

# INNOVATION OF BINAHONG (*Anredera cordifolia*) SEED EXTRACT INDICATOR PAPER AS AN ALTERNATIVE IN ACID BASE TESTING

Cica Susilawati<sup>1</sup>, Tety Sudiarti<sup>2</sup>, Ade Yeti Nuryantini<sup>3\*</sup>

<sup>1,2,3</sup> Program Pascasarjana Tadris IPA, Universitas Islam Negeri Sunan Gunung Djati Bandung, Indonesia

E-mail: \*ade.yeti@uinsgd.ac.id

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**Abstract:** The use of synthetic chemicals in acid–base testing has raised concerns about their environmental impact. The use of natural materials to produce natural indicator paper is an attempt to reduce the negative impact on the environment. Binahong (*Anredera cordifolia*) seeds can replace conventional chemical synthetic materials. This study aims to develop Binahong seed indicator paper as an alternative environmentally friendly material for acid–base testing. The method used in this research is experimental and involves the extraction of Binahong seeds, making indicator paper with a certain formulation, and testing the performance of the indicator paper using standard acid and base solutions. Data collection was performed via the observation and measurement of certain parameters. The test results of the Binahong natural indicator paper revealed a significant color change when 95% ethanol was used, which produces a red or purple color at pH 1–7, a blue color at pH 8–10 and a yellow color at pH 11–14. Binahong seeds have potential as an environmentally friendly alternative in acid–base testing because they respond well to variations in the acidity and basicity of the solution.

**Keywords:** acid–base, Binahong seeds; extraction; indicator paper

**Abstrak:** Penggunaan bahan kimia sintetis dalam pengujian asam basa telah menimbulkan kekhawatiran terhadap dampak lingkungan. Penggunaan bahan alam untuk menghasilkan kertas indikator alami merupakan upaya untuk mengurangi dampak negatif terhadap lingkungan. Biji Binahong (*Anredera cordifolia*) dapat menggantikan bahan-bahan sintesis kimia yang konvensional. Penelitian ini bertujuan untuk mengembangkan kertas indikator biji Binahong sebagai alternatif bahan ramah lingkungan dalam pengujian asam basa. Biji Binahong dipilih karena memiliki sifat yang dapat memberikan respons terhadap perubahan pH. Metode yang digunakan pada penelitian ini adalah eksperimental yang melibatkan tahapan ekstraksi biji Binahong, pembuatan kertas indikator dengan formulasi tertentu, dan pengujian kinerja kertas indikator tersebut menggunakan larutan asam dan basa standar. Pengumpulan data dilakukan dengan metode observasi dan pengukuran parameter tertentu. Hasil pengujian kertas indikator alami Binahong memberikan perubahan warna yang signifikan menggunakan pelarut etanol 95% yaitu menghasilkan warna merah atau ungu pada pH 1–7, biru pada pH 8–10 dan kuning pada pH 11–14. Biji Binahong memiliki potensi

sebagai alternatif ramah lingkungan dalam pengujian asam basa dengan menunjukkan respons yang baik terhadap variasi tingkat keasaman dan kebasaaan larutan..

**Kata kunci:** Asam-Basa; Biji Binahong; Ekstraksi; Kertas Indikator

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## INTRODUCTION

Synthetic chemicals commonly used in acid–base testing in educational institutions, such as phenolphthalein and litmus paper, pose hazardous effects and are costly (Leba, 2022). The most widely used litmus paper is an imported product (Widiani, 2019), which contributes to its relatively high price. The financial burden of using synthetic chemicals in acid–base testing, particularly in vocational high schools in the East Bandung area and its surroundings, underscores the need for research into more affordable and environmentally friendly alternatives (Vadivel & Chipkar, 2016). To mitigate economic and environmental impacts, it is essential for educational researchers and practitioners to explore cost-effective and safe alternatives. For example, (Mulyaningtyas et al. 2020) conducted a study on the use of pH paper as a learning medium in education. This research is crucial for vocational schools, as it not only addresses immediate needs but also supports efforts to develop sustainable solutions for acquiring test materials for acid–base testing.

