pH Indicator from *Kamias* (*Averrhoa bilimbi L.)* Flower Extract

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Abstract

The acidic or basic nature of materials is usually determined using pH meter and commercially-sold or synthetic indicating means. In this study, an eco-friendly pH indicator was prepared from the aqueous and ethanolic extract of *kamias* (*Averrhoa bilimbi L.*) flowers containing anthocyanin pigment. The *Kamias* pH indicator paper was calibrated using buffer solutions pH 0 to 14 to come up with a colored chart. This served as reference in the subsequent effectiveness and acceptability tests conducted of the product. The indicator produced showed a highly acceptable response from the respondents proving that it can be a good alternative to commercially-sold pH indicating means. The shelf-life test also revealed that it could withstand the storage time of 3 months without losing its effectivity. These findings have significant implications to further refine the product and prolong its storage time, and consequently, the production of *kamias* pH indicator paper.

Keywords: kamias, pH indicator, anthocyanin, shelf-life

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Technically, the acidity or alkalinity of solutions is determined through its pH. The "power of hydrogen" or simply pH uses the mathematical logarithm of the concentration of hydrogen ions in solutions (Brown, Le May, Bursten, & Murphy, 2009). This chemical measurement makes use of various indicating ways ranging from the sophisticated bench tester digital, pH meter, and hydron paper which can be used in school laboratory activities, in analyzing commonly used household materials, in farming, and in small scale industries. The most popular and common litmus and pH hydron paper establishes as the acidic or basic nature of substances and solutions by causing a chemical reaction that produces visual specific color change to the chemical in the paper thus, "indicating" the nature of the material being tested (Brady & Holum, 1988).

Sold in the market are several types of expensive commercial pH paper test strips that can read pH with multiple color changes, indicating pH increments of even 0.5 and 1.0. These test strips have been prepared by infusion in colored extracts from plant parts. For hundreds of years these isolated natural indicators had been utilized even before chemists manufactured acid-base indicators. In fact, Robert Boyle first reported the use of natural dyes as acid-base indicators in 1664 in his collection of essays Experimental History of Colors (Bhise et al, 2014).

The prominent colors in plant extracts are due to the presence of naturally occurring organic substances such as anthocyanins, azo compounds, flaovenes, flavonol, xanthine, among others. Literature reveals that extracts containing anthocyanin are pH sensitive in that they exhibit different color reactions when added to an acid or base (Khan & Farooqui, 2011**).** In the Philippines there are plenty of plants that can be sources for preparing indicators. One of these is *kamias*, *Averrhoa bilimbi Linn*. of the Family Oxalidaceae and locally called *iba.* This plant does not seem to have varieties. However, it has been reported to have a sweet nature occurring in the cultivated and semi cultivated state throughout the country (Quisumbing, 1978). This tropical attractive, long-lived tree reaches 5-10 meters in height and is abundant in Iloilo. It has a short trunk which divides into a number of upright branches (Padua, 1996). Its flowers are 10-22 mm long, borne in small hairy panicles emerging directly from the trunk and oldest, thickest branches and on some twigs, as do the cluster of its fruits (World Agro Forestry, 2015). They are small, fragrant, auxiliary or cauliflorous, 5-petalled, yellowish-green or purplish marked with dark purple color, the source of pigments that contain chemicals from the naturally-colored anthocyanin family of compounds (Ecocrop FAO, 2007**).** Anthocyanins are a large group of naturally colored red-blue family of flavonoids found in pigments, responsible for the attractive colors of plant parts. They constitute the largest group of pigments in nature. They are the universal water soluble red, violet and blue pigments of flowers, fruits, vegetables and leaves (Sharp, 1987). Chemically, they are glycosides and their aglycones, the sugar free pigments, are identified as the anthocyanidins (Khan & Farooqui, 2011). The known anthocyanin isolated from *kamias*, *Averrhoa bilimbi* is the cyanidin-3-*O*-beta-D-glucoside (as cited in Gunasegaran, 1992).This anthocyanin cation is a cyanidin cation linked to a ß-D- glucosyl moiety at position 3 (Chemical Entities of Biological Interest [ChEBI], 2014)

