ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF EDAMAME PEEL AND SEEDS (*Glycine max* (L.) Merr) AGAINST THE GROWTH OF *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus is the cause of acne disease that is resistant to antibiotics in the long term. Edamame is one of the natural ingredients used as an alternative treatment. Edamame seeds have the potential as antibacterial because they contain saponin compounds, in addition edamame peel waste can also be developed as an antibactetrial. The study aims to identify the profile of compound content from edamame peel and seeds extracts, to identify and analyze the antibacterial activity of the extract against *Staphylococcus aureus* bacteria. Ethanol extract of edamame peel and seeds were obtained from the *Ultrasonic Assisted Extraction* (UAE) method using 96% ethanol. Each extract was screened for phytochemicals and than made into concentrations of 10%, 20% and 30% to test antibacterial activity using the well diffusion method. Then continued with the *One Way Anova* test. Ethanol extracts of edamame peel and seeds obtained an average yield of 17,31%±0,57and 17,10%±0,51. Edamame peel and seeds extracts contain saponins, tannins, steroids,terpenoids, flavonoids dan phenols but edamame seeds do not contain steroid compounds. Edamame peel extract at concentration of 10%, 20% and 30% can inhibit *Staphylococcus aureus* with an average inhibition zone diameter of 10,13mm±0,54; 12,80mm±0,67 and 14,74mm±0,78 while ethanol extract of edamame seeds produces an average inhibition zone diameter of 9,32mm±0,67; 11,55mm±0,83 and 13,83mm±0,96. The *one way Anova* test produced a value (Sig.) 0,000 followed by the *Post Hoc LSD* test showing that there were significant differences between each group. Edamame peel and seeds extracts contain phytochemical compounds such as flavonoids,steroids, phenols, tannins, terpenoids and saponins where each compound has a mechanism of inhibiting the growth of *Staphylococcus aureus*. The antibacterial activity of edamame peel extract is classified as strong at each concentration, while edamame seed extract is classified as strong at concentration of 20% and 30%, than at the concentration of 10% it is classified as moderate.

Key words: Antibacterial, Edamame, Seeds, Peel, *Ultrasonic Assisted Extraction*

INTRODUCTION

Acne is a skin disease that can occur due to excess oil production in the skin which causes blockage of the pores. If the pores are blocked, it will triger excess growth of bacteria causing infection. One of the bacteria that can cause infection is *Staphylococcus aureus*. Inhibiting the growth of *Staphylococcus aureus* bacteria can be one way to treat acne. In general, acne is treated using antibiotics which can inhibit inflammation and kill bacteria. However long term use of antibiotics will cause resistance because they contain chemicals (Rasydy *et al*., 2019).

Edamame is one of the leading commodities in Jember Regency because it has high nutritional content and productivity. In general, edamame seeds are often used for consumption and are developed into several preparations such as edamame milk and brownies (A, 2022). The production of various processed edamame seeds will increase the amount of edamame peel waste which can cause enviromental pollution and unpleasant odors if left untreated. So far it has been reported that edamame peel waste has the potential to be a source of feed for livestock and there has not been much research on the potential of edamame peel extract as an antibacterial compound like edamame seeds extract (Nurkholis *et al.*, 2021).

Several studies report that apart from being a food ingredient, edamame seeds alsa have antibacterial potential. The ethanol extract of edamame seeds produced through the remaceration process shows that there is very weak inhibition of the growth *Escherichia coli* bacteria *in vitro* (Akhita, *et al.*, 2020). The secondary metabolite compounds contained in edamame, namely flavonoids, tannins, polyphenols dan saponins can create an inhibitory zone for bacteria (Chaleshtori *et al*., 2017). These secondary metabolite compounds can be obtained by extraction, one of which is using the *Ultrasonic Assited Extraction* (UAE) method. UAE is a modern extraction method that uses ultrasonic waves and is starting to be used frequently because of its advantages, namely higher efficiency and shorter time (Ramadhani *et al*., 2023).

Based on the description above, this research will analyze the antibacterial activity of ethanol extract edamame peel and seeds (*Glycine max* (L.) Merr) against *Staphylococcus aureus* bacteria. The study aims to identify the compound profile of each edamame peel and seeds extract (*Glycine max* (L.) Merr) as well as identify and analyze the antibacterial activity of each edamame peel and seeds extract (*Glycine max* (L.) Merr) against *Staphylococcus aureus* bacteria.

