Preparation of Acid – Base Indicator Papers Using Calyces of *Habiscuss Sabdariffa* (Zobo)

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ABSTRACT

This research focuses on developing and assessing the performance of greenish-blue and pink paper indicators made from *Hibiscus sabdariffa* (zobo) calyces. The calyces were collected, processed, and ground into powder, which was then used to produce anthocyanin-based extracts. These extracts were treated with acidic and alkaline solutions to create pink and greenish-blue indicators, respectively. Paper strips were immersed in these solutions to produce the colour-changing indicators. The effectiveness of the prepared indicators was tested against standard litmus papers (SLP) in both acidic and basic environments. The pink *Hibiscus sabdariffa* indicator demonstrated 98.24% efficiency compared to red SLP, while the greenish-blue indicator showed 97.36% efficiency compared to blue SLP. Statistical analysis revealed that the pink indicator did not significantly differ from red SLP at the 95% confidence level but showed agreement at higher confidence levels. The greenish-blue indicator was consistent with blue SLP across all levels of confidence. In conclusion, the study suggests that *Hibiscus sabdariffa* indicators provide high performance and could be a cost-effective alternative to imported litmus papers. With large-scale production, these indicators could reduce import dependency and even be exported, contributing to foreign exchange earnings.

Keywords: Hibiscus sabdariffa, indicators, anthocyanins, greenish blue, pink and papers.

INTRODUCTION

Indicators are compounds or substances that change colour to indicate whether a solution is acidic or alkaline, mark primary reaction points, or reveal the presence of specific substances or analytes in a mixture [1][2][3]. However, indicators do not provide information on the strength of acids or bases or the quantity of identified analytes; this is accomplished through pH meters and quantitative analyses. Inorganic indicators are commonly used for the quantitative identification of gases, such as using acidified dichromate paper to detect sulfur dioxide (SO_2) or lead acetate paper for hydrogen sulfide (H_2S) [4][5][6].

Hibiscus sabdariffa, as noted by [7][8][9][10], is a shrub that is classified within the *Malvaceae* family [11][12][13]. This species is believed to be indigenous to West Africa and is utilized for the production of bast fibre and the preparation of a beverage called carcade, according to [14][15][16]. [17][18][19] have reported that *Hibiscus sabdariffa* can exist as either an annual or perennial herb, or as a woody-based subshrub, with heights ranging from 2 to 2.5 meters (7 to 8 feet). The leaves are deeply lobed, typically featuring three to five segments and measuring between 8 to 15 cm (3 to 6 inches) long, and are arranged alternately along the stems [14][15][16]. [17][18][19]. The flowers, which are 8 to 10 cm (3 to 4 inches) in diameter, are white or pale yellow with a dark red spot at the base of each petal [14][15][16]. [17][18][19]. They feature a thick, fleshy calyx at the base, measuring 1 to 2 cm (0.39 to 0.79 inches) wide, which expands to 3 to 3.5 cm (1.2 to 1.4 inches) and becomes fleshy and bright red as the fruit ripens, typically taking around six months to fully mature [20]. Fig. 1 is a pictorial presentation of *Hibiscus sabdariffa* plants.

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Hibiscus sabdariffa is thought to have originated in India and Malaysia, where it is extensively grown, before making its way to Africa at an early point in time [21]. In English-speaking regions, it is commonly known as roselle, sorrel, or red sorrel [22][23]. In North and East Africa, it is typically referred to as karkade or carcade, and similar names are used in Europe [24][25]. Various studies have documented the morphology of Hibiscus sabdariffa, examining its growth patterns and structural features [26][27][28]. Different parts of the plant have been utilized for treating a range of health concerns, such as hypertension, fever, and liver diseases [29][30]. Research indicates that aqueous extracts of H. sabdariffa can help lower hypertension and offer cardioprotective effects in animal studies [31][32], while its anti-cancer properties have also been investigated, yielding promising findings from multiple researchers [33][34][35]. The phytochemistry of *Hibiscus sabdariffa*, including its bioactive compounds, has been further explored by [36].

