RGB-based colorimetric method for the analysis of water alkalinity using *Cordyline fruticosa* extracts as visual indicator

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ABSTRACT

Leaf extracts of *Cordyline fruticosa* was used as a natural visual indicator for a digital image-based analytical procedure for the measurement of water alkalinity. A smartphone and ImageJ were used to capture and process the images, respectively. This work sought to develop and evaluate a greener strategy of using of *Cordyline fruticosa* leaf extract as natural indicator, in a digital image-based colorimetric set-up using smartphone. Under optimized conditions, the linear working range was from 40 to 160 ppm of $CaCO_3$, with a calibration sensitivity of 5 x 10^{-3} ppm and a coefficient of correlation (r^2)=0.998. The limit of detection and quantification were 39 ppm and 117 ppm, respectively. A 10,000 ppm $CaCO_3$ solution was tested using the proposed method and the results statistically agree with the results obtained from titrimetric methods. The study presented a simple analytical procedure that can be develop and apply for the assessment of pollution that could pose serious human and environmental health hazards.

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1. INTRODUCTION

Low water quality is a global health concern since it is a risk factor for the occurrence of various illnesses ranging from stomach aches, diarrhea, urinary tract infections, typhus, dysentery, and malnutrition, to dehydration [1], [2]. Water quality is generally referred to the biological, chemical, and physical characteristics of water that define its drinking potability and suitability for various applications in fishing, agriculture, and industrial processes [3]. Assessment of the water quality is based on the presence and levels of physicochemical parameters such as pH, total dissolved solids, hardness, conductivity, anions and cations, biological components, and heavy metals [4], [5].

Among the following parameters, pH in terms of acidity and alkalinity is one of the fundamental parameters often tested and measured and characterizes the chemical processes that can influence water quality [6]. The alkalinity of water (pH>7) is due to the presence of bicarbonate (HCO_3-), carbonate (CO_3^{2-}), and hydroxide (OH-) ions [7] while the acidity (pH<7) is based on the significant amount of hydrogen ion (H+) concentrations [6]. At high pH, metals present in water tend to precipitate out and the presence of ammonia is toxic to aquatic organisms. Alkaline water tends to have unpleasant odors and tastes [4]. In lakes, water alkalinity is vital in maintaining the structural stability of aquatic ecology [7]. The level of pH in water may also indicate the amount of heavy metals dissolved in water, thus, an indicator of environmental contamination [4]. Water is used in drinking, bathing, cleaning, and food preparation, and it can be a medium for the presence and transmission of pathogens, microorganisms, and chemical contaminants. Therefore, ingestion of contaminated water can lead to serious waterborne illnesses and even death [8]. From the stated facts, routine monitoring and evaluation of water alkalinity, especially for potable water is important.

A standard method for determining alkalinity is the titration of the sample with a standard acid solution using a pH indicator solution [8]. In seawater and natural terrestrial waters, the alkalinity can be analyzed using a spectrophotometric technique using a bromocresol green indicator. The method has the advantage of using only a small volume of water samples and reagents, as compared with the volume required for titrimetric methods [9]. Another reported method for water alkalinity detection is the use of a combined selective proton pump and pH probe that is applicable for on-site testing, with comparable results to standard volumetric methods [10]. One report on the measurement of alkalinity is a reagentless electroanalytical procedure that is based on the use of polyaniline films as a material for the release of hydrogen ions for efficient and controlled acidification [11]. The developed procedure is fast and reusable up to 74 times for 2 weeks and the method validation presented results that agree with the results obtained from classical titration. The procedures yield accurate and precise results, however, the methods require the use of laboratory apparatus, expensive chemical reagents and instrumentation, and elaborate laboratory set-up. Therefore, the development of a simple and green procedure for the determination of water alkalinity that can be developed and optimized for routine analysis is relevant.

In this present investigation, a digital image colorimetric procedure for the analysis of water alkalinity is developed. The proposed method is composed of a smartphone camera for image acquisition, ImageJ for the RGB image analysis, and *Cordyline fruticosa* leaf extracts as the visual indicator and colorimetric reagent. Digital image colorimetry (DIC) is a current technology used in the quantification of various analytes that is based on the analysis of the basic red, green, and blue (RGB) color layers in a digital image [12]. DIC is a low-cost and simple technique that basically relies on the conversion of the pixel intensities of the images captured using digital imaging devices to values using image processing software [13].