MATERIAL AND METHODS

The tools used is this research were an *oven* (*memmert)*, analytical scales, blender (*miyako*), *beakerglass* 100 mL(*Iwaki)*, measuring cup 10 mL(Iwaki), stir bar, *ultrasonic homogenizer* (*Benchmark)*, glass funnel, erlenmeyer 250 mL (*Iwaki)*, porcelain cup, *rotary evaporator* (*Intra*), vial, test tube (*Iwaki)*, tube rack, *hotplate stirrer*, dropper pipette, L rod, tube wire, spektrofotometer UV-Vis (*Shimadzu* 1900i), cuvvete, petri dish, bunsen, autoclave, *Biological Safety Cabinet (Biobase)*, *cork borer*, micropipette (*DragonLAB)*, incubator (*memmert)*, *vortex* dan caliper.

The materials used in this research were edamame peel and seeds which were processed from PT. Mitra Tani Dua Tujuh, ethanol 96% (Merck), *Nutrient Agar* (*himedia)*, *Mueller Hinton Agar (himedia)*, DMSO 10%, Chloramphenicol, HCl 2%, *mayer's* reagent, magnesium powder (Mg), concentrated HCl, distilled water, $BaCl_2$ 1%, H_2SO_4 1%, sterile NaCl, *Staphylococcus aureus* ATCC® 25923™ bacteria, $FeCl_3$ 1%, anhydrous acetic acid, chlorofom, filter paper, cotton, sterile gauze, alumunium foil dan brown paper.

Making Simplicity

Fresh edamame in picked and package condition obtained from PT. Mitra Tani Dua Tujuh as many 7 kg were sorted and then peel and seeds were found to have a wet weight of 3,25 kg and 3,75 kg. The edamame peel and seeds are each washed under running water, then chopped and dried using an oven at 50^{o}C until they become simplicia. The skin simplicia and edamame seeds obtained were 1,89 kg and 2,1 kg. Then each simplicia is refined using a blender and then sieved

(Lady *et al*., 2020). The simplicia peel powder and edamame seeds produced were 1,5 kg and 2 kg.

Extraction of edamame peel and seeds

The extraction process between simplicia and solvent is using the UAE method in a ratio of 1 : 10, 100 grams of simplicia powder is extracted with 1.000 mL of 96% ethanol solvent at a temperature of 40^{o}C for 30 minutes. This extraction was carried out 3 times in replication. The filtrate is then filtered using filter paper and then thickened using a rotary evaporator at a temperature of 40^{o}C. The thick extract then calculates the yield (E S Syamsul *et al.*, 2020).

Phytochemical Screening

Alkaloids

A total of 0,1 gram of edamame peel and seed extract each was put into a test tube and 2 mL of 2% HCl was added, then 2-3 drops of *Mayer's* reagent were added. The presence of alkaloids when a white precipitate is formed (Agustina & Handayani, 2017).

Terpenoids

A total of 0,5 grams of edamame peel and seed extract for each extract was put into a test tube and dissolved with 0,5 mL of chloroform then added with 0,5 mL of anhydrous acetic acid and concentrated H_2SO_4. The appearance of a brownish or violet color at the border of the solution indicates the presence of terpenoids (Kursia *et al*., 2016).

Steroids

A total of 0,5 grams of edamame peel and seed extract for each extract was put into a test tube and dissolved with 0,5 mL of chloroform then added with 0,5 mL of anhydrous acetic acid and concentrated H_2SO_4. The appearance of a greenish blue color in the solution indicates the presence of steroids (Kursia *et al*., 2016).

Flavonoids

A total of 0,5 grams of edamame peel and seed extract of each extract was dissolved in 2 mL of 96% ethanol and filtered. Then magnesium powder (Mg) was added and shaken until homogene. Once homogeneous, 1 mL of concentrated HCl was added. The presence of flavonoids results in a red or orange color(Purwaningtiyas, 2023).

