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CHARACTERISTICS OF STAR FRUIT EXTRACT (*Averrhoa Bilimbi* Linn) AT ETHANOL SOLVENT TREATMENT AND EXTRACTION TEMPERATURE

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ABSTRACT

Starfruit is a type of plant that contains a lot of acids. In addition to containing acids, star fruit also contains bioactive compounds such as polyphenols, flavonoids, alkaloids, saponins, and tannins that have the potential to inhibit bacterial activity. This study aims to determine the effect of ethanol solvent concentration and extraction temperature on the characteristics of star fruit extract, to determine the ethanol solvent concentration and the best extraction temperature to produce star fruit extract. The experiment conducted was a factorial experiment with two factors designed using a Randomized Design Group (RAK). The factor I is the concentration of ethanol solvents which consists of two types, namely 96% ethanol and 70% ethanol. Factor II is the extraction temperature consisting of 3 types, namely 30oC, 40oC, and 50oC. The data were analyzed using variance analysis and further tested with Honest Real Differences. The results showed that ethanol solvent concentration and extraction temperature and their interactions affected the amendment, total flavonoids, total tannins, and total acids. The highest yield, total flavonoids, tannins and acids were shown at the treatment with an ethanol concentration of 96% and an extraction temperature of 30°C. The conclusion of this study is that the treatment with an ethanol solvent concentration of 96% and an extraction temperature of 30 °C is the best treatment to obtain star fruit extract with yield characteristics of 71%, total flavonoids of 7.10%, tannins total 0.914 mg QE /g, and total acid 34.24%.

Keywords: *star fruit wuluh (Averrhoa bilimbi L), extract, ethanol, extraction temperature*

INTRODUCTION

Starfruit is a type of plant that contains a lot of acids, which function as an antimicrobial. Acidic substances can disrupt bacterial metabolism. Acidic conditions will make many bacteria not metabolize (not adapting well to the acidic atmosphere) so that bacterial growth does not occur (Sulistyo, 2019). Some organic acids include acetic, citric, formic, lactic, and oxalic acids. In addition, star fruit contains a lot of natural vitamin C which is useful as an immune enhancer and protection against various diseases.

Research by Sitompul et al (2020) showed that the citric acid content in star fruit can improve the characteristics of cob fish, including chemical characteristics (moisture content 71.5%, pH value 2, *total volatile base* 21.56 mg N/100g) and sensory characteristics, namely color, smell, texture and scales, eye state, gills, and body surface mucus that can be accepted by the panelists as well as the overall preferred acceptance. The results of the research Andayani et al (2014), showed that star fruit contains antibacterial compounds such as triterpenoids, saponins, tannins, flavonoids, and alkaloids. This study was conducted to examine the factors that affect the characteristics of star fruit extract produced by the maceration method. The solvent used is ethanol because ethanol is a safe and non-toxic solvent.

Irawan (2014) states that patikan kebo leaf extract is very optimal using 70% ethanol solvent compared to 96% ethanol solvent with the highest flavonoid content of 4.33%. Research by Sulistyo (2019) showed that the highest total flavonoid levels in sawo duren leaf

extract were obtained using 85% ethanol solvent compared to methanol solvent, which was 0.0079 mg QE.

Generally, the maceration extraction method uses room temperature in the process, but using room temperature has the disadvantage that the extraction process is less than perfect which causes the compound to become less perfectly dissolved. Research Putri (2016) showed that the extraction process using the maceration method using ethanol solvent at 40ºC for 24 hours resulted in higher levels of ginger extract flavonoids at 6.7% compared to 30ºC temperature of 3.11%. Research Hradaya & Husni (2021) also showed that the extraction process using the maceration method with a temperature of 55 ° C for 24 hours quantitatively produced higher flavonoid levels of *E. spinosum* extract of 789.21 mg QE / g compared to a temperature of 65 ° C which was 587.43 mg QE / g.

This study aims to determine the effect of ethanol solvent concentration and extraction temperature on the characteristics of star fruit extract, to determine the ethanol solvent concentration and the best extraction temperature to produce star fruit extract.

METHOD

Materials and Tools

The materials used in the study consist of raw materials and chemicals. The raw material is star fruit that has been prepared (green color, size 3-5cm), with the criteria of green fruit skin obtained from Jimbaran Village, Badung Regency. The chemical used as a solvent is an ethanol (*Merck bratachem*) with a concentration of 96% and 70%. Akuades, NaNO2, AlCl3, NaOH.