Researchers are working to increase the quality of natural indicators for acid–base testing by developing them in paper form. This approach is seen as a promising solution to the limitations associated with using liquid indicators. Converting indicators into paper form is expected to increase their durability and usability over the long term, offering a more efficient and environmentally responsible option for chemical practicums in educational settings. Despite its potential, there has been limited research on the production of natural indicator paper. However, it is possible to produce indicator paper from local plants such as turmeric, which can last up to five months (Leba, 2022), as can beetroot, red spinach (Sari & Nilmarito, 2019), butterfly pea flowers (Wiyantoko & Astuti, 2020) (Widiani, 2019), hibiscus flowers (Siregar, 2012), and starfruit flowers (Lestari, 2016) These plants contain anthocyanins, organic compounds that dissolve in polar solvents and impart a wide range of colors—including orange, red, purple, blue, and black (Mojica et al. 2017)—to various parts of higher plants, such as

flowers, fruits, grains, vegetables, and tubers. Anthocyanins, a subclass of flavonoids, are primary plant pigments commonly used in natural dyes, medicines, and cosmetics (Luiza Koop et al. 2022). They consist of an aglycone called anthocyanidin, which is soluble in polar solvents and esterifies with one or more sugar molecules. (Priskilia et al. 2022) identified several common anthocyanin structures in nature, including pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin.

The novelty of this research lies in its contribution to the development of natural indicator paper using the seeds of the *Binahong* plant (*Anredera cordifolia*), a plant traditionally recognized for its medicinal properties. This plant is characterized by very short stems and simple leaves with pinnate venation. The seeds of *Binahong* are black and, when crushed, produce a natural pigment that can be used as a dye (Sriatun et al., 2017) In addition to their potential as a natural dye, *Binahong* seeds are also utilized as photosensitizers in dye-sensitized solar cells (DSSCs) (Cahyani & Sanjaya, 2021). The seeds contain various compounds, including *betalains*, *antioxidants*, *flavonoids*, *alkaloids*, and *saponins*, which impart a red to purple

color (Cahyani & Sanjaya, 2021) Among these, *betanin* is the most stable red compound, known for its antioxidant activity through hydrogen and electron donation (Coy-Barrera, 2020). This study presents a unique opportunity, as no previous research has explored the application of *Binahong* seeds in acid–base testing.

Whatman filter paper is recognized as an ideal choice for creating natural indicator paper, offering durable and cost-effective color changes (Widiani, 2019) In this study, high-quality paper No. 93, coarse paper No. 91, and chromatography paper were used for indicator development. The selection of these paper types is crucial for assessing the validity of the resulting indicators. Given that *Binahong* seeds contain *anthocyanins*, which are hydrophilic and dissolve readily in water (Singh et al. 2017) and polar organic solvents such as ethanol, methanol, acetone, and chloroform, the soaking process uses 95% and 70% medical-grade ethanol. The use of ethanol not only adds a practical dimension to the study but also provides insight into the factors influencing the efficiency of the indicator paper. The primary objective of this study is to compare the effectiveness of indicator papers produced using different

paper types and solvents. By varying paper types and solvents, this research strategically aims to identify significant differences that may arise in acid–base testing. Consequently, this study is not only an exploration of materials and technology but also a systematic effort to understand how these factors interact and impact the final outcomes.

Research has demonstrated that acid–base tests can be conducted via natural materials or traditional materials, including paper flowers, purple cabbage (Fenger et al. 2021), mangosteen peel, turmeric, butterfly pea flowers (Widiani, 2019), purple tubers (Bria, 2021), and hibiscus flowers (Siregar, 2012). While these indicators are typically prepared as liquid extracts, they present certain limitations, particularly regarding their long-term usability. These natural indicators often suffer from changes in color and fragrance over time, which restricts their effectiveness. Consequently, there is a significant need for innovation in the development of natural indicators that maintain their stability and functionality over extended periods. This motivates ongoing research aimed at creating durable solutions for the practical use of natural indicators.

## **METHOD**

This experimental study investigated the impact of *Binahong* seed extraction on the properties of natural indicators, with the extraction procedure used as the independent variable and the acid–base solution used as the dependent variable. The focus of the study is on natural indicators derived from *Binahong* seeds, which were collected from the Cimekar, Cileunyi, Bandung, and West Java regions.

