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ACTIVITY ANALYSIS OF ANTHOCYANIN FROM *SYZYGIUM CUMINI* (L.) SKEELS AS A NATURAL INDICATOR IN ACID-BASE TITRATION

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ABSTRACT

Syzygium cumini (L.) skeels (*SC*) is a fruit containing anthocyanin pigment that can be used as an indicator in the acid-base titration. An indicator is required in an acid-base titration in order to show a color change based on the endpoint of titration with specific pH range. Standard indicators used so far have some disadvantages such as an environmental pollution, toxic, and expensive. This study was conducted to test the anthocyanin activity of *SC* fruit as a natural indicator that can be used as the application of standard indicators in the acid-base titration. The absorption of UV-visible wavelength characterized the anthocyanin showed in the range between 465 nm and 550 nm. Then, the titration proceeds with acid-base titration of four types of titration. All titrations were compared between the titrations using a natural indicator and the titration using standard indicators, phenolphthalein, and methyl orange. The results indicated that the natural indicator from *SC* fruit was capable to show the color changes at the end point of the titration process that gave almost the same activity as standard indicators in acid-base titrations. Therefore, the extract of *SC* fruit containing anthocyanin can be used widely as an indicator of acid-base titrations in daily laboratory experiments that is environmentally friendly, non-toxic, and cheap.

Keywords: *Syzygium cumini*, Anthocyanin, Natural Indicator, Acid-Base, Titration

INTRODUCTION

The natural acid-base indicator is a promising alternative to standard indicators. Standard indicators are expensive, environmentally hazardous, and poisonous.[1] Acid-base indicators are used as a colorimetric aid to measure changes in pH at equivalence points in chemical titrations.[2] Some natural indicators that have been reported include Copper leaf,[3] wild Sunflower,[4] Caesar Weed flower,[5] Rose, Golden Trumpet, Hibiscus flowers,[6] Jacaranda flower,[7] Golden Beet root,[8] Cactus Pear fruit[9] and many other fruits and flowers.

The fruits that have high quantities anthocyanin are Blackberry, Blackcurrant, Chokeberry, Cherry, Eggplant, Blue Grape, and Red Cabbage.[10] Anthocyanin contained in fruits and flowers is natural pigments that are usually purple, blue, red, and slightly blackish in color, and are soluble in water.[10] Anthocyanin is cheaper, more readily available, simple to extract, safer, and environmentally friendly. Taken together, these favorable characteristics suggest that anthocyanin may replace the widespread use of standard acid-base indicators.[11] Anthocyanin is known as a flavonoid that is widely distributed in the plant. Anthocyanin can be found in all parts of plant tissues including stems, root, leaves, flowers, and fruits. Anthocyanin consists of two aromatic rings linked by three carbons in an oxygenated heterocyclic. Anthocyanin pigment consists of flavylium ring, sugars, and acylating groups (Fig.-1b). The types of anthocyanin are petunidin, delphinidin, pelargonidin, peonidin, cyanidin, and malvidin.[12]

One of the fruits containing anthocyanin is *Syzygium cumini* (L.) Skeels (*SC*) from *Myrtaceae* Family often called *Jamblang* in Indonesia. This plant has several synonyms such as *Eugenia jambolana* (L.), *Syzygium jambolana* D.C., *Syzygium jambolanum* (L.), Blackberry India, Jambolan, Jamun, and others.[13] This plant usually grows in the territory of Indonesia, India, Burma, Nepal, Pakistan, Sri Lanka, Malaysia, South America, Madagascar, and the United States of America. The fruit is round-shaped elongated from

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1.5 cm to 3.5 cm, purple or dark purple, has a fruit flesh and a single seed (Fig.-1a).[14] This fruit is healthy for consumption because of various health benefits such as anti-oxidant,[15,16,17] anti-inflammation,[15,16,17] anti-diabetic,[18] anti-bacterial,[19,20] anti-viral,[19,20] inhibit cancer cells,[21] cure diarrhea,[22] cleaner of dirty blood,[20] and many other diseases.

