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PHYTOCHEMICAL SCREENING AND ANALYTICAL ASSESSMENT OF ACID-BASE INDICATOR PROPERTIES OF RED AND WHITE KOLA NUTS EXTRACTS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author AEA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EJU and PEA managed the analyses of the study. Author AEO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In Nigeria, the most commonly used indicator for acid-base titration is synthetic indicator, which is non biodegradable when exposed to the environment. The need to develop acid-base indicators from natural sources to serve as an effective alternative for synthetic indicators is the demand for environment green chemistry. This research work is aimed at the extraction, phytochemical screening, UV/visible spectroscopic analysis of different extracts of red and white kola nuts and application of the extracts as eco-friendly, cost effective and sustainable indicators for acid- base titrations. Extraction was done for 24 hours using acetone, ethanol, cold and hot water separately. The results revealed that all the extracts contained varying amount of phytochemicals, except anthraquinone and phlobatannins which were absents in all the extracts. UV/visible spectroscopic analysis carried out at 600 nm showed that all the extracts have varying absorbance in acidic (HCl) and basic (NaOH) mediums. The potentials of the acid-base indicator of the extracts were evaluated using the titrimetric reactions of strong acid/strong base (HCl/NaOH), strong acid/week base (HCl/NH_4OH), week acid/strong base (CH_3COOH /NaOH) and week acid/week base (CH_3COOH/ NH_4OH). The results revealed that the extracts of red kola nut give remarkable colour changes in the titration of strong acid/strong base and strong acid/ weak base. While white kola nut extracts showed remarkable colour changes for all titrations, except in the titration of weak acid with strong base using ethanol and hot water extracts. However, kola nuts extracts which are non toxic, environmentally friendly and biodegradable can serve as effective alternative to synthetic indicator for acid-base titrations.

Keywords: Acid- base titration; kola nuts extracts; indicator; phytochemicals.

1. INTRODUCTION

The high level of environmental pollution posed by synthetic acid-base indicator cannot be over emphasized. The search for natural compounds as acid-base indicators started increasing interest due to the environmental pollution and high cost effectiveness associated with synthetic indicator [1-3].

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The advantages of using natural indicator from plant extract as acid – base indicator is that it is cheap, locally available, non toxic, and hence environmentally friendly since it can easily degrades when released to the environment. Natural indicators have been used for hundreds of years, long before chemists made synthetic acid-base indicators [1]. The choice of an indicator for a particular titration depends on the characteristic of the neutralization curve. The end point in traditional titrimetry analysis is usually indicated by change in colour of indicator in the analyte solution [4]. The majority of indicators in use today are synthetic. A number of commonly used indicators in the laboratories are methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, bromophenol blue, thymol blue etc. Many flowers such as blue, purple or red in colour contain a class of organic pigment called anthocyanins that can change colour with changes in pH [5,6]. Reports have been made by several authors on the effectiveness of the extract from natural sources in acid-base titrations. Some of these natural plants are *Dianthus plumarius* and *Antirrhium majus* [7], *Ipomoea biloba* [8]. *Jacaranda acutifolia* flower [2], pride of barbados [9], guinea corn leaves [10], *Basella alba* and *Hibiscus sabdariffa* [11]. Odiongeyi et al. [12] reported the use of methanol and acetone extracts of *Dissotis rotundifolia, Centrosema pubescens* and *Allium cepa* as acid-base indicator. Onwuachu et al. [3] reported the workability of ethanol extract of Hibiscus flower, mango seed, ginger stem and kola nuts as natural indicator. Research work on the utilization of different extracts of red and white kola nuts as acid base indicator has not been reported.

