The pH indicator capability of *Basella rubra* (alugbati) stem peels aqueous extract

ELISHA L. EUSEBIO, KAIZEN P. NAQUITA, JOHN EARL S. SETIAS, and RAMON ANGELO N. SINCO

Philippine Science High School Western Visayas Campus - Department of Science and Technology (DOST-PSHS WVC), Brgy. Bito-on, Jaro, Iloilo City 5000, Philippines

Article Info

Submitted: Apr 8, 2022
Approved: Aug 01, 2022
Published: Aug 08, 2022

Keywords:
pH
indicator
Basella rubra
stem peels
phytochemicals

Abstract

Synthetic pH indicators pose hazardous effects to humans and the environment. *Basella rubra* (alugbati) stems contain phytochemicals which are pH-sensitive. Thus, this study aimed to assess the capability of *Basella rubra* stem peel aqueous extract as a plant-based pH indicator. The extract was obtained through aqueous maceration of *Basella rubra* stem peels and was tested to solutions with pH levels 3, 5, 7, 9, and 11; then, color and pH changes were observed and analyzed. The trend of color changes observed from acidic to basic pH were red to yellow. Also, the mean pH difference of the solution with pH 9 was relatively higher compared to other pH levels. The mean pH differences were lower in pH 3-7 due to stability of betacyanin in the said range. Hence, the aqueous extract of *Basella rubra* (alugbati) stem peels is capable as a plant-based pH indicator at pH levels 3 to 7.

Introduction. - pH indicators are halochromic substances that change in color when they bind to hydrogen and hydroxide ions in a solution [1]. When this occurs, they undergo changes in ion equilibrium which alters the color from colorless to a distinct color depending on the acidity or alkalinity of a solution [2]. Commercial synthetic indicators are man-made chemical substances that change in color with respect to pH and are used to determine the pH of a specific solution. However, the production and use of these commercial synthetic indicators such as methyl red, methyl orange, and phenolphthalein pose hazardous effects to both the environment and humans, and are also costly [3]. Furthermore, compared to plant-based indicators, synthetic indicators are more hazardous to produce as they involve the use of toxic chemicals such as phthalic anhydride and p-toluenesulfonic acid monohydrate in phenolphthalein [4].

Considering these factors, plant-based pH indicators have been studied as alternatives for commercial synthetic indicators as they are easily available, eco-friendly, less hazardous, and low-cost [5]. These plant extracts contain various phytochemicals and some of these, specifically anthocyanins and betacyanins, have been shown to be sensitive to pH [6,8]. Anthocyanins usually present in plant-based indicators have been identified to be less toxic and environmentally compatible [7]. Also, plant extracts are capable of showing a spectrum of color at different pH values, from acid to alkaline [8]. Most of the known plant-based indicators use the flowers, fruits, leaves, and seeds of plants. However, these are usually considered either as food sources or ornamental plants. Hence, plant-based extracts that possess similar properties with these known indicators are being further investigated.

In the Philippines, *Basella rubra* is cultivated for its leaves while its stems, however, are considered as domestic kitchen waste. Furthermore, the plant extract of *Basella rubra* had been screened to contain similar phytochemicals which contribute to its pigmentation, especially in its stem [9,10,11]. The fruits of *Basella rubra* have also been found to contain betacyanin and anthocyanins which are natural pigments causing the purple color in its fruits, flowers, leaves, and stems [12]. These fruits have already been proven to be effective as plant-based pH indicators [13,14].

This study aimed to assess the capability of *Basella rubra* stem peel aqueous extract as a plant-based pH indicator. Specifically, the study aimed to:

(i) Determine the mean pH of the solutions before and after the application of the indicator at pH 3, 5, 7, 9, and 11;

(ii) Determine if there is a significant difference between the mean pH of the solutions before and after the application of indicator at pH 3, 5, 7, 9, and 11;

(iii) Construct color scales to visually compare the colors identified on the solutions with pH 3, 5, 7, 9, and 11; and

(iv) Identify the pH range capability of the *Basella rubra* stem peel aqueous extract as a plant-based pH indicator.