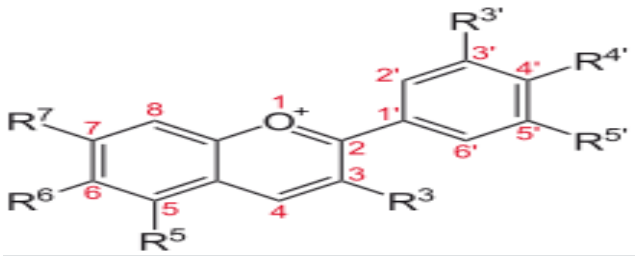
Figure 1: An Image of *Hibiscus sabdariffa* Plant

Source: [14][15][16]

The flowers and calyces of *Hibiscus sabdariffa* are rich in anthocyanins, which are the pigments responsible for the plant's colour [37][38]. These pigments typically produce pink, red, purple, violet, green, and blue shades in flowering plants [39][40]. [41] along with [42] found that the pigment extracted from hibiscus changes colour based on the hydrogen ion concentration of the solution, allowing it to be used as an acid-base indicator in neutralization titrations. A key feature of pH indicators is that their colour transitions from a predominantly alkaline hue occur gradually rather than abruptly, within specific pH intervals [43][42][43]. This phenomenon is known as the colour-change intervals of indicators [44].

[45] noted that the colour of hibiscus tea is influenced by pH levels. The anthocyanins present in hibiscus are similar to those found in red cabbage and other red-hued plant parts [46][47]. [48], along with [49] and [50] reported that hibiscus tea appears red at low pH but shifts to green or blue as the pH increases. *Hibiscus* tea itself is acidic, contributing to its red colour [51]. When an acid is added, there is generally no significant colour change, except for possible dilution effects [52]. In contrast, adding a base causes the colour to change to green or blue, but the addition of acid will return the colour to red [52][53]. Anthocyanins have a general structure illustrated in Figure I. [54] also emphasized that anthocyanins can serve as pH indicators due to their colour changes with pH levels: they are red or pink in acidic solutions (pH < 7), purple in neutral solutions (pH ~ 7), greenish-yellow in alkaline solutions (pH > 7), and colourless in highly alkaline solutions, where the pigment is entirely reduced [55].

Figure 1: General Structure of Anthocyanins



Source: [56][57][58][59]

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The 'R' groups determine which anthocyanin and these groups will be some mix of R = H-, HO- or CH_3O- (e.g., cyanidin: R^3, $R^{5,}$ R^7, $R^{3'}$& $R^{4'}$ = HO-, R^6 & $R^{5'}$ = H-, cyanidin is found in grapes and berries such as blackberry, blueberry… and is red with ph<3, violet at ph = 7–8, and blue with ph>11) **[56][57][58][59]**

For many years, blue and red litmus papers have been imported for use in Nigerian educational institutions, commercial analytical laboratories, industries, factories, and research centers, and their cost has become increasingly high. Litmus, derived from the plant *Roccella tintoria* or *Orchilla* weeds [60][61], is not commonly found locally. However, *Hibiscus sabdariffa* (commonly known as zobo) is abundant and can be cultivated almost anywhere in Nigeria. With this in mind, the local production of affordable zobo-based paper indicators, using plain A4 paper to qualitatively identify acidic and basic solutions, has been developed as a potential alternative to traditional blue and red litmus papers. Studies are currently comparing the effectiveness of these zobo paper indicators with imported standard litmus papers (SLP) used as controls, and the findings are presented in this report.

MATERIALS AND METHODS

Samples collection and selection

The procedure described by [62][63][64] was adopted. Approximately 500 grams of *Hibiscus sabdariffa* (zobo) calyces were obtained from a vendor at the central market in Nasarawa, Nasarawa State. The calyces were placed in a clean, rinsed plastic container, labelled, and taken to the laboratory. Any foreign or undesirable materials, such as stones, stems, dead insects, leaves, and seeds, present in the calyces were identified and removed.

Sample preparation

This was done in line with the method reported by [65][66][67]**.** Two hundred grams of *Hibiscus sabdariffa* calyces were measured and immersed in 500 cm^3 of double-distilled water at room temperature, allowing them to stand for 30 minutes. The resulting extract was then poured into a Pyrex beaker. To further extract the remaining anthocyanins, another 500 cm^3 of double-distilled water was added, and the mixture was stirred. This extract was combined with the previous one and heated at 105 °C to evaporate the water until dry. However, this concentration did not yield the anticipated results, possibly supporting the findings of [68][69], who noted that temperature influences anthocyanin recovery. They suggested that the optimal recovery of anthocyanins is achieved using rotary evaporators or freeze dryers. In the absence of these methods, the calyces could be ground into a powder to serve as a crude dye. Therefore, 200 grams of dried *Hibiscus sabdariffa* calyces were ground using a ceramic pestle and mortar until they reached an amorphous state and then sieved to obtain a fine, smooth powder suitable for dyeing (Plate 1).

