



Effect of Solvent Concentration of (*Tamarindus Indica L.*) Ethanol Leave Extract on Secondary Metabolite and Antibacterial Activity Against *Staphylococcus Aureus*

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Abstract

Pimple is a disease the biggest contributor to the problem health skin. Most pimple caused by the bacterium *Staphylococcus aureus*, seen from prevalence infection skin consequences the bacteria reach 62.8–90.0%. Tamarind leaves (*Tamarindus indica L.*) in a few study own activity hinder growth of bacteria Because There are potential flavonoids and phenolic compounds as antibacterial. The amount of the content is influenced by the solvent used. The research objective was to determine the effect of variations in solvent concentration on total flavonoid levels, total phenol levels and antibacterial activity against *Staphylococcus aureus bacteria* from tamarind leaf extract 5%, 10%, 15%, 20%, 25%. Tamarind leaf extract was obtained by maceration using various ethanol concentrations of 50%, 70%, 96%. Followed by the determination of the total levels of flavonoids and phenols using method colorimetry of $AlCl_3$ and folin ciocalteu in a manner *Uv-vis spectrophotometry*, while the antibacterial test was carried out using the well method. The results showed that the total levels of flavonoids and phenols at concentrations of 50, 70, 96% is 2.3241; 2.6251; 4.55 mgQE/g and 12.9237; 22.0794; 27.3647 mgGAE/g for phenolic value ($p < 0.05$). Antibacterial testing from concentration extract 5, 10, 15, 20, 25% for 50% solvent, namely 13.28; 14.34; 15.8; 16.61; 18.03 mm, at 70% solvent, namely 13.18; 14.77; 16.71; 17.52; 18.76 mm, whereas in 96% ethanol it is 13.30; 16.08; 17.42; 17.63; 18.94 mm with ($p > 0.05$). The more big concentration solvent the more big total levels of flavonoids, phenols, but not for activity its antibacterial.

INTRODUCTION

Pimple is one disease marked skin inflammation and swelling freckles red on the skin so that can interfere with appearance (Adianti, 2023) . in Indonesia disease common skin happen ranges from 85-100%. on women skin acne appear up to 83-85%, whereas condition acne in men reach 95-100% (Astrid Teresa, 2020) . One of the bacteria that often causes disease is *Staphylococcus aureus*. Bacteria *Staphylococcus aureus* can also cause mastitis infections, dermatitis (Dewa et al., 2019) . According to (Schaumburg et al., 2014) the prevalence of skin infections due to *Staphylococcus aureus* found ranged from 62.8–90.0%. Antibiotics are one of the therapies given to kill or inhibit the spread of bacterial infections, but inappropriate use can cause resistance . (Suyasa, 2020) . This is evidenced by as much as 45-91%

resistant to clindamycin (Madelina & Sulistiyaningsih, 2018) .

Tamarind leaves (*Tamarindus indica L.*) is one of them plant originating drug from Indonesia believed capable hinder growth bacteria Because own flavonoids and phenolic compounds (Yuliana et al., 2021) . According to research (Yuliana et al., 2021) compounds that play an active role as antibacterial in tamarind leaves is a group of polyphenolic compounds including flavonoids and tannins which have the potential to prevent bacteria from growing . The results of the study reported by (Yuliana et al., 2021) said that the ethanol extract of the leaves at levels of 50%, 60%, 70%, 80%, 90%, 100% could prevent bacteria that cause infection by as much as 7.98mm; 8.10 mm; 9.10 mm; 10.15 mm; 15.20 mm; 20.12 mm. The study results were also obtained from



