenolic content using the Folin-Ciocalteu method

Determination of maximum wavelength. A solution with a concentration of 5 ppm was made, then a 0.2 mL pipette was added and 15.8 mL of distilled water was added and 1 mL of Folin-Ciocalteau reagent was added and shaken until homogeneous. The solution was then allowed to stand for 10 minutes then added 3 mL of 20% Na_2CO_3 solution and then homogenized. measured using a UV-Vis spectrophotometer with a wavelength of 400-900 nm with an interval of 0.5 nm. Then the maximum wavelength is determined based on the highest absorbance value.

Preparation of gallic acid calibration curve. Gallic acid solutions were made with concentrations of 5, 10, 20, 30, 40, and 50 mg/L from the mother liquor using the Folin-Ciocalteau reagent method. Then a calibration curve was made by connecting the gallic acid concentration (mg/L) with the absorbance.

Determination of total phenol content. Weighed 0.1g of the extracted sample and then dissolved it to 10 mL with distilled water and then determined using the Folin-Ciocalteau reagent method. Repeat 3 times so that the phenol content obtained was obtained as mg gallic acid equivalent/g fresh sample.

Antibacterial test

Pure culture rejuvenation. The test bacteria in the form of *S. aureus* and *E. coli* were taken 1 ose of aseptically available agar medium, then inoculated by scratching on nutrient agar (NA) medium, and then incubated at 37°C for 24 hours.

Preparation of test microbial suspension. 2 oses of rejuvenated bacteria were taken from the available agar medium aseptically, then put into a test tube containing 3 mL of 0.9% NaCl solution, then vortexed.

Test sample preparation. Each extract of methanol, ethyl acetate, and water was then made at a concentration of 5% which was made by weighing 0.5 g of extract powder and then dissolved with 10 mL of distilled water and shaken until homogeneous.

Antibacterial activity testing with agar diffusion method. A total of 7.5 g of NA medium was dissolved in 150 mL of distilled water and sterilized in an autoclave at 121°C with a pressure of 1 atm for 20 minutes. Next, the medium was poured into a petri dish. After the NA medium was prepared, the paper disc was put into a 5% extract solution. Then proceed with the determination of MIC at an extract concentration of 2.0%; 1.5%; 1.0%; 0.5%; 0.1%; 0.05 and

0.01. Let stand for 15 minutes so that the extract seeps into the paper disc. Each suspension of the test bacteria *S. aureus* and *E. coli* was scratched using a cotton bath on the surface of the media so that it was evenly distributed. Then the paper disc that has been soaked in the extract solution is placed carefully and aseptically on the surface of the agar media that has been scratched by the test bacteria. Then it was incubated for 24 hours at 37°C. As a positive control, antalgic and aquabidest were used as a negative control. The test was carried out in triples. After the incubation period, the antibacterial activity was indicated by the presence of a clear zone around the paper disc and then measured using a caliper.

Result and Discussion

Test of active compounds

Based on the results of phytochemical tests, it is known that the extract of water, methanol, and ethyl acetate of *C. odorata* L. leaves contain alkaloids, flavonoids, and tannins, saponins, and terpenoids. The result of phytochemical test can be seen in the Table 1.

Table 1. Phytochemical Test

Chemical compound	Reagent	Extract Results		
		H_2O	Methanol	Ethyl acetate
Alkaloids	Meyer	+	+	+
	Wegner	+	+	+
Flavonoids	HCl p.a + Mg powder	+	+	+
Terpenoids	Lieberman-Burchard	+	+	+
Steroids		-	-	-
Tannins	$FeCl_3$ 1%	+	+	+
Saponins	Hot water	+	+	+

Note: (+) : Positif (-) : Negatif

Analysis of total phenolic content

The maximum wavelength is carried out to get the maximum absorption wavelength. The theoretical maximum wavelength for the phenolic test is 764 nm (Pourmorad et al., 2006). The maximum wavelength is 762 nm (Figure. 1a). The following is the spectrum of the maximum wavelength measurement results obtained:

