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Study of Ethanol Extract of Karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk) as Acid-Base Bioindicator

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Abstract

Karamunting or Rhodomyrtus tomentosa (Aiton) Hassk is a fruit that contains anthocyanins. This study aimed to determine the efficacy of the ethanol extract of this fruit as an acid-base bioindicator. The Karamuting fruit was macerated with three solvents (n-hexane, ethyl acetate, and ethanol) for a duration of 24 hours. The extract of the karamunting fruit was evaluated as an indicator in acid-base and buffer solutions, and compared with phenolphthalein and methyl orange for acid-base titrations involving strong acids with strong bases, weak acids with strong bases, and weak bases with strong acids. The findings indicated that the ethanol extract of karamunting fruit appeared brown in strong acids, red in strong bases, dark green in mild acids, pink in weak bases, and light green. The kara munting fruit indicator extract buffer solution exhibited four distinct color changes: red at pH 1, pink from pH 2 to 6, light green from pH 7 to 10, and dark green at pH 11 to 12. The ethanol extract of karamunting fruit serves as an acid-base indicator, yielding findings comparable to those of phenolphthalein and methyl orange.

Keywords: Karamunting fruit, anthocyanin, extract, acid-base indicator

Introduction

The acid-base indicator is an organic compound that distinguishes an acidic or alkaline solution by changing colour (Yusraini, 2009). Acid-base indicators often used are synthetic indicators such as phenolphthalein indicators, orange methyl, red methyl, blue bromintol, and others (Maulika et al., 2019).

Synthetic indicators are often acid-base because they have a very clear colour change. The existence of limited synthesis indicators restricts their users; besides that, synthesis indicators are quite expensive and can cause chemical pollution that can pollute the environment (Pathade et al., 2009). Consequently, it is essential to seek alternative measures (natural indicators) that are readily accessible and environmentally sustainable. Synthetic indications may be substituted with natural indicators..

Natural indicators use coloured plants as a basic material, such as stems, leaves, flowers, and fruits. Plants with fruits or flowers with more anthocyanin content can be used as natural indicators (Kurniati et al., 2017).

According to Yulfriansyah & Novitriani (2016), Natural indicators can be made by utilising dyes in plants. Dyes in plants are organic compounds that are coloured like those possessed by synthetic indicators. The plant used to make it must have colour characteristics so that the extract from the plant can give different colour changes at each pH.

Plants used as natural acid-base indicators generally have colour pigments or anthocyanins (Mahmud et al., 2018). Anthocyanins are secondary metabolites belonging to the flavonoid family, present in significant concentrations in fruits and vegetables. (Talavera et al., 2006).

Anthocyanins are plants' basic formers of red, purple and blue pigments (Harborne, 1958). In addition, anthocyanins are pigments soluble in water (Ondagau et al., 2018). Stable anthocyanin compounds impart a vivid hue at acidic pH levels and diminish in color as pH levels increase. The color stability of anthocyanin compounds is influenced by pH levels, exhibiting greater stability in acidic environments. (Alvionita et al., 2016).

Based on the findings of Nuryanti et al. (2010), hibiscus extract can be used as a bioindicator of acid-base acid. The colour change in the acidic atmosphere is red, and the alkaline is green. In addition, research by Kurniawati et al. (2015) shows that the ethanol extract from the johar flower can be used to indicate acid-alkaline. The discolouration in the acid is yellow, and the base is orange. According to Khairunnisa et al. (2017), the ethanol extract from the Chinese ketepeng flower can be used as a bioindicator of alkaline acid. The discolouration in the acid is yellow, and the base is

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orange. The results of research conducted by Afandy et al. (2017) show that purple sweet potato extract can be used as an indicator of acid-base, where in an acidic atmosphere, it is red. In an alkaline atmosphere, it is green. Like the findings on these flowers and fruits, karamunting fruit is also one of the plants that have the potential to be used as a natural indicator because of its red fruit and its anthocyanin compounds (Rifkowaty et al., 2018).

Since karamunting plants are generally only used as ornamental plants and are easy to obtain in the surrounding environment that has not been utilised optimally, researchers want to research karamunting fruit as an indicator of acid-base acid.

This paper is intended to find out whether the ethanol extract of karamunting fruit can be used as an indicator of acid-base. In addition, this research is expected to help shift the use of synthetic indicators to natural indicators that are more economical and environmentally friendly.

Methods

Tools and materials

This study used beakers, measuring cups, spatulas, shakers, drip plates, droppers, burettes, clamps and states, centrifuges, analytical balances, aluminium foils, Erlenmeyer, and pH meters.