(Hardianti et al., 2015) tamarind leaves concentration 1% can prevent MRSA bacteria from growing up 6.10 mm. The existence of variations in solvent concentration affects the amount of secondary metabolite content that can be extracted from tamarind leaves. Based on study from (Riwanti et al., 2020) mention variation concentration solvent ethanol 50%, 70%, 96% effect to total flavonoid content of ethanol extract *Sargassum polycystum* where the results showed that the highest total extract flavonoid content was found in 70% solvent. This achievement is also in line with studies from (Yunita & Khodijah, 2020) which states that variations in the concentration of ethanol solvent in macerated extraction affect the quercetin levels of tamarind leaves which produce quercetin levels of 24.684 mg/g using 70% ethanol solvent and 31.328 mg of 96% ethanol /g. Based on description above, then this research aims to determine the optimal variation in ethanol solvent concentration in attracting flavonoids and phenolic compounds, with variations in solvent concentrations it aims to see the ability of tamarind leaf extract to inhibit growth of *Staphylococcus aureus* bacteria.

RESEARCH METHODS

Equipment and Materials

laboratory glassware (pyrex[®]), digital scales (Ohaus), aluminum foil, stir bar, tissue, funnel, filter, 125 ml and 225 ml porcelain cup, filter paper, spatula spoon, rotary evaporator (Heidolph®, Germany), bottom flask (Duran Schott), label paper, test tubes, tube racks, measuring pipettes, dropping pipettes, loops, incubators, autoclaves, spirit lamps, waterbaths, moisture analyzers, petri dishes, calipers, flasks, LAF.

Tamarind leaves (*Tamarindus indica* L.), 50% ethanol, 70% ethanol, 96% ethanol, distilled water, *Staphylococcus aureus* bacteria, media MHA, McFarland 0.5, HCl 2N, dragendorff reagent, magnesium powder, methanol, FeCl₃, gelatin salt, aquadest, ethanol pa, AlCl₃ 10%, sodium acetate 1M, quercetin powder, DMSO 10%, clindamycin, Folin Ciocalteu reagent 10%, Na₂CO₃ 7.5%, crystal violet, lugol solution.

Research procedure

1. Determination

Determination plant tamarind leaves (*Tamarindus indica* L.) was carried out for know truth plant, sure the plants used isa plant tamarind and avoid mistakes when choosing samples. Determination plant carried out in the Laboratory Biology FMIPA Semarang State University

by preparing a section roots, stems, and leaves tamarind plant.

2. Extract Manufacturing Tamarind Leaves

Powder from young tamarind leaves 4 00 g am macerated with solvent ethanol 50%, 70%, 96% as much as 4 liters for 3x24 hours with stirring. The maceration results were then filtered to obtain a filtrate. Next evaporate with a rotary evaporator with a temperature of 50°C to obtain desired thickness.

3. Screening Test Phytochemicals

Alkaloids

Extract as much as 0.5 grams dissolved with 5 ml of 2N HCl, heated on a bath and filtered when cold. On the alkaloid test added reagent dragendorff if brick red precipitate formed, extract alkaloid positive (Meilia et al., 2022)

Flavonoids

0.5 gram of ethanol extract of tamarind leaves was dissolved in 2 ml of methanol, then stirred and mixed with 0.5 gram of magnesium powder and 3 drops of concentrated HCl. Tamarind leaf extract is positive which has flavonoids if it forms orange red (flavones), pale red (flavonols), dark red (flavonones) (Meilia et al., 2022)

Saponins

As much as 0.5 gram of extract was dissolved with 10 ml of distilled water, after cold it was shaken vigorously for 10 seconds. A positive gain is seen if within 10 minutes foam is seen with a height of 1-10 cm, and on the addition of 1 drop of 2N HCl, the foam does not disappear (Meilia et al., 2022)

Tannins

Enter the ethanol extract of tamarind leaves as much as 0.5 grams in a test tube and 10 ml of distilled water, then the sample is added with gelatin salt. In this test a positive gain can be seen from the appearance of a yellowish white precipitate (Abriyani et al., 2022).