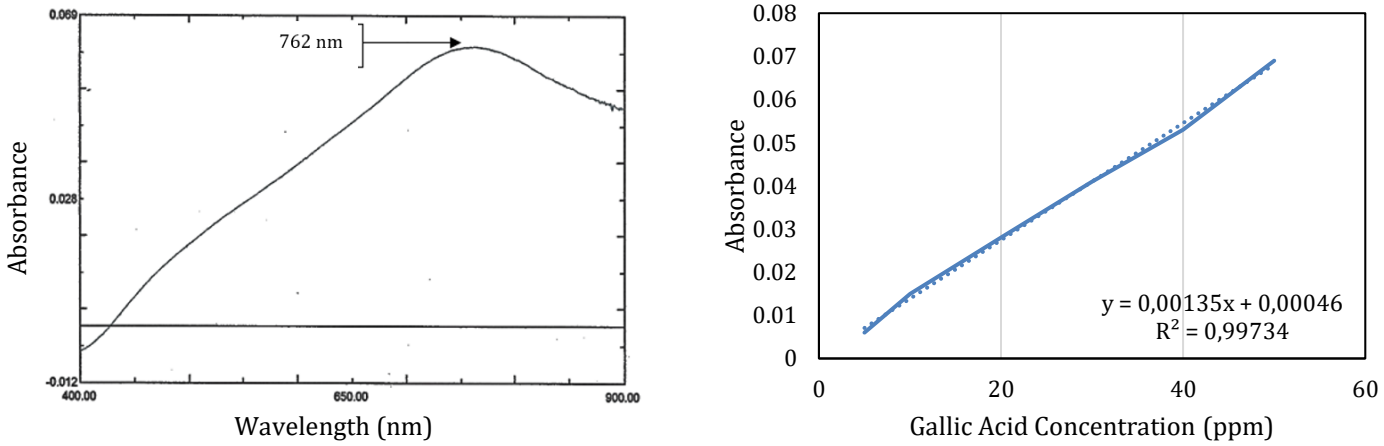

Figure 1. (a) The maximum wavelength spectrum of gallic acid; (b) Gallic acid standard curve

The total phenolic content in the Siam weed leaf extract obtained was determined as the equivalent mass of gallic acid. The maximum wavelength spectrum of gallic acid and Gallic acid standard curve could be seen in Figure 1. Gallic acid was used as a comparison compound because gallic acid is a heteropoly acid which has 3 phenolic hydroxy groups. The phenolic hydroxy groups will be oxidized by the Folin-Ciocalteu reagent under alkaline conditions. Folin-Ciocalteu reagent will oxidize gallic acid in its phenolic hydroxy group to form a molybdenum-tungsten complex which has a blue colour (Alfian and Susanti, 2012). During the reaction, the molybdenum ion (Mo^{6+}) is reduced to Mo^{5+} which causes the colour of the solution to turn blue (Prior, 2005). The reaction could be seen in Figure 3.

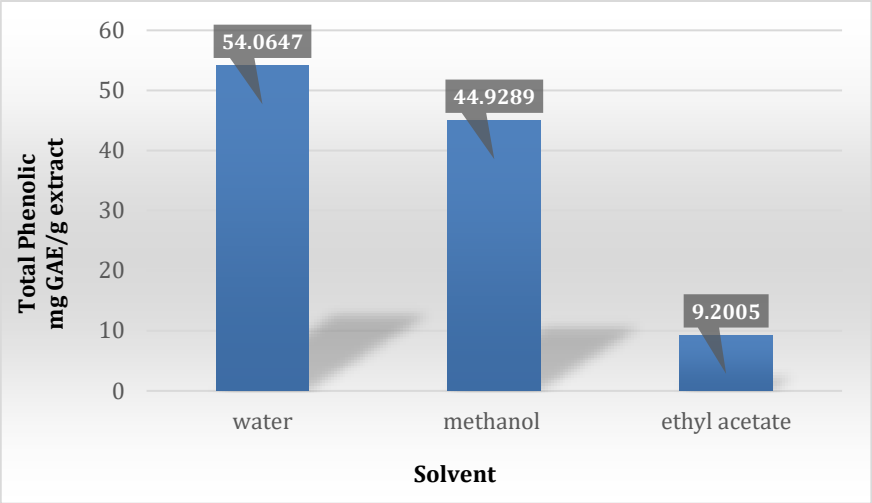

Figure 2. Graph of Total Phenolic Content.

