Application of Butterfly Pea \((\textit{Clitoria ternatea} \text{ Linn})\) extract as an indicator of acid-base titration

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ABSTRACT

Flowers of butterfly pea \((\textit{Clitoria ternatea} \text{ Linn})\) contain anthocyanins. The color of anthocyanins change according to the pH solution. The aim of this study is to determine the application of butterfly pea extract as an indicator of acid-base titration. The results showed that the butterfly pea extract has the refractive index from 1.382±0.25 to 1.390±0.30, specific gravity from 0.975±0.20 to 0.993±0.25, maximum wavelength at 572 and 614 nm, and discoloration from violet to blue at pH 4, blue to green at pH 9, and green to yellow at pH 12. We concluded that the butterfly pea extract can be applied as an indicator of acid-base titration.

Keywords: \textit{Clitoria ternatea} Linn, flower extract, anthocyanin, acid-base indicator

INTRODUCTION

Butterfly pea \((\textit{Clitoria ternatea} \text{ Linn})\) are used as folk medicines. The roots and seeds are used as thenerves tonic and laxative. The leaves and roots are used in the treatment of urinogenital disorders, anthelmintic and antidote to animal stings [1]. Flowers of butterfly pea contain anthocyanins. Anthocyanins are plant pigment, which are responsible for red violet-blue color in plant flowers [2]. There are six major anthocyanins ternatins (A1, A2, B1, B2, D1 and D2) [3] which were characterized as malonylated delphinidin 3,3’,5’-triglucosides having 3’,5’-side chains with alternating D-glucose and \(p\)-coumaric acid [4].

The various shades of flower color are due to a very small number of different pigments. These pigments contain the same carbon skeleton, and different only in the nature of the substituent groups [5]. The color stability of anthocyanins depends on structure of anthocyanins, pH, temperature, oxygen, light and water activity [6]. The color of anthocyanins tends to red in very acidic solution and blue in basic solution [2, 7]. The application of blue anthocyanin-contained flower, likes butterfly pea, has not been optimal yet. This study was aimed to identify the anthocyanins in the flower of butterfly pea and apply its extract as an indicator in acid-base titration based on anthocyanin features.

EXPERIMENTAL SECTION

Materials

Flowers of butterfly pea obtained from Subang, Indonesia. Sodium chloride (NaCl), hydrochloric acid (HCl), potassium chloride (KCl), potassiumhydrogen phthalate, citric acid, sodium citrate, sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)), sodium tetraborate decahydrate (Na\(_2\)B\(_4\)O\(_7\)), boric acid (H\(_3\)BO\(_3\)), sodium bicarbonate (NaHCO\(_3\)), disodium hydrogen phosphate (Na\(_2\)HPO\(_4\)), methyl orange, and phenolphthalein were obtained from Merck (Germany).

Instrumentation

The instruments were pH-meter (Boeca), magnetic stirrer, analytical balance (Shimadzu), UV-Vis
spectrophotometer (Shimadzu), and refractometer (Innotech).

Methods

Extraction of Butterfly Pea Flowers
Fresh flowers (100 g) were cutted to 1-2 mm [8] and extracted with 100 mL of distilled water at 25 °C for 24 hours and at 60 °C for 30 minutes. Each extract was filtered to volumetric flask then rounded up to 100 mL.

Qualitative Analysis
Extracts spotted on silica gel GF254 plate (Merck), then eluted with n-butanol: glacial acetic acid: water (BAW) with ratio 5: 1: 2, 4: 1: 5, 3: 2: 5, and 4: 2: 4. The patterns of chromatograms were observed [2].

Determination of the Maximum Wavelength
Extract (1 mL) was diluted to 5 mL, wavelength is measured using visible spectrophotometer at 500-700 nm [2].

Physical Properties Determination

Organoleptic Test
Organoleptic test consists of determination of the color, smell and taste of the extract [9].

Specific Gravity Determination
The empty pycnometer is weighed, then filled with distilled water and extract, respectively. Extract specific gravity is obtained by dividing the weight of the extract with distilled water at 25 ºC [9].

Refractive Index Determination
Refractometer was calibrated with NaCl solution, then 1-3 drops of extract dripped and read the scale. Readable concentration is brix (%), then converted to the refractive index from the correlation table between brix and refractive index [9].

Chemical Properties Determination
The extract (1 mL) was added to 5 mL buffer solution of pH 1 to pH 14, then observed the color change[10].

Butterfly Pea Extract as An Acid-Base Indicator
Weak base-strong acid titration
Solution of 0.1 N NaHCO₃ (25 mL) and 10 drops of extract were putted in erlenmeyer and the solution turned green. The mixture was titrated with standardized 0.1 N HCl. The titration was stopped when the color of solution turned pink. The same procedure was conducted using methyl orange as indicator [11].

Weak acid-strong base titration
Solution of 0.1 N acetic acid (25 mL) and 10 drops of extract were putted in erlenmeyer and the solution turned pink. The mixture was titrated with standardized 0.1 N NaOH. Titration was stopped when the color of solution turned green. The same procedure was conducted using phenolphthalein as indicator [11].