Phenol

Extract as much 0.5 gram dissolved in 3 ml ethanol. The filtrate solution can be added with FeCl₃, polyphenol positive colored, blue, green black, brown black (Abriyani et al., 2022)

4. Determination of Total Flavonoid Content (TFC)

Wavelength Determination

Take 1 ml of serial solution lowest ppm quercetin added 3 mL of ethanol, 0.2 mL of 10% AlCl₃ and 0.2 mL of CH₃COO Na 1M, added 5.6 mL of distilled water leave for



30 minutes. Absorbance was measured at a wavelength of 400-450 nm (D. Nofita et al., 2020)

Determination Quercetin Standard Curve

Weigh 50 mg of quercetin dissolved in 50 ml of distilled water so that obtained liquor 1000 µg/ml. Furthermore preparation of concentration series from mother liquor as much 10, 20, 30, 40, 50 ppm.

Determination of Total Flavonoid Extract Levels

Weigh out 10 mg extract each concentration dissolve in 10 mL of ethanol pa. Take 1 ml of standard solution series quercetin and samples ethanol extract of tamarind leaves in a tube reaction. Each added 3 ml of ethanol , 0.2 ml AlCl₃ 10%, 0.2 ml of 1M anhydrous CH₃COONa, plus 5.6 ml of distilled water (Rusley et al., 2022) . Then homogenize, incubate for 30 minutes and do it readings at maximum wavelength using UV-Vis Spectrophotometry (Chang, 2022)

5. Determination of Total Phenol Content (TPC)

Wavelength Determination

Take 1 ml of gallic acid solution at the lowest ppm add 4.5 ml of reagent Folin Ciocalteu (1:10), then shaken and leave for 3 minutes. Insert 4.5 mL of 7.5% Na₂CO₃ solution . Shut up for 30 minutes, measure the absorbance at 700–800 nm (CN Putri et al., 2022) .

Determination Gallic Acid Standard Curve

As much 50 mg of gallic acid plus 50 ml of distilled water, dissolve until homogeneous to obtain a mother liquor of 1000 µg/ml. Then make a series of gallic acid solutions 10, 20, 30, 40, 50 ppm (D. Nofita et al., 2020) (CN Putri et al., 2022) .

10 mg of extract each sample dissolve 10 ml of aquadest (1000 ppm), make a sample solution concentration of 100 ppm. Then a lot of 1 ml of standard solution gallic acid series and each sample dissolved in 4.5 mL folin ciocalteau reagent (1:10), shaken. Leave it for 3 minutes, add 4.5 mL of 7.5% Na₂CO₃ solution. (D. Nofita et al., 2020) . Then the solution was incubated for 30 minutes and read at λ 757 nm with UV-Vis Spectrophotometry (Vongsak et al., 2013)

6. Antibacterial Activity Test Against *Staphylococcus aureus* s

Identification Bacteria

One ose bacteria *Staphylococcus aureus* put it on *the object glass* and fixed on Bunsen. drops prep with crystal violet and wait until 1 min. Next throw away remainder color and rinse it off using water drops prepare with Lugol's solution and let stand for 1 minute. Fade the color

with 96% alcohol until the dye disappears and wash it off with water. Then wash return using running water and dry, the preparation is diluted with 96% alcohol and let stand for 1 minute. The next step is dripping with safranin dye, which is then allowed to stand for 1 minute. Wash using running aquadest and dry using a tissue attached to the side review then let dry up. Identification carried out using a microscope with a magnification of 100x (Nugroho et al., 2022)

Creating Media Controls

On the media scratch 0.1 ml of bacteria *Staphylococcus aureus* to evenly. Then incubate the petri dish with a temperature of 20-25° C for 3-5 days in an inverted position until the media is solid. Observe and count the number of bacteria that grow (A. Putri et al., 2020)

Antibacterial Activity

Antibacterial test was performed using the well-diffusion method with MHA test media. MHA media was inoculated with 0.1 ml of *Staphylococcus aureus* bacteria until blended. C petri cloud that has a well made is filled 50 µl concentration (5%, 10%, 15%, 20%, 25%), DMSO as negative control, Clindamycin served as a positive control and was replicated 5 times. Then each petri dish was incubated for 24 hours at 37° C and observed the inhibition zone. (AD Nofita, 2021)

Data Analysis

Data results tested using SPSS with a non- parametric test that is *Kruskal Wallis*. Is known test results show value (p<0.05) so can followed by *Mann Whitney*, Coefficients Determination, as well *Pearson Correlation*.

