

# Phyllanthus (*Phyllanthus multiflorus* Willd.) Fruit as Natural pH Indicator

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**Abstract**— A substance property as an acid or a base is determined in terms of quantity of pH. It makes use of various tools ranging from simple litmus paper to the sophisticated benchtop pH meter. Natural indicators on the other hand are utilized as an alternative to synthetic acid-base indicators. This paper relates to the preparation of natural indicator from *Phyllanthus* fruit containing anthocyanin, and the establishment of its effectiveness. The experimental method of this study is composed of the preparation of raw materials, standardization of solutions, calibration, testing for effectiveness, and development of a color chart. The developed pH indicator consists of *Phyllanthus* fruit, water, and ethanol. The process of producing the pH indicator comprises of the following steps: collecting; washing; weighing; crushing; mixing with water; boiling the mixture; filtering; cooling down; mixing with ethanol; soaking; and drying. The calibration of the indicator involves different solutions with pH 0 to 14 to come up with a color chart. This serves as a reference in the effectiveness test. Treatment 2 of the indicator produced a distinctive color that is comparable to the existing pH measurement tool. This finding indicates that the pH indicator from *Phyllanthus* fruit consisting of a 1:1 ratio of fruit to water, and a 1:1.5 ratio of the mixture to ethanol can be a cheaper alternative tool in measuring pH that can be used in laboratory testing, as instructional material, and in the household.

## I. INTRODUCTION

In science, a substance property as an acid or a base is one of the main concepts being discussed. The acidity and basicity of solutions are determined in terms of quantity of pH. The “power of hydrogen” is a scale ranging from 0 to 14, with lower numbers than pH 7 representing higher acidity while numbers higher than pH 7 indicate basic solutions [1]. Chemistry titrations, water quality testing, testing of proper composition of biotechnology, food products preservation, and tasting are few of the various activities that need the measurement of acidity and alkalinity of a substance. It makes use of various tools ranging from simple litmus paper to the sophisticated

benchtop pH meter. The litmus paper is effective at indicating the acidity or basicity of a substance by changing its color due to chemical reaction when exposed to acid as it turns red, or a base as it turns blue, but cannot report an exact pH value. It works the same with pH test strips but the packages come with a color-coded scale as a reference when paper turns a certain color. A typical pH meter on the other hand can be made of a moving coil or a digital meter, and either one or two probes. It works by dipping the two electrodes into a solution and by ion-exchange it creates a different degree of hydrogen ion activity showing a pH measurement [2]. The sophisticated pH meter from the group of benchtop pH meters, wireless

pH transmitters, and portable pH meter offers great accuracy and convenience but is costly.

Natural indicators on the other hand are utilized as an alternative to synthetic acid-base indicators. These indicators are dyes and pigments that are isolated from groups of plants, fungi, and algae. Plant pigments such as flavonoids with common types including anthocyanins, aurones, chalcones, flavonoids, and proanthocyanidins are commonly produced in many colors in flowers and plants such as red cabbage, berries, eggplant, turmeric, curry powder, cherries, tomato, and citrus fruits. Different studies revealed that various plants such as antirrhinum, cotton tree, marigold, dahlia, sunflower, China rose, beach morning glory, santan, fern tree, white mulberry, and pomegranate show good acid-base indicator activity against various synthetic pH indicators [3].

Phyllanthus is a common weed in the Philippines that is synonymous with *Phyllanthus reticulatus* [4]. It is a small spreading shrub with rough bark from brown to grey and bears a depressed-globose bluish-black berry when ripe usually with dark purplish pulp [5]. There are more than 510 known organic substances isolated from Phyllanthus, the majority of which are flavonoids, tannins, lignins, and triterpenoids [6]. Thus, this paper relates to the development of pH indicator from Phyllanthus fruit extract and the establishment of its effectiveness.