Anthocyanin is present in the purple peel of *SC* fruit.[23,24] The green peel has no anthocyanin content, the red peel has anthocyanin of 0.19 mg/g, the reddish-purple peel has anthocyanin of 2.67 mg/g, and full purple peel has anthocyanin of 3.79 mg/g.[23] Anthocyanin can be used in acid-base titration as a natural indicator to determine pH from acid or base solutions. The natural indicator can provide a marker of the end point of acid-base titration with changing the color of the solution.[9,8] Anthocyanin is tested for its ability as a natural acid-base indicator and is capable of providing a marker of the titration endpoint at the time of acid-base titration activity. In addition, testing of its ability compared with standard indicators needs to be done. The most commonly used standard indicators in every acid-base titration experiment are phenolphthalein (PP) and methyl orange (MO) depending on the specific pH range of color change in the titrant solution. Therefore analysis of anthocyanin activity from *SC* fruit is very important in order to obtain clear information about the similarity of the activity of standard indicators even able to be used widely in acid-base titrations.

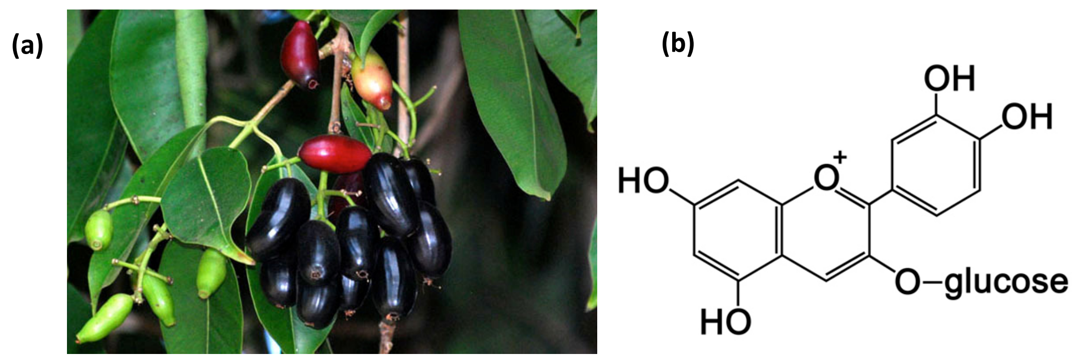

Fig.-1: (a) Fruit of *Syzygium cumini* (L.) Skeels[25] and (b) Anthocyanin structure[26]

EXPERIMENTAL

Materials and Apparatus

SC fruit was obtained from the plantation in Krueng Raya village, Aceh Besar, Indonesia. The selected fresh fruits have been washed and separated from seed, flesh, and peel. The peels were dried for five days at room temperature to reduce water content as well as durable. Hydrochloric acid (HCl), acetic acid (CH_3COOH), sodium hydroxide (NaOH), and ammonium hydroxide (NH_4OH) were used as acid and base solutions with a concentration of 0.1 M, respectively. PP and MO were used as standard indicators.[5] pH meters, digital balance, beaker, knife, burette, pipette, clamp, stand, spray bottle, spatula, stirrer, Soxhlet extractor, Whatman filter paper, measuring cylinder, conical flash, and polystyrene spot plate were used for apparatus.[46] All samples and apparatus were used in all experiment steps and titrations using natural indicator and standard indicators.

Extraction of *SC* Dried Fruit

20 g of dried peels were weighed for both macerated and soxhlet extraction methods. For soxhletation, the sample was wrapped with filter paper and inserted into thimble attached to the condenser and boiling flask. 500 mL of ethanol was put into the boiling flask. The temperature was set at 60°C for 3 hours of extraction.[27] The pigment was extracted and mixed with the solvent in the boiling flask.[28] For maceration, the sample was fed into a beaker containing 1000 mL of ethanol. The sample was stirred to facilitate the mixing of sample and solvent. The beaker was covered with aluminum foil to prevent the interaction of the sample with the environment and also to prevent solvent evaporation. The sample kept overnight at room temperature.[27] Then, the sample was filtered and poured into a beaker. The residual was rinsed back

and extracted using 20 mL of ethanol. All samples were concentrated by using Rotary Evaporator (Rotavor R-215) for 2 hours until the solvent was removed from the desired extracts. The desired extracts were put in the desiccator to cool down and then were stored at 4˚C in the refrigerator.[6]

Testing of *SC* Extract as Acid-Base Natural Indicator

The extracts were dropped into each of acid-base solutions to test whether it can change the color of the solution or not. The acid solutions used in this test were HCl and CH_3COOH, while the base solutions were NaOH and NH_4OH.[29,3] Three drops of extracts were added to each solution provided in the polystyrene spot plate. The extract serves as a natural indicator if the initial color extract in solutions would be changed.