RESULTS AND DISCUSSION

Determination Plant

Determination plant done for know the plant is a tamarind plant and avoids mistakes when selecting samples (Diniatik, 2015) . The results of the determination in the study showed that the tamarind plant used was the same as the expected family I, namely Fabaceae and was a species of *Tamarindus indica* L.

Extraction

Extraction on study This use method maceration with variation concentration solvent ethanol ie 50%, 70%, 96 % . There is use variation concentration solvent for know the most optimal concentration in extract metabolites secondary. Ethanol including type solvent are polar capable extract content compound metabolites polar



secondary compounds such as flavonoids and phenols (Riwanti et al., 2020) .

Yield results on the extract leaf sour java 50,70,96% of 2.65; 5.275; 6.575%. Yield value show many compound metabolites soluble secondary in the extract. The more Lots mark yield obtained _ so the more Lots content substance contained active in the extract. Acquisition yield on ethanol 96 % have mark the highest yield Because 96% ethanol is included in the solvent organic own ability For dissolve more compounds. Factor big its small yield influenced by type solvents , comparison

amount sample to amount solvent as well as the long processing time extraction (Sunnah et al., 2021)

Screening Phytochemicals Extract Tamarind Leaves

Objective from perform a screening test phytochemicals know content class compound metabolites existing secondary in the extract. Screening test results phytochemicals extract can seen in Table I. Based on results that have been done extract tamarind leaves positive have content alkaloid compounds, flavonoids, tannins, saponins, phenols

Table I_ Screening Phytochemicals

Test Type	Method	Reagent	Result (+)	Test results		
				50%	70%	96%
Alkaloids	Tube	Dragondorff	Brick metastases	+	+	+
Flavonoids	Tube	Mg, Concentrated Hcl	Orange red	+	+	+
Saponins	Tube	Aquadest, Hcl 2N	Formed foam	+	+	+
tannins	Tube	Gelatin salt	Yellow white precipitate	+	+	+
Polyphenols	Tube	FeCl ₃	Blackish blue/green , brown black	+	+	+

The results show testing screening phytochemicals has aligned with previous studies of (Putri, 2023) who also reported that tamarind leaves contains alkaloids, flavonoids, saponins, tannins, and phenols .

Total Flavonoid Content (TFC)

Determination total levels of flavonoids used method colorimetry with reagents specific AlCl₃. In principle AlCl₃ reagent will form complex stable acids _ between aluminum chloride with a keto group on the C-4 atom and a C3 or C5 group hydroxyl from changing flavones and flavonols color the original sample colored chocolate become yellow. This is due Because exists reaction reduction oxidation Where reducing agent of flavonoids and AlCl₃ as oxidizer so that happen shift long wave

toward ray visible (*visible*) (Susilowati & Sari, 2021) . Determination long quercetin solution waves obtained by 435 nm and obtained equality regression $y = 0.1339x + 0.0423$.

Acquisition results extract can seen in Table II. Research results show that 96% ethanol has highest total flavonoid content namely 4.5686 mgQE /g ± 0.0087 . Viewed from By their nature , flavonoid glycosides are present types of flavonoids glycones (binds to sugar) and aglycones (not binds to sugar) (Suwanditya et al., 2020) . Compound quercetin belong characteristic flavonoid compounds aglycone own solubility not easy dissolves in water, however more easy dissolve in compounds alcohol and solvents organic (Yunita & Khodijah, 2020) .

