Journal of Health Management and Pharmacy Exploration
https://shmpublisher.com/index.php/johmpe
p-ISSN: 2985-4814
e-ISSN: 2985-5543

Comparison of ethyl acetat fracination of ganitrie *(Elaeocarpus sphearicus)* seeds as anti bacterial of *Staphylococcus aureus* and determination of total flavonoid content

Yunita Kurnia Sari[1], Ririn Suharsanti [2], Wulandari [3*]
[1,2,3]Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang, Indonesia

Article Info

Article history:

Received February 04, 2024
Revised February 28, 2024
Accepted February 29, 2024

Keywords:

Ganitri seeds
Exctract
Fraction
Total flavonoid
Staphylococcos aureus

ABSTRACT

Infectious disease is a disease coused by the presence pf pathogenic microbes. Stahpylococcus aureus is a gram positive aerobic bacterium that couses phyogenic infections on humans. Antibiotics are the best choise to treat an infections. The purpose of this study was to determine the antibacterial activity of ganitri seed (Elaeocarpus sphearicus) to determine the exstract ratio, the ethyl acetat fractination of ganitri seeds woth a concetration of 35% on the growth of staphylococcus aureus bacteria and to determine the total flavonoid content. Exctraction was carried out by maceration method for 3x24 hours with 96% ethanol as solvent. Exctraction fractination using 2 methods, the first method is using 30% ethanol, cloroform, and ethyl acetat as solvent. The second fractination method uses hot destiled water and ethyl acetat as solvents. The test result of total flavonoid content in ganitri (Elaeocarpus sphearicus) seeds in ethanol exstract were 38.009 mgEQ / g, fraction 1 of 99.512 mgEQ / g, fraction 2 of 68.235 mgEQ / g. From the result of the determinatoin of the ethyl acrtat fraction 1 had highest yield with an average value of 99,512 mgEQ/g. The antibacterial activity test was 35% in each sample ang using a positive control of ciprofloxcacin and a negative control of DMSO. Based on the result of the clear zone of the ethanol extract was 1.118 cm, the ethyl acetate 1 fraction was 1.170 cm, and the ethyl acetat 2 fraction was 0,956 cm.

Corresponding Author:
Wulandari,
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang,
Indonesia
Email: wulandari@stifar.ac.id
DOI: https://doi.org/10.52465/johmpe.v2i1.291

1. Introduction

An infectious disease is a disease caused by the presence of pathogenic microbes [1], [2]. *Staphylococcus aureus* [3] is a gram-positive bacterium. Infections caused by *Staphylococcus aureus* [4] are such as ulcers, pastula, pemphigus neonatorum, mastitis and food poisoning [5].

Antibiotics [6] are the best choice for an infection. The use of antibiotics often causes side effects to users so it is necessary to alternative natural antibiotics [7] that become antibacterial compounds and have few side effects for users. One plant that can be used as a natural antibiotic is ganitri seeds *(Elaeicarpus sphearicus).*

Based on research [8] states that ganitri seeds have abilities as sedative, antihypertensive [9], antiepileptic, antidepressant, antidiabetic, analgesic [10], anti-inflammatory and antibacterial. From the results of the study [11] Compounds contained in ganitri seed extract that can be used as antibacterial are flavonoid compounds, alkaloids, tannins, and glycosides.

2. Method

2.1. Tools and materials

UV-Vis spectrophotometer, LAF, petri dish, measuring flask, Erlenmeyer, beaker glass, micropipette, incubator, autoclave, Bunsen, test tube, cola fabric, split funnel, stative, clamps, chamber, silica gel GF 254nm, Ganitri seed simplicia (Elaeocarpus sphearicus), ethanol 96%, ethyl acetate, chloroform, distilled water, Mannitol Salt Agar (MSA), Dimethyl sulfoxide (DMSO) Nutrient Agar (NA), Nutrient Broth (NB), Staphylococcus aureus, ciprofloxacin, Mc solution .Farland 0.5, quercetin standard, ethanol p.a , AlCl3, sodium acetate.

2.2. Extraction ganitri seeds

How the extraction of ganitri seeds (Elaeocarpus sphearicus) that have been powdered is weighed and put in a vessel, add 96% ethanol, let stand for 5 days, then filter and evaporate to get a thick extract of ganitri seeds *(*Elaeoarpus sphearicus.

2.3. Fractionation method of ethyl acetate

Method 1 : 96% ethanol extract weighed 10 grams, added 30% ethanol as much as 25 ml, input the split funnel added chloroform (1: 1) ethanol phase added ethyl acetate to produce clear ethyl acetate, ethyl acetate [12] phase concentrated and obtained ethyl acetate fraction [13].

Method 2 : 96% ethanol extract weighed 10 grams, add 200 ml of hot aquadest. Put a split funnel plus 200 ml of ethyl acetate, corner for 1 minute there is a separation between the ethyl acetate phase and the water phase, add ethyl acetate until it produces clear ethyl acetate. The ethyl acetate phase is removed and the ethyl acetate fraction is obtained [13].