### ***A pH 1--14 test solution was prepared***

A pH 1–6 solution was made by dissolving diluted hydrochloric acid (HCl), while a pH 8–14 solution was prepared by dissolving diluted sodium hydroxide (NaOH). A pH 7 solution was created by mixing solutions of sodium hydroxide (NaOH) and hydrochloric acid (HCl). To ensure accuracy, all the diluted solutions were tested with a pH meter and universal pH paper to confirm that their pH values ranged from 0–14 (Lloyd, 2000; Leba, 2022)

For sample preparation and extraction, fresh black *Binahong* seeds collected from the local environment were used. The seeds were weighed and then macerated in ethanol solutions with concentrations of 95% and 70%. Maceration was performed on ground

*Binahong* seeds for 30 minutes with these ethanol concentrations at a 1:1 ratio on the basis of previous studies with butterfly pea flowers (Widiani, 2019). Specifically, 50 grams of *Binahong* seeds were combined with 50 ml of ethanol. After 30 minutes of maceration, the extract was filtered through filter paper and prepared for subsequent analysis.

#### ***The Binahong seed extract was tested in a pH 1–14 solution***

Three drops of the prepared pH 1–14 test solution were placed on a drop plate to test the *Binahong* seed extract. One drop of *Binahong* seed extract was then added to each mixture. This method, which is based on prior research by Bria (2021), uses color changes to indicate the reaction.

#### ***Immobilization of Binahong Seed Extract on Whatman Filter Paper***

Wasito et al. (2017) described the adsorption technique used to immobilize the *Binahong* seed extract. The extract was poured into a container or beaker, and fine-grade No. 93 Whatman filter paper was cut into 1 cm × 5 cm pieces. The container was then kept in a shaded area for 24 hours. After the adsorption process, the filter paper was gradually removed and allowed to air dry indoors, following the method reported by Bria, (2021). Once dried, the indicator paper is

stored for use in subsequent stages. This process is also applied to different types of paper and 70% ethanol.

#### ***The efficacy of Binahong seed indicator paper in the test solution was assessed***

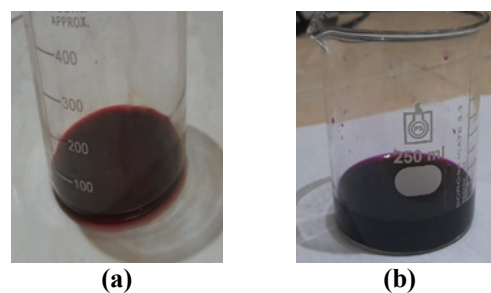
To assess the efficacy of *Binahong* seed indicator paper, the test solution was applied to a drop plate containing three separate drops. One sheet of the prepared *Binahong* seed indicator paper was then immersed in each solution. For comparison, conventional indicators, such as red and blue litmus paper, were also used (Leba, 2022).

## **RESULTS AND DISCUSSION**

### ***Sample extraction***

In the *Binahong* seed extraction process, ethanol is employed because it is an organic compound that is soluble in nonpolar solvents and consists of carbon (C), hydrogen (H), and oxygen (O). Ethanol is widely used as an organic solvent in various industrial, pharmaceutical, and laboratory applications because of its ability to dissolve organic compounds. Its solubility in nonpolar liquids makes it particularly suitable for extracting organic chemicals from natural sources or for use in chemical purification processes. Furthermore, ethanol is considered a universal and highly

effective solvent (Gil-Martín et al. 2022). The use of different medical-grade ethanol concentrations, specifically 70% and 95%, results in varying coloration, with higher ethanol contents producing more intense colors (Cheng et al. 2021), as shown in Figure 1.



**Figure 1.** *Binahong* seed extract with (a) 70% ethanol, (b) 95% ethanol

**Table 1.** Color of *Binahong* seeds extracted at pH 1–14

Color of <i>Binahong</i> seed extraction in pH 1-14							
pH solution	1	2	3	4	5	6	7
70% ethanol extract	Red	Red	Red	Red	Red	Red	Red
Figure							
95% ethanol extract	Violet	Violet	Violet	Violet	Violet	Violet	Violet
Figure							
pH solution	8	9	10	11	12	13	14
70% ethanol extract	Pink	Greenish red	Dark ash	Green to brass	Yellowish green	Yellow Bright	Deep yellow
Figure							
pH solution	8	9	10	11	12	13	14
95% ethanol extract	Blue	Blue	Greenish ash	Yellow	Yellow	Bright Yellow	Deep yellow
Figure							

The 70% ethanol concentration yields a lighter color and is effective at dissolving polar compounds (Priskilia et al. 2022) In contrast, 95% ethanol, which is effective at dissolving nonpolar compounds such as alkaloids, produces a more intense color when used as a solvent in the extraction of *Binahong* seeds. The test results for *Binahong* seed extract across a pH range of 1–14 are presented in Table 1.

