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Spectrophotometric Determination of Total Anthocyanin Content (TAC) in *Costus afer* Flower Extract and its Application as pH Indicator in Acid/Base Titrations

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ABSTRACT

This paper reports for the first time, the spectrophotometric determination of the total anthocyanin pigments in the extract of the flowers of the wild plant, *costus afer* Ker Gawl (called bush cane) growing abundantly in the lowland rainforests of the Niger Delta Region of Nigeria. The anthocyanin-rich extract was obtained by subjecting the dried flowers to extraction using absolute ethanol acidified with 0.1% HCl. The total anthocyanin content (TAC) was spectrophotometrically determined using the pH differential method. The total anthocyanin content was 268.58 µg/g cyanidin-3-glucoside equivalent. This parent extract was subsequently concentrated and employed as indicator in acid/base titrations of varying concentrations: 0.1 M, 0.5 M and 1.0 M. The acid/base pairs employed in this study were hydrochloric acid sodium hydroxide, hydrochloric acid/ammonia, acetic acid/sodium hydroxide and acetic acid/ammonia. The mean titre values at equivalence points of the titrations were compared with corresponding values obtained using standard synthetic indicators: phenolphthalein and methyl red. The results revealed that the equivalence points for the various titrations using the flower extract as indicator showed no significant difference ($P > 0.05$) when compared to the values obtained when standard synthetic indicators were employed. These results, thus; indicate that the under-utilized flowers of this wild plant is a rich source of anthocyanin pigments which could be explored as cost- effective eco-friendly pH indicators as well as other potential industrial applications.

Keywords: *Costus afer* flower extract, Anthocyanin, pH Differential method, pH Indicator.

Introduction

Anthocyanins are hydrophilic polyphenolic plant pigments present in flowers, fruits and leaves of higher plants. They belong to a parent class of molecules called flavonoids. Structurally they consist of aglycones (anthocyanidins), sugar(s) and in many cases acyl group(s) [1-3]. Consumption of plants rich in anthocyanins have also been associated with protection against various ailments, since a number of studies have revealed that that they provide dominant anti-inflammatory, carcinogenic activities, and possess very strong anti-oxidant properties leading to a variety of health benefits [4-6]. These pigments are responsible for the red, salmon pink, purple and dark blue of these plant parts. Anthocyanins have been reported to have the potential to act as suitable alternatives to synthetic indicators [7-8]. This is due to the difference in the chemical structures that occur in response to changes in pH leading to sharp and distinct color changes in both acid and basic media. These color changes with pH occur when anthocyanins like synthetic indicators donate or accept protons and it has been reported that steadily increasing the pH results in an alteration of anthocyanin colors from red towards blue [9]. Pure anthocyanin standards for diverse applications are typically sourced from berries, grapes, blood oranges, calyces of hibiscus sabdariffa,

red cabbage and other anthocyanin-rich plants. Raspberry fruit has a long history of been used as a natural colorant and dye due to its rich anthocyanin content [10]. Brightly colored plants parts with little economic value, are presently being investigated to ascertain their anthocyanin content for possible application as indicators of endpoints in the quantitative analytical technique: volumetric analysis

Costus afer, the plant been investigated in this study is a perennial rhizomatous herb that belongs to a genus Costus [11]. It is naturalized in Nigeria, Ghana, Niger, Guinea, South Africa and Senegal [12]. It is commonly called bush cane, ginger lily, spiral ginger. The Ogbia people of Bayelsa State call it "Obokoloman" while Cameroonian Anglophones call it "Monkey sugar cane" [13]. It grows up to 4 m tall [14]. The leaves are spirally arranged, tubular sheath with green purple blotches. The spikes are conical with a length of 2.5-7.5 cm long [15]. The stems, seeds and leaves are reported to contain several metabolites. Hence they are used as remedy for cough, inflammation, arthritis, as laxative diuretic and in the treatment of several other ailments [12]. Costus afer flower typically possess white and reddish pink petals. These brightly colored petals are due to the presence of anthocyanins in the vacuoles of the cell sap. However, there is no report on the total monomeric anthocyanin content in the flower extract of this plant to ascertain if it is a rich source of these pigments for possible use as natural pH indicators as well as other industrial applications. Thus the objective of this study is to determine the total anthocyanin content of the shade-dried flower extract of this wild plant. The resultant extract will subsequently be investigated for its pH indicator potential in acid/base titrations.