The materials used in this study are karamunting fruit, equates, ethanol, ethyl acetate, n-hexane, HCl, NaOH, CH3COOH, NH4OH, orange methyl indicator, and aluminium foil.

Preparation of karamunting fruit extract

50 g of karamunting fruit and put it in erlenmeyer then add 100 mL of n - hexane. The mixture is then macerated for 24 hours. The extract was then centrifuged, and the residue was re-macerated with 100 mL of ethyl acetate for 24 hours. The extract is then centrifuged, and the residue is further - re-macrified with 100 mL of ethanol for 24 hours. After that, the extract is centrifuged and ready to be used as an acid-base indicator (Afandy et al., 2017).

Testing of karamunting fruit indicator extract on acid and base solutions

3 drops of karamunting fruit, then put into the drip plate and tested with each solution of HCl 0.1 M, NaOH 0.1 M, CH_3COOH 0.1 M and NH_4OH 0.1 M. Observed the colour change that occurred (Rahmawati et al., 2016).

Testing of karamunting fruit indicator extracts in ph 1 - 12 buffer solution

Five drops of buffer solution were then put into a drip plate with different pH levels, namely pH 1 to pH 12, after which three drops of karamunting fruit extract were added to the buffer solution. The resulting colour change was observed (Rahmawati et al., 2016).

Testing on acid-base titration

Strong acid-base titration

20 mL of 0.1 M HCl solution was then put into Erlenmeyer, and three drops of karamunting plant fruit extract were added. Then, the mixture was titrated with NaOH 0.1 N until colour change occurred. Titration was carried out 3 times, and the titer volume was used. For each addition of 2 mL of titer, the pH value of the mixture is measured until a colour change occurs. Furthermore, this titration was replaced with a phenolphthalein indicator as a comparison (Afandy et al., 2017).

Strong weak-base acid titration

20 mL of CH_3COOH 0.1 M solution was then put into Erlenmeyer, and three drops of karamunting plant fruit extract were added. Then, the mixture was titrated with NaOH 0.1 N until colour change occurred. Titration was carried out 3 times, and the titer volume was used. For each addition of 2 mL of titer, the pH value of the mixture is measured until a colour change occurs. Furthermore, this titration was replaced with a phenolphthalein indicator as a comparison (Afandy et al., 2017).

Weak base titration – strong acid

20 mL of 0.1 M NH_4OH solution was then put into Erlenmeyer, and 3 drops of karamunting plant fruit extract were added, then titrated with HCl 0.1 N until colour change occurred. Titration was carried out 3 times, and the titer volume was used. For each addition of 2 mL of titer, the pH value of the mixture is measured until a colour change occurs. Furthermore, this titration was replaced with a methyl orange indicator as a comparison (Afandy et al., 2017).

Results and Discussion

Karamunting fruit extract

Fresh karamunting fruit is extracted by maceration using 3 types of solvents with different levels of polarity: n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol (polar). Extraction is a process that aims to separate the desired components from a plant so that active compounds with high purity are obtained (Hidayah et al., 2016).

The maceration method was chosen because it can extract active compounds well through immersion without heating to avoid damage to the components of compounds that are labile and do not withstand heat (Hidayati & Harjono, 2017). Fifty grams of karamunting fruit were weighed, and then the sample was macerated using n-hexane at as much as 100 mL for 24 hours. N-hexane is a nonpolar solvent that will attract compounds in karamunting fruit that can be dissolved in nonpolar solvents. The sample is then centrifuged to separate the filtrate and its residue after the resulting residue is inflated for a few moments.

This aims to evaporate the remaining n-hexane solvent that is still contained in the

karamunting fruit, after which the residue obtained is re-evaporated using 100 mL of ethyl acetate for 24 hours. This aims to attract semipolar components that do not act as indicators (Afandy et al., 2017).

After that, the sample is then centrifuged again to separate the filtrate and residue. Next, the filtrate and residue are aerated for a few moments and then - macerated using 100 mL of ethanol for 24 hours. Ethanol functions as a polar solvent that will dissolve anthocyanin compounds. The selection of ethanol solvents is based on optimal screening, so using ethanol as a solvent is expected to extract many anthocyanins (Pratiwi et al., 2016). The sample was then centrifuged again to obtain a filtrate from karamunting fruit extract ready to be used as an indicator.