The equipment used is equipment for the blender extraction process (*Phillips*), extraction flask, *hot plate, magnetic stirrer*, stainless steel knife, oven (Blue M, USA), *rotary evaporator* (*Janke & Kunkel RV 06 – ML, Switzerland*), UV-Vis spectrophotometer (*Thermo Scientific, USA*), colorimeter (*PCE-CSM4, Spain*), analytical scales (*Mattler Toledo AB 204 and Shimadzu, Japan*), sieve 60 mesh (*Retsch, Germany*), pH meter (*Hanna HI98107*) and glassware (*Pyrex).*

Method

The experimental design used in the phase I study was the Group Randomized Design (RAK). This experiment is a factorial experiment with two factors. The first factor is the concentration of solvent (P) which consists of 2 levels, namely: P1 (Ethanol 96%**),** P2 (Ethanol 70%). The second factor is the extraction temperature (S) which consists of 3 levels, namely S1 (30 o C), S2 (40 o C)**,** and S3 (50ºC). Based on both factors, 6 treatment combinations were obtained and grouped into 3 based on the time of implementation so that 18 experimental units were obtained.

Star fruit wuluh (*Averrhoa bilimbi* L) is prepared which is green, size 3-5 cm, and cleaned of inherent impurities using running water, the sample is sliced first and then dried using an oven at a temperature of 50ºC until the star fruit is marked easily broken. Furthermore, crushing is carried out using a *blender* and then sifted using a 60-mesh sieve to obtain a homogeneous powder. The manufacture of star fruit extract is carried out using maceration. A sample of 10 g was put into a 1000 ml Niemeyer plus a 200 ml solvent, then macerated at a temperature according to the treatment for 24 hours. During extraction, stirring is carried out with a magnetic stirrer at a speed of 300 rpm. Next is carried out filtering with coarse filter paper. The filtrate is accommodated (filtrate I) while the pulp is added solvent until a volume of 100 ml is

shaken and filtered with coarse filter paper (filtrate II). Filtrates I and II are mixed and filtered with Whatman No. 1 filter paper. The filtrate has then evaporated the solvent with a *rotary vacuum evaporator* at a temperature of 40^{o} C, a pressure of 110 bar at a speed of 100 rpm until all the solvents evaporate and a viscous extract is obtained (Naufalin, 2005).

The star fruit extract that has been obtained is then analyzed. The variables analyzed were amendment, total flavonoids, total tannins, and total acids. Based on the results of the analysis, an effectiveness test was then carried out to determine the best treatment.

Data Analysis

The data obtained were analyzed by a variety of analyses and further tests with Tukey's real difference test (BNJ) with a 95% confidence interval with *Minitab* 17 software. The best treatment is selected from the highest value of each treatment.

RESULTS AND DISCUSSION

Amendments

The results of the various analysis showed that the ethanol concentration treatment and extraction temperature had a very noticeable effect ($P<0.01$), while the interaction between treatments had no real effect ($P>0.01$) on the yield of star fruit extract. The average value of the yield of star fruit extract is shown in Table 1.

Table 1. The average yield value (%) of star fruit extract was at ethanol concentration treatment and extraction temperature.

Ethanol Concentration	Temperature		
	30^{o}C	40^{o}C	50^{o}C
Ethanol 96%	0.77 ± 0.025a	0.73 ± 0.000a	0.61 ± 0.015c
70% ethanol	0.65 ± 0.036b	0.52 ± 0.036s	0.47 ± 0.064e

Description: Different letters behind the average value indicate a noticeable difference in the BNJ Tukey test with a 95% confidence interval.

Table 1 shows a 96% ethanol concentration treatment with a temperature of 30 o C resulting in the highest yield of 77%, but it does not differ markedly from a 96% ethanol concentration treatment with a temperature of 40 o C, while 70% ethanol with a temperature of 50^{o}C produces the lowest yield of 47%. The extracted star fruit powder has a moisture content of 17.18% (Andayani et al., 2014b).

The higher the ethanol concentration results in a higher average value of the amendment, but the higher the extraction temperature the lower the average value of the amendment. This is likely because the compounds in star fruit have a polarity that is most similar to the polarity of 96% ethanol. The results of this study are in accordance with the results of research conducted by Lydia (2001) on the extraction of rambutan fruit peel using ethanol solvents with various concentrations. In the use of 95% ethanol solvent, the highest yield was 13.67% compared to 70% ethanol solvent (11.86%), 75% ethanol (7.39%), 80% ethanol (10.74%), 85% ethanol (6.42%), and 90% ethanol (9.62%). The more components of the compound dissolved in the solvent will result in a higher yield. According to the research of Lidiyawati et al (2013) The percentage of amendments obtained from sweet potato extraction at the use of 65% ethanol

solvent was 2.98%, 75% ethanol was 3.75%, 85% ethanol was 3.80% and 95% ethanol resulted in the highest yield of 4.08% with the same extraction time of 24 hours.

