Acid-Base Indicator Paper Extracted from Rose (*Rosa damascena Mill*) and Asoka Flower (*Ixora occinea Linn*) Ethanols

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Abstract

This research aims to develop paper indicators extracted from rose (*Rosa damascena Mill*) and Asoka flower (*Ixora coccinea Linn*) ethanols. These flowers have anthocyanin pigment and are sensitive toward acidity (pH). The contents make rose and Asoka flower to have potentials as natural indicators. The anthocyanin pigment extraction from both flowers was done by maceration method with 96% ethanol solvent. It was done with 24 -hour soaking period. The ethanol extracts from rose and Asoka were calibrated by buffer solution with pH 1-12. Then, it was continued by cutting Whatman 42-paper sized ±5x1 cm. The paper was soaked in rose ethanol extract (*Rosa damascena Mill*) and Asoka flower (*Ixora coccinea Linn*) with a ratio of 1:1 for 24 hours. The indicator papers were dried then tested in acid-base solution. The indicator papers showed a colour change from pink, found in acid solution, to green-brownish, found in base solution. The findings showed that the rose and Asoka flower indicator papers could be used as an acid-base indicator. The produced indicator papers have several superiorities. They are made from natural materials, less-polluted, more economical, more durable, and have contrast colour gradation.

Keywords:

Acid Indicator Paper, Ethanol Extract, Rose, Asoka Flower

1. Introduction

An acid-base indicator is a substance in which the colour can be changed as acid or base solutions. Indicator functions to indicate the acid or base features of a solution. An acid-base indicator is an organic compound in which the colour can be changed due to changing pH. It is usually used to differentiate acid or base solution by providing different acid or base solutions (Fesseden & Fesseden, 1999). An acid-base indicator is complex compound and can react with acid or base features with colour changes based on the hydrogen ion concentration with titration process (Reshetnyak et al., 2013; Zoromba, 2017; Sukhanov et al., 2016; Petrossyan et al., 2018; Udugala-Ganehenege et al., 2015 ; Baldigo et al., 2018; Akbar, 2019) The function is based on the indicator features. They can provide different colours when they are added to acid or base solutions.

Indicators are dyes or pigments that can be isolated from a variety of sources, including plants, fungi, and algae and nearly all flowers that are red, blue, or purple in color contain a class of organic pigments called anthocyanins that change color according to their pH (Pawar et al., 2021). The colour changes on indicators occur due to the changing pH. The titration process's applied indicators show the colour changes, labelled good in certain pH interval (Singh et al., 2011). Theoretically, the indicators of acid-base are halochromic compound. They are dropped into a certain solution so that the solution's pH could be determined visually due to its colour changes. Thus, pH indicators are chemical detectors of hydronium ion (H_3O^+) or hydrogen ion (H^+) (Zumdahl, 2009). The indicators are mostly from weak acids or base in the ion forms with a little disassociation when they are dissolved in water (Sharma et al., 2013). Acid-base pH indicators are weak acids (indicator acids) or weakly basic organic substances that give different colors to their protonated and deprotonated forms meaning they change color according to pH and can be classified according to the mechanism of change or the type of titration and discoloration extract showed good activity having the same pH indicator (Torres Brilhante et al., 2015).

Acid-base indicators are mostly used in a chemical laboratory in the forms of synthesized indicators. Each synthesized indicator has specific characteristics in the forms of pH trajectories. They are shown by colour changes within acid and base conditions along with the constant indicator value. The synthetic indicators are commercially available, such as phenolphthalein, red phenol, red methyl, blue bromophenol, blue thymol, etc. They are mostly preferred as pH indicators with acid-base titration (Nair et al., 2018; Sabnis, 2007). The availability of synthesized indicators are limited and so do the implementations. Besides that, synthetic indicators are expensive and polluting the environment (Nuryanti et al., 2010). Thus, alternative indicators (natural indicators) that can be easily obtained and environmentally green are needed. Commercial indicators have several weaknesses. For examples, they are expensive, toxic, and polluting the environment (Saati, 2015). Thus, the intention to find an alternative - natural indicator sources should be realized (Singh et al., 2011; Abbas, 2012; Onwuachu, 2014). The natural material indicators are such as colourful flowers. They can be used as natural indicators, such as rose, Asoka flower, etc.