Plate I: Fine and Smooth Powder of Hibiscus sabdariffa Calyces Dye

Preparation of pinkish and greenish-blue anthocyanins solution

Approximately 10 grams of anthocyanins were dissolved in 500 cm^3 of double-distilled water at room temperature, resulting in a pink solution (see Plate IIE). The pH of the resulting filtrate was then measured. This filtrate was split into two portions of 400 cm^3 each. To one portion, a few drops of sodium hydroxide solution (NaOH) were added, while hydrochloric acid (HCl) was added to the other portion, in accordance with the method described by [70][71]. The alkaline mixture changed to a greenish-blue color (see Plate IIb), whereas

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the acidified solution remained pinkish (see Plate IIA). Both solutions were filtered, and their pH levels were measured.

Plate II: Pink Acidic Extract (E), Alkaline (B) and Acidified (A)

Hibiscus sabdariffa calyces dye Solutions

Stripping and sewing of papers

This procedure followed the methods described by [70][71]. The paper was cut into strips measuring 60.0 mm in length and 10.0 mm in width (see Appendix I). Six strips were grouped together and sewn at one end using a needle and thread, leaving an excess length of approximately 300 mm of thread for handling during the preparation of the paper indicators (Appendix II).

Preparation of greenish - blue and pink hibiscus sabdariffa papers

This procedure was conducted following the methods outlined by [70][71]. Strips of paper, with threads that were greenish-blue, were immersed in an alkaline greenish-blue solution of anthocyanins derived from *Hibiscus sabdariffa*, allowing the strips to absorb the solution until they achieved a uniform greenish-blue colour (see Plate IV). The papers were then dried at room temperature by hanging them from the extra thread length. In a similar manner, strips of paper with pink threads were dipped into the acidified pink solution of anthocyanins from *Hibiscus sabdariffa* and soaked until they turned uniformly pink (see Plate V). These papers were also dried at room temperature using the extra thread for hanging. Finally, the greenish-blue and pink cover pages (see Appendix III) were labelled and sewn according to the colour of the *Hibiscus sabdariffa* papers.

Plate IV: Greenish - Blue *Hibiscus sabdariffa* Paper Indicators

Plate V: Pinkish *Hibiscus sabdariffa* Paper Indicators

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Calibration (performance analysis) of prepared hibiscus sabdariffa papers

This procedure was carried out following the methods outlined by [70][71]. Solutions of 1M HCl and NaOH were prepared, with 50 cm³ of each dispensed into ten 100 cm³ beakers. A piece of red standard litmus paper (SLP) was utilized to test the acid solution in the first beaker, after which it was removed and used to test the alkaline solution in the same beaker. This process continued until the SLP became ineffective or faded and could no longer function as a litmus paper. The number of successful tests was recorded.

The same procedure was repeated using the prepared pink *Hibiscus sabdariffa* paper, with the results documented accordingly. Additionally, a piece of blue SLP was tested as described above, followed by testing with the prepared greenish-blue *Hibiscus sabdariffa* paper.

The formula,

$$\%\ efficiency = \frac{Number\ of\ times\ by\ which\ prepared\ (zobo)\ paper\ functioned}{Number\ of\ times\ by\ which\ SLP\ functioned}\ x\ 100 \quad (1)$$

was used to measured performance of the prepared *Hibiscus sabdariffa* papers.

Data treatment and statistical analysis

Data collected the test and control analyses were analyzed using several statistical methods: computation of mean ($\bar{\mathbf{x}}$=$\sum$ **x/n**), standard deviation (SD = $\sqrt{\Sigma(xi-\bar{x})2n-1}$) and statistical student test ($t = \frac{X_1 - X_2}{Spooled\sqrt{\frac{n_1 n_2}{n_1+n_2}}}$) and $Spooled = \sqrt{\frac{S1^2\ (n_1-1)+S2^2\ (n_2-1)}{n_1+\ n_2\ -2}}$. This analysis aimed to determine whether the two methods - the test method (utilizing the prepared *Hibiscus sabdariffa* indicator papers) and the control method (employing standard litmus indicator papers) - showed a significant agreement at a 95% confidence level, using the student's t-test method as reported by [72][73][74][75][76].