For this study, smartphones were used as digital imaging devices since smartphones are ubiquitous in modern society and they are powerful devices for the collection and analysis of data. Moreover, smartphones have the potential in popularizing analytical chemistry through the simplification of complicated laboratory procedures [14]. For the image analysis, ImageJ was used due to it being a multiplatform public domain application [15]. In this platform, the image is divided into red, green, and blue channels, and a whole number is assigned for each of the three colors in a given tone of the image [13]. Due to its advantages, DIC has been applied in the colorimetric analysis of various analytes in different sensing platforms such as hydrogen peroxide in aqueous solutions [16], iron in dietary supplements [17], aspirin and salicylic acid [18], and peroxide value in edible oils [19]. For the visual indicator, leaf extracts of *Cordyline fruticosa* were used as it is reported to contain pigments [20] that can be used for the digital image analysis of water alkalinity. Traditionally, *Cordyline fruticosa* is a medicinal plant in the Philippines reported to possess anti-inflammatory and is used as plaster for sprains [21]. There are also reports that *Cordyline fruticosa* is traditionally used in ethnic rituals where the plants are whisked in the air and are believed to be helpful in driving away evil spirits [22]. Even though the plant material has been used in the community for various purposes, its local availability and the presence of plant pigments make it a good candidate as a visual indicator for this study. The presence of various pigments in plants such as anthocyanins and chlorophyll allowed observable color changes in the test solutions when the samples have varying pH values thus can be used to test the level of alkalinity of samples [23]. Overall, the study aimed at developing and testing a colorimetric procedure based on digital image analysis and plant extracts as a visual indicator to test the alkalinity levels of water samples, and to provide alternative protocols with acceptable precision and accuracy that can be used for routine analysis and monitoring.

2. METHOD

2.1. Chemicals and materials

Sodium carbonate (Na_2CO_3), sodium hydroxide, phenolphthalein, sulfamic acid, and 95% ethyl alcohol were purchased from Dalkem Corporation. The reagents that were used are of high purity and were used without further purification. All the solutions were prepared as aqueous solutions. The different concentrations of Na_2CO_3 were prepared using freshly boiled distilled water and expressed as calcium carbonate ($CaCO_3$) concentration.

2.2. Preparation and characterization of *Cordyline fruticosa* extracts

The *Cordyline fruticosa* specimen was subjected to a taxonomic identification at the Department of Biology, Central Luzon State University (CLSU), Science City of Muñoz, Nueva Ecija. Fresh plant leaves were cut into small pieces and soaked in 95% acidified ethanol (0.1% v/v HCl) for 24 hours and later filtered to obtain the leaf extracts. The acid was added to improve the solubility of the organic compounds in ethanol by ionizing them [24]. Perkin Elmer Lambda 365 UV-Vis's spectrophotometer was used for the spectral characterization of the extracts.

2.3. Construction of light-box for digital image analysis

An enclosed system or chamber was made from cardboard with the dimensions of 260×190×300 cm and a 10 W LED light bulb for the accommodation of 3 mL vials, where the internal illumination of the chamber was homogeneous which ensures the reproducibility of the method as shown in Figure 1. The power of the lamp increases the brightness of the captured image which also results in greater applicability [25], [26].

