PREPARATION AND USE OF *ACANTHUS SENNII CHIOVENDA* FLOWER EXTRACT AS A GREEN SUBSTITUTE FOR SYNTHETIC ACID-BASE INDICATORS

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ABSTRACT

Due to environmental friendliness, availability and cost, the search for natural compounds as acid-base indicators has continued. Certain highly coloured pigments obtained from plants produce changes in colour with varying pH. This study has been conducted to investigate the indicator property of ethanolic and acidified ethanol extracts of *Acanthus sennii chiovenda* flower and compare the results with that of already existing synthetic indicators (phenolphthalein, bromothymol blue and methyl red). Flower extracts of the plant were characterized with ultraviolet-visible spectrophotometer and phytochemical screening test. The colour change with respect to varying pH was also examined by adding four drops of flower extracts of this plant to definite volume of solutions at various pH. The flower extracts of the plant were applied in titrimetric analysis for four types of acid-base titrations. Phytochemical analysis and spectral studies confirmed presence of anthocyanins and flavonoids in ethanol and acidified ethanol *Acanthus sennii chiovenda* flower extracts responsible for accurate and sharp color change at the end point. The results indicated that flower extracts of this plant can be used as acid-base indicators. Statistical tests and comparisons are made with performances of the synthetic indicators. *[African Journal of Chemical Education—AJCE 9(2), July 2019]*

INTRODUCTION

Acanthus is a genus of flowering plants that belong to the family *Acanthaceae* [1] which is a large family of dicotyledonous flowering plants including about 4300 species and 346 genera all over the world. Most are tropical shrubs, herbs and twining vines while some are epiphytes. The family is known for a wide variety of its tropical and subtropical habitats. Some species are found in temperate regions. The four main regions of its distribution are Malaysia and Indonesia, Brazil, Africa and Central America while also found in Asia [2]. There is an increasing interest in indigenous medicinal plants for correct identification, taxonomy and medicinal applications. Chemically, the acanthaceae family contained some important secondary metabolites such as glycosides, flavonoids, alkaloids, triterpenoids, fatty acid methyl esters and fatty acids. These compounds play an important role in many biological reactions and work against many lethal diseases. Leaves, roots and other parts are used as anti-pyretic, anti-inflammatory, anti-spasmodic, antiseptic, antifungal, cytotoxic, antioxidant, antiviral, insecticidal, hepatoprotective, immunomodulatory, anti-platelet aggregation and anti-diabetics. Some other important plants belong to this family are used to treat skin diseases, cough, eye infections, wounds, pneumonia, anti-diarrhea, edema etc [1, 2].

Acanthus sennii chiovenda with a generic name ‘Kosheshile’ in Amharic is endemic to Ethiopia. Besides their medicinal uses, these plant species are hypothesized to be good substitutes for synthetic acid-base indicators which are disadvantageous due to their toxicity and expensiveness, because the *Acanthus senni chiovenda* species are rich in polyphenols like flavonoids, phenols, tannins and terpenoids, [1] that are sensitive to changes in pH [3].

Many studies have been carried out on the use of plant extracts as green substitutes to synthetic indicators in acid-base titrations. Natural indicators have been extracted from Rose (*Rosa*

setigera), Allamanda (*Allamanda cathartica*), and Hibiscus (*Hibiscus rosa-sinensis*) flowers by Okoduwa *et al*. [4], Golden beet root by Thote *et al.* [3], *Eichhornia crassipes* root by Nhapi [5], *Opuntia ficus indica* (*L.*) fruit by Suva [6], Petal sap of *Delonix regia* by Jain *et al*. [7], *Argyreia cuneata* flower by Pimpodkar *et al.* [8], flowers of *Ipomoea biloba* by Abbas [9], *Ixora coccinea* flower by Deshpande *et al*. [10], *Euphorbia milii* flower by Trivedi *et al.* [11], *Aspilia Africana* (oramejula) flowers by Eze and Ogbuefi [12], Dahalia flower sap by Gupta *et al.* [13].