Methods. - *Basella rubra* stems were cut and its peels were macerated in distilled water for 24 hours. The obtained extract was applied to solutions of pH 3, 5, 7, 9, and 11, which were obtained by dilution of distilled water with lemon juice and bleach. A paired samples t-test was conducted to determine if there is

How to cite this article:
CSE: Eusebio EL, Naquita KP, Setias JES, Sinco RAN. 2022. The pH indicator capability of *Basella rubra* (alugbati) stem peels aqueous extract. Publiscience. 5(1): 39–43.
APA: Eusebio, E.L., Naquita, K.P., Setias, J.E.S., & Sinco, R.A.N. (2022). The pH indicator capability of *Basella rubra* (alugbati) stem peels aqueous extract. *Publiscience*, *5*(1), 39–43.

For supplementary data, contact: publiscience@wvc.pshs.edu.ph.

a significant difference between the pH of the solutions before and after the extract application. The color changes observed in each replicate per pH level were also recorded and pH scales were constructed to determine the pH range capability of the extract.

Preparation of Plant Samples. Freshly harvested samples of *Basella rubra* were collected from Brgy. Bayunan, San Joaquin, Iloilo and were sent to the Department of Agriculture in Santa Barbara, Iloilo for species confirmation.

Preparation of Extract. The purple parts of the epidermis of the aerial *Basella rubra* stems were collected using a peeler with an approximate thickness of 1 mm. They were soaked in 7% NaOCl solution for 15 minutes and rinsed with distilled water thrice. After rinsing, the peels were cut into small square pieces approximately 2 x 2 mm in size. Forty grams of the stem peels were macerated with 40 mL of distilled water for 24 hours at room temperature (25°C). The aqueous extract was filtered using a cheesecloth, its pH was measured using a pH paper, and it was packed in a cooler for transport.

Preparation of Acidic and Basic Solutions. Lemon juice and bleach were added to distilled water to obtain solutions of varying pH values with volumes of 20 mL each. Lemon juice was diluted with distilled water to prepare the acidic solutions with pH 3 and 5, and bleach was diluted with distilled water to prepare the basic solutions with pH 9 and 11. Distilled water was used as the neutral solution with pH 7. All of the solutions prepared were colorless.

Application of Extract to Solutions. One mL of the extract was added to the 50 mL beaker containing 20 mL of the solutions of varying pH values. The solutions were then stirred 20 times for 10 seconds.

Stability Test of Extract. To determine the stability of the aqueous extract, three sets of pH measurements were conducted at different time intervals after the collection of the aqueous extract. The pH measurements at pH 3, 5, 7, 9, and 11 were performed at 0, 25, and 51 hours after extraction.

Observation of Color Change. The colors of the resulting solutions were identified and documented in each replicate three (3) times. Using the Color Grab mobile application Version 3.9.2, the hexadecimal values of the colors of the solutions were obtained.

Measurement of pH. The pH of the resulting solutions in each of the three replicates were measured using a calibrated pH meter.

Data Analysis. A Shapiro-Wilk test was performed to determine the normality of the data. A Paired Samples T-test at $\alpha=0.05$ was performed using IBM SPSS Statistics for Windows Version 28.0.0.0 to determine if a significant change was caused by the extract before and after its application.

Colors identified from each resulting solution in each of the three replicates were visually compared to evaluate the consistency of the occurrence of color change. Three color scales were constructed based on the hexadecimal codes of the colors obtained from each solution in each replicate.

Safety Procedure. Due to the pandemic, proper health protocols were observed during the conduct of the experiment such as the wearing of face masks. Personal protective equipment was worn at all times, and physical contact between the chemicals and open areas of the body were avoided. The bleach solution and lemon juice solution were neutralized with distilled water then flushed down the drain with running water after use.

Results and Discussion. - This study aimed to evaluate the pH indicator capability of *Basella rubra* stem peel aqueous extract to solutions with pH 3, 5, 7, 9, and 11. After the extract application, a statistical analysis for their pH means was performed. The colors of the solutions identified using the Color Grab mobile application were used to create color scales.

After the extraction of *Basella rubra* stem peels, a total yield of 33.5 mL was extracted from 40 grams of the plant stem peels. The pH of the *Basella rubra* stem peel aqueous extract was then measured using a pH paper to be approximately 6.

Mean Difference of pH Means. Table 1 shows that the mean difference of the pH means ranges from 0.10 to 2.06.