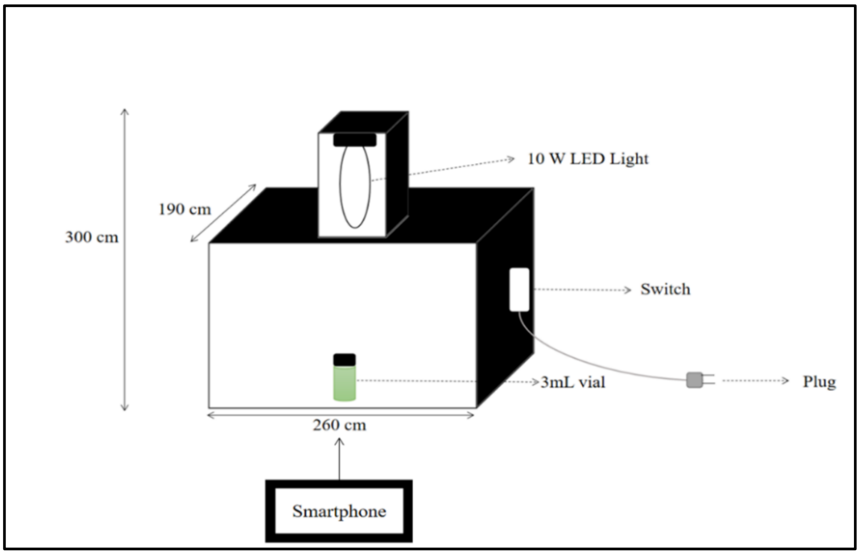

Figure 1. Light-box for the digital image analysis

2.4. General procedure and testing of analytical performance

A 500 μL of 1:1 leaf-to-ethanol ratio was mixed with 3 mL of the test solutions in a clear vial and placed inside the custom-built lightbox. The images were captured from a distance of 12 cm using Vivo Funtouch OS version 10 and were processed using ImageJ. The images were imported into the software and a defined number of pixels were selected for the RGB approach and the values of mean that were collected were transferred to an Excel spreadsheet. The blue channel signals were used for the calibration curve and the absorbance was determined using the formula as in (1).

$$A = -\log {}^{I}/_{I_0} \quad (1)$$

Where: I is the signal of the sample and I_0 for the blank [26].

The calibration curve for the total alkalinity was obtained in triplicate from digital images captured for calcium carbonate solutions with a concentration range from 40 ppm to 160 ppm using the blue channel. The detection limit (LOD) and quantification limit (LOQ) of the method were determined using the formula as in (2) and (3), respectively:

$$LOD = {}^{3.3s}/_{m} \quad (2)$$

$$LOQ = {}^{10s}/_{m} \quad (3)$$

Where: s is the standard deviation and m is the slope of the calibration curve.

A $CaCO_3$ solution in distilled water were used as sample solutions for the testing of the proposed method. Classical titration using HCl solution as titrant and phenolphthalein as indicator was used as the reference method to compare the results collected from the DIC method [27], [28].

2.5 Statistical Treatment of Data

Experimental design was used to gather the results of the experiments. The t-test was used to determine if there is a significant difference between the results of the concentrations of $CaCO_3$ in distilled and tap water using DIC method and classical method. The % relative standard deviation (% RSD) was also used to determine the precision of the methods.

3. RESULTS AND DISCUSSION

3.1. Characterization of *Cordyline fruticosa* extracts

Figure 2 shows the absorption spectra of the *Cordyline fruticosa* extracts and *Cordyline fruticosa* in a basic pH environment. The *Cordyline fruticosa* extracts as is showed a maximum absorption wavelength of 662 nm. The maximum absorption wavelength is within the absorption range of chlorophyll which is from 630 – 700 nm [29]. Chlorophylls are photosynthetic pigments composed of four pyrrole rings joined through a carbon bridge to an aromatic macrocycle [30]. Moreover, the obtained maximum absorption wavelength in the present study is near to the obtained maximum absorption wavelength in the previous study [31]. Absorption in the 600-700 nm region is classified as the "chlorophyll" region [32]. Whereas the shift in the maximum absorption wavelength of the *Cordyline fruticosa* in a basic pH environment is at 597 nm. Therefore, the color changes of the *Cordyline fruticosa* extracts showed their colorimetric response to the changes in the pH of the environment and can be used as a visual indicator, as can be observed from the inset photo in Figure 2.

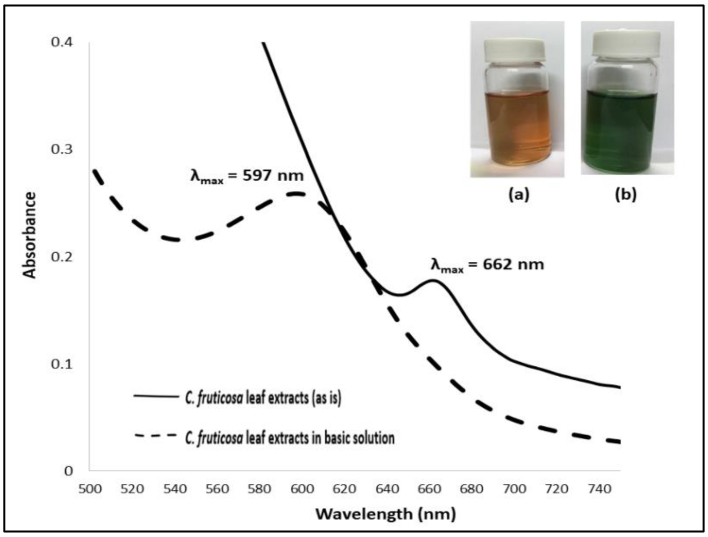

Figure 2. Absorption spectra of *Cordyline fruticosa* leaf extracts and *Cordyline fruticosa* leaf extracts in basic pH. (Inset photo: (a) *Cordyline fruticosa* leaf extracts and (b) *Cordyline fruticosa* leaf extracts in basic pH