Materials and method

Material

The plant material used in this work as a source of anthocyanin are flowers of *Costus afer* commonly known as Ginger lily, Bush cane or Monkey sugar cane. They were collected from natural habitats in and around the Federal University Otuoke, Bayelsa State; a higher institution located in the South-South zone of Nigeria. The plant was identified by botanists in University of Port Harcourt.

Figure 1: Costus afer plant

Figure 2: Fresh and dried *Costus afer* flowers

Reagents

Analytical grade reagents (ethanol, acetic acid, hydrochloric acid, sodium hydroxide, ammonium hydroxide, potassium chloride, citric acid and sodium citrate) were used for this study.

Apparatus and Instruments

The apparatus used in the course of this work are; Beakers, Amber bottles, Conical flask, Burettes Pipettes, Pipette fillers, Micro pipettes, Sample bottles, Buckner funnels.

The instruments were: Hand-held pH meter (HANNA, China); pH meter (JENWAY 3505, England) UV-Vis Spectrophotometer (JASCO V-730 Spectrophotometer, Japan); Analytical balance (OHAUS Pioneer PA413, Japan), Stage Vacuum pump (TW-1A, China)

Extraction of anthocyanins

An extraction method previously developed was employed with slight modifications [16]. 50 ml of absolute ethanol acidified with 0.1% HCl was applied to 10 g of dried macerated flower sample. Acidified absolute ethanol was used in order to maximize extraction and minimize degradation, by maintaining pH around 3. The pH measurements were made using a digital pH-meter (HANNA) calibrated with pH 1 and 7 buffers. The resultant extract was filtered by suction, preserved in air- tight amber bottle and stored prior to further analyses.

Qualitative test for anthocyanin

To 2 ml of the flower extract, 1 ml of 2 M sodium hydroxide was added and heated for 5 min at 100 °C. Formation of persistent bluish green colour indicates the presence of anthocyanins.

Determination of total anthocyanin content (TAC)

In other to determine the total anthocyanin content in the flowers of *Costus afer*, the pH differential method which determines total monomeric anthocyanin content based on the structural changes of chemical forms of anthocyanin as a function of pH was employed [17].

A UV-vis. spectrophotometer was used for the quantification of total anthocyanin contents. The buffers were prepared as follows: 50ml of 0.2 M KCl and 134 ml of 0.2 M HCl were mixed in order to have a pH 1.0 buffer solution while 44.5ml of 0.1M citric acid and 55.5 ml of 0.1 M sodium citrate were also mixed to prepare a pH 4.5 buffer solution in order to have the sample in pH 1.0 and 4.5 respectively. The pH of the solutions was measured using a pH meter calibrated with pH 1 and 4 buffers.

The absorbance at 538 nm for the extract diluted in the respective buffers (pH 1.0 and pH 4.5) was recorded. To determine the appropriate dilution factor for the samples, 2 ml of sample extract was diluted in 100 ml of pH 1.0 and 4.5 respectively, which gave a dilution factor of 51. The dilution was to ensure that the absorbance was within the linear range of the spectrophotometer. Quartz cuvettes of 1 cm path length were used.

The total anthocyanin content (TAC) in this sample was calculated using the mathematical relation stated in equation 1 & 2 based on the pH-differential method [14].

$$\mathbf{TAC = (A}_{\lambda vis\text{-}max} \mathbf{- A_{700})}_{pH1} \mathbf{- (A}_{\lambda vis\text{-}max} \mathbf{- A_{700})}_{pH4.5} \mathbf{* F} \quad \text{..... Eq. 1}$$

$$F = \frac{MW*DF*CF1*CF2}{\varepsilon*l} \quad \text{.....Eq.2}$$

$$= \mathbf{(A}_{\lambda vis\text{-}max} \mathbf{- A_{700})}_{pH1} \mathbf{- (A}_{\lambda vis\text{-}max} \mathbf{- A_{700})}_{pH4.5}$$