Performing Acid-Base Titrations

25 mL of 0.1 M of titrant was put into an Erlenmeyer. Three drops of extract were added to the titrant. 50 mL of titrant was added into the burette. The titration was done slowly by dropping the titrant into the titrant. The titrand was shacked several times during titration process. All titrations were done for triplicate titrations of each. Each titration also was performed by using standard indicators, PP and MO, to compare their results with natural indicator from *SC* extract. These procedures were used for strong acid-strong base titration, strong base-strong acid titration, weak base-strong acid titration, and weak acid-strong base titration.

Measurement of UV-Vis Absorption of Anthocyanin

UV-Vis absorption of *SC* fruit extract containing anthocyanin was measured using a UV-1800 spectrophotometer (Serial No. A114550, Shimadzu Corp.). 100 ppm of the sample was fed into the cuvette. The wavelength was set from 200 nm to 700 nm. The measurement was performed three times for the accuracy of the spectrum results. The spectra results were then inserted into OriginLab Software for graphics creation plotting the wavelength with absorbance rates. Pure water is used as a reference for calibration standard of UV-Vis instrumentation.

RESULTS AND DISCUSSION

Extract Obtained from *SC* Fruit

A pure extract of *SC* fruit was obtained by performing evaporation system and the solvent was separated from the extract. 28 mL of extract was obtained for Soxhlet extraction. For maceration extraction, 22 mL of pure extract was obtained by using rotary evaporator (Table 1). The extract was put into a labeled bottle and stored at 4˚C to maintain the extracted quality from sample degradation. The differentiation of these two extraction methods is the efficiency of solvent, required time, and extract amounts that can be obtained. Soxhlet extraction method is more efficient in using solvent and time, and it can do the extraction better than maceration. Although, soxhletation cannot be run as simple as maceration process.

Table 1. The extract volumes of *SC* fruit peel.

Extraction Method	Mass of Dried Fruit Peel	Extract Volume
Maceration	20 g	22 mL
Soxhletation	20 g	28 mL

Extract of *SC* Fruit as Natural Indicator

The purple pigment of *SC* extract obtained after extraction was indicated as anthocyanin. The solution of strong acid, weak acid, strong base, and weak base were used to test the natural indicator ability with three drops of the extract in the solutions. Color changes occur in strong and weak base solutions and there are no color changes in strong and weak acid solutions from extract initial color. By adding extract to NaOH solution, the color changed from red to olive green, while the color changed from red to pine green in NH_4OH solution. In contrast with the strong and weak acid solutions, the color did not change from initial extract color (Fig.-2 and Table-2). It is indicated that the *SC* fruit extract containing anthocyanin can be used as a natural indicator for an acid-base solution. The extract can distinguish the pH characteristics of acid-base solutions clearly.

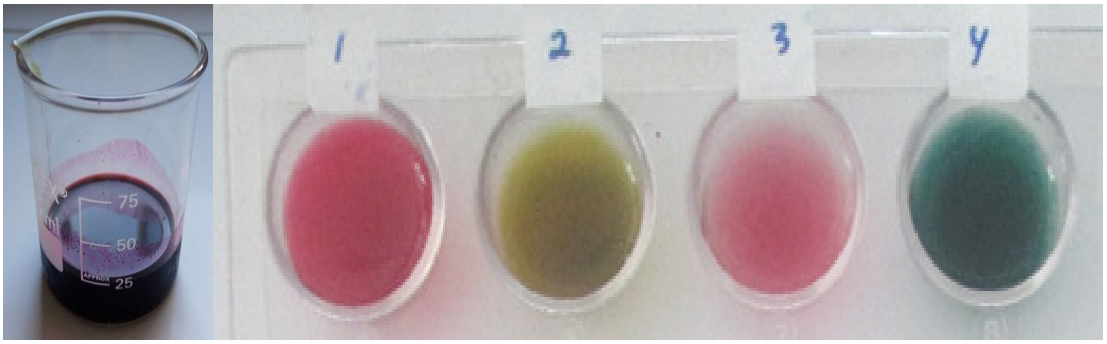

Fig.-2: Color changes of acid-base solutions after adding extract of SC fruit into: (1) Hydrochloride acid, (2) Sodium hydroxide, (3) Acetic acid, and (4) Ammonium hydroxide.