Construction of protective packets and packaging

Packets for the greenish-blue *Hibiscus sabdariffa* papers were made using greenish-blue cardboard, while those for the pink *Hibiscus sabdariffa* papers were crafted from pink cardboard (see Appendix III). Four bundles of each colour of *Hibiscus sabdariffa* papers were packed into the corresponding packets, following the colour scheme and labels on the packets.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 present the percentage of moisture content in the *Hibiscus sabdariffa* calyces, the confirmatory tests for the prepared greenish-blue and pink *Hibiscus sabdariffa* papers, and the performance evaluation of the prepared Hibiscus sabdariffa papers, respectively.

Table 1: Percentage moisture content *Hibiscus sabdariffa* calyces

Sample	% Moisture Content
Calyces of *Hibiscus Sabdariffa*	11.64 ± 0.03

Table 2: Confirmation of the prepared *Hibiscus sabdariffa* papers

Indicator	pH	Acid	Base
Greenish - blue paper	8.5	Pink	Greenish - blue
Pink paper	3.7	Pink	Greenish – blue

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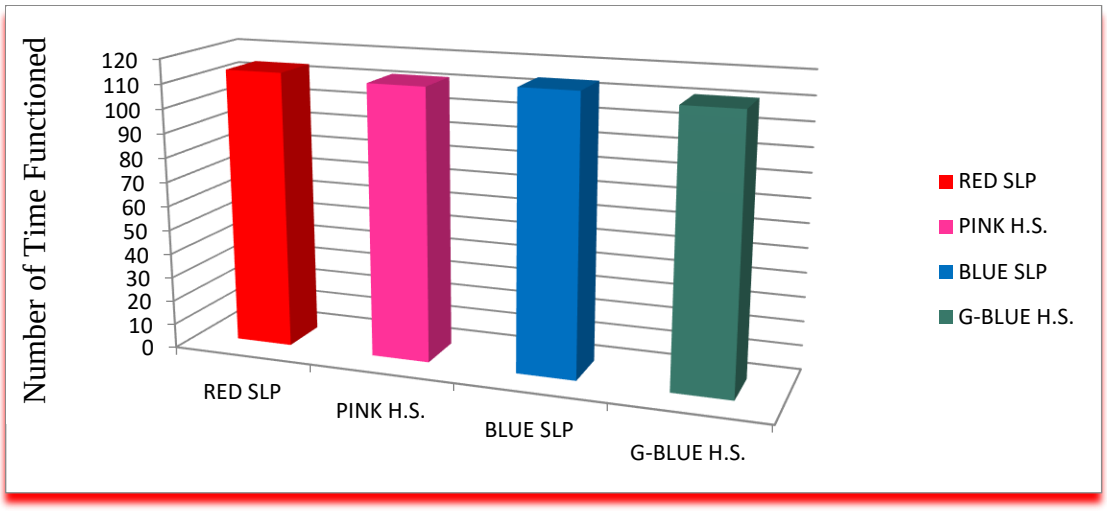
Table 3: Number of times SLP, pink and greenish blue *Hibiscus sabdariffa* papers functioned

Indicator	Number of Time Functioned			
	1st	2nd	3rd	Mean ± SD
Red SLP	114	113	114	113.6±0.583
Pink *Hibiscus sabdariffa*	113	113	112	112.6±0.583
Blue SLP	113	114	114	113.6±0.583
Greenish-blue *H. sabdariffa*	113	112	113	112.7±0.579

Table note:

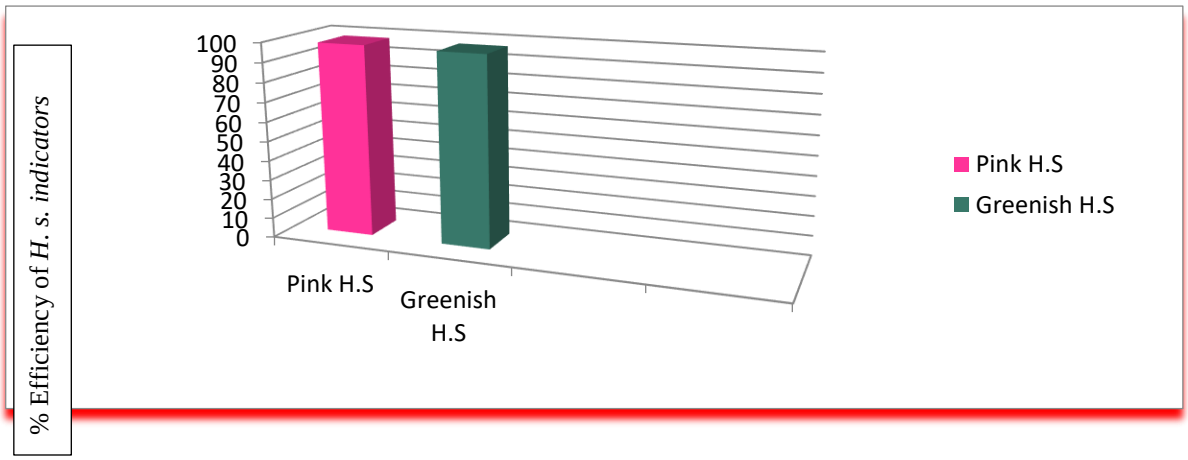
SLP = Standard Litmus Paper

Figures 2 and 3 illustrate the numerical data from Table 3 and evaluate the performance of the prepared pink and greenish-blue *Hibiscus sabdariffa* paper indicators.



Note: H. S. = *Hibiscus sabdariffa*

Figure 2: Chart of Number of Time Functioned by SLP, Pink and Greenish-blue *Hibiscuss Sabdariffa* Papers



Note: H. S. = *Hibiscus sabdariffa*

Figure 3: Chart of Efficiency of Pink and Greenish - blue *H. sabdariffa* Papers

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The moisture content of the calyces was 11.64 ± 0.03%. pH of the extract from *Hibiscus sabdariffa* calyces was 4.5, while that of the greenish-blue *Hibiscus sabdariffa* solution was 8.5, and pH of the acidified pink *Hibiscus sabdariffa* solution was 3.7. The greenish-yellow solution, with a pH of 9.8 (basic), is appropriate for preparing greenish-blue *Hibiscus sabdariffa* paper indicators. Likewise, the pink solution, which had a pH of 3.9 (acidic), is suitable for producing pink *Hibiscus sabdariffa* paper. Strips of both greenish-blue and pink *Hibiscus sabdariffa* papers were individually submerged in 0.2 M HCl and 0.2 M NaOH. The outcomes of these tests are detailed in Table 2. The results indicated that the greenish-blue paper changed to pink in 0.2 M HCl and remained greenish-blue in 0.2 M NaOH, confirming it as a greenish-blue *Hibiscus sabdariffa* indicator. In contrast, the pink paper turned greenish-blue in 0.2 M NaOH but retained its pink color in 0.2 M HCl, thereby verifying it as a pink *Hibiscus sabdariffa* indicator.

The red standard litmus paper (SLP) exhibited a functionality of 114, 113, and 114 times in 50 cm^3 solutions of acid and base, while the pink *Hibiscus sabdariffa* paper showed a functionality of 113, 112, and 113 times. Conversely, the blue SLP functioned 114, 113, and 114 times, whereas the greenish-blue *Hibiscus sabdariffa* paper operated 111, 112, and 113 times. The triplicate values, along with their averages and standard deviations, are presented in Table 3.

Equation 1 was used to determine the efficiency of the prepared *Hibiscus sabdariffa* papers. The pink *Hibiscus sabdariffa* papers performed 98.24 % $(112\ times_{(prepared)}/114\ times_{(SLP)} \times 100)$ of the red SLP. However, the prepared greenish - blue *Hibiscus sabdariffa* papers performed 97.36 % $(111\ times_{(prepared)}/114\ times_{(SLP)} \times 100)$ of the blue SLP. These are interpreted in Fig. 2. The sum total performance of the prepared *Hibiscus sabdariffa* papers $(98.24 + 97.36)\%$ is 195.6 %. The average performance $(195.6\ \%)/2$ is 97.8 %.

