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The Acid-Base Indicator Paper of Extracted Ethanol from Ashoka Flower (*Ixora coccinea Linn*) with Variances of Solvent and Soaking Period

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Abstract: This research developed a paper indicator made from extracted Ashoka flower, *Ixora coccinea Linn*, with solvent and the soaking period variances. Ashoka flower is a plant containing anthocyanin pigment. The plant is sensitive to acidic levels of pH. The content of the Ashoka flower brings significant potential as a natural indicator. The researchers extracted the anthocyanin pigment of the lower with the maceration method with 96% and 70% ethanol solvents for 24 hours. The researchers calibrated the extracted ethanol with buffer solution under pH 1-12. Then, the researchers cut 42 Whatman papers sized $\pm 5x1$ cm. The researchers put the papers into the mixtures of the extracted ethanol within 48 - 72 hours of the soaking period. After that, the researchers dried out the papers and examined the solution in terms of acid-base level. The papers made of extracted ethanol from Ashoka flower on solutions of HCl, CH₃COOH, NH₄OH, NaOH had their colors changed into brighter pink, pink, light green, and green-brownish. The color changes on the developed paper indicators made of extracted ethanol solution and 72 hours of soaking period.

Keywords: Paper Indicator; Acid-Base; Ashoka flower extract; Solvent variances; Soaking period

Introduction

The acid-base indicator refers to chemical compounds to measure the acidic and base levels. This indicator will change its color under certain pH levels (Pimpodkar et al., 2014). This indicator is useful to indicate the acidic and base features of a solution. The indicators of acid and base are complex compounds. These compounds react with acid or base, indicated with color changes based on the hydrogen ion concentration due to the titration process (Reshetnyak et al., 2017; Zoromba, 2017; Sukhanov et al., 2016; Mchedlov-Petrossyan et al., 2018; Udugala-Ganehenege et al., 2015; Baldigo et al., 2018; Akbar, 2019).

The acid-base indicators in chemical laboratories, especially at Senior High School levels, are synthetic indicators. The indicators work within lower concentrations and could detect the acid-base levels at the final titration point. The observable changes of the indicators include color changes. Each synthetic indicator has certain characteristics, such as pH trajectories and color change indications within the acidbase situation and applied indicator constant. The commercial synthetic indicators include phenolphthalein, red phenol, red methyl, blue bromophenol, blue thymol, etc. These indicators are preferable pH indicators during acid-base titration. Pawar et al. (2021) explain that synthetic indicator is expensive. This indicator can also make the environment polluted. Thus, finding alternative or natural and green indicators is important. The drawbacks of synthetic indicators include expensive costs, toxic effects, and polluting the environment (Saati, 2015). Therefore, efforts to find alternative indicator sources from nature

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are important (Singh et al., 2011; Abbas, 2012; Onwuachu et al., 2014).

Many plants contain anthocyanin-based components the natural colors. Anthocyanin can be extracted from waters or other solvents of plants, including the leaf, flower, fruit, and trunk. The anthocyanin pigments are varied, depending on the pH levels of the medium (Bendaali et al., 2022). The natural indicators can be synthesized from the surrounding plants. Natural indicators include pigment substances isolated from various plants, fungi, and algae. People can make natural indicators from colorful plants. Most colored plants can be used as indicators. However, some of them have unclear color changes. One of the natural indicators is the Ashoka flower. Ashoka flower shown Figure 1.



Figure 1. Ashoka Flower (*Ixora occinea Linn*) (Personal Documentation)

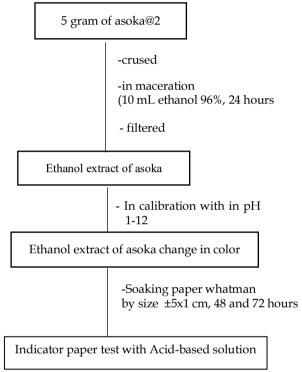
Ixora coccinea Linn (*Rubiaceae*), also known as the Jungle of geranium from Ayurveda, refers to bushes with blossoming flowers. These plants are mostly found in Asia. These plants have dark green colors with heights of about 4-6 feet, 1.2 - 2 m. The plant's leaf has an oblanceolate or elliptical shape with opposite strips. The length of the leaf ranges from 3 - 10 cm. The plant has a green color with integrated, sharped, and elliptical petals with 9 - 14 mm lengths. The flowers have orange colors due to flavonoid and anthocyanin contents ((Deshpande et al., 2010). Ixora coccinea is a plant that contains polyphenols that can counteract free radicals (Bose et al., 2013).