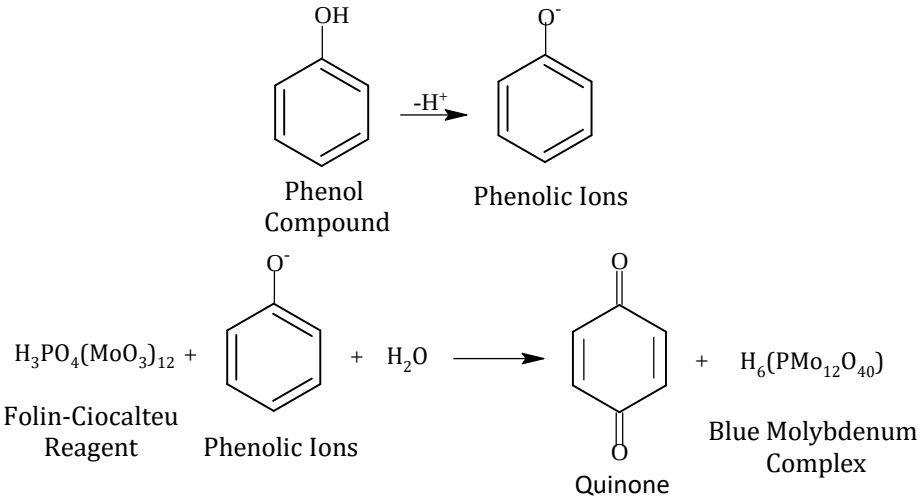

Figure 3. The reaction of Follin-Cioucalteu reagent with phenol compounds (Tursiman and Risa, 2012).

The total phenolic content in the Siam weed leaf extract obtained was determined as the equivalent mass of gallic acid. The calculation results showed that the highest total phenolic content of Siam weed leaf extract was found in water, methanol, and ethyl acetate extracts, respectively, at 54.0647 mg GAE/g extract, 44.9289 mg GAE/g extract, and 9.2005 mg GAE/g extract as shown in Figure 2. The results showed that the number of phenolic compounds obtained in polar solvents was greater than in semipolar solvents. The high yield in polar solvents indicates the ability to extract more bioactive components which have higher polarity. Phenolic compounds are polar because their structure is composed of several hydroxyl groups. Therefore, in general, phenolic compounds are more likely to dissolve in polar solvents.

Antibacterial activity testing

This test aims to determine the antibacterial activity of extracts of water, methanol and ethyl acetate of Siamese weed (*C. odorata* L.) leaves on the growth of *E. coli* and *S. aureus* bacteria. Antibacterial activity is influenced by several factors, including the concentration of the extract, the content of antibacterial compounds, the diffusion power of the extract, and the type of bacteria inhibited (Jawetz et al., 1996). From the test of secondary metabolite compounds on several Siamese weed leaf extracts, it is known that Siamese weed leaves contain alkaloids, saponins, phenolic compounds, flavonoids, and terpenoids. These compounds are known to have antibacterial properties (Maliana et al., 2013).

The mechanism of inhibition of bacterial growth by a class of phytochemical compounds has different activities. According to Trease and Evans (1978), the class of flavonoid compounds can denature proteins which cause the metabolic activity of bacterial cells to stop. The availability of alkaloids can

interfere with the formation of the constituent components of peptidoglycan in bacterial cells so that it can cause bacterial cells to lyse. Terpenoids can cause the lysis of bacterial cells by binding to proteins, lipids, or carbohydrates contained in cell membranes (Harborne, 1987). Saponin compounds can work as bacteriostatic by damaging the cytoplasmic membrane (Robinson, 2005).

Based on the results of the preliminary test of water extract, methanol ethyl acetate at a concentration of 5% was able to inhibit the growth of *E. coli* and *S. aureus* bacteria in Figure 4. The three types of Siam weed leaf extract tend to be more active in inhibiting *E. coli* bacteria than *S. aureus*.