Table 1 also shows that at the extraction temperature treatment of 30 o C produces a markedly different average value of amendment compared to a temperature of 50 o C, but the solvent treatment of temperatures of 30 o C and 40ºC does not differ markedly. The increasing temperature decreases the resulting amendment. This is caused by the destruction of compounds contained in materials, namely compounds that are not resistant to high temperatures. The results of this study are in accordance with the results of research conducted by Cahayanti et al (2016) on the extraction of natural dyes of pandanus fruit, the highest amendment was produced at a temperature treatment of 60 o C of 3.48%, 75 o C of 3.29% and the lowest amendment obtained at a temperature of 90ºC of 1.92%.

Total Flavonoids

The results of the various analysis showed that the solvent concentration treatment and extraction temperature had a very noticeable effect (P<0.01) and the interaction of the two treatments had a real effect (P>0.01) on the total flavonoids of star fruit extract. The average value of total flavonoids of star fruit extract is shown in Table 2.

Table 2. The average value of total flavonoids (%) of star fruit extract was at ethanol concentration treatment and extraction temperature.

Ethanol Concentration	Temperature		
	30ºC	40ºC	50ºC
Ethanol 96%	7.10 ± 0.65a	4.96 ± 0.29c	4.38 ± 0.38s
70% ethanol	6.60 ± 0.24b	6.48 ± 0.40b	6.47 ± 1.35b

Description: Different letters behind the average value show a noticeable difference in the BNJ Tukey test with a 95% confidence interval

The highest average total flavonoid value was obtained from a combination of 96% ethanol concentration treatment at a temperature of 30 o C of 7.10% and the lowest average total flavonoid value was obtained from a combination of 96% ethanol concentration treatment at a temperature of 50 o C with a value of4.38%. Based on Table 3 on the treatment of 70% concentration of 30 o C and 40 o C temperatures, it did not differ markedly from the temperature treatment of 50º C but a significant decrease in total flavonoids occurred in the ethanol solvent treatment concentrations of 96% and 70%. This is thought to occur because differences in ethanol concentrations can result in changes in solvent polarity, thus affecting the solubility of bioactive compounds such as flavonoids. According to Harborne (1987), each type of flavonoid has a different polarity depending on the amount and position of the hydroxyl group of each type of flavonoid so it will affect the solubility of flavonoids in the solvent.

Table 2 shows a decrease in total flavonoids that occurs with the addition of ethanol concentrations above 70% with increasing extraction temperatures. This is thought to occur because the addition of ethanol concentration above 70% followed by an increase in extraction temperature causes the polarity level of ethanol solvents to decrease so that the binding of flavonoid compounds to star fruit extract is not optimal due to incompatibility of polarity levels. Total flavonoids decrease with the increasing temperature of the extractions used. The total

flavonoids in an extract can decrease in levels as the extraction temperature increases, this can happen because flavonoids are easily damaged at high temperatures (Sa'adah & Nurhasnawati, 2017). The results of this study are in accordance with the results of research conducted by Hradaya & Husni, (2021) on the effect of extraction temperature on E. spinosum extract (red algae) total flavonoids of *E. spinosum* extract extracted with temperatures of 55 °C, 65 ° C, and 75° C respectively are 789.21±17.25; 587.43±10.63; and 403.50±49.09 mg QE / g. A decrease in total flavonoid compounds along with increasing temperatures can also occur because high temperatures can damage the structure cell material so that existing components are easily migrated and become easily damaged by various chemical reactions that include light and oxygen (Zainol, 2009).

Total Tannins

The results of the various analysis showed that the extraction temperature treatment had a very noticeable effect (P<0.01), but the solvent concentration and the interaction of the two treatments had a real effect (P>0.01) on the total tannins of star fruit extract. The average value of total tannins of star fruit extract is shown in Table 3.

Table 3. The average value of total tannins (mg QE/g) of star fruit extract was at ethanol concentration treatment and extraction temperature.