Figure 1. Rose **Figure 2**. Asoka flower
(*Personal Documentation*)

The natural indicators in the form of solutions are the most frequently used. However, these indicators are easily damaged and cannot be stored for a longer time. Contrary, paper or granule formed indicators are relatively durable than the natural soluble indicators. This research aims to develop paper indicators extracted from rose (*Rosa damascena Mill*) and Asoka flower (*Ixora coccinea Linn*) ethanols. The benefit of this research is to provide information to the community, especially the teachers and practitioners in school that interest Asoka can be used as a natural indicator so it can used as a substitute for indicators of synthesis in practicum acid and base at school.

1.1 Objectives

This research aims to develop paper indicators extracted from rose (*Rosa damascena Mill*) and Asoka flower (*Ixora coccinea Linn*) ethanols

2. Literature Review

Rose is a shrub crop plant from the genus of *rosa*. It also refers to the name of the plant's flower. The Rosaceae family, commonly known as the rose family, is one of the most important families that serve as a food source and ornamental tree and shrub. Rosa damescene of the family Rosaceae, is one of the most important commercial flower crops with over 150 species, more than 20 000 cultivars (Elfina, et al., 2020). Rosa damascena factory can also be used as a priority crop of economic importance for the country (Minera et al., 2020). Rosa damascena Mill is grown for its flowers, which contain essential oils, including rose oil (Mineva & Stoyanova, 2020). The flowers are characterized by their full appearance and dense blooms which make them outstanding as houseplants (Elhawary et al., 2021). Damask roses (Rosa damascena Mill) usually used to make candy in addition, rose flowers have been used as traditional Chinese medicine to treat various ailments and Roses are rich in polyphenols and flavonoids which have significant levels of antioxidants (Liu et al., 2020). The most dominant components in fresh-rose petals are such as water (83-85%), vitamin, β-carotene, cyanin (anthocyanin), total sugar 8-12%, essential oil - approximately 0.01 until 1.00% citronellol, eugenol, gallic acid, and linalool (Visita&Widya, 2014). Roses contain quite a lot of chemicals including tannins, geraniol, nerol, citronellol, geronic acid, terpenes, flavonoids, polyphenol pectin, vanillin, carotenoids, stearopten, farnesol, eugenol, phenyletilacohol, vitamins B, C, E, and K (Ardian et al., 2018).

Rose flower is known as an astringent, stomach, and is used traditionally as an agent to activate blood circulation to relieve blood stasis, and ward off toxins. The color of roses is caused by the presence of anthocyanins and carotenoids (Kumari et al., 2017). Rose petals have been consumed as food ingredients in teas, cakes, and flavoring extracts as well as medicines for various diseases (Li et al., 2014).
Red rose flower extract (Rosa damascena Mill) contains tannin, geraniol, nerol, citronellol, flavonoids which have antibacterial effects (Pasril & Okasari, 2020). Roses are known to contain anthocyanin pigments which are classified as flavonoids, and the types are pelargonidin and cyanidin, which can work as free radical scavengers or antioxidants, while dark red roses contain cyanidine, and pink roses have pelargonidin (Saati et al., 2021; Pawar et al., 2020).

A red-rose anthocyanin pigment could be used as an indicator. The use of pigments of some common flowers as acid/base indicators has been demonstrated using common flowers such as Hibiscus rosa-sinensis, containing various anthocyanin pigments of purple-red color, the acetone extract of the pigment turns red in the presence of acid and shows max absorption. at 500 nm H. rosa-sinensis (Vankar & Bajpai, 2010). In addition, roses are rich in anthocyanins which may be used as PH sensing indicators for the development of colorimeter films (Kang, et al., 2020). Paristiowati et al., (2019) found that rose and Rosella could be used as acid-base indicators. The extract of rose provides pink and green colours on an acid-base solution; Maryanti et al. (2011) found that extract of rose with titration had colour changes in acid-base solution from red to yellow. The colour changes happen since rose has anthocyanin. It has roles as colour-formation substances, so it could be used as indicators (Askar, 2015). Anthocyanin is a natural pigment. It is mostly found in fresh fruits, vegetables, and flowers. It is a water-soluble compound and has various colours. It also has been proved to be a very excellent antioxidant (Patil et al., 2010 Roses are rich in anthocyanins which have the potential as pH sensing indicators (Suzihaque & Mohammad, 2021). According to (Park et al., 2016), rose petals and roots contain organic molecules that can resist microbial attack. Flower color investigation of roses so far have shown that four anthocyanins, 3-glucosides and 3,5-diglucosides of cyanidin (Cy) and peonidin (Pn), can be detected in flowers of wild Rosa species, and also pelargonidin (Pg) 3-glucoside and Pg 3,5-diglucoside are detected in Rosa cultivars, the structure of anthocyanins can be seen in Figure 2.