Colour testing of karamunting fruit extract against acid-base solution

Results of Testing of karamunting fruit extract in alkaline acid solution. The color of karamunting fruit extract tested on a strong acid solution (HCl 0.1 M) was red, weak acid (CH_3COOH 0.1 M) was pink, strong base (NaOH 0.1 M) was dark green and weak base (NH_4OH 0.1 M) was light green. This can be seen in **Figure 1.**

Figure 1. Colour of karamunting fruit indicator extract in acid-base solution

The ability to change the colour of karamunting fruit extract in acidic and alkaline conditions can be caused by the presence of anthocyanins. Anthocyanins are amphoteric compounds that can react with acids and bases (Maulika et al., 2019). In pH, anthocyanins are red-orange; in pH, alkaline, anthocyanins are blue, green or sometimes yellow.

Amphoteric compounds can react with acids and bases (Maulika et al., 2019). In pH, anthocyanins are red-orange; in pH, alkaline, they are blue, green, or sometimes yellow. The color stability of anthocyanin compounds is influenced by pH levels, exhibiting greater stability in conditions with low pH (Alvionita et al., 2016).

Testing on phosphate buffer solutions 1 - 12

A buffer solution is a solution of weak acids and their conjugate bases. The main property of the buffer solution is its resistance to pH changes due to the addition of small amounts of strong acids or strong bases (Goldberg, 2003). Karamunting fruit indicator extract was tested by dripping karamunting fruit indicator extract in a pH buffer solution of 1 - 12. The results can be seen in **Table 1.**

Table 1. Results of colour testing of karamunting fruit extract indicator in buffer solution

Buffer Solution (pH)	Colour of Karamunting Fruit Extract solution
1	Red
2	Pink
3	Pink
4	Pink
5	Pink
6	Pink
7	Light green
8	Light green
9	Light green
10	Light green
11	Dark green
12	Dark green

The test results obtained in Table 1 show that karamunting fruit extract gives various colour changes in the pH 1 - 12 buffer solution, including red pH 1 buffer solution, pink pH 2 - 6 buffer solution, light green pH 7 - 10 buffer solution, and dark green pH 11 - 12 buffer solution. The results suggest that karamunting fruit extract can be utilized to ascertain the pH value.

Anthocyanins contain flavylium cations in their structure, which can change colour due to structural changes by experiencing anhydrase equilibrium caused by the influence of acidity (pH) (Yazid, 2018). The state of equilibrium form of anthocyanin is seen in **Figure 2.**

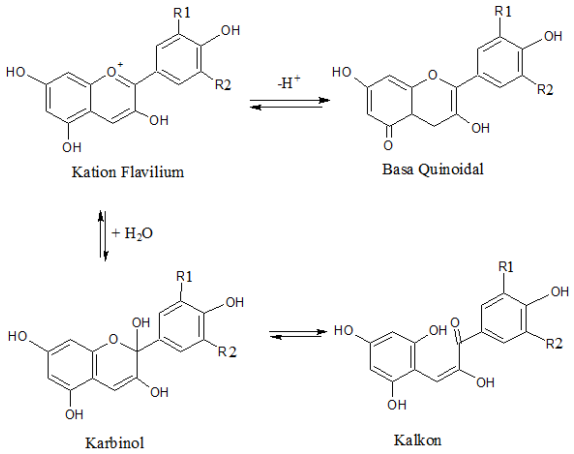

Figure 2. Anthocyanin equilibrium in solution (Rein, 2005)

Figure 2 shows four forms of anthocyanin equilibrium in the solution: flavilium cathode, quinoidal base, carbinol (pseudo base) and chalcone (Bakowska-Barczak, 2005). Under low pH, anthocyanins are in the form of red flavilium cations. When the pH is elevated (more than 5). The acceleration of proton loss will lead to the formation of quinoidal bases, which are typically blue or

purple, while the increase in pH promotes the hydration of flavylium cations, resulting in the formation of carbinol (pseudobases) or colorless chalcones (Rein, 2005).

Testing on acid-base titration

The investigation on strong acid-base titration with karamunting fruit indicator extract revealed that the titration endpoint was reached upon the addition of 20 mL of 0.1 M NaOH, with an average pH of 8.76, accompanied by a color change from pink to colorless at the endpoint. The phenolphthalein indicator is utilized as the comparative metric. The titration endpoint was reached with the addition of 19.8 mL of 0.1 M NaOH, resulting in an average pH of 8.79 and a color change from colorless to purple (**Figure 3.**).