The kola nuts are a caffeine-containing nut of evergreen trees of the genus *Cola*. *Cola nitida* and *Cola acuminata* are among the various species of cola [13]. Kola nut has a bitter flavor. Voelcker [14] reported that the colour of the cotyledon of *C. nitida,* may be red, white or intermediate shades of pink. The red is the most common, white are rare in Nigeria. The colour of the nuts may vary from follicle to follicle, from tree to tree, and, on the same tree, from year to year. Trees grown from white nuts produce only white nuts when the flowers were self–fertilized and produce red, pink or white when the flowers are fertilized by pollen from trees grown from red nuts. Cola nut is chewed in many West African countries by elderly people in both private and social settings; it is also used ceremonially, presented to chiefs or guests. It contains about 2% caffeine, hence, it is considered useful for aiding digestion when ground and mixed with honey, and are used for coughs [15]. Cola nut is chewed by many people as a stimulant, it is also used in the manufacturing of dye [13]. The proximate and phytochemical composition has of two varieties of kola nut have been reported by Dawole et al. [16]. The phytochemical results revealed that both species contain varying percentage of alkaloids, phenol, tannin, flavoniod and saponins. However, this present work is aimed at evaluating the phytochemical contents and acid-base indicator potentials of ethanol, acetone, cold and hot water extracts of white and red varieties of kola nuts.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh samples of white and red kola nut were bought at Itam main market, Uyo in Akwa Ibom State, Nigeria. The kola nut seeds were cut into chips and extraction was done using different solvents.

2.2 Extraction Procedures

10% kola nuts extracts were prepared by soaking 10 g of each kola nut chips separately in 100 ml of acetone, ethanol, cold water, and hot water. After 24 hours, the extracts were filtered using whatman filter paper and the filtrates obtained were used for titration to evaluate their acid-base indicator properties. The colour of each filtrate in acidic (HCl) and basic (NaOH) medium were determined and the absorbance of the filtrate in each medium was also determined using UV/visible spectrophotometer at wavelength of 600 nm [17].

2.3 Phytochemical Screening

The qualitative phytochemical composition of red and white kola nuts extracts were determined using standard methods reported by Shabi et al. [18] and Sawant and Godgnate [19]. The results obtained are presented in Table 1.

2.4 Titrimetric Analysis

25 ml of 0.5 M solution of the bases (sodium hydroxide and ammonium hydroxide) were measured separately into a conical flask and about 0.5 -1.0 ml of the kola nut extract was added as indicator using a 1.0 ml dropper. A 50 ml burette was filled with the 0.5 M acid solution and titrated against the base until a sharp colour change was observed signifying the end of the titration reaction. Each titration sequence was done using either the standard indicator (methyl orange and phenolphthalein) or the plant extract. Each titration was carried out in triplicate and the average values of the end points for each set of titration and its standard deviation was calculated and recorded. The titration was carried out at room temperature for the strong

acid against strong base (HCl/NaOH), strong acid against weak base (HCl/NH_4OH), weak acid against strong base (CH_3COOH/NaOH) and weak acid against weak base (CH_3COOH/NH_4OH). The results are presented in Tables 3 – 5.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of the phytochemical screening of kola nuts extracts revealed that the extracts contain alkaloids, saponins, tannin, flavoniod, glycoside, steroids, coumarins, phenols, quinone, sugars, terpenes and volatile oil in varying quantities. Anthraquinone and phlobatannins were absent in all the extracts, cardiac glycoside was absent in acetone extract of both red and white kolanut, while volatile oil was absent in ethanol extracts of the two samples. Phytochemicals played an important role in acid-base titrations using plant extract as indicator because its chemical structures and colours changes with change in pH of the medium. The colours of some phytochemicals in different medium as reported by Patil et al. [20] are presented in Table 2.

The colour of the phytochemicals depends on the number and position of hydroxyl and methoxyl group. When these are fixed, the colour then depends upon the pH and solvent [21].