Saponins

A total of 0,5 grams of edamame peel and seed extract for each extract was added to 10 mL of distilled water then shaken vigorously for 10 seconds. If stable foam forms for no less than 1 minute, then 2% HCl is added. If the foam does not disappear then the sample is declared to contain saponin (Septia *et al.*, 2020).

Tannins

A total of 0,5 grams of edamame peel and seed extract for each extract was put into a test tube and 10 mL of distilled water was added. Then add a few drops of 1% $FeCl_3$ solution, if there is a greenish black color change then it can be said that there is tannin content (Septia *et al.*, 2020).

Phenols

A total of 50 mg of edamame peel and seed ethanol extract of each extract was put into a test tube and 3-4 drops of 1% $FeCl_3$ were added, the presence of phenol content if the color changes to dark black or bluish black (Septia *et al.*, 2020).

Sterilization of tools and media

Tools and media to be used in testing must be sterilized first by autoclaving at 121ºC for 15 minutes. The NA media made for rejuvenation was placed in a test tube and the MHA media for the antibacterial test was placed in an Erlenmeyer flask, both were tightly closed and tools such as petri dishes, wire tubes, L-rods, stirrers, dropper pipettes and tweezers were wrapped in brown paper. The mouth of the test tube, Erlenmeyer flask and beakerglass is covered using cotton wrapped in sterile gauze. The use of wire loops, L rods and tweezers are sterilized again when they are ready to be used by lighting them with a bunsen flame (Berliana & Pujiyanto, 2020).

Rejuvenation *Staphylococcus aureus*

Rejuvenation of bacteria using sterile slanted *Nutrient Agar* media by taking one sterile ossicle that has been spawned and then implanted by scratching on the slanted agar surface in a zig-zag manner and incubating using an incubator at 37ºC for 24 hours (Lestari *et al*., 2020).

***Mc. Farland* 0,5 Standard Manufacturing**

Mc. Farland 0,5 standard manufacturing is carried out by adding 0,05 mL of 1% $BaCl_2$ in distilled water with 9.95 mL of 1% H_2SO_4. Then the Mc density test was carried out. Farland 0,5 by measuring the absorbance using a spectrophotometer with a wavelength of 625 nm (Berliana & Pujiyanto, 2020).

Preparation of *Staphylococcus aureus* Suspension

Staphylococcus aureus bacterial culture was taken from 1 colony loop from *Nutrient Agar* media into a test tube containing 10 mL of sterile NaCl and then vortexed for 15 seconds. The absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 625 nm, then compared with the turbidity of the *Mc. Farland* 0,5 standard solution (Lestari *et al*., 2020).

Antibacterial Activity Test

The *Staphylococcus aureus* bacterial suspension was put into a petri dish containing MHA media by taking 100 µL then leveled using an L rod. A well was made in the media then ethanol extract of edamame peel and seeds at a concentration of 10%, 20%, 30%, positive control using 1% chloramphenicol and negative controls using 10% DMSO each were put into 50 µL wells using a micropipette aseptically. Petri dishes containing bacteria with various extract concentrations were placed in an incubator at 37ºC for 24 hours so that the compounds could diffuse (Fitriyanti *et al*., 2019). Next, the bacterial clear zone was measured in mm using a caliper. Then the diameter of the inhibition zone for the antibacterial compound was calculated (Hasanah, 2023).

Data Analysis

The data in this research was tested for normality using *Shapiro-Wilk*, the data was said to be normally distributed if the significance >0,05, than a data homogenity test was carried out using *lavene's test.* If the data is normally distributed then proceed with a parametric statistical test, namely *one way Anova*. Next to find out which groups have signifcant differences use the *Post Hoc* test, namely *Least Significanly Difference* (LSD).

RESULT AND DISCUSSION

Extraction of Edamame Peel and Seeds

Ethanol extract of edamame peel and seeds was obtained using the *Ultrasonic Assisted Extraction* (UAE) method with 96% ethanol solvent. The yield results of edamame peel and seeds extract can be seen in table 1.