2.4. Identification of flavonoid compounds

The sample was added with 0.1 grams of magnesium powder, 1 ml of hydrochloric acid, 2 ml of amyl alcohol, shaken and allowed to separate. Identification by thin layer

chromatography using the eluent butanol:acetic acid:water (4:1:5) and spotting of ammonia vapor gives a yellow stain.

2.5. Determination of total flavonoid levels of ganitri seed extract

As much as 100 mg dissolved in 10 ml of methanol, taken 1 ml add 3 ml of methanol, 0.2 ml of 10% AlCl3, 0.2 ml of potassium acetate, add with aquadestilate to 10 ml, let stand 30 minutes in a dark place at room temperature, measured absorbance on UV-Vis spectrophotometry with a wavelength of 431 nm. The sample solution is made in three replications.

2.6. Antibacterial activation test

Sterile Mannitol Salt Agar (MSA) measured as much as 10 ml, wait for solidification, put a cylinder cup, pour back MSA media as much as 20 ml that has been mixed with 5µl of bacterial suspension after The cylinder cup solidification media is lifted, put each sample into the well hole as much as 20 µl, add ciprofloxacin positive control and DMSO negative control into the contribution, incubated at 37°C for 24 hours, measured the inhibitory zone using a caliper.

3. Results and Discussion

Based on the research that has been carried out, the extraction yield was obtained at 4.166%, ethyl acetate fraction method 1 obtained yield results of 8.54% and in method 2 as much as 8.042%. From the results of phytochemical screenings in all samples contained steroid compounds, flavonoids, tannins, alkaloids, and saponins. In KLT testing all samples were also positive for steroid compounds, flavonoids, tannins, alkaloids and saponins. For testing total flavonoid levels in ganitri seed samples, the equation y = 0.0047x + 0.2387 with a correlation coefficient value of 0.9994. The following is a table of test results of flavonoid compounds in ganitri seeds *(Elaeocarpus sphericus).*

Table 1. Total flavonoid content test results

Replication	Extract (mg EQ/g)	Fraction 1 (mg EQ/g)	Fraction 2 (mg EQ/g)
1	36.577	99.104	69.644
2	38.264	99.881	68.254
3	37.346	99.754	66.628
4	38.966	99.361	68.869
5	38.891	99.463	67.780
Average	38.009 ± 0.921	99.512 ± 0.278	68.235 ± 1.017

Based on the results of the total flavonoid test, flavonoid levels were obtained with average results, namely in extracts of 38.009 mgEQ / g, fraction 1 of 99.512 mgEQ / g, fraction 2 of 68.235 mgEQ / g. Judging from these average results, the highest flavonoid level value is in the ethyl acetate fraction method 1 with an average of 99.512 mg EQ / g. Flavonoids are compounds commonly found in plants and have certain chemical properties that influence their solubility in various solvents. The solubility of flavonoids in certain solvents depends on their chemical structure. Among commonly used solvents such as n-hexane, ethyl acetate, and water, flavonoids tend to be more soluble in ethyl acetate solvent. This is

caused by the polarity of the solvent and the chemical properties of flavonoids. Ethyl acetate is a semi-polar solvent. Flavonoids have possible polarity properties interacts well with semi-polar solvents such as ethyl acetate. The hydroxyl groups (-OH) and carbonyl groups in the structure of flavonoids allow polar interactions with ethyl acetate, thereby increasing their solubility in this solvent [14].

Antibacterial activity test on ganitri seed samples (*Elaeocarpus sphearicus*) based on sample orientation results carried out at a concentration of 35% of samples can inhibit the growth of Staphylococcus aureus bacteria with a positive control, namely ciprofloxacin at a concentration of 0.005% and a negative control of DMSO. The results of the antibacterial activity test are shown in Table 2.

Table 2. Antibacterial activity test results

Replication	Extract (35%)	Fraction 1(35%)	Fraction 2 (35%)	Control +	Control -
1	1,140	1,130	0,950	1,470	0,000
2	1,100	1,200	0,970	1,480	0,000
3	1,100	1,180	0,950	1,490	0,000
4	1,130	1,170	0,970	1,480	0,000
5	1,120	1,170	0,940	1,480	0,000
Average	1.118 ± 0.016	1.170 ± 0.022	0.956 ±0.012	1.480 ± 0.006	0

Based on the results of antibacterial activity tests shown in Table 2. The extract and fraction at a concentration of 35% has antibacterial inhibitory power on Staphylococcus aureus bacteria. The results of antibacterial activity test values of ethanol extract and ethyl acetate fraction in ethyl acetate fraction results method 1 have the highest inhibitory value of 1.170 cm. Based on these data, ethyl acetate fraction 1 has the highest inhibitory power because there is method 1 using 96% ethanol suspended into 30% ethanol which is more likely to be polar. So it is expected that these flavonoid compounds can inhibit the growth of *Staphylococcus aureus bacteria*[13]. The antibacterial activity of the sample was smaller compared to the positive control Ciprofloxacin. Ciprofloxacin is a bactericidal antibiotic in the fluoroquinolone class of drugs. This drug works by inhibiting bacterial DNA topoisomerase and DNA gyrase replication. This drug is effective against several gram-positive bacteria [15].