Table 1. Phytochemicals present in different extracts of kula nut

Phytochemicals	Red Kola nuts				White Kola nuts			
	Hot water extract	Cold water extract	Acetone extract	Ethanol extract	Hot water extract	Cold water extract	Acetone extract	Ethanol extract
1. Alkaloids								
a) Mayers test	+++	++	++	+++	+++	++	++	+++
b) Hagers test	+++	++	++	+++	+++	++	++	++
2. Saponins (froth test)	+++	++	+	+++	++	++	+	++
3. Tannin ($FeCl_3$ test)	+++	++	+	+++	++	++	+	++
4. Flavonoid								
a) Alkaline test	+++	++	+	+++	+++	++	+	++
b) $FeCl_3$ test	+++	++	+	++	++	+	+	++
5. Glycosides	+++	+++	++	++	++	++	++	++
6. Steroid	++	+	++	+	+	+	++	+
7. Anthraquinone	-	-	-	-	-	-	-	-
8. Coumarins	+	+	+	+	+	+	+	+
9. Phenols	+++	++	+	+++	++	++	+	++
10. Quinone	+++	+++	+++	+++	++	++	++	++
11. Sugar (Fehling's)	++	++	++	++	++	++	++	++
12. Cardiac Glycoside	+	+	-	+	+	+	+	+
13. Volatile oil	++	+	++	-	++	+	++	-
14. Diterpenes	+++	++	+	+	+++	++	-	-
15. Triterpenes	+	+	+	+	+	+	+	+
16. Terpenoids	+	+	++	+	+	+	++	+
17. Phlobatanins	-	-	-	-	-	-	-	-

Table 2. Colours of phytochemical in acidic and basic medium

Phytochemicals	Colour with aq. NaOH	Colour with Conc. H_2SO_4	Colour with Mg-HCl
Anthocynins	Blue violet	Yellow orange	Red (fades to pink)
Flavones	Yellow	Yellow to orange	Yellow to red
Flavonols	Yellow to orange	Yellow to orange	Red to magenta
Flavonones	orange (cold)	Crimson	Red, magenta,
	Red to purple (hot)	Orange	violet, blue
Iso flavones	Yellow	Yellow	Yellow
Leucoantho cyanins	Yellow	crimson	Pink

Source: Patil et al. [20]

3.2 Titrimetric Analysis

The results of the titrimetric analysis of different extracts of kola nuts are presented in Tables 3 – 5, while the absorbance of different extracts of kola nut is presented in Table 6.

As presented in Table 3, all the extracts of red kola nuts give a sharp colour change in the titration of strong acid/weak base and strong acid/strong base, and there was no colour change when these extracts were used as indicator for the titration of weak acid/weak base and weak acid /strong base. This may be due to the percentage of the phytochemicals especially anthocyanin present in these extracts, or the strength of the acid/base to change the structural functional group (chromophore) phytochemicals present in these extract.

On the other hand there was a remarkable colour change in all titrations when acetone and hot water extracts of white kola nuts were used as indicator. Ethanol and cold water extracts of white kola nuts could not give a remarkable colour change in the titration of weak acid against strong base, hence their extracts are not recommended for this titration.

The reason for the observed colour changes in different mediums are due to the presence of plant pigments primarily anthocyanin [22]. Anthocyanin is responsible for the attractive colours of plants [1]. Anthocyanins are generally degraded at higher pH (basic medium) and due to this; they are more stable in acidic medium.

On the other hand, methyl orange and phenolphthalein give a sharp colour change in all the titrations except in the titration of weak acid and weak base. Comparing the end point of the kola nuts extracts with that of the synthetic indicators, it can be seen that in the titration of strong acid and weak base, the end points for the titration with synthetic indicators were higher than that of the natural indicators. The end point values for titration with synthetic indicator and natural plant indicator compares favourably for the titration of strong acid and strong base. However, in all the titrations, cold water extract gives higher value of the end points, meaning that the titration with this extract will consume much acid before the end point is reached. This may be because of the less amount of phytochemical present in the extract.

3.3 Titrimetric Equations

Equations 1 - 4 were considered to be the reactions which occur during the titration process, in the presence of either kola nuts extracts or synthetic indicator.

$$HCl + NaOH \longrightarrow NaCl + H_2O \quad (1)$$

$$HCl + NH_4OH \longrightarrow NH_4Cl + H_2O \quad (2)$$

$$CH_3COOH + NaOH \longrightarrow CH_3COONa + H_2O \quad (3)$$

$$CH_3COOH + NH_4OH \longrightarrow CH_3COONH_4 + H_2O \quad (4)$$

Table 3. Titrimetric analysis using red kola nuts extracts as indicators

Acid/base	End point (ethanol extract)	End point (acetone extract)	End point (cold water extract)	End point (hot waterextract)
HCl/NH_4OH	16.00 ± 0.03	20.06 ± 0.02	24.05± 0.13	21.02 ± 0.05
HCl/NaOH	27.05± 0.01	27.05 ± 0.01	29.09 ± 0.08	28.09 ± 0.03
CH_3COOH/NH_4OH	No colour charge	No colour charge	No colour charge	No colour charge
$CH_3COOH/NaOH$	No colour change	No colour change	No colour change	No colour change