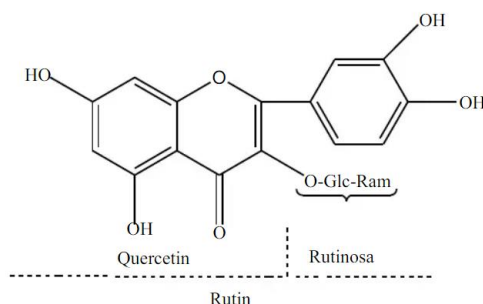


Figure 1. Flavonoid Glycosides

Solvent 96% ethanol is not more polar than ethanol 50% and 70% which means that the solvent has more water mix A little compared to other concentrations (Riwanti et al., 2020) .So the possibility of many flavonoid compounds contained in the extract tamarind leaves characteristic aglycone as in Figure 1. compound quercetin will easy late in the solvent 96% ethanol because characteristic from these compounds and their presence OH group (group hydroxyl) on the inclined quercetin more easy binds to the OH (hydroxyl) group on ethanol (Riwanti et al., 2020) . This is also supported previous research by (Yunita & Khodijah, 2020) who reported that highest total flavonoid content from extract tamarind leaves in 96% ethanol is 3.133 ± 0.007 % while extract 70% ethanol, is 2.468 ± 0.004 %. There is increase concentration solvent influential to total levels of flavonoids in a extract . (Yunita & Khodijah, 2020) state the more big concentration solvent ethanol, then the more the high levels of flavonoids contained in the extract tamarind leaves. Results of analysis with *Mann Whitney* show that there is significant difference between group concentration solvent ($p < 0.05$)

Total Phenol Content (TPC)

In principle Folin will react with compounds phenolic in the extract to be form a colorless solution be measured its absorbance (CN Putri et al., 2022) . During reaction going on happen reaction oxidation from reactor folin ciocalteu with compounds phenol in atmosphere base

that uses Na_2CO_3 7.5% which will produce compound complex colored blue due to reducing phenolic ions heteropoly acid (phospho molybdate phospho tungstate) to molybdenum tungsten complex (Irawan et al., 2020) . The greater the concentration of phenolic compounds in the sample, the more concentrated the blue color will be. Determination long wave at λ 757 is obtained equality linear regression $y = 0.1595x + 0.0658$ with coefficients regression $r = 0.9928$. Result of total phenol content can contained in Table II Based results obtained total phenol levels the largest in 96% ethanol is $27.3647 \text{ mgGA E/g} \pm 0.16$. It is not far different from results determination where is the total level of flavonoids rate largest in extracts 96% ethanol. Ethanol is capable solvent dissolve compound from less polar to polar. on structure phenol own group capable hydroxyl bond with clusters derived hydroxyl from ethanol so that cause enhancement solubility compound phenolic (Yasa et al., 2019) . The same thing was also reported by (Yasa et al., 2019) that total phenol levels highest from extract leaf betel red was in 90% ethanol 106.00 mg GAE/g and the lowest was in 50% ethanol which was 53.56 mg GAE/g . There is difference concentration solvent influence rate obtained phenolic (Prayitno et al., 2016) . (Nisa et al., 2014) state that increase concentration compared directly with the total phenol content obtained. Results of analysis with *Mann Whitney* show that there is significant difference between group concentration solvent ($p < 0.05$).

Table II. Results of Total Flavonoid and Phenol Levels

Solvent Concentration	Rep.	TFC (mgQE /g) \pm SD	T P C (mg GA E /g)
50%	1	2.3241	12.8589
	2	2.3249	12.9559
	3	2.3234	12.9530
	X \pm SD	2.3241 ± 0.0007	12.9237 ± 0.05



70%	1	2.6229	21.9812
	2	2.6229	22.1191
	3	2.6296	22.1379
	X±SD	2.6251 ± 0.0039	22.0794 ± 0.08
96%	1	4.5728	27.2539
	2	4.5586	27.2915
	3	4.5743	27.5486
	X±SD	4.55743 ± 0.0087	27.3647 ± 0.16

Extract Antibacterial Test Tamarind Leaves

Identification Bacteria

Identification bacteria by gram stain confirmed the bacteria enter into the gram positive or negative bacteria. From the results identification on a microscope show color purple, form round and clustered like a fruit wine. Cell bacteria *Staphylococcus aureus* colored ones purple prove that the bacteria enter into the class gram positive

bacteria. Color purple because bacteria capable guard color first, namely crystal violet (Nugroho et al., 2022).

