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 Roccella 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, diarylethe, 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 bifiid 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- α-galloyl-β-d-glucose, 3,6-di-α-galloyl-d-glucose, chebulagic acid, quercetin, chebulinic acid, corilagin, and isoorientin [20], nirammhi, nirtetalin, hinokiniiin, and geraniin compounds [21], roseoside, and byzantioneside 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.**

| pH Level | 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 |
|-----------------|----------------|
|                 | Litmus paper   | Paper strips | Phyllanthus indicator | Portable pH meter |
| Hydrochloric acid| Red            | Red          | Dark Red              | Red               |
| Acetic acid     | Red            | Red          | Dark Red              | 2                 |
| Carbonic acid   | Red            | Red          | Orange                | Light Red         |
| Tomato juice    | Red            | Red          | Light Orange          | Pink              |
| Black Coffee    | Red            | Red          | Yellow Orange         | Light Brown       |
| Milk            | Red            | Red          | Light Brown           | Light Pink        |
| Distilled water | Red            | Blue         | Dark Yellow           | No reaction       |
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

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