A thick extract can be said to be good if the yield value is >10% (Kusuma & Dwi, 2022). Based on table 1, the average yield of ethanol extract from edamame peel and seeds was 17,31% 0,57 and 17,10% 0,51 respectively. The yield of the thick extract of edamame peel and seeds was said to be good because the extract yield was more than 10%. This can happen due to modern extraction methods, namely UAE with 96% ethanol solvent. Where in the extraction process using UAE there is involvement of ultrasonic amplitude waves in the UAE method. Amplitude waves that pass through the material medium can cause pressure on the solvent molecules, under which conditions the massa transfer between phases increases so that the yield also increases (Wisyasanti *et al.*, 2018).

In previous research, extracting cocoa pod peel from maceration resulted in an extract yield of 8,03%. Extraction using the UAE method in this study produced a higher yield value compared to the maceration method (Khoiriyah *et al.*, 2022). A similar thing was also seen in other research where the yield produced by the UAE extraction method was greater than the yield value produced by the conventional extraction method (Ramadhani *et al.*, 2023). The UAE extraction method has the advantage that the extraction process does not take a long time and uses less solvent compared to the maceration method, but the UAE method is able to produce extracts with higher yield values.

A good yield value in this study can also be influenced by the solvent used, where the solvent used in making edamame peel and seed extracts is 96% ethanol. Ethanol 96% is a universal solvent that is selective, non-toxic, has good absorption and high filtering ability so that it is able to attract all secondary metabolite compounds. This is in accordance with previous research that the yield produced using 96% ethanol solvent was greater than that with 70% solvent (Yunita & Khodijah, 2020). Previous research showed that the high yield found in 96% ethanol solvent was able to extract more than the solvents ethyl acetate and n-hexane.

Phytochemical Screening

Phytochemical screening was carried out using the tube method to determine the content of secondary metabolite compounds contained in the peel and seeds pf edamame (*Glycine max* (L.) Merr) the results of the phytochemical screening test can be seen in table 2.

Based on the results of research that has been carried out, it shows that the secondary metabolite compounds contained in the ethanol extract of edamame peel are flavonoids, phenols, terpenoids, steroids, saponins, tannins and do not contain alkaloid compounds, while the compounds contained in the ethanol extract of edamame seeds are flavonoids, phenols, terpenoids. , saponins, tannins but does not contain alkaloids and steroid compounds. Flavonoids, phenols, terpenoids, saponins and tannins are polar while non-polar compounds are steroids. The

disinterest in alkaloid and steroid compounds in this study could be caused by differences in the polarity of these compounds and the solvent used. In the extraction process, edamame peel extract produces a higher yield value compared to the yield value of edamame seed extract. Where the greater the yield value produced, the higher the content of compounds attracted to the extract.

This is in accordance with previous research where the yield can influence the presence of bioactive compounds (Senduk *et al.*, 2020). The same results were shown in previous research, namely that edamame seed extract (*Glycine max* (L.) Merr) contained flavonoids, tannins, phenols and saponins, but did not contain alkaloids, terpenoids and steroids. The secondary metabolite compounds contained in edamame peel and seed extracts can be completely attracted by the ethanol solvent because these compounds are polar. Alkaloid secondary metabolite compounds are semi-polar compounds and cannot be screened because the 96% ethanol solvent is not able to attract alkaloid compounds so no precipitation reaction occurs because there is no ligand replacement in the alkaloid compounds (Kartikasari *et al*, 2022). This is in line with other research, where extracts using 96% ethanol solvent did not dissolve completely compared to using semi-polar solvents (Hidayah *et al*., 2016). The ethanol extract of edamame seeds is negative for alkaloid and steroid compounds, this is due to the difference in polarity in the ethanol solvent, while the steroid compound is a non-polar compound so this compound cannot be extracted completely and the ultrasonic extraction method uses ultrasonic waves to increase mass transfer between two phases, but is not always effective for extracting steroid compounds. Therefore, ultrasonic extraction methods may not be effective for extracting steroid compounds from solids (Kusumo *et al*., 2022). This is in accordance with other research where yellow frangipani flower extract does not contain steroid compounds with 96% ethanol solvent (Arianta & Datu, 2022).