3.2. Analytical performance and method validation

To test the specificity and compare the colorimetric responses of the *Cordyline fruticosa* extracts in acidic and basic environments, the plant extracts were separately mixed with dilute acid and base solutions. As can be observed from Figure 3, the *Cordyline fruticosa* extracts in acidic pH resulted in a red-colored solution as shown in Figure 3(a) whereas the *Cordyline fruticosa* in basic pH yielded a green-colored solution as shown in Figure 3(b). In reference to the initial orange color of the plant extracts, the colorimetric response of the *Cordyline fruticosa* used in the proposed method is specific to samples with basic pH. Analysis of acidity and alkalinity of water is important as it determines the ecological community since it has a direct effect on the growth rate of aquatic organisms and to the activity of non-metals and metals [7]. Moreover, alkalinity in water is suggested to be due to natural and anthopogenic processes, and human activities and watershed properties [33].

The linear working range was between 40 to 160 ppm of $CaCO_3$ with a calibration sensitivity of 5 x 10^{-3} ppm and a coefficient of correlation (r^2) = 0.998 as shown in Figure 4. The obtained r^2 value showed the linear relationship between the sample solution concentration and the RGB values from the image analysis since the obtained r^2 is within 0.998 to 1.000 [34], [35]. The LOD, which is the lowest concentration detected but not exactly quantified [34], [35] was 39 ppm. While the LOQ or the quantifiable concentration usually higher than LOD [34], [35] was 117 ppm.

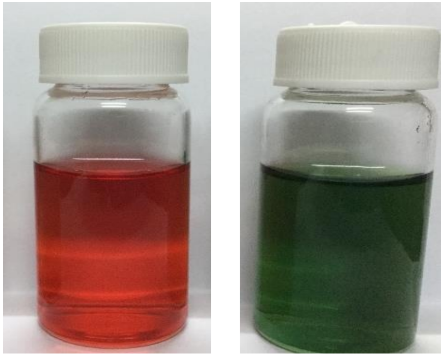

Figure 3. *Cordyline fruticosa* leaf extracts in (a) acidic and (b) basic solutions

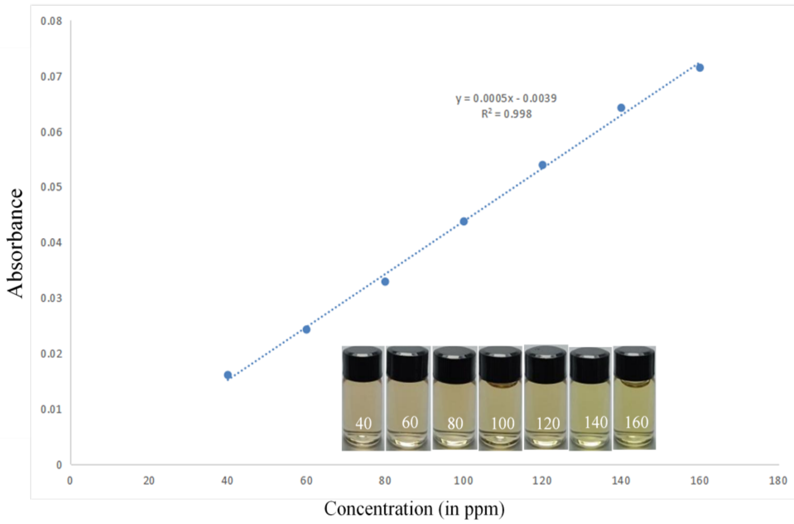

Figure 4. Calibration curve for the colorimetric response vs. concentration of $CaCO_3$ (in ppm)

The percent recovery for the proposed method was 89.79% which falls within the acceptable criteria for the analysis of anions in drinking water (85%-115%) [27]. The computed relative standard deviation (RSD) for the proposed method was 11.54% which is within the acceptable RSD for an analytical procedure (10-25%) [36] as presented Table 1. Statistical analysis using paired sample t-test was employed to test the agreement between the results of the two methods of analysis. The t-test showed that the $t_{stat}<t_{crit}$ (-3.7<4.3) which means that there is no significant difference between the determination of the total alkalinity using the DIC method and the titrimetric method.