## II. LITERATURE REVIEW

### pH Measurement

The term pH means power of hydrogen or hydrogen ion exponent was first used by the Danish biochemist Søren Peter Lauritz Sørensen in 1909. It is a measure of the acidity and alkalinity of a substance. Specific pH value gives the same relative point of reference and is defined in terms of the hydrogen ion activity which equals the negative logarithm of the hydrogen ion activity [7]. The activity is referred to the effective concentration of the hydrogen ion in the solution. Litmus paper is the most common pH indicator used in the laboratory. It is a paper that has been treated with natural dyes obtained from lichens *Rocella tinctoria* [8] and is usually sold in strips. Red litmus paper will turn red in acid and blue in basic substances. The pH paper strip is used in the same way as the litmus paper but comes with a color-coded reference indicating the pH level when the paper turns a certain color. This is due to the presence of flavin. Flavin is an anthocyanin that is water-soluble and turns red in acidic solution, greenish in basic solution while purple in neutral solution [9]. Another accurate pH measurement tool consists of three parts: a pH measuring electrode which is a hydrogen ion sensitive glass bulb, a reference electrode, and a high input impedance meter that displays

the results directly. Modern technology offers sophisticated means of measuring pH from the group of wireless, handheld, portable, and benchtop pH meters which are commercially available at a high cost. Wireless pH meter used to remotely monitor pH with that easily streams data to a mobile device. The portable pH meter combines advanced digital processing technology and software design.

Measuring pH has significant importance in food quality and production which is used to produce products with consistent well-defined properties, efficiently produce products at optimal cost, avoid causing health problems to consumers, and meet regulatory requirements. pH is important in agriculture, biological process, and corrosion research [10]. In agriculture, determining the pH of the soil aids in the identification of the type of fertilizer to be used and the types of crops to sow. In the biological process, pH can adjust the medium in fermentation, enzyme hydrolysis, sterilization, etc. In corrosion research, measuring the pH of seawater is also a determining factor on the material to be used for building ships and submarines.

### Natural Indicator

Major plant pigments include chlorophyll, carotenoid, carotene, xanthophyll, astaxanthin, flavonoid, aurone, chalcone, proanthocyanidin, betalain, betacyanin, betaxanthin, flavanol, acylated flavonoid, anthocyanin, glycosylated acylated anthocyanin, quinine, imine, polymethine, naphthoquinone, anthraquinonoid, indigoid, dihydropyran, diarylethene, and carotene are compounds responsible for the color property of parts of the plants.

The use of natural indicators can be an alternative for synthetic indicators due to the presence of these colored pigments that change in color with a variation of pH used in acid-base titrations to show sharp endpoints such as with *Combretum indicum* ethanolic extract found to be nearly closed with equivalence point by standard indicators for four types of titrations equivalence using methyl red, methyl orange, phenolphthalein, and mixed indicator [11].

The utilization of natural indicators is not only limited to chemistry experiments but it can be developed to formulate a pH test kit. Extracts from butterfly pea flower, roselle red flower, and dragon fruit peel can be used for effluent measurement and provide similar results produced by commercial pH test kit [12]. Also, the utilization of natural indicators serves a significant role in the food industry. Anthocyanin in dragon fruit skin (*Hylocereus costaricensis*) can be utilized as an indicator of the presence of formalin and borax in food [13]. The same with *Hibiscus esculentus* that is a better substitute for methyl orange and *Telfairia occidentalis* to phenolphthalein in research analyses [14]. *Tagetes erecta*,

*Impatiens balsamina*, *Tecoma stans*, white rose, and hybrid tea rose were found to give positive results at neutralization and are comparable with methyl orange and phenolphthalein [15]. *Quisqualis indica* L., *Pentas lanceolata*, *Melastoma malabathricum* L., and *Impatiens acaulis* ethanolic extract also proved to be very close with an equivalence point obtained by standard phenolphthalein [16]. Kamias (*Averrhoa bilimbi* L.) flower extract was found to be highly acceptable as a substitute to pH indicating mean [17]. *Hibiscus rosa sinensis* flower sap's methanolic and aqueous extract shows very little variation in acid-base titration [18].