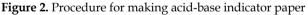
Ashoka flower, *Ixora coccinea L*, is a plant that produces pigments. *Ixora coccinea L* extract substances contain flavonoids, triterpenoids, and tannin (Okhale et al., 2018). Flavonoid and tannin produce sharpest-color changes at certain pH compared to non-flavonoid and tannin derivatives (Abugri et al., 2012). The photochemical of *Ixora coccinea Linn* with orange colors include methyl ester palmitate, stearate, oleate and linoleic acid, and octadecadienoic acid (Finlaysoniana et al., 2018).

Natural indicators in the form of a solution are preferable. However, the natural indicators in the form of solvent have some shortcomings, such as being easily damaged and unstorable for a longer time (Andarias, 2019). From the explanations, this research developed a paper indicator made from extracted Ashoka flower, *Ixora coccinea Linn*, with variances of solvent and the soaking period.

Method

The applied method used Ashoka flower, *Ixora coccinea Linn*, as the main ingredient to create a natural acid-base indicator. The researchers found the flower around the surrounding environment. The process of creating the indicator used the extraction method.





First, the researchers washed the fresh Ashoka flowers. Then, the researchers determined the size, 5 grams of Ashoka flower. The researchers did this process four times before grinding the flowers with mortar and pestle. Then, the researchers macerated the ground flowers by adding 10 mL of 96% and 70% alcohol. After that, the researchers stored the macerated results in a dark container for 24 hours. The researchers screened the extracted ethanol from the Ashoka flower with screening paper. Then, the researchers calibrated the results with pH 1-12 buffer solutions. The calibration results showed color changes of Ashoka flowers, Ixora coccinea Linn. The next step required the researchers to cut the numbered 42 Whatman paper sized \pm 5x1 cm. The researchers soaked the paper into the extracted ethanol of Ashoka flowers with 70% and 90% alcohol for 48 and 72 hours. The final step was drying the Whatman paper. Then, the researchers examined the papers with acid-base solutions to identify the acid-base features observed from the indicator papers.

Result and Discussion

This research developed a paper indicator made from extracted Ashoka flower, *Ixora coccinea Linn*, with solvent and the soaking period variances. The researchers washed and ground the flowers with mortar and pestles to enlarge the particle surfaces. Thus, anthocyanin's pigment substance will be dissolved in the solvent (Jafari et al., 2016). The particle sizes of the extracted ingredients get smaller with simpler molecular structures. These results have the enlarged pores of the ingredients. Thus, the solvent will be easily diffused into the extracted cells and create more dissolved substances within the solvent.

The researchers macerated the ground Ashoka flowers for 24 hours. (Pratama, 2015) Found the best soaking period of extracted teak leaf in ethanol-HCL was 24 hours. This soaking period was the optimum period because the process oxidized the thick extract that made the plant could not survive for a longer storing process. (Março *et al.*, 2011) found maceration process of rose and Rosella for natural acid-base indicators needed 96% ethanol solvent stored at room temperature. The storing place had to be capable of absorbing lights because high temperatures influenced the instability of anthocyanin. This situation would damage anthocyanin.

Ashoka Flower Ethanol Extract Calibration

Ethanol was also useful as a solvent during the maceration process due to its polarity. Thus, ethanol was more efficient in gaining the extracted result. The extracted results of 70% and 96% ethanol solvent of the sample showed that the extracted flowers of ethanol fraction had pink and green brownish colors. The researchers calibrated each extract with pH 1-12, as shown in Figure 3.



Figure 3. The extracted color of 96% ethanol of Ashoka flower at pH 1-12

Figure 3 shows the calibration results of the extracted 96% ethanol of Ashoka flower in solutions with pH 1-2, which are pink. Then, the color fades at pH

3 until pH 6. The color change appears at pH 7 until pH 9, indicated by lighter brown. Then, at pH 10-11, the color change appears to be brown-reddish, while at pH 12, the color change is green brownish. These color changes were due to anthocyanin structure changes due to pH changes. Anthocyanins are unstable and bring brighter colors at acid pH. The color fades along with increased pH, and the color changes along with the increased base pH. The acidity or pH level influences the stability of the anthocyanins compound. The compound is more stable at pH situation (Choi et al., 2017); (Ge & Ma, 2013); (Puértolas et al., 2011).