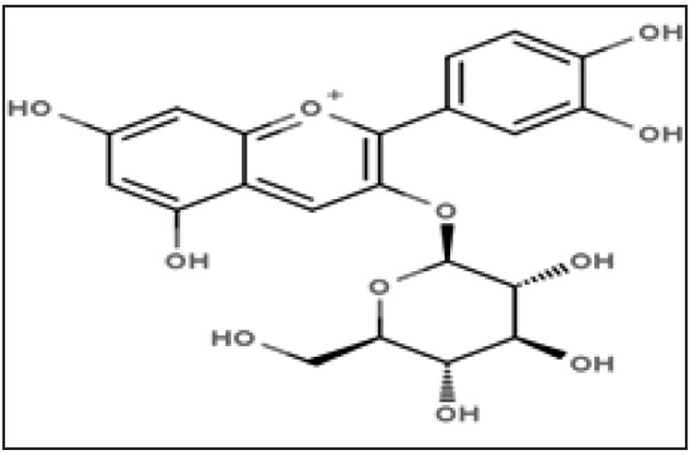

Figure 1. Chemical structure of cyanidin-3-O-beta-D-glucoside

From these evidences and owing to its abundance, it is viable and beneficial to prepare an eco-friendly and affordable *kamias* pH indicator paper as a substitute for expensive pH indicating means. Such will be used as an instructional material for household or domestic activities and local industries. Explicitly, this study aimed to (1) prepare pH indicator from *kamias* (*Averrhoa bilimbi L.*) flower extract; (2) establish the effectiveness of the indicator; and (3) determine the acceptability and shelf-life of the product.

Methodology

The present work is both experimental and descriptive. The experimental component involved the following steps: preparing, storing, calibrating, and testing for effectiveness and shelf-life of *kamias* pH indicator paper. The subsequent descriptive part employed the cross-sectional survey to assess the product's efficiency and acceptability with respondents coming from West Visayas State University and some family households from two towns in Iloilo who agreed to participate after securing a permit. One survey instrument was utilized to gather data which were encoded, analyzed and interpreted.

Experimental Component

Preparation and storing. The indicator was prepared by collecting and weighing about 5 grams of fresh and clean *kamias* flowers; grinding the sample and then mixing the mush with 5 mL water, the first extractant. The mixture was heated until half its original volume to obtain a concentrated amount of pigment and then filtered. To this, 37.5 mL of 80% ethanol, second extractant was added. The resulting mixture served as soaking medium for the strips of filter paper cut into 6cm. x1.5cm. These strips were oven dried for 2 hours at 75^{0}C, packed in a dry 5/16 cmx 1.5cm, and eventually stored under controlled temperature in the refrigerator for calibration, shelf-life, and acceptability testing.

Preparation and standardization of pH 0 to 14 buffer solutions. The different buffer solutions to be used for calibrating *kamias* pH indicator paper were prepared ahead according to the desired pH 0 to 14 (Shugar, Shugar, Baseman, & Baseman, 1981). These were then standardized by using three methods: (1) by Electrometric Method, SMEWW (2005) 4500-H at the Department of Science and Technology (DOST) Reg. VI, Region VI; (2) using commercially sold hydron paper with pH scale 0 to14; and (3) using pH paper with scale of 1to11. The accompanying color scales for hydron and pH paper were utilized as basis for comparison in calibrating the prepared buffer solutions and eventually of the actual *kamias* indicator.

Calibration of *kamia*s pH indicator paper. *Kamias* indicator paper was calibrated against the standardized buffer solutions at the WVSU Central Laboratory. Individual strip of paper was dipped in each pH buffer from 0 to 14 to determine the specific color reaction change. Results were noted by taking the photograph of every strip of paper indicator a minute after it was dipped in the different buffer solution. The resulting fifteen color reactions prepared into a chart served as *kamias* pH reference indicator chart where colors were arranged corresponding to pH scale 0 to 14. This also served as point of comparison for succeeding investigations on the effectiveness and shelf-life testing. Since the product is expected to function like the commercially sold hydron paper, its resulting color chart was also compared with the color scale of the commercial indicator.