### Phyllanthus

*Phyllanthus multiflorus* Willd is commonly known as a black-honey shrub that grows up to about 3m tall. The stems are hairy when young and the leaves are simple, alternate, and stipulate. The stipules are lanceolate and minute with petiole up to about 5mm long. The blade is elliptic, 1-5cm x 0.7 – 3cm, membranous, base obtuse, apex acute, and with 5-7 pairs of secondary nerves. The inflorescences are axillary fascicles. The calyx includes 5-minute sepals. The androecium includes 5 stamens. The ovary develops 3 bifid styles. The fruits are globose berries and about 5mm in diameter, dark purplish, and contain numerous minute seeds. Phyllanthus in Bangladesh and West Bengal is used to clean teeth and treat malaria. Extracts of the plant exhibited anti-diabetic, anti-inflammatory, analgesic, and anti-plasmodial activities [19]. The fruit, whole plant, and root of Phyllanthus contain gallic acid, ellagic acid, 1- *o*- galloyl- $\beta$ -d-glucose, 3,6-di-*o*-galloyl-d-glucose, chebulagic acid, quercetin, chebulinic acid, corilagin, and isotricitinin [20], niranthin, nirtetralin, hinokinin, and geraniin compounds [21], roseoside, and byzantionoside B [22].

## III. METHODOLOGY

### Experimental Method

The method is composed of the preparation of raw materials, standardization of solutions, calibration of Phyllanthus fruit ethanolic extract, testing for effectiveness, and development of color chart. The preparation of raw materials includes the steps of: collecting ripe Phyllanthus fruit which is bluish-black in color; washing; weighing; measuring of distilled water; crushing of Phyllanthus fruit; mixing with water; boiling the mixture; filtering the mixture; cooling down the filtrate; mixing the filtrate to ethanol- a mixture of sugar cane, wheat, and barley; soaking the filter paper strips; and drying. Calibrating and testing the effectiveness of the indicator comprises the following steps: dipping of an

individual strip of paper in each solution; developing a color chart; and comparison to other pH measuring tools.

For standardization of solutions, different solutions measured with pH 1 to 14 were calibrated using the portable pH meter as shown in Table 1.

Table 1. pH Level of Different Solutions.

Solutions	pH
Hydrochloric acid	1
Acetic acid	2
Carbonic acid	3
Tomato juice	4
Black Coffee	5
Milk	6
Distilled water	7
Seawater	8
Sodium bicarbonate	9
Milk of magnesia	10
Dishwashing Liquid	11
Ammonia solution	12
Sodium hypochlorite	13
Sodium Hydroxide	14

### Composition

Treatments in three different formulations as shown in Table 2 are composed of selectively hand-picked ripe Phyllanthus fruit which is bluish-black in color, distilled water, and carefully milled and fermented ethanol which is a mixture of sugar cane plant, wheat, and barley.

Table 2. Treatments of the Composition of Natural pH Indicator.

Composition	Treatment 1	Treatment 2	Treatment 3
Phyllanthus fruit	33%	29%	22%
distilled water	17%	29%	45%
Ethanol	50%	42%	33%

## IV. RESULTS

### Development of Color Chart

The best formulation of the study that produced distinctive colors for pH 1-14 is Treatment 2 comprises 29% Phyllanthus fruit, 29% water, and 42% ethanol as shown in Table 3, pH 1 resulted in color red; pH 2 dark red; pH 3 light red; pH 4 pink; pH 5 light brown; pH 6 light pink; pH 7 no reaction; pH 8 violet; pH 9 yellow-

green; pH 10 green; pH 11 dark green; pH 12 light green; pH 13 blue; and pH 14 light blue. It shows that the color reaction of Treatment 1 and Treatment 3 is indistinguishable. For Treatment 1, pH 5 and 6 are light pink; pH 8 and 9 are violet; and pH 10-12 are green. For Treatment 3, pH 1 and 2 are dark red; pH 3 and 4 are pink; and pH 5 and 6 are light pink.

Table 3. Color Reactions at Different Formulations.