4. Conclusion

In ethanol extract, ethyl acetate fraction 1 and ethyl acetate fraction 2 in ganitri seed samples *(Elaeocarpus sphearicus)* have inhibition of *Staphylococcus aureus* bactri growth and there is a significant difference between ethanol extract and fraction at a concentration of 35%, namely in extracts of 1.118 cm, fraction 1 1.170 cm, and fraction 2 0.956 cm. In testing flavonoid levels in the ethyl acetate fraction has the highest value of 99.512 mgEQ / g.

REFERENCES

[1] S. Alminsyah, Indria Hafizah, "Uji Daya Hambat Ekstrak Daun Tapak Kuda (Ipomoea pes caprae (L) R. Br.) Terhadap Staphylococcus aureus," *Medula*, vol. 2, no. Vol 2, No 1 (2014), hal. 91–96, 2014.

[2] N. Rübsamen, V. K. Jaeger, A. Zapf, M. Chaturvedi, dan K. Denise, "The impact of inaccurate assumptions about antibody test accuracy on the parametrisation and results of infectious disease models of epidemics," *Epidemics*, vol. 46, no. January, 2024, doi: 10.1016/j.epidem.2024.100741.
[3] A. Khatoon *et al.*, "Emerging novel sequence types of Staphylococcus aureus in Pakistan," *J. Infect. Public Health*, vol. 17, no. 1, hal. 51–59, 2024, doi: 10.1016/j.jiph.2023.10.036.
[4] A. Abdollahi, M. Nojomi, Y. Karimi, dan M. Ranjbar, "Heliyon Mortality patterns in patients with Staphylococcus aureus bacteremia during the COVID-19 pandemic : Predictors and insights," *Heliyon*, vol. 10, no. 2, hal. e24511, 2024, doi: 10.1016/j.heliyon.2024.e24511.
[5] Entjang, *Mikrobiologi dan Parasitologi untuk Akademi Keperawatan.* Bandung, 2001.
[6] M. E. P. Rijkx *et al.*, "Evidence for the use of peri - and post - operative antibiotic prophylaxis in autologous breast reconstruction : A systematic review," *J. Plast. Reconstr. Aesthetic Surg.*, vol. 83, hal. 404–414, 2023, doi: 10.1016/j.bjps.2023.04.038.
[7] J. Wang, L. Deng, M. Chen, Y. Che, L. Li, dan L. Zhu, "Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade," *Anim. Nutr. J.*, 2024, doi: 10.1016/j.aninu.2024.01.012.
[8] P. K. Dixit, S. Dixit, M. Bhardwaj, B. Chauhan, dan J. Sahoo, "Review Article A Review on Traditional and Ethnomedicinal Uses of Elaeocarpus ganitrus (Rudraksha)," *Int. J. Pharm. Sci.*, vol. 52, no. 01, hal. 1–7, 2018.
[9] A. G. U. Heisel *et al.*, "The use of specific antihypertensive medication and skin cancer risk : A systematic review of the literature and meta-analysis," *Vascul. Pharmacol.*, vol. 150, no. April, hal. 107173, 2023, doi: 10.1016/j.vph.2023.107173.
[10] L. M. Chen, C. Mirkazemi, dan F. C. Veal, "Patient Education and Counseling Are we educating patients about postoperative analgesics following orthopaedic surgery ? A scoping review," *Patient Educ. Couns.*, vol. 120, no. December 2023, hal. 108123, 2024, doi: 10.1016/j.pec.2023.108123.
[11] S. Krunal, R. P. Shaival, K. Rao Shaival, dan N. P. Jivani, "Pharmacognostical standardization of elaeocarpus ganitrus leaf. Family: Elaeocarpaceae," *Int. J. Pharmacogn. Phytochem. Res.*, vol. 4, no. 3, hal. 97–98, 2012.
[12] L. Lv *et al.*, "Catalytic oxidation of ethyl acetate over Y (Y = Cu , Mn , Co) -modified CeO 2 derived from Ce-MOF," *Catal. Commun.*, vol. 186, no. 1, hal. 106832, 2024, doi: 10.1016/j.catcom.2023.106832.
[13] suwijiyo pranomo Agustina dian puspita, "No Title," *perbandingan Metod. pembuatan ekstrak terpurifikasi bee Prop. dari lebah madu (Apis mellifera) berdasarkan kadar flavonoid Total dihitung sebagai rutin*, vol. 20, 2015.
[14] J. B. Harborne, *Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan*. 1996.
[15] A. Shariati *et al.*, "The resistance mechanisms of bacteria against ciprofloxacin and new approaches for enhancing the efficacy of this antibiotic," *Front. Public Heal.*, vol. 10, 2022, doi: 10.3389/fpubh.2022.1025633.