The statistical analysis on whether the two methods agree with each other or not, t_{cal} for the red litmus paper and pink *H. sabdariffa* paper was 3.875 while for 3 + 3 – 2 = 4 degree of freedom (d.f.) on the student t_{table}, it is 2.776 at 95 % confidence level and, because the t_{cal} (3.875) > t_{table} (2.776) at 95 % confidence level, the two methods do not agree with each other significantly at 95 % confidence level however, at 99.0 even at 99.5 % confidence level where t_{cal} (3.875) < t_{table} (4.604), (5.598) respectively, there is no significant difference between the two methods. They both therefore agree with each other significantly at 99.0 and 99.5 % confidence levels.

Also, t_{cal} for the blue litmus paper and greenish-blue *H. sabdariffa* paper was 1.231 while for 3 + 3 – 2 = 4 degree of freedom (d.f.) on the student t_{table}, it is 2.776 at 95 % confidence level and, because the t_{cal} (1.231) < t_{table} (2.776) at 95 % confidence level, the two methods agree with each other significantly at 95 % confidence level however through to 99.0 and 99.5 % confidence level where t_{table} are 4.604 and 5.598 respectively.

CONCLUSION

Greenish-blue and pink *Hibiscus sabdariffa* paper indicators were created using the calyces of *Hibiscus sabdariffa*. The pink *Hibiscus sabdariffa* paper changed to greenish-blue in alkaline solutions, while the greenish-blue *Hibiscus sabdariffa* paper turned pink in acidic solutions. The performance of the greenish-blue *Hibiscus sabdariffa* paper was 96.49% when compared to the blue standard litmus paper (SLP), and the pink *Hibiscus sabdariffa* paper performed at 97.37% relative to the red SLP. Overall, the average performance of both papers was 96.93% in comparison to the blue and red SLP. If produced on a larger scale, these *Hibiscus sabdariffa* papers could reduce the need for imported litmus papers, making them more affordable and conserving funds that would otherwise be spent on imports. It is anticipated that locally prepared *Hibiscus sabdariffa* papers could eventually be exported and become a source of foreign exchange.

While the red litmus paper and pink H. sabdariffa paper did not show significant agreement at a 95% confidence level, they did agree significantly at 99.0% and 99.5% confidence levels. In contrast, the blue litmus paper and greenish-blue H. sabdariffa paper demonstrated agreement from a 95% confidence level up to a 99.5% confidence level.

Author's contribution: DEM conceptualized, designed and drafted the methodology and wrote the article;

JEE supervised and acquired funds; AAU was in charge of resources while YCE and EOO; prepared solutions, indicators and conducted experiments. All authors read and agreed to the published version of the manuscript.

Conflicts of interest: The authors declare no conflict of interest.

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APPENDIX I

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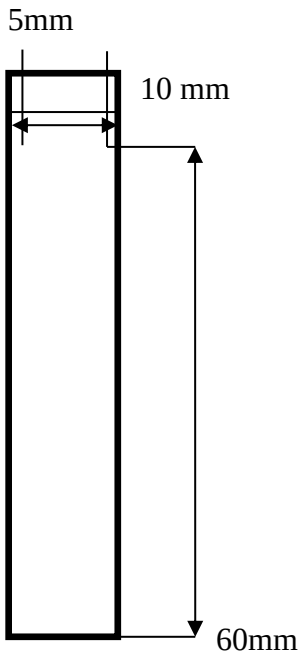
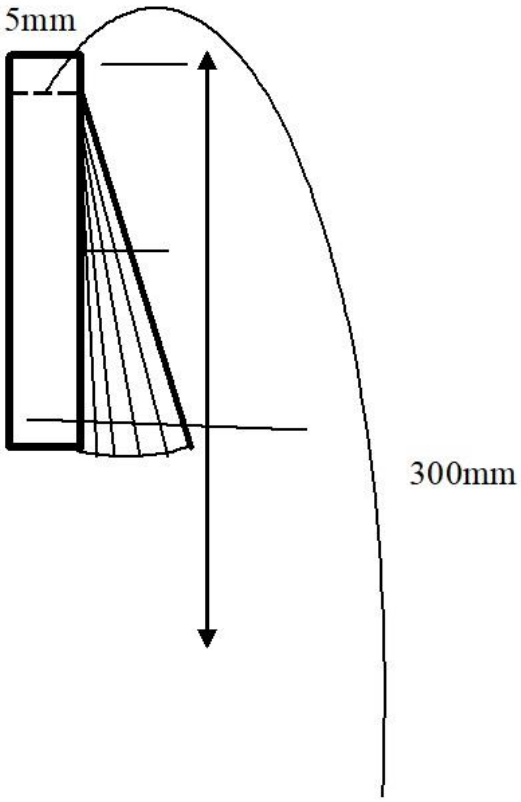