Solutions	Color Reaction		
	Treatment 1	Treatment 2	Treatment 3
1	Red	Red	Dark Red
2	Dark Red	Dark Red	Dark Red
3	Light Red	Light Red	Pink
4	Pink	Pink	Pink
5	Light Pink	Light Brown	Light Pink
6	Light Pink	Light Pink	Light Pink
7	No reaction	No reaction	No reaction
8	Violet	Violet	Violet
9	Violet	Yellow Green	Yellow Green
10	Green	Green	Green
11	Green	Dark Green	Dark Green
12	Green	Light Green	Dark Green
13	Light Blue	Blue	Blue
14	Light Blue	Light Blue	Light Blue

### Comparison of Results

Results of comparison of different pH measurement tools as shown in Table 4 revealed that the developed pH indicator is comparable with the existing pH measuring tools. It works the same with the popular line of compound pH indicators as it offers distinctive colors for each pH level.

### Shelf-life Analysis

The developed pH indicator was stored in a room temperature and tested once every month for 5 months. The indicator can stay for a period of 4 months in the case of the present study. Results revealed that the storing of the developed indicator did not affect its effectiveness.

## V. CONCLUSION

Natural pH indicators have been developed as an alternative to existing pH measurement tools. This study shows that Phyllanthus fruit can be used as a natural indicator. It can also be concluded that the developed product is comparable with the existing pH measurement tools such as pH meter and pH paper strips due to its distinctive color reaction in different pH levels. Most importantly, this study can be a reference to other research related to natural indicators.

Table 4. Comparison of Results of Different pH Measuring Tools.

Solutions	Color Reaction				Portable pH meter
	Litmus paper		Paper strips	Phyllanthus indicator	
	Red	Blue			
Hydrochloric acid	Red	Red	Dark Red	Red	1
Acetic acid	Red	Red	Red	Dark Red	2
Carbonic acid	Red	Red	Orange	Light Red	3
Tomato juice	Red	Red	Light Orange	Pink	4
Black Coffee	Red	Red	Yellow Orange	Light Brown	5
Milk	Red	Red	Light Brown	Light Pink	6
Distilled water	Red	Blue	Dark Yellow	No reaction	7

Seawater	Blue	Blue	Yellow Green	Violet	8
Sodium bicarbonate	Blue	Blue	Green	Yellow Green	9
Milk of magnesia	Blue	Blue	Dark Green	Green	10
Dishwashing Liquid	Blue	Blue	Dark Blue	Dark Green	11
Ammonia solution	Blue	Blue	Blue Violet	Light Green	12
Sodium hypochlorite	Blue	Blue	Blue Green	Blue	13
Sodium Hydroxide	Blue	Blue	Yellow	Light Blue	14