Antibacterial Activity Test

The antibacterial activity test begins with measuring the absorbance of the *Mc.Farland* 0,5 standard and *Staphylococcus aureus* bacterial suspension. The *Mc.Farland* 0,5 absorbance was compared with the absorbance of the bacterial suspension using a UV-Vis Spectrophotometer with a wavelength of 625 nm. In this study, the *Mc.Farland* absorbance was 0,088 and the bacterial suspension was 0,089. The absorbance of the bacterial suspension should be close to the *Mc.Farland* 0,5 absorbance where a good absorbance result in the *Mc.Farland* 0,5 standard measurement must be in the range of 0,08 – 0,1 which is equivalent to a bacterial count of 1,5 x 108 CFU/ mL (Yanti & Rosmania, 2020).

The antibacterial activity test was carried out using the well method where the diameter of the inhibition zone was measured using a caliper. The results of the average diameter of the inhibition zone can be seen in table 3.

Based on this research, it was found that the average diameter of the inhibition zone of edamame peel ethanol extract with concentrations of 10%, 20%, 30%, and K+ respectively was 10,13mm±0,54, 12,80mm±0,67, 14,74mm±0,78, and 22,26mm±0,92, while the average diameter of the inhibition zone of the ethanol extract of edamame seeds was 9,32mm±0,67, 11,55mm±0,83, 13,83mm±0,96, and 22,49mm±1,03. Antibacterial activity can be categorized as weak if the diameter of the inhibition zone is <5 mm, moderate 5-10 mm, strong 10-20 mm and very strong >20 mm (Emelda *et al*., 2021). In this research, the average diameter of the inhibitory zone of edamame peel ethanol extract at concentrations of 10%, 20% and 30% shows that the antibacterial activity against *Staphylococcus aureus* bacteria has strong antibacterial power. The ethanol extract of edamame seeds at concentrations of 20% and 30% has strong antibacterial power while the 10% concentration has moderate antibacterial power. This may happen because in edamame seed extract there are steroid compounds that are not completely attracted compared to the

compounds that are attracted in edamame peel extract, so that edamame seed extract has 2 different categories of antibacterial strength. This is in accordance with previous research that the absence of steroid content in the extract will indicate antibacterial activity in the moderate category (Firas, 2017).

This research has a difference in the strength of activity with previous research which stated that the ethanol extract of edamame seeds from remaceration showed very weak antibacterial activity. Differences in antibacterial activity results may occur due to differences in the extraction methods used. In this study, the *Ultrasonic Assisted Extraction* (UAE) extraction method was used. The UAE method is an *ultrasonic assisted extraction* method that utilizes the principle of acoustic cavitation to create spontaneous bubbles in the liquid phase below the boiling point. This process will penetrate the cell walls so that the solvent can enter the material and attract secondary metabolite compounds. The UAE method has several advantages compared to the maceration method, namely better extraction results, smaller solvent volume and shorter time (Norhaliza *et al*., 2022). Compared to maceration, using the UAE extraction method is able to attract all secondary metabolite compounds found in plants so that this can increase the ability of a material to provide antibacterial activity. The temperature factor in the extraction process can increase the rate of transfer of metabolic substances into the solvent.

Judging from the average diameter of the inhibition zone for *Staphylococcus aureus*, it shows that the higher the extract concentration, the greater the value of the diameter of the inhibition zone. An increase in concentration will be followed by an increase in the active substances contained in the extract so that the antibacterial activity is higher (Emelda *et al*., 2021).

Ethanol extracts of edamame peel and seeds from all concentrations against *Staphylococcus aureus* bacteria showed the presence of an inhibition zone. The inhibition zone can occur due to the presence of secondary metabolite compounds contained in edamame peel extract, namely flavonoids, phenols, terpenoids, steroids, saponins and tannins, while edamame seed extract contains flavonoids, phenols, terpenoids, saponins and tannins. The mechanism of flavonoid compounds as antibacterials is by inhibiting cell membrane function and bacterial energy metabolism, where flavonoids will form complex compounds with extracellular proteins which are able to damage bacterial cell membranes, followed by the release of intracellular compounds. Inhibition of energy metabolism by flavonoids occurs because oxygen is inhibited so that bacterial molecules cannot develop into complex molecules (Zamzani & Triadisti, 2014). Saponins have a role in antibacterial activity by causing leakage of bacterial proteins and enzymes resulting in lysis (Norhaliza *et al.*, 2022). Tannin has the ability to inactivate bacterial enzymes and disrupt the flow of proteins in the inner layers of cells (Norhaliza *et al*., 2022). Steroids work by causing sensitivity in the lipid membrane which causes leakage in bacterial liposomes (Kirtanayasa, 2022). Terpenoids work by causing the growth of bacteria to be inhibited or die by damaging the transmembrane proteins on the outside of the bacterial cell wall which will reduce permeability so that the bacterial cells lack nutrition (Norhaliza *et al*., 2022). Phenolic compounds are able to damage and penetrate cell walls which disrupt the formation of bacterial peptidoglycan so that cell wall layers do not form and cause bacteria to die (Norhaliza *et al*., 2022).