Table-2: Discoloration of extract of *SC* fruit peel (Initial color: red)

No.	Solution	+ Extract	Discoloration
		Acids	
1	HCl	+ Extract	Red
2	CH_3COOH	+ Extract	Pink Red
		Bases	
3	NaOH	+ Extract	Olive Green
4	NH_4OH	+ Extract	Pine Green

UV-Vis Spectral Characterization of Anthocyanin

To characterize whether the extract of *SC* fruit contains anthocyanin, we measured it via UV-Vis absorption spectrophotometry. Anthocyanin showed maximum absorption bands an in the range 465-550 nm. A peak locating in the wavelength range of visible region between 465 nm and 550 nm indicated of the anthocyanin (Figure 3).[30,31] These results provided evidence of the anthocyanin nature of the pigments produced in the *SC* fruit. The major anthocyanins found in *SC* fruit were delphinidin 3,5-diglucoside (45%), petunidin 3,5-diglucoside (32%) and malvidin 3,5-diglucoside (15%).[32]

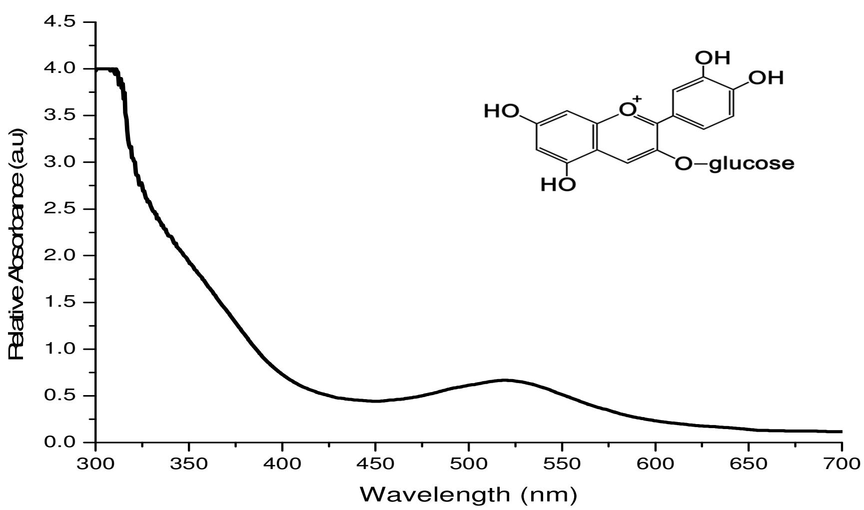

Fig.-3: UV-Vis spectrum of *SC* fruit extract containing anthocyanin.

SC Fruit Extract Performance on Acid-Base Titrations

SC fruit extract is able to be a good natural indicator as a marker of acid-base solutions. Acid-base titration was performed to see how this extract can be used as an indicator. This extract was compared to the standard indicators, phenolphthalein and methyl orange. The concentration of acid and bases were 0.1 M. Four different types of titration performed were strong acid/strong base, strong base/strong acid, strong acid/weak base, and strong base/weak acid. The titrant v/s titrand were NaOH v/s HCl, HCl v/s NaOH, NaOH v/s CH_3COOH, and HCl v/s NH_4OH. All of these titration types were tested by adding three drops of extract, PP, and MO. The titrand volumes were 25 ml. The volume of titrant from titrations with natural and standard indicators were compared. The initial color was recorded after dropping the indicators into titrands. The results of titration showed that fruit extract can be a good indicator in accordance with showing the endpoint of the titration. The end points of all titrations using *SC* extract indicator were almost the same as the end point of titrations with standard indicators based on their color changes with the similarity of added volumes. The color changed from pink to green in the case of adding the extract of *SC* fruit containing anthocyanin as a natural indicator. The other color changes and pH after titration with different titrands and titrants can be seen in Table 3. The obtained results showed that the routinely used indicator can be replaced successfully by *SC* fruit extract indicator.