Testing for effectiveness. An experiment was performed using *kamias* pH indictor paper and its color chart for reference to screen its effectiveness and acceptability compared to the commercially sold hydron and pH paper. This practical activity has shown and identified the nature of some commonly used chemicals in the laboratory.

Twelve pieces of watch glass were arranged horizontally in three sets: A, B, and C on the working table at the WVSU Central Laboratory. In set A, the first watch glass contained *kamias* pH indicator paper; the second had red litmus, the third had blue litmus, and the fourth had hydron indicator paper. The same procedure of indicator distribution was done in Sets B and C. The initial colors of the different indicators were recorded before they were tested on acid, basic solution, and distilled water. For this test, 0.1 M Hydrochloric acid, HCl was added in set A, 0.1 M Sodium Hydroxide, NaOH in B, and distilled water in C. All results obtained were recorded and tabulated.

Testing for product's shelf-life or degradation. The shelf-life of a material or product is the time or period within which the material is safe to use and or has an acceptable quality or usefulness to the consumers. The calibration and testing for shelf-life of *kamias* pH indictor paper were repeated every 2^{nd} week of the month for 3 consecutive months: September, October, and November 2014. The color outcomes obtained in every analysis was also compared with the first calibrated color chart result. Literature shows that the shelf-life of a commercially sold pH paper is 2 to 2.5 years (Sanders, 2014).
Descriptive Component

Acceptability testing. An activity "*Kamias pH Indicator Paper*" was performed from October to December 2014 by the randomly selected 153 students with their subject teachers from West Visayas State University. The respondents included forty one (41) B.S. Ed. 2C Physical Science & Physics; twenty seven (27) B. S. Ed. 2D Biology; five (5) B.S. Ed. 3C Physical Science; forty five (45) B.S. N. 1C; and thirty five (35) B. S. N. 1B students. This cohort of students was taking up General and Inorganic Chemistry or Analytical Chemistry subjects. They agreed to participate after the intention of the study was explained to them. Moreover, the activity was scheduled in such a way that no classes were disrupted in as much as it was already towards the closing of the first semester and early part of the second semester. In addition, eight household respondents from 2 towns in Iloilo also readily agreed to participate in the study after their permission was sought.

In the activity, the students determined the indicator's usefulness as an instructional material and as substitute in identifying the nature of some commonly used materials in the laboratory. They answered the guide questions and were requested, together with their subject instructor to evaluate the product and give their feedback by accomplishing the Acceptability Checklist Survey Form. In the case of household participants, each of them did the experiment using only the materials frequently used at home. Thereafter they also answered the survey questionnaire. These feedbacks were gathered, encoded, tabulated, analyzed and interpreted as follows:

Scores	Interpretation
3.51 – 4.00	*kamias* indicator is *highly acceptable*
2.51 – 3.50	*kamias* indicator is *acceptable*
1.51 – 2.50	*kamia*s indicator is *slightly acceptable*
1.00 – 1.50	*kamia*s indicator is *not acceptable*

Results and Discussions

Aqueous and ethanolic extract of *kamias* (*Averrhoa bilimbi L.*) flowers containing anthocyanin was prepared and used to infuse strips of filter paper to be utilized as pH indicator. These were stored in sealed plastic tube with cover under controlled temperature and set for three calibration and shelf-life testing procedures.

Calibration of the Indicator Paper

The standardization of the locally prepared indicator paper showed a clear picture presentation of its different color reactions with buffer solutions from pH 0 to 14. Figure 2 illustrates the color chart produced for the indicator. This representation consequently served as reference for the succeeding investigations on the effectiveness and shelf-life testing of the indicator paper.