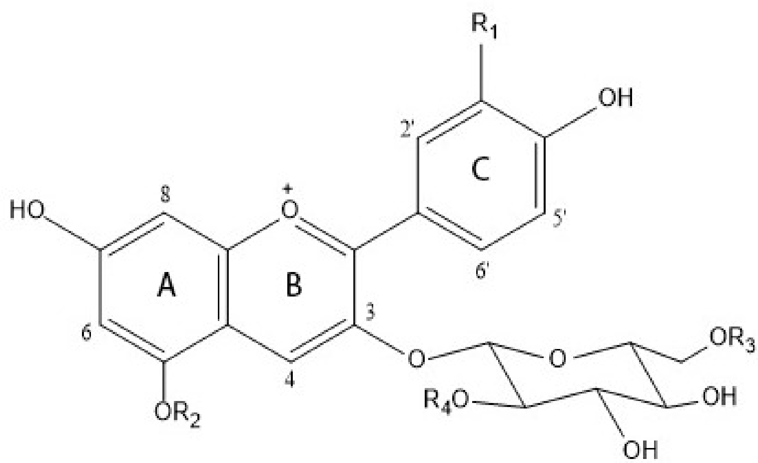

Figure 2. *Rosa* anthocyanins
(Mikanagi et al., 2000)

The other applicable natural material as an acid-base indicator is Asoka flower. Ixora coccinea Linn known as Jungle of Geranium or flame is a common flowering shrub native to Asia, belonging to the family Rubiacae, is a common flowering shrub native to Asia (Saha et al., 2008; {Formatting Citation}. There are about 400 species spread from Africa to South Asia. They differ in leaf size, plant height, flower size and flower color, are well-cultivated common flowering shrubs (Takarkhede et al., 2019). Ixora grows in tropical climates, flowers round, blooms and is easy to grow every year. Table 1, Ixora coccinea is an important plant used in gardens or outdoor gardens, because of its flower color and potential for other uses such as hedges or single plants (Tun et al., 2017).

Table 1. Ixora coccinea L.Taxonomic Hierarchy

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Subkingdom	Viridaeplantae – green plants
Infra kingdom	Streptophyta – land plants
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanerogames
Infradivision	Angiospermae – flowering plants, angiosperms
Class	Magnoliopsida
Superorder	Asteranae
Order	Gentianales
Family	Rubiaceae – madders, rubiacees
Genus	*Ixora*
Species	*Ixora coccinea* L.

(Castle & Herd, 2018)

The name Ixora usually used for Ixora coccinea is a dense, multi-branched evergreen shrub generally 1.2-2 m tall. The leaves are simple, opposite, usually elliptical 3-10 cm long, dark green. Ixora is a handsome plant in cultivation with bright and attractive flowers (Arafa et al., 2019). The flowers are used for the treatment of dysentery, vaginal discharge, dysmenorrhea, hemoptysis, and cataract bronchitis (Ghani, 2003) and the leaves can be used to treat diarrhea (Annapurna et al., 2003). According to Nuria et al., (2009) these compounds can function as antibacterials. Flavonoid compounds are lipophilic which will damage bacterial membranes and flavonoids work as antibacterials to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and are followed by the release of compounds. Intracellular. Ashoka flower (Ixora coccinea L) is an ornamental plant that grows a lot in Indonesia and has properties for several diseases (Shobana, 2021) and phytochemical screening tests of asoka flower show that methanol extract contains a class of flavonoid compounds, saponins, and alkaloids containing compounds of asoka flower extract positive for triterpenoid compounds, tannins and glycosides (Hikmah & Astuti, 2020; Hendra & Yuni, 2016). The extract of *I.coccinea* flowers contains triterpenoid and ursolic acid (Latha & Panikkar, 2000).