From the *One Way Anova* test for each sample, a significance value of 0,000 ($p<0,05$) was obtained so that the results of testing the antibacterial activity of the ethanol extract of edamame peel and seeds against *Staphylococcus aureus* from each test group were significantly different. In this research, all treatments were significantly and meaningfully different for each group. This is indicated by the sign (*) in the comparison of treatments given to each group which can be seen in the *Post Hoc LSD* data in the Mean Difference section.

CONCLUSION

Edamame peel and seeds extracts contain phytochemical compounds such as flavonoids, steroids, phenols, tannins, terpenoids and saponins where each compound has a mechanism of inhibiting the growth of *Staphylococcus aureus*. The antibacterial activity of edamame peel extract is classified as strong at concentrations of 20% and 30% than at a concentration of 10% it is classified as moderate. From the results of the study, it is recommended to conduct further research related to the antibacterial activity of the combination of edamame peel and seed extracts and its potential to be formulated into a dosage from such as *Facewash*.

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TABLE

Table 1. Ethanol Extract Yield of Edamame Peel and Seeds

Type of Extract	Powder (grams)	Replication	Yield (%)	Average±SD (%)
Peel	100	1	17,96	17,31±0,57
		2	17,70	
		3	16,87	
Seeds	100	1	16,57	17,10±0,51
		2	17,15	
		3	17,58	

Table 2. Phytochemical Screening Test Results

Compound	Reagent	Test Results		Conclusion	
		Peel	Seeds	Peel	Seeds
Alkaloids (Agustina & Handayani, 2017)	*Mayer* dan HCl 2%	No white precipitate was formed	No white precipitate was formed	(-)	(-)
Flavonoids (Purwaningtiyas, 2023)	Ethanol 96%, Mg powder dan concentrated HCl	The color becomes orange	The color becomes orange	(+)	(+)
Phenol (Septi *et al.*, 2020)	$FeCl_3$	The color changes to dark black	The color changes to dark black	(+)	(+)
Terpenoids (Kursia *et al.*, 2016)	Chlorofom, anhydrous acetic acid dan H_2SO_4	A brownish color appears at the border of the solution	A brownish color appears at the border of the solution	(+)	(+)
Steroids (Kursia *et al.*, 2016)	Chlorofom, anhydrous acetic acid dan H2SO4	The color changes to greenish blue	The color do not changes to greenish blue	(+)	(-)
Saponins (Septia *et al.*, 2020)	Distilled water dan HCl 2%	The formation of foam witha height of 1-3cm is stable for > 5 minutes	The formation of foam witha height of 1-3cm is stable for > 5 minutes	(+)	(+)
Tannins(Septia *et al.*, 2020)	$FeCl_3$	The color changes to greenish black	The color changes to greenish black	(+)	(+)

Keterangan : (+) = contains secondary metabolite compounds
(-) = does not contains secondary metabolite compounds

Table 3. Results of the Inhibition Zone Diameter of Ethanol Extract of Edamame Peel and Seeds against *Staphylococcus aureus* Bacteria

Sample	***Clear Zone Diameter Staphylococcus aureus* (mm)**				
	Average±SD Extract Group			**Rata-rata±SD Control Group**	
	10%	**20%**	**30%**	**K+**	**K-**
Ethanol Extract of Edamame Peel	10,13±0,54	12,80±0,67	14,74±0,78	22,26±0,92	0
Ethanol Extract of Edamame Seeds	9,32 ± 0,67	11,55±0,83	13,83±0,96	22,49±1,03	0

Keterangan : K+ = Positive control
K- = Negative control