A = (0.36076 – 0.03088) – (-0.07477 – (-0.08913))

A = 0.32988 – 0.01436

A = 0.31552

$$F = \frac{449\ gmol{-}1*51*10\text{^}6\mu g/g*1L/1000ml}{\frac{26900L}{mol.cm}*1cm}$$

$$F = \frac{22899000}{26900}$$

F = 851.26 µg/g

TAC = (0.31552) * 851.26

TAC = 268.59 µg/g = 268.59 mg/kg cyanidin-3-glucoside equivalent

The anthocyanin content was expressed as cyanidin-3-glucoside equivalent since it is the most abundant anthocyanin in nature.

Application of extract as pH indicator

Slight modifications were applied to an earlier established method [18]. 10 ml of titrant with 2 drops of indicator was used for the acid/base titration. The titrations were carried out in triplicates and results recorded were mean values. This procedure was repeated for phenolphthalein and methyl red indicators for 0.1 M, 0.5 M and 1 M of all acid/base strengths.

Statistical analysis

The data was analyzed and expressed as mean ± standard deviation for triplicate determinations. A one-way ANOVA was used to determine significant differences in the equivalence points in the titrations with the flower extract as indicator and the standard synthetic indicators. Significance was fixed at $P< 0.05$. All statistical analyses were performed using SPSS 16.0

Result and discussion

Extraction of anthocyanin from sample

45 ml of the flower extract was recovered by vacuum filtration, and the standard qualitative test for anthocyanins carried out on the extract

From the spectra, the λ_{max} was shown at 538 nm, which falls within the standard maximum absorption spectra for anthocyanins in the visible region of the electromagnetic spectrum. Figure 4 shows the pH dependency of the absorption spectra of anthocyanins in the

was positive [19]. This confirmed the presence of anthocyanin pigments.

Spectrophotometric analysis of Costus afer flower extract

The absorption spectra of anthocyanins in the flower extract was achieved by subjecting it to spectrophotometric measurement using a UV-vis. spectrophotometer. Figure 3 shows the spectra for the flower extract in 0.1%HCl absolute ethanol.

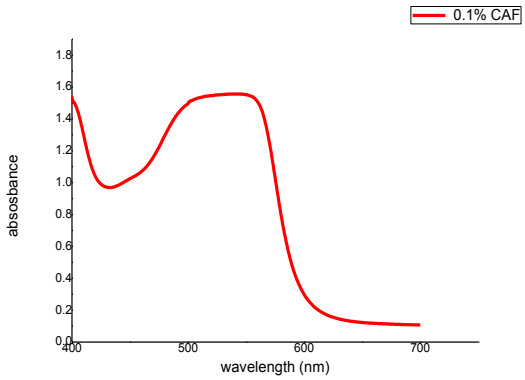

Figure 3: UV-vis Spectrum of *Costus afer* flower extract.

extract.

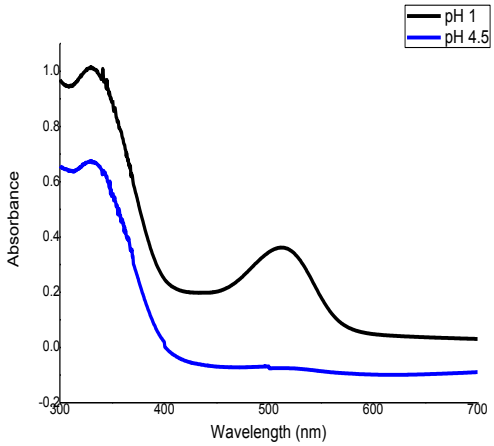

Figure 4: Absorption spectrum of Costus *afer* flower extract in pH 1 and pH 4.5 buffers.

The Total Anthocyanin Content(TAC)

The total monomeric anthocyanin content was 268.59 µg/g cyanidin-3-glucoside equivalent

based on the pH differential method

At pH 1 the red flavylium form exits, while at pH 4.5, the colorless hemiketal form exit. There is no absorbance of monomeric anthocyanin in pH 4.5. The difference in absorbance of the anthocyanin solution between these two pH values allowed for an accurate and rapid determination of total monomeric anthocyanin content in the sample.