Table 1. Mean pH of solutions before and after application of extract and their significant differences

pH Level	3	5	7	9	11
Mean Initial pH	3.05	5.05	6.98	9.02	10.97
Mean Resulting pH	3.41	5.15	6.36	6.96	10.10
Mean pH Difference	0.36	0.10	0.62	2.06	0.87
SD	0.11	0.05	0.05	0.07	0.33
p-value	0.03*	0.08	0.002*	<0.001*	0.045*

*pH groups that showed significant difference ($\alpha=0.05$) before and after the application of the extract

As shown in Table 1, a significant pH change can be observed in the solutions with a mean initial pH of 9.02 after the application of the *Basella rubra* stem peel aqueous extract. Their mean pH change of 2.06 is relatively higher than the other pH groups. This result may be attributed to the stability of betacyanin at pH 3-7 which undergoes color and structural variations at alkaline conditions [15,16]. With this, more alkaline pH groups experienced greater pH changes.

Significant Difference of pH Means. Furthermore, only the solutions with initial pH 5 showed no significant pH changes after the application of the aqueous extract.

Saptarini et al. [17] and Yao et al. [6] have previously used buffer solutions of various pH levels to observe the color changes, unlike our experimental set-up which used household chemicals, and this led to a significant change in the pH levels of 3, 7, 9, and 11 after the application of the extract. The Paired Samples T-test showed that only the solutions with initial pH of 5 showed no significant pH changes. This result is in accordance with the studies of Woo et al. [18] and Wong and Siow [19] wherein betacyanin was found to be most stable and have the highest concentration at pH 5.

Figures 1, 2, and 3 show the colors of the solutions after the application of the *Basella rubra* stem peels aqueous extract in the three replicates. The solutions of pH 3, 5, 7, 9, and 11 changed into colors from red to yellow, respectively. Moreover, these colors became lighter after every replicate.

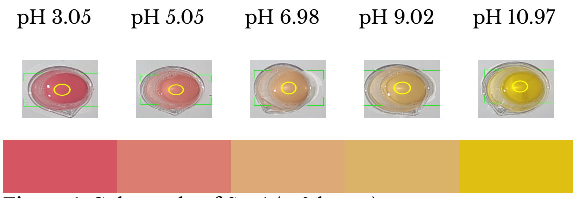

Figure 1. Color scale of Set 1 (t=0 hours)

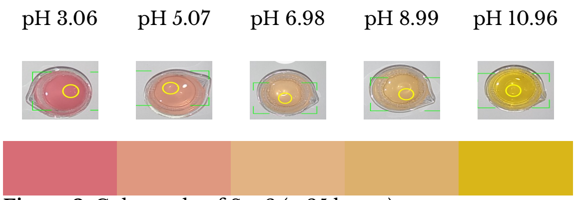

Figure 2. Color scale of Set 2 (t=25 hours)

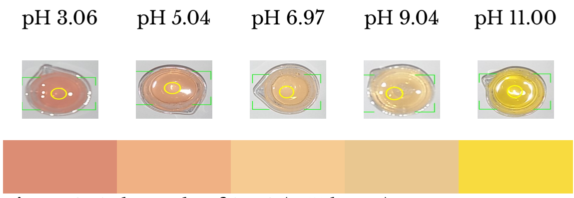

Figure 3. Color scale of Set 3 (t=51 hours)

These color changes reflect a similar result to the study of Kapilraj et al. [5] wherein the betacyanin-containing aqueous extract of *Bougainvillea glabra* gave colors of red to brownish yellow. Comparing the color changes to the studies of Ezati and Rhim [20] and Qin et al. [15], the colorant properties of the *Basella rubra* stem peel aqueous extract can be most likely attributed to the presence of betacyanin as its major colorant. Betacyanin is stable at weakly acidic and neutral conditions which is represented by the red color [21]. When exposed to alkaline conditions, it undergoes color and structural variation. Betacyanin degrades into betalamic acid as shown in Figure 4 [15], which undergoes condensation with amines and/or their derivatives that generate betaxanthins, giving off the yellow color [16]. The degradation of betacyanins is caused by the aldimine bond hydrolysis when subjected to basic solutions, and the recondensation of betalamic acid and colorless betaxanthins or cyclo-Dopa-5-O-β-glucoside at acidic pH [18,22]. These mechanisms might be occurring in our study based on the observed color changes.