Extraction of the active ingredients using appropriate solvents, filtration and use of the crude extract as an indicator [7] are the basic methodological steps that were employed. Deshpande *et al*. [10], Eze and Ogbuefi [12] and Patrakar *et al.* [14] dried their samples away from the direct sunlight as they tried to prevent photo-degradation and oxidative loss of the dye, but Gupta *et al.* [15] dried the flower samples on direct sunlight and they both found positive results.

Of all studies that were carried out, very few isolated the active components that give the acid-base indicator properties. The compounds that were being isolated from the crude samples were anthocyanins. A colour change of natural indicators at different pH values has been attributed to the presence of anthocyanins and flavonoids which are pH sensitive [13].

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and belong to the family of compounds known as flavonoids which are part of an even larger group of compounds known as polyphenols [16]. Chemically, they are polyhydroxy and polymethoxy glycosides of anthocyanidin, derived from 2-phenylbenzopyrylium, also named flavylium cation [17]. The various anthocyanins were shown to possess the same carbon skeleton and differed only in the nature of substituent groups [18] shown in Figure 1 where R basically means that it can be occupied by almost any organic group like a methoxyl group, sugar, and the number of R that are occupied by specific substitutions would determine the colour of the anthocyanin [19]. The

stability of anthocyanin is dependent on pH, light, temperature, and its structure. Most of the anthocyanin pigments have a high stability in acidic conditions compared with bases, and degradation occurs at higher pH [20]. The anthocyanin belongs to the group of natural dyes responsible for several colours in the red – blue range [19]. Almost any plant that has blue, violet, purple or red flowered colours contains organic pigments, anthocyanins that changes colour with change in pH [5]. Anthocyanins are intensely red or orange under acidic conditions (below pH 2), but at higher pH they are colourless and in alkaline conditions change their colour into bluish. These pigments can be found in fruit, stems, flowers and leaves, and their levels may fluctuate [21].

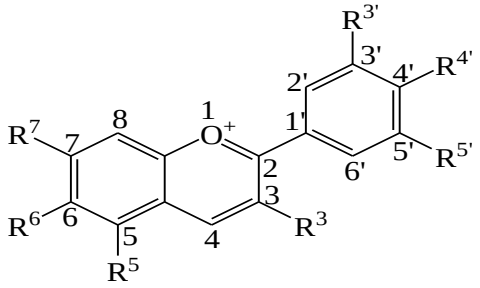

Figure 1: Structure of anthocyanin [19]

Structural transformation of anthocyanins in aqueous solutions

The color of a solution containing anthocyanins is dependent primarily on the structure of anthocyanin present. Non-acylated and mono-acylated anthocyanins behave like pH indicators, since they are red at a low pH, bluish at an intermediate pH and colorless at high pH [22]. Anthocyanins exist in solution as various structural forms in equilibrium through hydration, proton transfer, and tautomerization reactions, and, depending on the particular structure, pH and temperature the relative amounts of each equilibrium form vary. The color of an anthocyanin solution is determined by the proportions of the different anthocyanin forms, namely, quinonoidal

base (A), flavylium cation (AH^+), pseudobase or carbinol (B), and *E*- and *Z*-chalcones (C_E and C_Z) [22, 23]. Of the four structures, the flavylium cation (AH^+) and the quinonoidal base (A) are the only coloured forms. At an acidic pH, the flavylium cation (AH^+) is red, the quinonoidal base or anhydrobase (A) is purple/violet, whereas the pseudobase or carbinol or hemiacetal (B) is colourless and the chalcone (C_E and C_Z) is pale yellow.

Therefore, the degree of coloring of a solution at a given pH is dependent upon the relative amounts of the four species present. At a low pH (approximately 0 to 1), the flavylium cation (AH^+) dominates with very little pseudobase (B) present to give a red solution. At pHs above 2.0 the flavylium cation (AH^+) loses protons (H^+) and as a result, blue/purple quinonoidal bases are formed. The AH^+ may also hydrate to colorless pseudobases or carbinols (B). These, in turn, equilibrate to the open chalcone form (C) [22]. As the pH value increases, kinetic and thermodynamic competition occurs between the flavylium cation hydration reaction and the proton transfer reaction, associated with the acidified groups of aglycon hydroxyls [17]. They further demonstrated that no hydration of the quinonoidal bases occurs and confirmed the existence of an open chalcone structure [22].