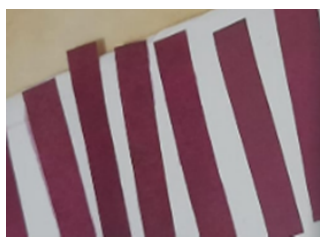
On the basis of the observations from the photos, trials using 70% and 95% ethanol produced similar indicator responses. Both solvents exhibited comparable behavior, with pH levels of 1–7 generating a purple tint and pH levels of 11–14 yielding a yellow color. However, 95% ethanol, owing to its ability to dissolve nonpolar molecules, was found to be more effective than 70% ethanol in producing color at pH 8, 9, and 10. Despite this, 70% ethanol can serve as a viable alternative for testing acidic and basic conditions, as it responds similarly in these environments. The preference for 70% ethanol is often due to its greater availability compared with 95% ethanol. The color changes observed in this indicator are attributed to the betalain compound present in *Binahong* seeds (Cahyani & Sanjaya, 2021).

Betalain is particularly prominent within the pH range of 4–6 and remains highly stable at pH 5. A decrease in pH causes the pigment to shift from red to purple, whereas an increase to neutral pH results in a color change from yellow to brown (Cahyani & Sanjaya, 2021).

### ***Binahong* Seed Indicator Paper**

The production of *Binahong* seed indicator paper involves the use of chromatographic paper with 70% ethanol and filter paper with 95% ethanol. The aim is to extract natural colors from raw materials such as flowers, leaves, or fruits. Figures 3–6 illustrate the process of manufacturing indicator papers from various types of papers.

These findings indicate that the type of paper used to produce *Binahong* seed indicator paper significantly impacts the results. The most effective paper is made from fine-grade filter paper combined with 95% ethanol (Leba, 2022). This paper has a color and texture comparable to those of red and blue litmus paper, with a hue that matches the purple color of the *Binahong* seed extract. The results of the solution test using both natural indicator paper and filter paper are presented in Table 2.



**Figure 3** Chromatogram paper with 70% ethanol content



**Figure 4.** Fine-grade Chromatogram Paper with 95% Ethanol Solvent



**Figure 5.** Fine-Grade Filter Paper 95% Ethanol Solvent

























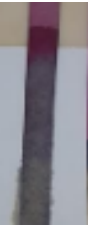





**Figure 6.** Coarse Grade Filter Paper with 95% Ethanol.

The test results using *Binahong* seed extract and *Binahong* seed indicator paper revealed distinct color changes: at pH 1–7, the indicator paper shifted from red to purple; at pH 8–9, the solution turned dark blue; at pH 10, it turned grayish-green; at pH 11, it turned light yellow; and at pH 12–14, it turned

intensely yellow. Similar results were reported by Cahyani & Sanjaya, (2021), who studied the purification of red-stemmed *Binahong* seed extract through column chromatography and reported color changes from yellowish-green to pink due to pH variations. This phenomenon is attributed to the presence of *betalain* derivatives in the red-stemmed *Binahong* seeds, which produce red to purplish-red colors. *Betalains* are generally stored in various plant tissues, including flowers, petioles, fruits (Smeriglio et al. 2019), stems, leaves, and roots (Coy-Barrera, 2020). Key *betalain* derivatives, such as betanidin ( $C_{18}H_{16}N_2O_8$ ) (Timoneda et al., 2019), betanin ( $C_{24}H_{26}N_2O_{13}$ ), and neobetanin ( $C_{24}H_{24}N_2O_{13}$ ) (Araujo-León et al., 2023), produce red colors within the pH range of 4–6 and are most stable at pH 5 (Cahyani & Sanjaya, 2021). *Betalains* are hydrophilic and stable at pH 3–7, where they are stored in vacuoles (Coy-Barrera, 2020). A decrease in pH causes a color shift from red to purple, whereas an increase to a neutral pH of 7 results in a transition from yellow to brown (Cejudo-Bastante et al. 2016) *Betalains* exhibit significant color changes across a wide pH range, shifting from red to purple at low pH and from yellow to orange at high pH (Azeredo, 2009).