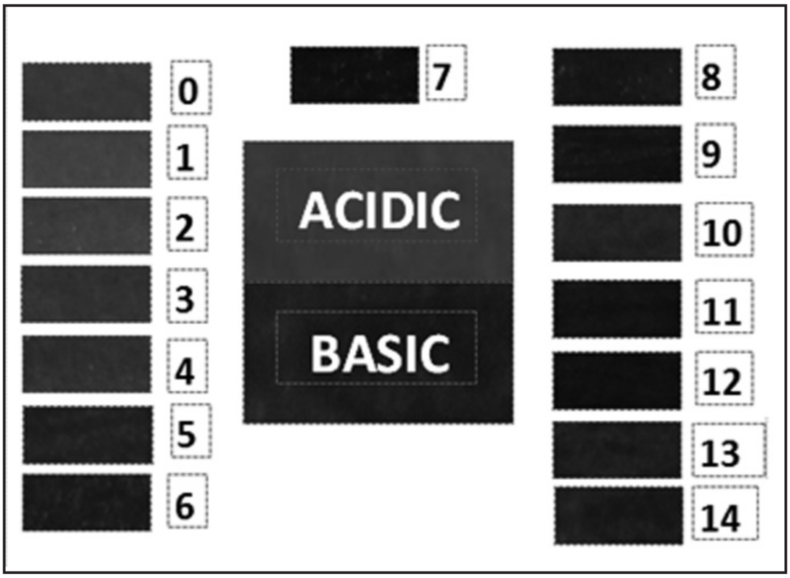

Figure 2. Kamias pH Indicator Color Chart

Establishing Effectiveness of *Kamias* pH Indicator Paper

Table 1 showed the effectiveness of the indicator in determining the acidity or alkalinity of some substances as compared with other indicting means. The reaction of *kamias* pH indicator and hydron paper on the test materials was specific and comparable. Litmus paper, on the other hand, gave a descriptive result, that is, the test material is either acidic or basic.

Table 1

pH of Some Common Laboratory Chemicals

Materials	*Kamias* Indicator Paper (base on the color chart)		Litmus Paper		Hydron Paper
	Color Change	pH Value	Red	Blue	
0.10 M Sodium Hydroxide (NaOH)	dark blue	11(basic)	blue	remained blue (basic)	11 (basic)
0.10 M Hydrochloric Acid (HCl)	dark red	1 (acidic)	remained red (acidic)	red	1 (acidic)
Distilled water	lighter shade of red violet	6 (slightly acidic)	No change in color	No change in color	6 (acidic)

Acceptability Testing

A product's acceptance can only be tested when consumers believe that it is useful. Table 2 shows that respondents generally have "highly accepted" (*M* = 3.63; *SD* = 0.485) the indicator as an alternative to commercially sold pH indicator after doing the experiment. They have also "highly accepted" the fact that it can be used satisfactorily in laboratory experiments and other related activities (*M* = 3.61; *SD* = 0.514). They further pointed out that it can be easily used by students and other practitioners (*M* = 3.79; *SD* = 0.409). However, though respondents strongly believed that the indicator gives an accurate result by producing distinct color change in acidic or basic solutions, this criterion got the bottom most result among the criteria presented (*M* = 3.54; *SD* = 0.601).

Table 2

Acceptability of kamias pH Indicator Paper

Criteria	Mean	Std. Deviation	Description
Kamias pH Indicator.			
1. Can be easily used by students and other practitioners.	3.79	0.409	Highly acceptable
2. Gives an accurate result by producing distinct color in acidic and basic solutions.	3.54	0.601	Highly acceptable
3. Can be used satisfactorily in laboratory experiments and other related activities as an indicator.	3.61	0.514	Highly acceptable
4. Can be a good substitute or alternative to commercially prepared pH indicator.	3.63	0.485	Highly acceptable

Note: 3.25 – 4.00 = Highly acceptable; 2.50 - 3.24 = Acceptable; 1.75 – 2.49 = Slightly acceptable; 1.00 – 1.74 = Not acceptable

Respondents were also asked to give remarks and observations and one hundred two of them readily gave their opinions. It was noted that 75.49% commented that k*amias* pH indicator paper can be a substitute to commercially sold pH indicator. They reasoned that it works better than the commonly used litmus paper. Making this as an alternative material accordingly is reasonable since the plant is abundant in the Philippines hence readily accessible.