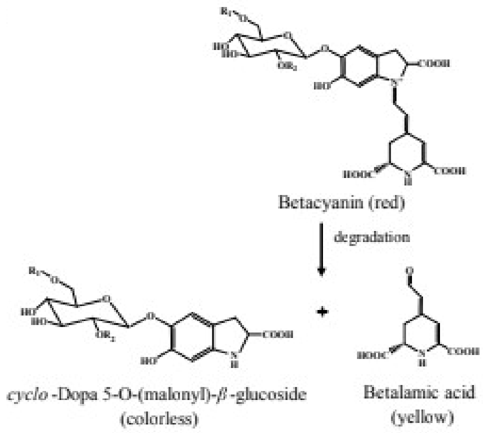

Figure 4. Degradation of betacyanin

According to the study of Priatni and Pradita [23], the betacyanin content of the red dragon fruit aqueous extract degraded by 22.58% after five hours at room temperature. It has also been stated that half-life time of the extract at 25°C was 23 hours and 90% shelf-life is 76 hours. In heat processing, betanin, the most abundant type of betacyanin may degrade leading to the reduction of the red color to a light brown color. Other phytochemicals such as anthocyanins have also been reported to degrade over time. In the study of Muche et al. [24], there is a direct correlation in the decrease of anthocyanin content and storage time especially in temperatures of 25°C and 35°C. Thus, external factors such as temperature and prolonged duration of storage caused the apparent degradation of the extract that resulted to the decreasing color intensities of the solutions from Set 2 and Set 3.

Limitations. The test solutions of varying pH levels were prepared differently in each replicate which may have influenced the mean pH changes. The experimental setups were also transported to different areas and conducted at different time frames by different researchers which might have affected the accuracy and consistency of the results obtained. Changes in temperature and storage time due to transportation may have affected our extract resulting in its degradation.

Conclusion. - The *Basella rubra* (alugbati) stem peel aqueous extract is capable as a plant-based pH indicator in identifying pH levels 3 to 7, showing color changes of red to yellow.

Recommendations. - The researchers suggest to conduct the experiment in one location for a more controlled environment. Buffer solutions may also be used as test solutions. Furthermore, UV-Visible spectroscopy may also be utilized to accurately determine and compare the color changes produced by the test solutions. Testing the aqueous extract of *Basella rubra* stem peels on a wider pH range and by manual titration could also be considered. Lastly, the stability of the *Basella rubra* stem peel extract in terms of color reversibility and storage could also be explored to determine its other chemical effects and shelf life, respectively. Phytochemical screening of the extract should also be performed to determine the active ingredient in the extract which gives the distinct color upon application of the test solutions.

Acknowledgment. - The researchers would like to extend their utmost gratitude to the personnel of the Department of Agriculture in Santa Barbara, Iloilo for their services in confirming our plant sample.

References

[1] Khalid KD, Idris MB, Muhammad NA, Bala A. 2016. Study of the acid-base indicator property of ethanolic extract of *Nerium indicum* flower. Br J Pharm Res . 9(1):1-4.

[2] Wiyantoko B, Astuti. 2020. Butterfly Pea (*Clitoria Ternatea L.*) Extract as Indicator of Acid-Base Titration. Ind J Chem Anal. 3(1):22-32.

[3] Garg S, Garg A, Shukla P, Mathew J, Vishwakarma D, Sen S. 2018. Investigation of plant sources as natural indicators for acid base titration to reduce the use of harmful chemicals in some extent. IJBPAS. 7(1): 64-70. https://bit.ly/3t82K2H

[4] Kiichi A, Nobuyoshi K. 2009. Phenolphthalein as organic teaching materials: small-scale preparation and modeling for some functional dyes. Chem Educ Journ, 13(1). https://bit.ly/3z5EBO5

[5] Kapilraj N, Keerthanan S, Sithambaresan M. 2019. Natural plant extracts as acid-base indicator and determination of their pKa value. Journ of Chem. 2019: 3-5. doi: 10.1155/2019/2031342

[6] Yao X, Qin Y, Zhang M, Zhang J, Qian C, Liu J. 2021. Development of active and smart packaging films based on starch, polyvinyl alcohol and betacyanins from different plant sources. Int J Bio Mac. 183(2021). doi: 10.1155/2019/2031342

[7] Byamukama R, Waninda EN, Mukama D, Namukobe J. 2016. *Hippeastrum hybridum* anthocyanins as indicators of endpoint in acid – base titrations. Int J Biol Chem Sci. 10(6): 2716-2727. doi: 10.4314/ijbcs.v10i6.25

[8] Ivankovic A, Bevanda AM, Martinovic M, Peteovic D, Peric MI, Drmac F, Talic S. 2018. Possibilities of applying natural extracts as acid-base indicators. J Phys Chem. 853-856. https://bit.ly/3POU1MF