Table-3: Color changes and pH at the end point of the titration.

Titrand	Titrant	Indicator	Color Changes	pH
HCl	NaOH			
25.00 mL	21.03 mL	Phenolphthalein	Colorless to pink	8.52
	20.60 mL	Methyl Orange	Orange to yellow	6.23
	21.23 mL	Extract Fruit	Pink to green	8.58
	20.50 mL	Extract Fruit	Pink to colorless	6.25
NaOH	HCl			
25.00 mL	28.70 mL	Phenolphthalein	Pink to colorless	6.83
	30.60 mL	Methyl Orange	Yellow to orange	3.86
	30.93 mL	Extract Fruit	Green to pink	3.72
	28.60 mL	Extract Fruit	Green to colorless	6.88
CH_3COOH	NaOH			
25.00 mL	21.10 mL	Phenolphthalein	Colorless to pink	8.40
	31.10 mL	Methyl Orange	Orange to yellow	4.23
	21.37 mL	Extract Fruit	Pink to green	8.34
	32.80 mL	Extract Fruit	Pink to colorless	4.26
NH_4OH	HCl			
25.00 mL	22.10 mL	Phenolphthalein	Pink to colorless	5.02
	22.77 mL	Methyl Orange	Yellow to orange	3.64
	22.13 mL	Extract Fruit	Green to colorless	5.05
	22.97 mL	Extract Fruit	Green to pink	3.62

Anthocyanin Activity on Acid-Base Titration

The results of titration with colors observed in acidic and alkaline solutions and volume of acid and base consumed are summarized in Table 3. The presence of anthocyanin was responsible for the color changes in the titrand occurred at the end point of the titrations. This natural indicator in acid-base titrations showed the color changes with variation of pH ranges. The result proved to be acceptable in introducing natural pigments as suitable acid-base indicators. The color change is due to ionization of the acid-base indicator. Based on Ostward theory, the unionized form has a different color from that the ionized form.[34] The ionization of the indicator is largely affected in acids and bases as it is either a weak acid or a weak base. In case, the indicator is a weak acid, its ionization is very much low in acids due to common H^+ ions while it is fairly ionized in bases similarly if the indicator is a weak base, its ionization is large in acids and low in bases due to common OH^- ions. Phenolphthalein (PP) as a weak acid, can be represented as HPh.[33] It ionizes in solution to a small extent as HPh (colorless) $\leftrightarrow$ H^+ + Ph^- (pink). The undissociated molecules of PP are colorless while Ph^- ions are pink in color. In the presence of an acid, the ionization of

HPh is practically negligible as the equilibrium shifts to left-hand side due to the high concentration of H^+ ions. Thus, the solution would remain colorless. In addition of alkali, hydrogen ions are removed by OH^- ions in the form of water molecules and the equilibrium shifts to the right-hand side. So, the concentration of Ph^- ions increases in solution and they impart a pink color to the solution. As well as PP, a natural indicator of anthocyanin is a weak acid.[35] Based on the results summarized in Table 3. The color changes from pink to green in base conditions indicated that the activity of anthocyanin from *SC* fruit is the same as PP on acid-base titration activity.

CONCLUSION

SC fruit extract can be used as a natural indicator for an acid-base solution. The extract can distinguish the characteristics of acid-base solutions clearly. The UV-Visible spectrum showed that the *SC* fruit contained the anthocyanin in a specific absorption wavelength range. Anthocyanin from *SC* fruit peel extract is able to have color changes when introduced into base solutions. The titration using natural indicator of *SC* fruit obtained almost the same results as is shown with standard indicators. Anthocyanin as a weak acid has the same activity as phenolphthalein (PP) in acid-base titrations that can change the color of base solutions. The results suggest that *SC* fruit extract containing anthocyanin is able to be used in acid-base titration as a natural indicator that is cheap, environmentally friendly, and non-toxic compound.

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