Table 1. Results of the analysis of alkalinity of the test solution using a titration method and the DIC method

Sample	Proposed Method (n=3)		Titrimetry (n=3)	
10000 ppm $CaCO_3$	ppm $CaCO_3$ ±SD	Spike recovery ± SD	ppm $CaCO_3$±SD	Spike recovery±SD
	8979±1036	89.79%	10982±149	109.82%
	(RSD=11.54%)		(RSD=1.36%)	

4. CONCLUSION

The study demonstrates the feasibility of using *Cordyline fruticosa* leaf extracts as a natural visual indicator for the analysis of alkalinity in water. The work utilizes smartphones and ImageJ as simple and affordable processing tools to convert the color channel intensities to analytical information. The *Cordyline fruticosa* leaf extracts exhibit different colorimetric responses to media with different pH. The proposed method has a linear response from tested concentration range and the results showed no significant difference from the samples tested via a titrimetric method. Based on the merits of the proposed method, the study can be an alternative to the classic procedure for the alkalinity determination of water samples. Moreover, the study will pave the way for the exploration and development of such colorimetric procedures for other analytes essential for the assessment of contamination for the benefit of human and environmental public health.

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REFERENCES

[1] A. B. Birawida, E. Ibrahim, A. Mallongi, A. A. Al Rasyidi, Y. Thamrin, and N. A. Gunawan, “Clean water supply vulnerability model for improving the quality of public health (environmental health perspective): A case in Spermonde islands, Makassar Indonesia,” *Gaceta Sanitaria*, vol. 35, pp. S601–S603, 2021, doi: 10.1016/j.gaceta.2021.10.095.

[2] S. O. Sangalang *et al.*, “Diarrhoea, malnutrition, and dehydration associated with school water, sanitation, and hygiene in Metro Manila, Philippines: A cross-sectional study,” *Science of The Total Environment*, vol. 838, p. 155882, Sep. 2022, doi: 10.1016/j.scitotenv.2022.155882.

[3] F. Mohseni *et al.*, “Ocean water quality monitoring using remote sensing techniques: A review,” *Marine Environmental Research*, vol. 180, p. 105701, Sep. 2022, doi: 10.1016/j.marenvres.2022.105701.

[4] B. M. Saalidong, S. A. Aram, S. Otu, and P. O. Lartey, “Examining the dynamics of the relationship between water pH and other water quality parameters in ground and surface water systems,” *PLoS ONE*, vol. 17, no. 1 1, p. e0262117, Jan. 2022, doi: 10.1371/journal.pone.0262117.

[5] L. Thokchom and K. S. Kshetrimayum, “Quality assessment of surface water and groundwater giving emphasis on water quality index and irrigational indicators in the southeastern part of Manipur Valley, north-east India,” *Geological Journal*, vol. 57, no. 12, pp. 5326–5344, Dec. 2022, doi: 10.1002/gj.4361.

[6] K. N. Brooks, P. F. Ffolliott, and J. A. Magner, *Hydrology and the Management of Watersheds*, 4th ed. Wiley, 2012. doi: 10.1002/9781118459751.

[7] L. Liang, Y. Deng, J. Li, Z. Zhou, and Y. Tuo, “Modelling of pH changes in alkaline lakes with water transfer from a neutral river,” *Chemosphere*, vol. 310, p. 136882, Jan. 2023, doi: 10.1016/j.chemosphere.2022.136882.

[8] G. Prasad, A. S. Reshma, and M. Vinodini Ramesh, “Assessment of drinking water quality on public health at Alappuzha district, southern Kerala, India,” *Materials Today: Proceedings*, vol. 46, pp. 3030–3036, 2021, doi: 10.1016/j.matpr.2021.01.302.