[9] Abubacker MN, Ganapathy G, Sebastin LM, Mondal KS. 2019. Histochemical and Shoot Induction Studies of *Basella Rubra* L. (Basellaceae). Biosci Biotech Res Asia. 16(1). doi: 10.13005/bbra/2736

[10] Adegoke GO, Ojo OA. 2017. Phytochemical, antioxidant and antimicrobial activities in the leaf, stem and fruit fractions of *Basella alba* and *Basella rubra*. Science PG. 5(5):73-79. doi: 10.1155/2019/2031342

[11] Deka J, Borah U, Dash B, Dash S, Kalita L. 2017. Preliminary phytochemical screening and in vitro antimicrobial activity of ethanolic extract of the stem of the herb *Basella alba* L. var *rubra* (L.) Stewart (Family-Basellaceae). Int J Curr Pharm. 9(3):91-94. doi: 10.22159/ijcpr.2017.v9i3.19599

[12] Kumar SS, Manoj P, Nimisha G, Giridhar P. 2016. Phytoconstituents and stability of betalains in fruit extracts of Malabar spinach (*Basella rubra* L.). J Food Sci Technol. 53(11): 4014-4022. doi: 10.1007/s13197-016-2404-8

[13] Mitra A, Das SK. 2016. Use of *Basella alba* fruit extract as a potent natural acid-base indicator. J Chem Pharm Res. 8(1): 663-667. https://bit.ly/3z8cIoM

[14] Poonam G, Pramod J, Pushpa J. 2017. Investigation of simple, reasonable and beneficial natural indicator for titration with go-green revolution. Int J Inf Res Rev. 4(3): 3852-3854. https://bit.ly/38QnOE9

[15] Qin Y, Liu Y, Zhang X, Liu J. (2020). Development of active and intelligent packaging by incorporating betalains from red pitaya (*Hylocereus polyrhizus*) peel into starch/polyvinyl alcohol films. Food Hyd. 100(2020): 105410. doi: 10.1016/j.foodhyd.2019.105410

[16] Miguel MG. (2018). Betalains in some species of the *Amaranthaceae* family: a review. Antioxidants. 7(53): 1-33. doi:10.3390/antiox7040053

[17] Saptarini NM, Suryasaputra D, Nurmalia H. 2015. Application of butterfly pea (*Clitoria ternatea* Linn) extract as an indicator of acid-base titration. J Chem Pharm. 7(2): 275-276. https://bit.ly/3t0zWsL

[18] Woo KK, Ngou FH, Ngo LS, Soong WK, Tang PY. (2011). Stability of betalain pigment from red dragon fruit (*Hylocereus polyrhizus*). Am J Food Technol. doi: 10.3923/ajft.2011.

[19] Wong YM, Siow LF. (2015). Effects of heat, pH, antioxidant, agitation and light on betacyanin stability using red-fleshed dragon fruit (*Hylocereus polyrhizus*) juice and concentrate as models. J Food Sci Technol. 52(5): 3086-3092. doi: 10.1007/s13197-014-1362-2

[20] Rhim JW, Ezati P. 2021. Recent Advances in Intelligent Food Packaging Applications Using Natural Food Colorants. Acsfoodcitech. https://bit.ly/3wQMioC

[21] Devi PS, Aravindhan KM, Reshmi SK. (2012). The effect of light, temperature, pH on stability of betacyanin pigments in *Basella alba* fruit. Asian J Pharm Clin Res. 5(4): 107-110. https://bit.ly/3t5uPHQ

[22] Herbach, K. M., Stintzing, F. C., & Carle, R. (2006). Betalain Stability and Degradation — Structural and Chromatic Aspects. J Food Sci. 71(4): R41–R50. doi: 10.1111/j.1750-3841.2006.00022.x

[23] Priatni S, Pradita A. (2015). Stability study of betacyanin extract from red dragon fruit (*Hylocereus polyrhizus*) peels. Pro Che. 16(2015): 438-444. doi: 10.1016/j.proche.2015.12.076

[24] Muche BM, Speers RA,Rupasinghe HPV. (2018) Storage temperature impacts on anthocyanins degradation, color changes and haze development in juice of "Merlot" and "Ruby" grapes (*Vitis vinifera*). Front Nutr. 5:100. doi: 10.3389/fnut.2018.00100