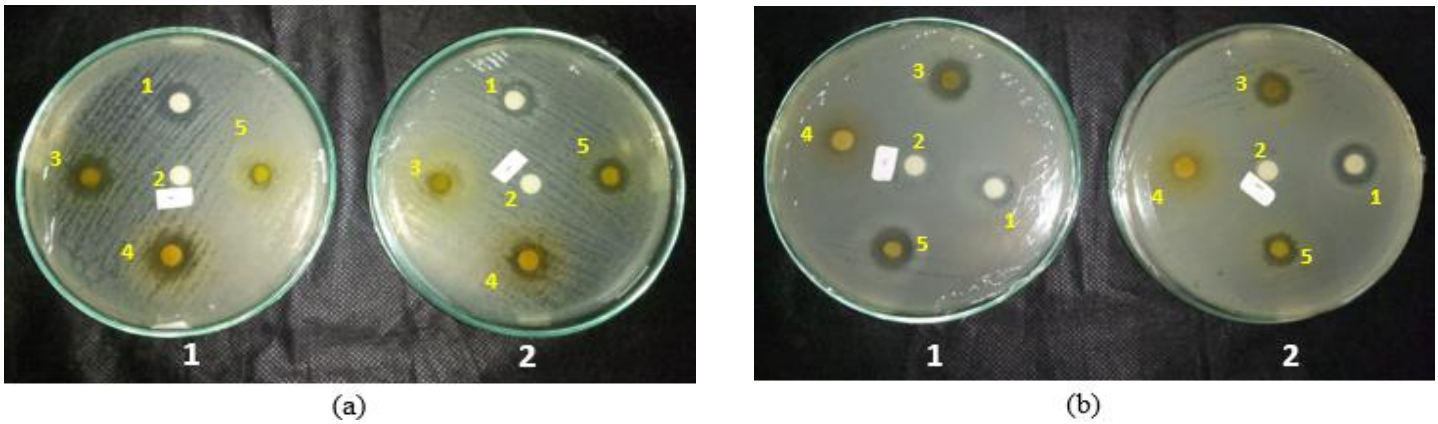

Figure 4. The preliminary test of (a) *E. coli* and (b) *S. aureus*. (1) Antalgin 5%, (positive control), (2) Aquades (negative control), (3) Methanol extract 5%, (4) Water extract 5%, (5) Ethyl acetate extract 5%.

Table 2. Inhibition of *C. odorata* L. Leaf Extract against *E. coli*

Extract	Barrier Diameter (mm)	
	1	2
Water extract 5%	15.75	13.55
Methanol extract 5%	15.25	13.65
Ethyl acetate extract 5%	14.55	12.15
Positive control (antalgin 5%)	14.95	13.15
Negative control (aquabidest)	-	-

Table 3 Inhibition of *C. odorata* L. Leaf Extract against *S. aureus*

Extract	Barrier Diameter (mm)	
	1	2
Water extract 5%	9.25	8.55
Methanol extract 5%	13.85	11.85
Ethyl acetate extract 5%	11.95	10.55
Positive control (antalgin 5%)	12.35	12.95
Negative control (aquabidest)	-	-

Based on the test results in Table 2 dan 3, the research obtained MIC against *E. coli* bacteria in 0.1% aqueous extract, 0.1% methanol and 0.5% ethyl acetate while against *S. aureus* 0.5% water extract, 0.5% methanol and ethyl acetate 0.5 %. Based on the test results, at a concentration of 0.1%, each extract could not inhibit the growth of *S. aureus* bacteria while the water and methanol extracts of *E. coli* bacteria still inhibited the growth of bacteria.

The results showed that water and methanolic extracts had a greater inhibitory ability against *E. coli* bacteria than ethyl acetate extract, which was indicated by a very small MIC value. Meanwhile, for *S. aureus* bacteria, the MIC of the three types of extracts was the same, but the inhibitory power of the methanol extract was greater. This result is supported by the theory which states that Gram-negative bacteria have thinner cell walls consisting of 10% peptidoglycan, while Gram-positive bacteria have 60% so that the cell walls of Gram-negative bacteria are more easily damaged when compared to Gram-positive bacteria. This ability is thought to be due to the content of secondary metabolites they contain, especially in

the phenolic compound group. Therefore, it is important to carry out further research related to the compounds contained in Siam weed leaves.