[9] H. Yang, T. Mishima, S. Katazakai, and M. Kagabu, “Analytical approach using a chemical equilibrium formula and geochemical modeling for alkalinity measurements of small natural water samples,” *Applied Geochemistry*, vol. 148, p. 105535, Jan. 2023, doi: 10.1016/j.apgeochem.2022.105535.

[10] M. G. Afshar, G. A. Crespo, and E. Bakker, “Thin-layer chemical modulations by a combined selective proton pump and ph probe for direct alkalinity detection,” *Angewandte Chemie International Edition*, vol. 54, no. 28, pp. 8110–8113, Jul. 2015, doi: 10.1002/anie.201500797.

[11] A. Wiorek, G. Hussain, A. F. Molina-Osorio, M. Cuartero, and G. A. Crespo, “Reagentless Acid–Base Titration for Alkalinity Detection in Seawater,” *Analytical Chemistry*, vol. 93, no. 42, pp. 14130–14137, Oct. 2021, doi: 10.1021/acs.analchem.1c02545.

[12] A. Choodum, W. Sriprom, and W. Wongniramaikul, “Portable and selective colorimetric film and digital image colorimetry for detection of iron,” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 208, pp. 40–47, Feb. 2019, doi: 10.1016/j.saa.2018.09.062.

[13] J. Caleb, U. Alshana, and N. Ertaş, “Smartphone digital image colorimetry combined with solidification of floating organic drop-dispersive liquid-liquid microextraction for the determination of iodate in table salt,” *Food Chemistry*, vol. 336, p. 127708, Jan. 2021, doi: 10.1016/j.foodchem.2020.127708.

[14] G. M. S. Ross *et al.*, “Best practices and current implementation of emerging smartphone-based (bio)sensors – Part 1: Data handling and ethics,” *TrAC Trends in Analytical Chemistry*, vol. 158, p. 116863, Jan. 2023, doi: 10.1016/j.trac.2022.116863.

[15] R. M. Caraballo, L. M. Saleh Medina, S. G. J. Gomez, P. Vensaus, and M. Hamer, “Turmeric and RGB Analysis: A Low-Cost Experiment for Teaching Acid–Base Equilibria at Home,” *Journal of Chemical Education*, vol. 98, no. 3, pp. 958–965, Mar. 2021, doi: 10.1021/acs.jchemed.0c01165.

[16] D. Fetalbero Reyes, “Green-synthesized silver nanoparticles as sensor probes for the naked-eye detection of hydrogen peroxide,” *Oriental Journal of Chemistry*, vol. 36, no. 04, pp. 640–644, Aug. 2020, doi: 10.13005/ojc/360407.

[17] J. I. Ballesteros, H. J. R. Caleja-Ballesteros, and M. C. Villena, “Digital image-based method for iron detection using green tea (Camellia sinensis) extract as natural colorimetric reagent,” *Microchemical Journal*, vol. 160, p. 105652, Jan. 2021, doi: 10.1016/j.microc.2020.105652.

[18] B. Jain, R. Jain, R. R. Jha, A. Bajaj, and S. Sharma, “A green analytical approach based on smartphone digital image colorimetry for aspirin and salicylic acid analysis,” *Green Analytical Chemistry*, vol. 3, p. 100033, Dec. 2022, doi: 10.1016/j.greeac.2022.100033.

[19] A. C. S. A. Anconi, N. C. S. Brito, and C. A. Nunes, “Determination of peroxide value in edible oils based on Digital Image Colorimetry,” *Journal of Food Composition and Analysis*, vol. 113, p. 104724, Oct. 2022, doi: 10.1016/j.jfca.2022.104724.

[20] M. A. M. Al-Alwani, N. A. Ludin, A. B. Mohamad, A. A. H. Kadhum, and K. Sopian, “Extraction, preparation and application of pigments from Cordyline fruticosa and Hylocereus polyrhizus as sensitizers for dye-sensitized solar cells,” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 179, pp. 23–31, May 2017, doi: 10.1016/j.saa.2017.02.026.