Asoka (*Ixora* occinea L) is a plant producing pigment. The substances contained in Asoka are such as glycosides, flavonoids, tannins, and saponins. Flavonoids and tannins can create contrast changing colours on a range of certain pH than the non-flavonoids and derivative tannins (Daniel et al., 2012). The orange-coloured Asoka flower is due to flavonoids and anthocyanin. Ixora flower extract is useful for weak acid and weak base titrations because it involves the use of mixed indicators, while flower extract can be used alone in titrations . The flower extract could be used as indicators for various acid-base titration types because flavonoids and anthocyanin of the flower are sensitive to pH (Deshpande, 2010).

According to Jadhav (2009), Ixora flower extract is useful for weak acid and weak base titrations because it involves the use of mixed indicators, while flower extract can be used alone in titrations and another benefit of this titration is the colorless end point at the equivalence point. titrant (base), gives a greenish-yellow color. Some indicators from nature, including color changes seen from the pH range. Catharanthus rosea and nerium oleander extracts in acidic water (pH 3.59 and 2.40) are red in color, a mixture of extract solution and saturated lead (II) acetate produces an intense blue green color; whereas in concentrated sodium hydroxide solution, the extract becomes green (Alexander, 2018).

3. Methods

This research was done using rose (Rosa damascena Mill) and Asoka flower (Ixora coccinea Linn) as the raw materials for producing natural acid-base indicators. The flowers are found in the surrounding environment. The production of these natural indicators was done by extracting them. Both rose, and Asoka flowers were washed well. Then, it was continued by measuring the flower weight, 5 grams. Then, they were finely ground. After that, they were macerated by adding 10 mL of 96% alcohol and stored in a dark place for 24 hours. The rose and Asoka ethanol extracts were screened by screening paper. Then, they were calibrated with pH 1-12 buffer solution. The calibration results showed changing colours of rose and Asoka flowers. They could also be used to mix both extracts with a ratio of 1:1. The next stage was cutting the Whatman 42 paper sized ±5x1 cm. Soaking the paper in Whatman paper in the extract solution for 24 hours. Drying the papers and examining results with acid-base solutions, identifying the acid-base feature of a solution.