A Stripped Paper

APPENDIX II



A bunch of Six Sewn Stripped Paper

APPENDIX III

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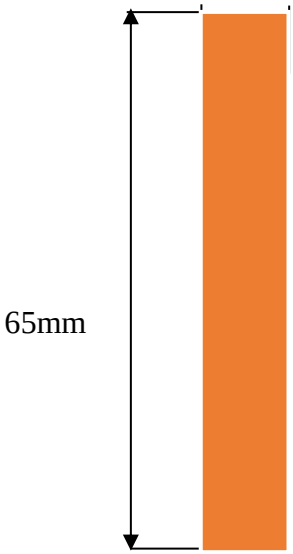
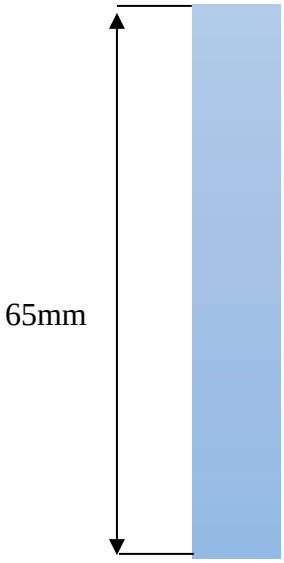


I

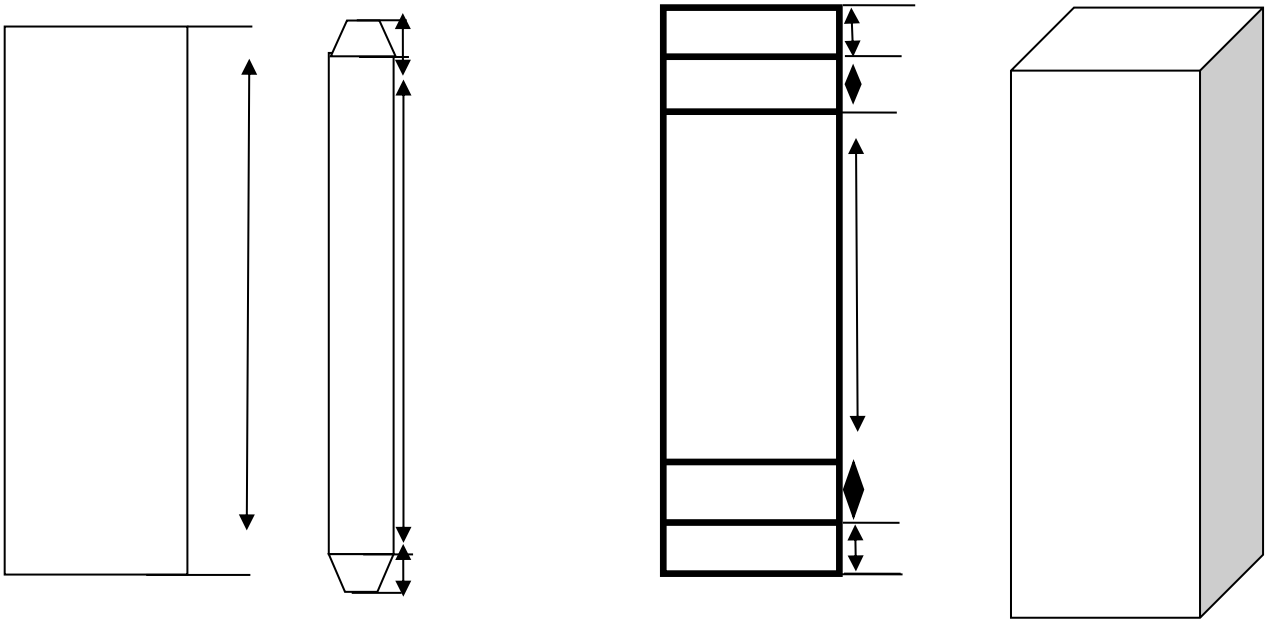
Cover Page for Pink *Hibiscus sabdarifa* Paper



II

Cover Page for Greenish - Blue *Hibiscus sabdarifa* Paper

APPENDIX IV



Steps 1 to 4 Show Designed and Constructed Package for *Hibiscus sabdarifa* Papers