Testing Antibacterial Activity

Extract antibacterial activity test results tamarind leaves to bacteria *Staphylococcus aureus* was obtained that exists variation concentration solvent show Antibacterial activity indicated by the clear zone formed. Clear zone diameter test results can seen in Table III.

Table III. Antibacterial Activity Test Results Against Bacteria *Staphylococcus aureus*

Const	(mm) ± SD		
	50%	70%	96%
5%	13.28 ± 0.45	13.18 ± 0.37	13.30 ± 1.06
10%	14.34 ± 0.78	14.77 ± 0.46	16.08 ± 0.60
15%	15.8 ± 0.6	16.71 ± 0.37	17.42 ± 0.48
20%	16.61 ± 0.94	17.52 ± 0.54	17.63 ± 0.34
25%	18.03 ± 0.52	18.76 ± 0.59	18.94 ± 2.43
Clindamycin 1%	43.686 ± 0.70 (susceptible)		
DMSO 10%	0		
Media	Note: there is bacterial growth		

Obtained antibacterial activity test showed extract 96 % ethanol tamarind leaves has a power diameter more inhibition big namely 18.942 ± 0.008 . Acquisition results by (Putri, 2023) show results Power inhibition on 96% ethanol of tamarind leaves 14.974 ± 0.46 mm. This is due Because 96% ethanol is selective, have Power good absorption as well ability high filtering. This solvent is more easy penetrate into cells sample compared to solvents ethanol which contain concentration more low

(Wendersteyt et al., 2021). If seen from results Power resistor from each concentration extract antibacterial test results show exists A little enhancement Antibacterial activity of tamarind leaves can formed Because exists metabolites existing secondary namely flavonoids and phenols (CN Putri & Ningrum, 2023).

Mechanism of alkaloids as antibacterial through use synthesis peptidoglycan and inhibition synthesis wall cell consequence group base on the alkaloids that can



change structure and arrangement of the constituent amino acids wall bacteria so that cause lysis of cells and will dead (Rifda & Lisdiana, 2022).

Mechanism of flavonoids and phenols For turn off bacteria in a way destroy cell membranes bacteria compound consequence formation bond hydrogen complexes with extracellular and denatures cell proteins so permeability wall cells are unstable and occur death (Azizah & Lingga, 2020).

Then abilities of saponins to prevent growth bacteria through decline voltage surface wall cell bacteria so that permeability from the cell membrane will disturbed cause leaks and happened lysis (Rifda & Lisdiana, 2022). Mechanism tannins can prevent bacteria to grow with agglomerate proplasm germs so that formed unstable bond _ (Rifda & Lisdiana, 2022) tannins also bind metal ions such as Fe and Cu which cause cell walls to break down (CN Putri, 2023) .

Mann-Whitney test results obtained mark *significance* ($p < 0.05$) indicating exists significant differences in each group treatment. However there is some data is not yet show significant result ($p > 0.05$). Acquisition the test results stated that there were none difference meaning variation concentration solvent to antibacterial activity. on analysis *Pearson Correlation* is found the results of $p=1$ show exists connection total levels of flavonoids, phenols with activity antibacteria.

Conclusion

There is influence variation concentration solvent to total levels of flavonoids and phenols, results largest in ethanol 96% that is 4.55 mgQE/g and 27.3647 mgGAE/g. Whereas activity antibacterial, power resistor the largest in 96% ethanol is 18.94 mm. The more big concentration solvent the more big total levels of flavonoids, phenols, but not for activity its antibacterial.

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