Table 4. Titrimetric analysis using white kola nuts extracts as indicators

Acid/base	End point (ethanol extract)	End point (acetone extract)	End point (cold water extract)	End point (hot waterextract)
HCl/NH_4OH	24.09 ± 0.02	25.05 ± 0.12	32.07 ± 0.03	25.00 ± 0.02
HCl/NaOH	29.00 ± 0.03	28.09 ± 0.04	29.03 ± 0.01	24.02 ± 0.03
CH_3COOH/NH_4OH	28.00 ± 0.02	27.05 ± 0.02	38.01 ± 0.13	25.00 ± 0.12
$CH_3COOH/NaOH$	No colour change	29.05 ± 0.03	No colour change	32.00 ± 0.00

Table 5. Titrimetric analysis using phenolphthalein and methyl orange as indicators

Acid/base	End point (Methyl orange)	End point (Phenolphthalein)
HCl/NH_4OH	37.02 ± 0.01	34.06 ± 0.00
HCl/NaOH	28.00 ± 0.03	28.03 ± 0.03
CH_3COOH/NH_4OH	No colour charge	No colour charge
$CH_3COOH/NaOH$	32.40 ± 0.02	30.32 ± 0.01

Table 6. Colour of the extracts in acidic and basic medium

Extracts	Colour of the extracts	Colour in acid	Colour in base
White kola nut			
Cold water extract	Orange	Light Yellow	Brown
Hot water extract	Orange	Brown	Deep brown
Acetone extract	Yellow	Light yellow	Orange
Ethanol extract	Yellow	Light Yellow	Red
Red kola nut			
Cold water extract	Brown	Yellow	Orange
Hot water extract	Brown	Yellow	Brown
Acetone extract	Yellow	Light Pink	Brown
Ethanol extract	Red	Pink	Green

Table 7. Absorbance of kola nuts extracts determined at λ_{max} of 600 nm

		Absorbance	
Cold water extracts	**Extracts**	**Acidic medium**	**Basic medium**
Red kola Nut	0.401	0.454	0.390
White kola Nut	0.208	0.214	0.097
Hot water extracts			
Red kola Nut	0.648	0.682	0.230
White kola Nut	0.722	0.736	0.388
Ethanol extracts			
Red kola Nut	0.237	0.176	2.510
White kola Nut	0.056	0.104	1.200
Acetone extracts			
Red kola Nut	0.033	0.019	6.690
White kola Nut	0.001	1.729	2.780

Plant extract showed different colours in different medium, this is because of the change in the chemical structure and functional groups (Chromophore) of its phytochemicals in different medium. Some of the kola nuts extracts give remarkable colour changes while others showed little colour changes.

3.4 Absorbance of Kola Nuts Extracts

The absorbance of the cold and hot water extracts of both red and white kola nuts increases in acidic medium and decreases in basic medium, while the absorbance of ethanol and acetone extracts of both sample decreases in acidic medium and increases in basic medium. This may be due to the change in functional group in these medium.

4. CONCLUSION

This study revealed that kola nuts extracts contained varying quantities of phytochemicals. The extracts of both white and red kola nuts function effectively as acid-base indicator. This is because they give sharp and intense colour changes in each titration medium. These colour changes are enhanced by the present of phytochemicals especially anthocyanin in the extracts. All the extracts of red kola nuts in this study are recommended for the titration of strong acid with strong base and strong acid with weak base, also extracts of white kola nuts are recommended for all titrations except ethanol and cold water extracts which showed no remarkable colour change in the titration of weak acid and strong base. However, kola nuts

extracts can serve as an inexpensive, and eco friendly alternative to synthetic indicator for acid-base titrations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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