The total anthocyanin content found in the flowers of *Costus afer* using absolute ethanol acidified with 0.1 % HCl was calculated to be 268.58 µg/g using the equation proposed by an earlier researcher [17]. This is in line with the TAC of 186.1 mg/kg cyanidin -3-glucoside obtained from industrial purple-fleshed sweet potatoes using 70% acidified methanol [20]. This shows that the anthocyanin content in *Costus afer* flower is high making it a potential source of these pigments.

Application of anthocyanin extract as pH indicator

The anthocyanin rich extract was used as an indicator in acid-base titrations of various strengths which are strong acid/ strong base (HCl & NaOH), strong acid/ weak base (HCl & NH_3), weak acid/ strong base (CH_3COOH & NaOH), and weak acid/ weak base (CH_3COOH & NH_3) using these concentrations: 0.1 M, 0.5 M, 1 M respectively. The resultant equivalence points were compared with values obtained in titrations using standard synthetic indicators: phenolphthalein and methyl red. Tables 1 gives a summary of the mean ± standard deviation values at the respective equivalence points reached.

Table 1: Mean and Standard Deviation of acid (in ml) at Equivalence point for all titrations

M	Hydrochloric acid v/s Sodium Hydroxide		Hydrochloric acid v/s Ammonia		Acetic acid v/s Sodium Hydroxide		Acetic acid v/s Ammonia	
	Phenol Phthalein	*Costus Afer* Extract	Phenol Phthalein	*Costus Afer* extract	Phenol Phthalein	*Costus afer* extract.	Methyl red.	*Costus afer extract*
0.1	7.95±0.07	7.93±0.04	8.45±0.07	8.45±0.07	8.45±0.07	8.35±0.07	17.95±0.07	17.15 ± 0.07
0.5	8.95±0.07	9.18±0.04	10.33±0.04	10.15±0.07	8.43±0.04	8.55±0.07	18.05±0.07	17.25 ±0.07
1	9.15±0.07	9.75±0.07	12.95±0.07	14.55±0.07	8.55±0.07	8.55±0.07	17.95±0.07	17.55+0.07

The end points obtained for all the titrations using the anthocyanins extract from *Costus afer* flower as indicator, were sharp for the respective concentrations. Additionally, the results as shown in Table 1 were accurate and precise. This resulted from pH changes that

occurred at the respective endpoints. These pH changes are in line with the conversion of the anthocyanins structure from the red flavylium ion form to the quinoidal base bluish-green form. This is also in line with the observations reported in a related study with anthocyanins of *Hippeastrum hybridum* as pH indicator [9]. There was no significant difference ($p>0.05$) in end points in acid/base titrations with the plant extract as indicator and those with synthetic standard indicators. This however, highlights the potential of the plant extract as an indicator in acid/base titrations. The results obtained showed that the routinely used indicator can be replaced successfully by the plant extracts. During the titrations, the plant extract changed from bluish-green in basic solution to reddish pink in acidic solution, phenolphthalein changed from pink to colourless while methyl red changed from yellow in base to red in acid.

Conclusion

This study shows that *Costus afer* flowers are rich in anthocyanins with total anthocyanin content: 268.58 µg/g cyanidin equivalent and are thus a potential source of pH indicator. In the application of this naturally abundant, cheap and environmentally friendly indicator in acid/base titrations in contrast to standard synthetic indicators, a negligible difference in equivalence points was observed. It was also noted that anthocyanins gave sharp color changes at the respective end points in line with the conventional synthetic indicators. Thus, the purified anthocyanin extract of this plant could be substituted for synthetic indicators in all types of acid-base titrations.

Conflict of Interest Statement

The authors declare that there is no conflict of interests.

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References

1. M. Jordheim; N. H. Giske and O. M. Anderson (2007**).** Anthocyanins in *Caprifoliaceae**.** Biochemical Systematics and Ecology***.*** 35: 153-159.