RESULTS AND DISCUSSION

Extraction
Anthocyanin, the flavanoid pigments, are polar. Its soluble in ethanol, methanol, water, and another polar solvents[12]. Distilled water is used as a solvent, because the extract will be used as an indicator of acid-base titrations, which performed in water environment. Extraction is done by maceration at room temperature (25 °C) for 24 hours, and by reflux at 60 °C for 30 minutes [13]. The aims of this variation is to determine the anthocyanin stability to heat and time of extraction in the water.

Qualitative Analysis
The aims of eluent variation is to identify the variation of chromatogram pattern. Figure 1 showed that more n-butanol in composition, more slower migration rate of the blue anthocyanin. Whereas, more water in composition, more faster migration rate of the blue anthocyanin (Fig. 1).
The Maximum Wavelength Determination
The color of extract of butterfly pea extract was blue. UV-Vis analysis (Fig. 2) gave absorption at the maximum wavelength of 572 nm and 614 nm. Anthocyanin can be distinguished with the other classes by observing the absorption region wavelength of 475-560 nm\cite{14}. The maximum wavelength is different from the literature (475-560 nm). This happens because there are another metabolites were extracted and able to absorb part of the light produced from the spectrophotometer. The color difference affect the maximum wavelength\cite{13}.

![Fig. 1. Chromatogram pattern in BAW in ratio (a) 5:1:2, (b) 4:1:5, (c) 3:2:5, and (d) 4:2:4](image)

![Fig. 2. The maximum wavelength of butterfly pea extract](image)

Physical Properties Determination
Table 1 showed that the color, taste, and smell are not affected by the extraction method, i.e. time and temperature. The specific gravity of extract of butterfly pea flower (0.975±0.20 and 0.993±0.25) is meet the literature, i.e. 0.915 to 1.115. This indicates that the extraction method does not affect the specific gravity of the extract. While the refractive index (1.382±0.25 and 1.390±0.30) is different from literature (1.450 to 1.470). This happens because the lower extract concentrations with literature, so extract capability to reflect light is lower.

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Extraction method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Maceration</td>
</tr>
<tr>
<td>Color</td>
<td>Bluish purple</td>
</tr>
<tr>
<td>Taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td>Smell</td>
<td>Specific</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>0.993±0.25</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.390±0.30</td>
</tr>
</tbody>
</table>

Chemical Properties Determination
It was observed that butterfly pea extract was blue at pH 7. UV-Vis spectrum (Fig. 2) also gave maximum wavelength at 614 nm. These indicated that butterfly pea extract contained anthocyanin and can be applied as acid-base indicator. Organic compound that can be applied as indicator in titration have characteristic in discoloration in various pH of solution. Discoloration was happen through equilibrium process of molecule and ion.
of the indicator. The butterfly pea extract had discoloration from violet to blue at pH 4, blue to green at pH 9, and green to yellow at pH 12 (Fig.3).

![Fig.3. Color change of the butterfly pea extract](image)

The color of anthocyanins are dependent on the acidity of the medium. Anthocyanins in butterfly pea extract had flaviliumcation, which was unstable in the change of pH solution. The change of pH might make the change of anthocyanins structure that led the discoloration (Fig.4). Anthocyanin (structure I), in acidic condition was red. When pH increased (pH < 4), colorless carbinolbase (structure III) would be formed. Tautomeration occurred to produce chalcone (structure IV). At pH < 6 the structure changed into anhydrobase (structure II). Extension of conjugation in this structure gave color change to be blue with stronger intensity and maximum wavelength at 610 nm[15].

![Fig.4. Equilibrium of flaviliumcation of anthocyanin in various pH](image)

**Butterfly Pea Extract as An Acid-Base Indicator**

Anthocyanin contained flaviliumcation, which had conjugated double bond, thus led delocalization of positive charge to the whole molecule and gave some structure resonance. The effect of resonance caused the pigment structure can be more stable as flaviliumcation [15]. If nucleophile attacks carbon atom 2 (step a), pseudobase carbinol (B) will be formed. This species would undergo isomerization into chalcone (c) via water
catalyzed-tautomerization. Then, if a base attacks hydrogen atom of hydroxyl group (step b), quinoid will be formed (Fig. 5). The formation of quinoid would extend the delocalization, thus, gave color change from red in acidic condition to green in basic condition[16, 17].

![Fig. 5. Reaction of flaviliumcation from apigenidin into quinoid (A), pseudobasecarbinol (B) and chalcone (C)[16, 17]](image)

The discoloration of indicator of butterfly pea extract in weak acid-strong base titration was from pink to green. Whereas, butterfly pea extract gave color change of green to pink in weak base-strong acid titration (Table 2). The pH range of the reference indicator of phenolphthalein and methyl orange are 8.0-9.6 and 4.4-3.1, respectively[11]. We concluded that butterfly pea extract could be applied as indicator in weak base-strong acid titration.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Weak acid – strong base</th>
<th>Weak base – strong acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>25 ± 0.35</td>
<td>Pink - green</td>
</tr>
<tr>
<td>Phenolphthalein</td>
<td>25 ± 0.15</td>
<td>Colorless - pink</td>
</tr>
<tr>
<td>Methyl orange</td>
<td>25 ± 0.20</td>
<td>25 ± 0.20</td>
</tr>
</tbody>
</table>

CONCLUSION

The butterfly pea extract can be applied as an indicator of acid-base titration.

REFERENCES

1970.