Conclusion

Based on the results of the study it can be concluded that siam weed leaf extract (*Chromolaena odorata* L.) has a total phenolic content of water extract at 54.0647 mg GAE/g extract, methanol extract of 44.9289 mg GAE/g extract, and ethyl acetate extract of 9.2005 mg GAE/g extract. the antibacterial activity of aqueous extracts was greater than that of methanol and ethyl acetate extracts against *E. coli* bacteria at an extract concentration of 5% with a minimum inhibitory concentration (MIC) of 0.1%. Meanwhile, against *S. aureus*, the antibacterial activity of methanol extract was greater than that of aqueous extract and ethyl acetate at an extract concentration of 5% with a minimum inhibitory concentration (MIC) of 0.5%. and the total phenolic content in Siam weed leaf extract (*C. odorata* L.) affects the inhibition of *E. coli* and *S. aureus* bacteria. Whereas against bacteria *E. coli*, the phenolic content is proportional to antibacterial inhibition, while against *S. aureus* the phenolic content is not comparable to antibacterial inhibition but still has an influence in terms of the ability to inhibit bacteria.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

Alfian, R., Susanti, H. (2012). Determination of Total Phenolic Concentration of Methanol Extract of Red Rosella Flower Petals (*Hibiscus sabdariffa* L.) with Spertrophotometric Growing Place Variations. *Jurnal Ilmiah Kefarmasian*, 2(1): 73-80.

Harbone, B.J. (1987). P*hytochemical Methods: A Guide to Modern Ways of Analyzing Plants*. Translators: Kosasih Padmawinata and Iwang Soediro. Bandung: ITB.

Hermawan, G. P. and Laksono. H. 2013. Soursop Leaf Extract Using Ethanol Solvent. *Jurnal Teknologi Kimia dan Industry*, 2(2) 111-115.

Ikewachi CJ and Ikewachi CC. (2011). *Alteration of Plasma Lipid Profiles and Atherogenic Indices by Staachytarpheta Jamaicensis L. (Vhal).* NSEB 71-77.

Jawetz. E., Melnick. J., Adelberg, E. (1996). *Medical Microbiology*, Edition 20. Jakarta: EGC.

Marliana, et al. (2005). Photochemical Screening and Thin Layer Chromatography Analysis of Chemical Components of Chayote (Sechium edule Jacq. Swartz.) Fruit in Ethanol Extract. *Biofarmasi,* 3(1): 26-31.

Ministry of Forestry. (2010). Regulation of the Minister of Forestry of the Republic of Indonesia Number P.49/Menhut-II/2010 concerning Village Forests. Jakarta: Ministry of Forestry.

Omokhua, A. G. (2015). Phytochemical and Pharmacological Investigations of Invasive *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae). Tesis Master in College of Agriculture, Engineering, and Science, University of KwaZulu-Natal. Afrika Selatan: University of KwaZulu-Natal.

Pourmorad, F., *et al*. (2006). Antioxidant Activity, Phenol, and Flavonoid Content of Some Selected Iranian Medicinal Plants. *African Journal of Biotechnology* 5(11) 1142-1145.

Prawidariputra. (2007). Kirinyu (*Choromolaena odorata* (L) R.M. King and H Robinson) Harmful Pasture Weeds. *Indonesian Animal Husbandry Science Bulletin* (*WARTOZOA*), 17(1): 46-53.

Prior, R.L., Wu, X., and Schaich, K. (2005). Standardized Method for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements, *J. Agric Food Chem,* 53: 4290-4302.

Robinson, T. (2005). *High Plant Organic Content*. Bandung: ITB.

Trease, G.E. and Evans, W.C. 1978. *A Textbook of Pharmacognosy*. 11th Edn, London BailliereTindal.

Tursiman, A. Puji and Risa, N. (2012). Total Phenol Ethyl Acetate Fraction from Kandis Acid Fruit (*Garcinia dioica* Blume). *Jurnal JKK*. 1(1): 45-48.

Weecharangsan, W. et. al*.* (2006). Antioxidative and neuroprotective activities of extracts from the fruit hull of mangosteen (*Garciniamangostana* Linn.). *Med Princ Pract,*15(4)281-287.

Wertheim, Heiman F. L. et al. (2005). *The Role of Nasal Carriage in Staphylococcus Aureus Infections.* Netherlands: Department of Medical Microbiology and Infectious Disease.

Yusriadi, Ahmad, A., Khaerah, N., Arfah, R., Karim, A. and Karim, H. (2019). Isolation, characterization and anticancer potential test of crude extract of L-asparaginase enzyme from siam weed leaf (*Chromolaena odorata* Linn): a novel source. *J. Phys.: Conf. Ser.,* 1341(032016): 1-10.