2. V. Gavrilova, M. Kajdzanoska, V. Gjamovski and M. Stefova (2011). Separation and quantification of phenolic compounds in Blueberries and red and black currants by HPLC-DAD-ESI-MS^n. *J. Agricultural and food chemistry.* 59**:** (8): 4009-4018

3. J. Lee and R. E. Wrolstad, (2004). Extraction of Anthocyanins from blueberry processing waste. *J. of food chemistry and Toxicology*. 69: 71-77.

4. A. Castaneda-Ovanda, M.D.L. Pacheco-Hernandez, M.E. Paez-Hernandez, J.A. Rodriguez and C.A. Galan-Vidal (2009). Chemical studies of anthocyanins: A review*. Food Chemistry.* 113: 859-871.

5. K. R. Christian; M. G. Nair and J. C. Jackson (2006). Antioxidant and cyclooxygenate inhibitory activity of sorrel *(hibiscus sabdariffa) j. of food composition and analysis.* 19: 778-783.

6. C. Welch, Q. Wu, and J. Simon (2008). Recent advances in anthocyanin analysis and characterization*. Current Analytical Chemistry.* 4(2)**:** 75–101.

7. D. A. Abrugri; O. B. Apea, and G. Pritchett (2012). Investigation of a simple and cheap source of a natural indicator for acid –base titration: Effects of system conditions on natural indicators. *Green and Sustainable Chem.* 2:117-122. DOI: http://dx.doi.org/10.4236/gsc.2012.23017

8. E. Pojer, F. Mattivi, D. Johnson and C. S. Stockley (2013). The case for Anthocyanin consumption to promote Human Health: A. Review. *Comprehensive Rev. Food Sci. and Food Safety.* 12: 483-508. DOI:10.1111/1541-4337.12024.

9. R. Byamukama, E. N. Waninda, D. Mukama and J. Namukobe (2016). *Hippeastrum hybridus* anthocyanins as indicators of endpoint in acid-base titrations. *Int. J. Biol. Chem. Sci.* 10 (6): 2716-2727.

10.V. Hong and R. E. Wrolstand (1990b). Use of HPLC Seperation / photodiode array detection for characterization of anthocyanins. *J. of Agriculture & food chemistry*. 38:708-715.

11. H. O. Edeoga and B. E. Okoli (2000). Chromosome numbers of *Costus lucanusianus* (Costaceae) in Nigeria. *Folia Geobotanica.* 35 315– 318.

12. A. N. Ezejiofor, C. N. Orish and E. O. Orish *(2013**).*** Effect of aqueous leaves extract of *Costus afer* Ker Gawl (Zingiberaceae) on the liver and kidney of male albino Wistar rat. *Ancient Science of Life J.* 33(1) 4–9.

13. T. Odugbemi (2008). Medicinal Plants from Nigeria, 1st edition, edited by (University of Lagos Press, Nigeria). ISBN-10 9784871297

14. Protabase-Plant Resources of Tropical Africa (2016). *Costus afer* [Online]. Available:http://www.prota.org [Accessed 12 July 2016].

15. G. Aweke (2007). Costus afer Ker Gawl. [Online] PROTA (Plant Resources of Tropical Africa / Resources Végétales De l'Afrique Tropicale), Wageningen, Netherlands. Available:http://www.prota4u.org/search.asp [Accessed 12 July 2017].

16. J. Chandrasekhar, M. C. Madhusudhan, and K. S. M. Raghavarao (2012). Extraction ofanthocyanins from red cabbage and purification using adsorption. *Food and Bioproducts Processing*, 90: 615-623.

17. F.S. Hosseinian, L. Wende and T. Beta (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry.* 109 (4):916-924

18. K.S. Pathade, S.B. Patil, M.S. Kondawar, N.S. Naikwade and C.S. Magdum, (2009). *International J. of Chem Tech Research*, **1(3)**: 549-551.

19.D. R. Kalpana, R. S. Vasantha, N.V. Rajesh, and A. Panneerselvan (2014). *Int. J. of pharmaceutical sciences review and research.,* 27(2): 408-412

20. E.N. Bridgers, M.S. Chinn, and V.D. Truong (2010). Extraction of anthocyanins from industrial purple-fleshed sweet potatoes and enzymatic hydrolysis of residues for fermentable sugars. *Industrial Crops and Products.* 32: 613-620.