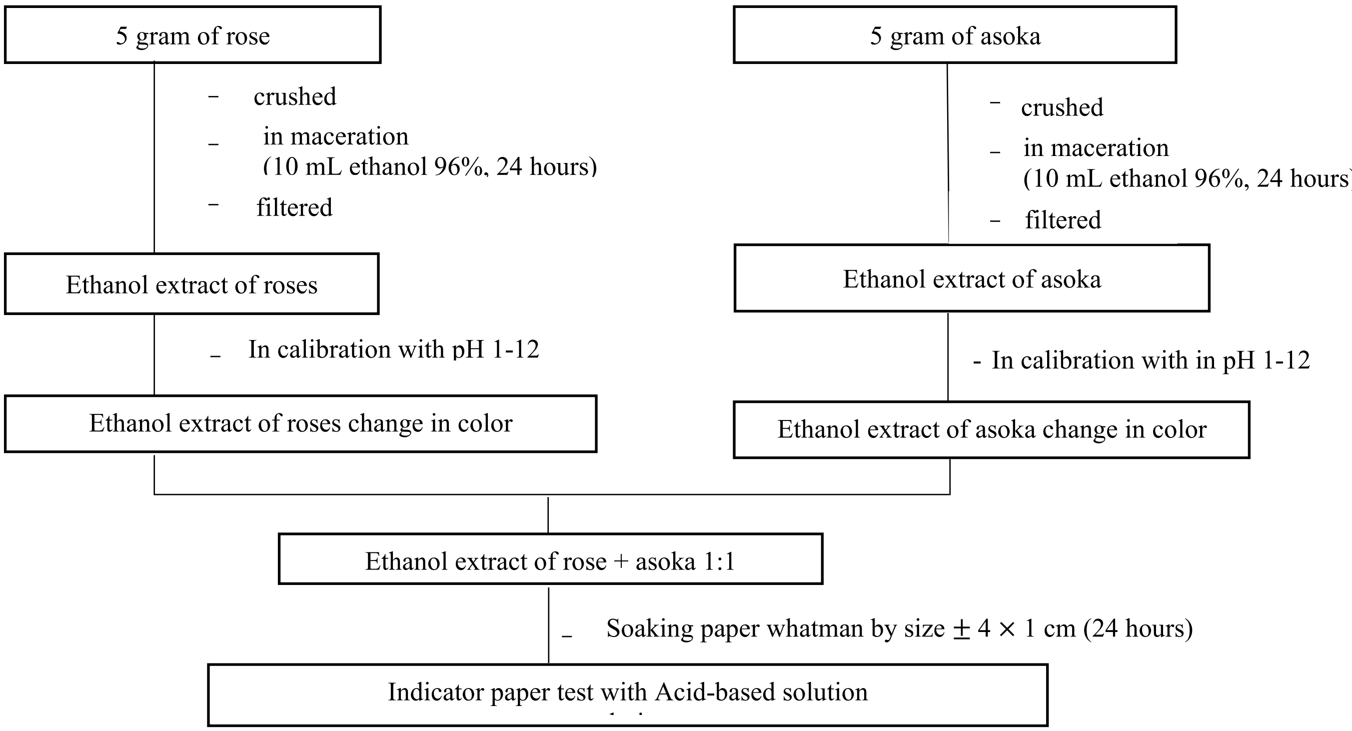

Figure 3. Procedure for making acid-base indicator paper

4. Results and Discussion

This research developed an acid-base indicator from natural materials by using rose and Asoka flowers. The flowers were washed then refined to expand the article surface area so the pigment, anthocyanin, would be dissolved in the solvent (Jafari et al., 2016). The extracted particle size would be smaller, and the molecule structures would be simpler. It caused porosities or the expanding pores of the materials. This situation made the solvent easily diffused into the cells of the extracted materials. Thus, there would be much-dissolved substance in the solvent. The process of macerating the flowers as an acid-base indicator was done by using 96% ethanol solvent at a room temperature and light-absorbing space because the stability of anthocyanin is influenced by temperature. Therefore, high temperature damages the anthocyanin (Henrique et al., 2011). The use of ethanol as a solvent during the maceration process was done because of the anthocyanin compound's polarity in rose. It was similar to ethanol solvent so that it would be more efficient to get the extract.

The extraction results of 96% ethanol solvent from both taken samples showed that rose extract in ethanol fraction had brownish colours, and so did the Asoka extract. Each extract was calibrated with pH 1-12. In the extracted-rose result, the test of the solution with pH, with rose indicator, showed pink colour and gradually faded at pH 6. Then, started at pH 7, the colour turned into yellowish. It turned again into yellow brownish until green brownish at pH 9-12. It showed that red rose with anthocyanin, which was acid, had a red colour.
On the other hand, when it was put in base condition, the colour would be green brownish. The stability of anthocyanin is influenced by pH, so rose can be used as alternative raw materials to substitute acid-base commercial indicator (Abou et al., 2011). The colour changes were seen in the solution with pH 1-12 after being added by rose indicator, as shown in Figure 4.

Figure 4. Color changes of the rose extract with pH 1-12

Similar things were shown in the test with Asoka indicator. The test of pH 1 solution showed a pink colour. In pH 2 solution, the colour turned into light pink. In pH3 until pH 8, the light pink colour faded. The colour change was seen in pH 9. The colour turned into yellow greenish. Then, in pH 10-11, the colour turned into yellow brownish. In pH 12, the colour showed green brownish. It was caused due to the anthocyanin structure. It easily changes due to pH changes. Anthocyanin is stable and provides bright colours in acid pH. The colour gradually changes along with the increased pH. Then, it has no colour in pH 4 until 5. The stability of anthocyanin compounds is influenced by pH or acid levels. It will then be more stable in the acid situation (Paristiowati et al., 2019; Choi et al., 2016; Ge & Ma, 2013; Puértolas et al., 2011).