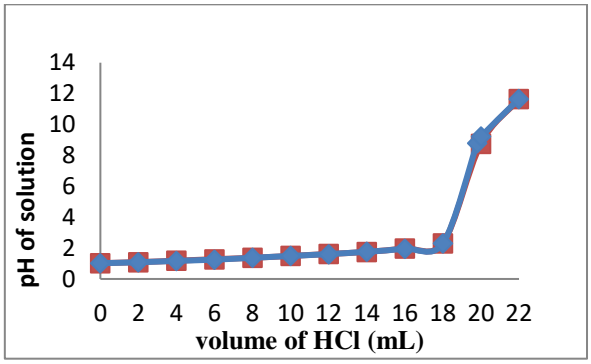

Figure 3. Strong acid - strong base titration curve

The titration curve of a strong acid-base utilizing karamunting fruit indicator extract, compared to phenolphthalein, revealed minimal variation in pH values between the two indicators. The pH values of the two indicators when adding 0.1 M NaOH solution from 0 mL to 22 mL are similar, resulting in minimal variation in the obtained pH values. This signifies that the karamunting fruit extract indicator is applicable for vigorous acid-base titration.

The investigation on strong weak-base acid titration with karamunting fruit indicators revealed that the titration endpoint was achieved with the addition of 20.2 mL of 0.1 M NaOH, resulting in an average pH of 9.11. The color transition from colorless to yellow at the titration endpoint. The comparable indicator employed was the phenolphthalein indicator at the titration endpoint, achieved with the addition of 20.4 mL of 0.1 M NaOH, resulting in an average pH of 9.17 and a color transition from colorless to pink at the endpoint. (**Figure 4.**).

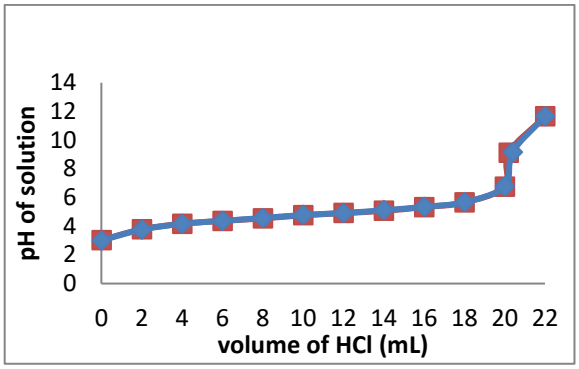

Figure 4. Weak - strong acid-base titration curve

The weak-base acid - strong acid titration curve using karamunting fruit indicator extract with phenolphthalein indicator as a comparison showed that the difference in pH value between the

Karamunting fruit with phenolphthalein indicators is not much different. The pH values of the two indicators in adding 0.1 M NaOH solution from 0 mL to 22 mL volumes tend to be close, so the pH values obtained are not much different. This indicates that the karamunting fruit extract indicator can titrate weak acids and strong bases.

Based on the results of the study on weak-acid base titration on the use of karamunting fruit indicator extracts, the titration endpoint was obtained in the addition of 20 mL HCl 0.1 M with an average pH of 3.68. The colour change occurs from green to pink at the titration endpoint. Meanwhile, the comparative indicator used was the methyl orange indicator of the titration endpoint obtained, namely at the addition of 19.8 mL HCl 0.1 M with an average pH of 3.69 and the colour change that occurred from yellow to orange at the titration endpoint (**Figure 5.**).

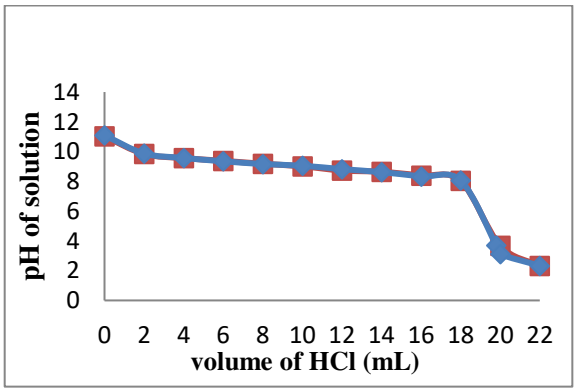

Figure 5. Weak-strong acid-base titration curve

The weak-base acid-strong acid titration curve using karamunting fruit indicator extract with methyl orange indicator as a comparison showed that the difference in pH value between the

Karamunting fruit with methyl orange indicator is not much different. The pH values of the two indicators in adding 0.1 M HCl solution from 0 mL to 22 mL volumes tend to be close, so the pH values obtained are not much different. This

indicates that the karamunting fruit extract indicator can be used for weak acid-base titration.

Conclusions

Ethanol extract from karamunting fruit can indicate acid-base. The karamunting fruit extract indicator can be used as an alternative to the phenolphthalein indicator, precisely in the titration of strong, weak, and strong acids. For the titration of weak bases, strong acids are good for replacing methyl orange.

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