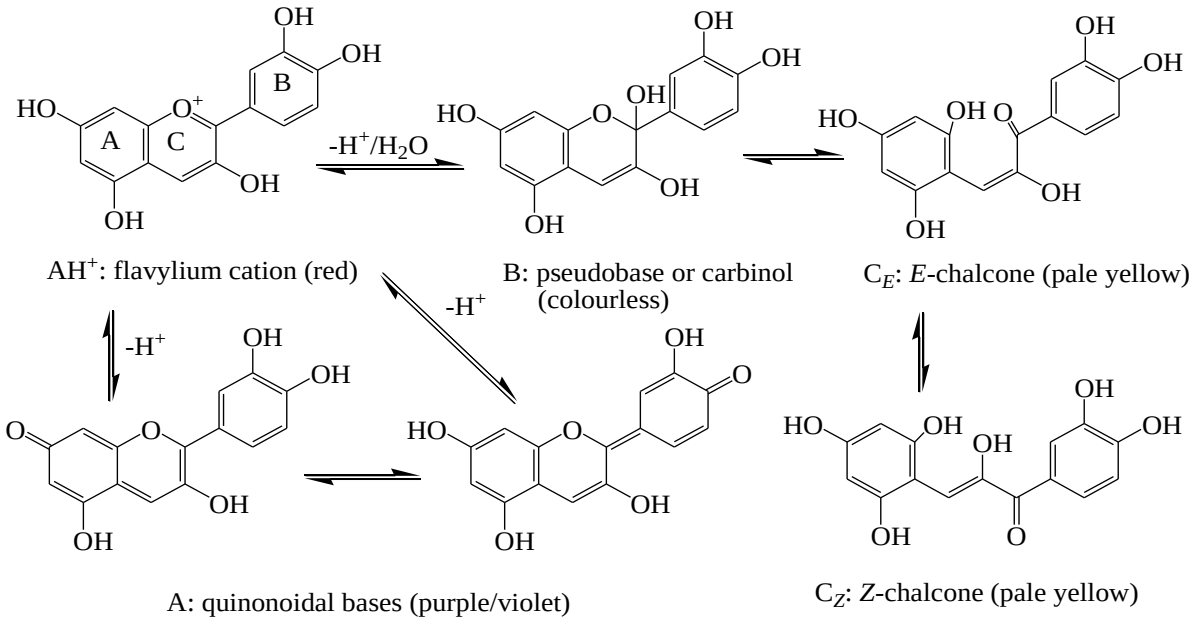

Figure 2: Anthocyanin forms in equilibrium [22]

The mechanisms for the interconversion between the quinonoidal base (A), the flavylium cation (AH^+), the pseudobase or carbinol (B), and the chalcone (C) occurred as is shown in Figure 2 [22]. Plant species containing anthocyanins can change color in solution by undergoing these transformations due to change in the acidity or basicity of the solution [5].

This research work aimed at preparing a natural acid-base indicator obtained from *Acanthus sennii chiovenda* flower extract. The study mainly answered the following questions:

1. What are the characteristics active components that give the acid-base indicator properties of *Acanthus sennii chiovenda* flower extracts?
2. What are the resulting colors of *Acanthus sennii chiovenda* flower extracts in the pH range of 1 to 14?
3. If there are any significant differences between the results of the extracted pH indicator with phenolphthalein, bromothymol blue and methyl red pH indicator in determining the end point in different types of acid-base titration?