On the other hand, 14.70% of respondents observed that the indicator can produce distinct results by producing a specific color in each test sample. According to them the outcomes of the experiment seemed accurate based on what they had seen. They added that it is efficient to use in determining whether the sample is acidic, basic or neutral.

About 29.40% remarked that it is good indicator. However, the resulting color reaction indicating pH 7 is not exactly the same with the one in the color chart. This is clearly noted and reflected in Table 2 since this particular criterion got the bottom most result among the standards presented.

Around 2.95% of respondents declared that they enjoyed using the indicator because aside from being practical and cheap, it is also an efficient tool to use in learning. They also noticed that the paper is thin and handy.

There were 3.92 % who also commented that using the indicator is a resourceful idea since it is an efficient way of assessing and determining the nature of different solutions. They added that one can gain profit from it if this will be commercially sold and accepted by the end users. For them this is a proof that there are many alternative materials aside from the ones customarily used in school and even for household material testing. They expressed hope that this study will be recognized by other people.

Some respondents also gave suggestions. They noticed that the degree of colors corresponding to pH 0 to 14 in the chart had a very slight difference. Thus one might make a mistake in giving the correct value when doing analysis if the observer is not so keen. Hence they recommended continuing the research to be able to come up with a reference chart where the colors corresponding to pH 0 to 14 are very distinct to facilitate ease of identification.

Testing for Product's Shelf-life

The calibration and shelf-life test of *kamias* pH indicator paper was done 3 times within 3 succeeding months to determine whether the product was still usable and had maintained its standard as an indicator. Findings revealed the same color reaction results with buffers pH 0 to 14 and thus affirming the original reference chart for the indicator prepared earlier. This further showed that the finished product was able to withstand the length of storage time, and that it did not lose or reduce its efficiency to detect the pH of the test materials. However, it still has to be improved to be comparable with the shelf-life of the other commercially sold pH indicating means.

Conclusions and Recommendations

Kamias (*Averrhoa bilimbi L.*) flowers containing cyanidin-3-O-beta-D-glucoside anthocyanin pigments extracted using water and ethanol can be prepared readily and cheaply as a natural and eco-friendly indicator considering the abundance and availability of the plant. Based on the results, the filter papers cut into strips infused in the indicator extract and consequently dried can be used as a gauge to determine the nature of a material.

The calibration of *kamias* pH indicator using prepared buffer solutions of pH 0 to 14 paper resulted to a color chart with corresponding pH scale. This can serve as a reference material to determine the acidity or alkalinity of some laboratory chemical. This is also analogous to the color chart of other pH indicating means like the hydron and Gumins' paper. Hence, it can effectively serve the purpose just like the mentioned indicators.

From the results of the acceptability testing, it could be assumed that respondents highly recognized *kamias* pH indicator paper as a substitute pH indicating mean that can be utilized in simple school laboratory activities as well as in water and other household material testing. This was affirmed by the positive comments and suggestions of the respondents implying that this natural indicator is beneficial, economical, and simple but efficient.

The indicator paper can stay for a period of time, 3 months in the case of the present study. The storing did not affect its quality and effectivity. The product though, needs to be properly stored and monitored under controlled temperature inside a refrigerator to ensure its longer shelf-life.

Hence, it is recommended that the experimental component of this research be continued-- the focus of which should be in the refinement of the color chart as reference for future analysis. Along this line, there is a need to investigate further the best proportion of the plant's flower extract to the extracting solvent to address the abovementioned issue. The shelf-life testing ought to be continued also for a year to really determine *kamias* pH indicator paper storage capacity. These steps would surely ensure the quality and usability of the product.

Lastly, the cost benefit analysis done in conducting this study attested that it will be economical to venture in the production of *kamias* pH indicator paper. The product cost per strip is cheaper than the other indicators sold in the market. Thus, going into this business will contribute to a positive financial outcome.

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