Ethanol Concentration	Temperature		
	30ºC	40ºC	50ºC
Ethanol 96%	0.914 ± 0.022a	0.646 ± 0.092c	0.434 ± 0.088s
70% ethanol	0.802 ± 0.134b	0.794 ± 0.128b	0.683 ± 0.080b

Description: Different letters behind the average value show a noticeable difference in the BNJ Tukey test with a 95% confidence interval

The highest total tannin mean value was obtained from the combination of 96% ethanol concentration treatment at 30 o C of 0.914 mg QE/g and the lowest total tannin average value was obtained from the combination of 96% ethanol concentration treatment at 50 o C with a value of 0.434 mg QE/g. A significant decrease in total tannins at a concentration of 96% accompanied by an increase in extraction temperature was in line with a decrease in total flavonoids caused by the level of solvent polarity getting more and more Decreased. The polarity of the ethanol solvent of each concentration also affects the solubility level of the tannin compound so the total tannins produced will be different.

Table 3 shows that the higher the extraction temperature, the more tannin compounds are extracted decrease. According to the research of Andriani et al (2019), total tannins obtained from the extraction of star fruit leaves with temperatures of 40 o C and 50º C respectively amounted to 402.27 mg TAE / g and 356.81 mg TAE / g. This is caused because tannins are damaged due to the hydrolysis process during the extraction and heating process that takes place continuously. The results of this study are also in accordance with the results of research conducted by Sumarni (2018), on the extraction of tannins from guava leaves as vegetable tanning materials. At a temperature of 65 o C, the highest total tannins were obtained, namely 54.73% compared to a temperature of 70 o C of 52.80% and 75ºC of 48.08%.

In general, with the increase in the concentration of ethanol solvents used to extract star fruit, the total tannins obtained are increasing, but as the total tannin extraction temperature increases, it decreases. The results of this study are also in accordance with the results of research conducted by Engel (2014), regarding the effect of ethanol solvent concentration on the characteristics of kesambi bark tannin extract, the total tannins obtained at the use of 20% ethanol solvent of 10.53 g, 40% ethanol of 18.83g and 60% ethanol of 20.21 g.

Total Acid

The results of the variance analysis showed that the extraction temperature treatment had a very noticeable effect (P<0.01), but the solvent concentration and the interaction of the two treatments had a real effect (P>0.01) on the total acid of star fruit extract. The average value of the total acid of star fruit extract is shown in Table 4.

Table 4. The average value of total acid (%) star fruit extract was at ethanol concentration treatment and extraction temperature.

Ethanol Concentration	Temperature		
	30ºC	40ºC	50ºC
Ethanol 96%	34.24 ± 0.554a	30.08 ± 1.109b	32.96 ± 0.554a
70% ethanol	10.88 ± 1,466s	13.44 ± 0.960c	14.40 ± 0.000c

Description: Different letters behind the average value show a noticeable difference in the BNJ Tukey test with a 95% confidence interval

The highest total acid means a value obtained from the combination of 96% ethanol concentration treatment at 30 o C of 34.24% did not differ markedly from the treatment of 96% ethanol concentration at 50 o C of 32.96% and the lowest total tannin average value obtained from the combination of 70% ethanol concentration treatment at 30 º C with a value of 10.88%. Table 5 shows the higher the extraction temperature the more the total acid increases despite a statistical decrease at 40ºC by 30.08%. This is because the increasing extraction temperature triggers intracellular evaporation of water which will generate pressure in the cells. The pressure will cause a rupture of the cell wall so that it can increase the diffusion compound to the solvent.

However, extraction temperatures exceeding the optimal time result in the degradation of compounds by such heat Silaban et al (2013). The results showed that the total acid in star fruit extract tends to be more effectively extracted in solvents with lower polarity as opposed to the increasing extraction temperature. According to the research of Krisanta *et al* (2021), total acids obtained from the extraction of waste leaves with ethanol concentrations of 60%, 70%, 80%, and 90% respectively amounted to 69.54 mg AAE/g, 79.01 mg AAE/g, 79.85 mg AAE/g, and 81.45 mg AAE/g. A solvent will effectively extract compounds if it has the same polarity as the solvent used (like *dissolves*) (Sulistyo, 2019).

Best Treatment

In this study, an ethanol solvent concentration of 96% at a temperature of 30ºC got the highest value of all treatments. This treatment is the best treatment to produce star fruit extract.

CONCLUSION

Based on the results of the study, several things were concluded as follows:

1. The treatment of ethanol solvent concentration and extraction temperature and its interactions affect yield, total flavonoids, total tannins, and total acids.
2. The treatment of 96% ethanol solvent concentration and extraction temperature of 30°C is the best treatment to obtain star fruit extract with yield characteristics of 77%, total flavonoids 7.10%, total tannins 0.914 mg QE/g, and total acid 34.24%.

Suggestion

Based on the study's results, it is recommended to use a solvent concentration of 96% ethanol with an extraction temperature of 30 °C to manufacture star fruit extract.

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