[21] L. A. Cajuday and E. N. Bañares, "Analysis of Traditional Knowledge of Medicinal Plants from Residents Near Kalikasan Park, Albay, Philippines," *Bicol University Research and Development Journal*, 2020, doi: 10.47789/burdj.mbtcbbgs.20192202.5.
[22] T. D. Balangcod and K. D. Balangcod, "Plants and culture: Plant utilization among the local communities in Kabayan, Benguet Province, Philippines," *Indian Journal of Traditional Knowledge*, vol. 17, no. 4, pp. 609–622, 2018.
[23] D. Reyes *et al.*, "Utilization of plant material extracts as natural acid-base indicators: an example of at-home lab experiment in the new normal learning Set-Up," *The QUEST: Journal of Multidisciplinary Research and Development*, vol. 1, no. 2, Dec. 2022, doi: 10.60008/thequest.v1i2.14.
[24] Courage T. Nhapi, "Application of Eichhornia Crassipes Root Extract As an Acid-Base Indicator," Midlands State University, 2016.
[25] H. Li, T. Fang, Q.-G. Tan, and J. Ma, "Development of a versatile smartphone-based environmental analyzer (vSEA) and its application in on-site nutrient detection," *Science of The Total Environment*, vol. 838, p. 156197, Sep. 2022, doi: 10.1016/j.scitotenv.2022.156197.
[26] I. S. A. Porto, J. H. Santos Neto, L. O. dos Santos, A. A. Gomes, and S. L. C. Ferreira, "Determination of ascorbic acid in natural fruit juices using digital image colorimetry," *Microchemical Journal*, vol. 149, p. 104031, Sep. 2019, doi: 10.1016/j.microc.2019.104031.
[27] US Environmental Protection Agency, *Determination of Inorganic Anions in Drinking Water by Ion Chromatography.* Cincinnati: US Environmental Protection Agency, 1993.
[28] American Water Works Association (AWWA), "Alkalinity," in *Standard Methods for the Examination of Water and Wastewater*, Washington D. C: American Public Health Association, American Water Works Association and the Water Environment Federation, 2017, pp. 2–36–2–38.
[29] M. A. M. Al-Alwani, N. A. Ludin, A. B. Mohamad, A. A. H. Kadhum, M. M. Baabbad, and K. Sopian, "Optimization of dye extraction from Cordyline fruticosa via response surface methodology to produce a natural sensitizer for dye-sensitized solar cells," *Results in Physics*, vol. 6, pp. 520–529, 2016, doi: 10.1016/j.rinp.2016.08.013.
[30] D. S. Amorim, I. S. Amorim, R. C. Chisté, J. T. Filho, F. A. N. Fernandes, and H. T. Godoy, "Effects of cold plasma on chlorophylls, carotenoids, anthocyanins, and betalains," *Food Research International*, vol. 167, p. 112593, May 2023, doi: 10.1016/j.foodres.2023.112593.
[31] A. K. Rosli, S. Suhaimi, M. A. Hashim@Ismail, N. M. Yatim, and N. Hamid, "Optimization of dye extraction from purple cabbage and cordyline fruticosa in dye-sensitized solar cell," *Solid State Phenomena*, vol. 307, pp. 192–200, Jul. 2020, doi: 10.4028/www.scientific.net/SSP.307.192.
[32] E. L. Ashenafi, M. C. Nyman, J. T. Shelley, and N. S. Mattson, "Spectral properties and stability of selected carotenoid and chlorophyll compounds in different solvent systems," *Food Chemistry Advances*, vol. 2, p. 100178, Oct. 2023, doi: 10.1016/j.focha.2022.100178.
[33] B. E, S. Zhang, C. T. Driscoll, and T. Wen, "Human and natural impacts on the U.S. freshwater salinization and alkalinization: A machine learning approach," *Science of The Total Environment*, vol. 889, p. 164138, Sep. 2023, doi: 10.1016/j.scitotenv.2023.164138.
[34] I. Taverniers, M. De Loose, and E. Van Bockstaele, "Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance," *TrAC Trends in Analytical Chemistry*, vol. 23, no. 8, pp. 535–552, Sep. 2004, doi: 10.1016/j.trac.2004.04.001.
[35] G. Abdel and M. El-Masry, "Verification of quantitative analytical methods in medical laboratories," *Journal of Medical Biochemistry*, vol. 40, no. 3, pp. 225–236, 2021, doi: 10.5937/jomb0-24764.
[36] D. Stöckl, H. D'Hondt, and L. M. Thienpont, "Method validation across the disciplines—Critical investigation of major validation criteria and associated experimental protocols," *Journal of Chromatography B*, vol. 877, no. 23, pp. 2180–2190, Aug. 2009, doi: 10.1016/j.jchromb.2008.12.056.

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