Ashoka Flower Ethanol Extract Indicator Paper

After calibrating the extracted ethanol of the Ashoka flower, the researchers cut the Whatman paper number 42, sized ±5x1 cm. Then, the researchers soaked the papers in 70% and 96% ethanol solutions for 48 and 72 hours. Then, the researchers dried the indicator papers at room temperature. Figure 4 shows the results.



Figure 4. The extracted ethanol 96% and 70% from Ashoka flower for acid-base indicator papers

The researchers used the applied Whatman paper because the paper was thick. The color absorption of the extracted plant by the paper is excellent, with better gradation than a screening paper or HVS paper. (Herdiana, 2013) Found that Whatman's paper had excellent graded colors than HVS papers and screening papers. The researchers dried indicator papers and examined the papers with strong-weak acid-base solutions, such as HCl, CH₃COOH, NaOH, and NH₄OH. Figure 5 shows the color changes of acid-base indicator papers from extracted Ashoka flowers with 70% ethanol.

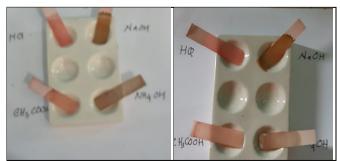


Figure 5. The colors of acid-base indicator papers made from extracted Ashoka flower with 70% ethanol (Soaking periods: 48 and 72 hours)

The examination of the indicator paper with four solutions, HCl, CH₃COOH, NaOH, and NH₄OH, indicated color changes. At 70% ethanol extract with 48 and 72 hours, all colors were similar in HCL. They were pink. Then, at CH₃COOH, the color was lighter pink. In NH₄OH, the color was lighter green brownish. Then, in NaOH, the color was lighter brown. From the results, the 72 hour soaking period had better results than 48 hour soaking period. Figure 5 shows the color changes of acid-base indicator papers from extracted Ashoka flowers with 96% ethanol.

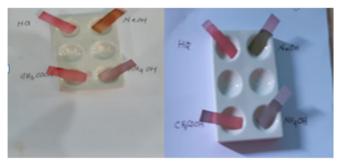


Figure 6. The colors of acid-base indicator papers made from extracted Ashoka flower with 96% ethanol (Soaking periods: 48 and 72 hours)

The examination results with four solutions, strong acid HCL, weak acid CH₃COOH, and weak base, indicated color changes. Table 1 shows the changes.

Table 1. The results of acid-based solution identification

 with indicator papers of extracted Ashoka flower

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Solutions	Color changes
HCL	Brighter pink
CH ₃ COOH	Pink
NH4OH	Lighter Green
NaOH	Green Brownish

The test results of indicator papers with four solutions, HCl, CH₃COOH, NH₄OH, and NaOH at 90% ethanol extract, showed that HCL had brighter pink color, CH₃COOH with pink NH₄OH with blue-greenish color, and NaOH with brownish-green color. The color changes indicated the best soaking time was 72 hours with 96% ethanol.

In this research, the researchers found the increased pH, strong base solution of NaOH, with brownish-green color. This result was equivalent to pH 10 or higher if the solution was examined with an indicator test of pH 1-12 (Putra *et al.*, 2017). From the analysis of the indicator papers of the extracted ethanol from Ashoka flower the examination results of acid-base solutions, the researchers found clear color changes on the extract with 96% alcohol and 72 hour soaking period. Thus, the indicator paper s because the papers showed clear color changes to identify the acid and base of a solution. The natural indicator papers had some superiorities, such as

economic, green material, and environmentally friendly. Thus, the extracted ethanol from the Ashoka flower was useful as an acid-base alternative indicator to replace the synthetic indicator.

Conclusion

The natural acid-base indicator of extracted ethanol from Ashoka flower is applicable to determine acid-base solutions. The calibration test showed the extracted ethanol with pH 1-12 had significant color changes within 70% and 96% ethanol contents. The color change of strong acid was bright pink, while the weak acid was light pink. The color change of the weak base was lighter green while the strong base was greenbrownish. The researchers also found the best color changes were at 96% ethanol concentration with 72 hours of soaking period. The analysis results showed the natural acid-base indicator had some superiorities, such as economic, green material, and environmentally friendly.

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