## REFERENCES

- [1] Hren C., & Mikulecky, P.J. (2017). Chemistry Workbook for Dummies 3rd Edition. For Dummies.
- [2] Woodford, C. (2018) pH meters. Retrieved from <https://www.explainthatstuff.com/how-ph-meters-work.html>
- [3] Garg, A., et al. (2013). Plant extracts as acid base indicator: An Overview. *InventiImpact: Planta Activa*.
- [4] Phuphathanaphong, L. & van Holthoon, F.L. (1804). *Phyllanthus reticulatus*. Plant Resources of South-East Asia. Protologue: Lamk, *Encycl. Meth. Bot.* 5: 298 Retrieved from [https://uses.plantnetproject.org/en/Phyllanthus\\_reticulatus\\_\(PROSEA\)](https://uses.plantnetproject.org/en/Phyllanthus_reticulatus_(PROSEA))
- [5] Naidu, V.S.G.R. (2012). Hand Book on Weed Identification Directorate of Weed Science Research, Jabalpur, India pp 354.
- [6] Mao, Xin et al., (2016). The Genus *Phyllanthus*: An Ethnopharmacological, Phytochemical, and Pharmacological Review. *Evidence-Based Complementary and Alternative Medicine* Volume 2016, Article ID 7584952, 36 pages. Retrieved from <http://dx.doi.org/10.1155/2016/7584952>
- [7] Westcott, Clark (1978) pH Measurements. University of Michigan. Academic Press. pp 1-3.
- [8] Helmenstine, A.M. (2019). What Is Litmus Paper? Understand the Litmus Test. Retrieved from <https://www.thoughtco.com/what-is-litmus-paper-3976018>
- [9] Taylor, Somer (2017) How Does PH Paper Work? Retrieved from <https://sciencing.com/ph-paper-work-4923429>
- [10] Oshunsanya, S.O. (2018). Introductory Chapter: Relevance of Soil pH to Agriculture, Soil pH for Nutrient Availability and Crop Performance, Suarau Oshunsanya, IntechOpen, DOI: 10.5772/intechopen.82551. Available from: <https://www.intechopen.com/chapters/64810K>
- [11] Mane, A.N., Koli, D.S., Kumbhar, V.B. (2016). Use of *Combretum Indicum Flower* Extract as a Natural Indicator in Acid-Base Titration. *International Journal of Institutional Pharmacy and Life Sciences* 6(3): May-June 2016. ISSN: 2249-6807.
- [12] Suppadit, T., Sunthorn, N., & Pongsuk, P., (2011). Use of anthocyanin extracted from natural plant materials to develop a pH test kit for measuring effluent from animal farms. *African Journal of Biotechnology* Vol. 10(82), pp 19109-19118.
- [13] Reyshan (2017). Utilization of Red Dragon Fruit Skin (*Hylocereus Costaricensis*) as an Indicator of Formalin and Borax in Food. Retrieved from <https://thebestscienceproject.com/utilization-red-dragon-fruit-skin-hylocereus-costaricensis-indicator-formali borax-food/>
- [14] Abuh, L. (2018). Ethanolic extract of *Telfairia Occidentalis* and *Abelmoschus osculentus* flowers as substitutes to hazardous synthetic indicators in acid-base titration. *International Journal of Chemical Science Online* ISSN: 2523-2843 Volume 2. Page 16-19.
- [15] Garg, S., Garg, A., Shukla P., Mathew, J., Vishkarma, D. & Sen, S. (2018). Investigation of Plant Source as Natural Indicators for Acid Base Titration to Reduce the Use of Harmful Chemicals in Some Extent. *International Journal of Biology, Pharmacy and Allied Science* ISSN: 2277-4998. January, 2018, 7(1): 64-70.
- [16] Vadivel, E and Chipkar S.D. (2016). Eco-Friendly Natural Acid-Base Indicator Properties of Flour Flowering Plants from Western Ghats. *International J Pharm Pharm Sci*, Vol 8, Issue 6, 250-252.
- [17] Jusayan, Shirley R. (2015). pH Indicator from Kamias (*Averrhoa bilimbi* L.) Flower Extract. *WVSU Research Journal* Vol. 4 No. 1 PP. 30-40.
- [18] Gupta, P., Jain, P., & Jain, P.K. (2012). Isolation of natural acid base indicator from the flower sap of *Hibiscus rosa sinensis*. *Journal of Chemical and Pharmaceutical Research*, 4(12):4957-4960.
- [19] Wiart, Christophe (2019) *Medicinal Plants of Bangladesh and West Bengal: Botany, Natural Products, & Ethnopharmacology*. 1<sup>st</sup> Edition. Botant, Natural Products, and Ethnopharmacology. CRC Press. 9781138735163 - CAT# K32320.
- [20] Thomas, M. D. Text book of biochemistry with clinical correlations 6th ed., Willey- Less., John Wiley and Sons Inc. Publication, Haboken, 2006, 91-97.
- [21] Huang, R.L., Huang, Y.L., Ou, J.C., Chen, C.C., Hsu, F.L., & Chang, C. (2003). Screening of 25 compounds isolated from *Phyllanthus* species for anti-human hepatitis B virus in vitro. *PubMed.gov. US National Library of Medicine National Institutes of Health. Phytoter Res.* 2003 May; 17(5):449-53. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/12748977>
- [22] Mao, Xin et al., (2016). The Genus *Phyllanthus*: An Ethnopharmacological, Phytochemical, and Pharmacological Review. *Evidence-Based Complementary and Alternative Medicine* Volume 2016, Article ID 7584952, 36 pages. Retrieved from <http://dx.doi.org/10.1155/2016/7584952>