**Table 2.** Test results of chromatogram paper of Binahong seeds with 70% solvent (KKBB) and fine paper of Binahong seeds with 95% solvent (KHBB) in pH 1–14 test solutions

Extraction color of <i>Binahong</i> seeds at pH 1-14 test solution							
pH solution	1	2	3	4	5	6	7
KKBB 70%	Red	Red	Red	Red	Red	Red	Violet
Figure							
KHBB 95%	Red	Red	Red	Red	Red	Red	Violet
Figure							
pH solution	8	9	10	11	12	13	14
KHBB 70%	Light violet	Gray-blue	Dark ash	Light yellow	Yellow	Bright Yellow	Deep yellow
Figure							
pH solution	8	9	10	11	12	13	14
KHBB 95%	Blue	Gray-blue	Blue	Yellow	Yellow	Bright Yellow	Deep yellow
Figure							

In addition to pH, color changes can also be influenced by the type of solvent used. During the production of natural indicator paper from *Binahong*

seeds, the use of 70% and 95% ethanol demonstrated distinct responses to pH variations. Notably, at pH values of 1–7, the indicator paper turned red with 70%

ethanol and purple with 95% ethanol. These differences can be attributed to the water content in the solvents, which affects the stability of the betalain compounds in *Binahong* seeds. A relatively high water content may lead to decreased stability of *betalains*, as water can facilitate chemical reactions that result in pigment degradation. At pH 11–14, where betalain degradation is already pronounced, the impact of the ethanol concentration on pigment stability becomes less significant, leading to a more uniform yellow color due to the instability of *betalain*. Factors influencing *betalain* instability include pigment content, chemical structure (Singh et al. 2017), pH, heat treatment (Luiza Koop et al. 2022), water activity, oxygen, light, and temperature during storage (Fu et al. 2020). *Betalains* can degrade under various processing and storage conditions, including temperature, pH, oxygen, and light exposure (Gaona-Ruiz et al. 2024)

The use of 95% ethanol was more effective in the extraction and maceration processes, as evidenced by the superior color results of the indicator paper produced. This higher ethanol concentration provides a more pronounced response than 70% ethanol does (Priskilia et al. 2022). Specifically,

at pH 8–10, the indicator paper with 95% ethanol clearly has a blue color, whereas with 70% ethanol, the color change is less distinct. While 70% ethanol facilitates a more complete extraction of polar and semipolar compounds, such as anthocyanins, resulting in a higher pigment content, 95% ethanol is more effective for extracting nonpolar compounds (Gil-Martín et al. 2022). The use of high ethanol concentrations in natural indicator production has been demonstrated in previous studies, such as those involving rose and rosella flowers (Paristiowati et al. 2019). Similarly, the optimal color change in natural indicator paper made from Asoka flowers was observed with a 96% ethanol concentration (Riyanti et al. 2022)

For the production of indicator paper, three types of paper were used: fine-grade filter paper, coarse-grade filter paper, and chromatographic paper. The results demonstrated that, compared with the other types of filter paper, fine-grade filter paper yielded the best and most effective performance in pH testing. This superior performance is attributed to the finer and more uniform pores of the fine-grade filter paper, which ensure a more even distribution of the indicator across the paper surface. Consequently, this results in a more consistent and readily

observable color change during pH detection. Previous research supports the use of fine-grade filter paper (Whatman) for natural indicator paper, noting its durability, cost-effectiveness, and optimal color change (Ahsana & Adriani, 2023). Additionally, Whatman filter paper has been successfully utilized in the preparation of natural indicators from jamblang fruit (Ahsana & Adriani, 2023)

## CONCLUSION

Research on *Binahong* seed-based indicator paper has demonstrated a positive response to variations in acidity and basicity, with performance comparable to that of traditional red and blue litmus paper. These findings indicate that *Binahong* seed indicator paper can be a viable alternative for acid–base testing. However, there are limitations to

consider, such as the availability of raw materials and the stability of pigments. *Binahong* seed pigments may be sensitive to environmental factors such as light, temperature, and pH, which can lead to undesirable discoloration over time and reduce the shelf life and effectiveness of indicator paper.

This study recommends the use of 95% ethanol as a solvent, as it produces more significant results, and suggests the use of finely graded paper for optimal performance. Future research should explore alternative plant materials for natural indicators to increase environmental sustainability and cost effectiveness. Additionally, this research has potential applications in project-based learning for junior and senior high school students, providing practical educational opportunities.

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