The colour changes were seen in the solution with pH 1-12 after being added by Asoka indicator, as shown in Figure 5.

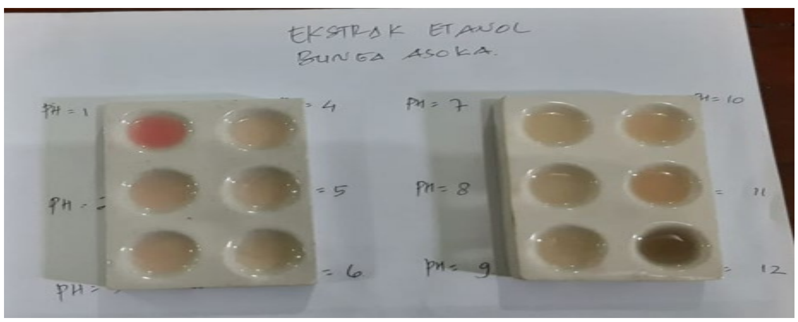

Figure 5. Color changes of Asoka extract with pH 1-12

After being tested on each extract and showing significant colour changes, those extracts were mixed with a ratio of 1:1. It was then continued by cutting Whatman 42 sized ±5x1 cm paper to create an indicator paper of the extract combination. The paper was soaked in the extract for 24 hours and dried. The dried indicator papers were then tested with HCl, CH3COOH, NaOH dan NH4OH. The colour changes in the produced indicator papers could be seen in Figure 6.

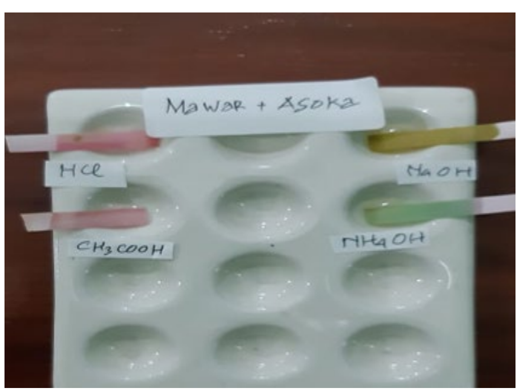

Figure 6. Indicator Paper Test with strong-weak, acid-based solution

The indicator paper test results with four solutions, strong acid, HCL, showed colour changes, as shown in Table 2.

Table 2. Acid-base liquid identification results with rose and Asoka indicator papers

Solution	Colour Changes
HCl	Pink
CH_3COOH	Faded Pink
NH_4OH	Faded light green
NaOH	Green brownish

From the table, with four solutions, HCL showed pink colour. The weak acid CH_3COOH showed a faded pink colour. The strong base, NaOH showed red-brownish. Then, the weak base NH_4OH showed faded green. PH changes influence the colour stability of anthocyanin. Thus, CH_3COOH solution had fading colours, but it is still in the acid situation. NH_4OH solution had faded light green. It showed that the pH increased, the base solution. Strong NaOH had a brownish-green colour. When it was referred on the previous indicator test results, in pH 1-12, the green colour showed that the solution had a pH higher than 10 (Putra et al., 2017).

The data analysis results about indicator papers of the flower extract combination, after being tested in acid-based solution, showed that clear colour changes. Thus, the indicator papers could be used as acid-base indicator papers. The indicator papers from natural materials had superiorities, such as more economical, reachable materials, and less contrast-colour degradation. The materials also could prevent environmental pollution and are more durable than natural liquid indicators.

5. Conclusion

Acid-base indicators made from natural materials rose, and Asoka flowers could be used to determine acid or base solutions' properties. The calibration results of rose and Asoka extracted ethanols in pH 1-12 showed significant colour changes. Thus, both extracts could be combined to create acid-base paper indicators by using Whatman 42 paper. It showed colour changes in strong acid (pink), a weak acid (faded light pink), weak base (faded light green), and strong base (green brownish). The indicator papers from natural materials had superiorities, such as more economical, reachable materials, and less contrast-colour degradation. The materials also could prevent environmental pollution and are more durable than natural liquid indicators.

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