MATERIALS AND METHODS

Reagents, apparatus and equipment

The raw materials used in the study are the powdered *Acanthus sennii chiovenda* flower. The reagents used for this work were of analytical grade and used without further purification. These include hydrochloric acid (HCl, 35-36%, UNI-CHEM Chemical Reagents, India), sodium hydroxide (NaOH, 98%, BLULUXR Analytical Reagents), glacial acetic acid (CH_3COOH, 99.5%, SCR-China), ammonia (NH_3, 30%, Merch, Germany), borax ($Na_2B_4O_7.10H_2O$, 99.0 – 103%, SAMIR TECH-CHEM PVT, LTD), iron (III) chloride ($FeCl_3$, 99.0%, Avi Chem Industries, India), magnesium ribbon (Mg, BUCK SCIENTIFIC PURO-GRAPHIC), zinc dust (Zn, BUCK SCIENTIFIC PURO-GRAPHIC), ethanol (CH_3CH_2OH, 97%, Fine Chemical General Trading, Ethiopia) and distilled water. The commercial indicators were phenolphthalein ($C_{20}H_{14}O_4$, SAMIR TECH-CHEM PVT, LTD), methyl red ($C_{15}H_{15}N_3O_2$, SAMIR TECH-CHEM PVT, LTD), bromothymol blue ($C_{27}H_{28}Br_2O_5S$, SAMIR TECH-CHEM PVT, LTD). The instruments that used in the study are pH meter (Elmetron CPI-501, Poland), double beam UV-Vis spectrophotometer (6705, Jenway), electronic analytical balance (AA200DS, Deriver Instrument Company, Germany) and magnetic stirrer (MS300, Germany). The following apparatus and equipment such as Erlenmeyer flasks, volumetric flasks, burette with volume size of 50 mL, micropipette (1-10 µL, 10-100 µL and 100-1000 µL), test tubes, beakers, graduated measuring cylinders with volume size of 10 mL and 25 mL, and Whatman filter paper No. 41 were used to carried out the experiment. All required reagents and volumetric solutions were prepared as per standard.

Collection and preparation of Acanthus sennii chiovenda sample

One kilogram of fresh *Acanthus sennii chiovenda* flower were collected from around Debre Markos, Ethiopia, at the beginning of December, 2018, as it is the blooming season of the plant.

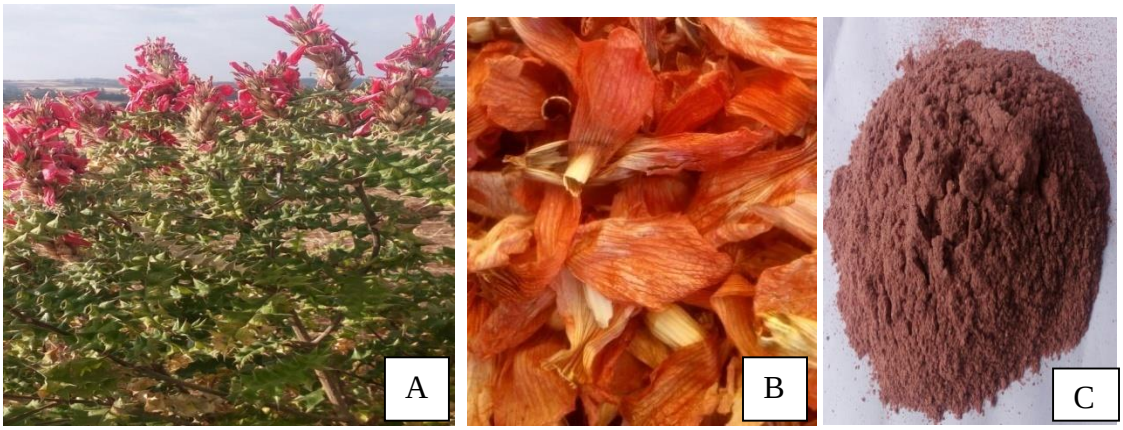

Figure 3: Acanthus sennii chiovenda flower (A), dried and powdered samples (B & C)

The fresh flower petals of *Acanthus sennii chiovenda* were separated from the whole flower by hand and washed with distilled water to remove dirt. The fresh *Acanthus sennii chiovenda* flower petals were air dried for three weeks without exposure to direct sunlight to minimize oxidative loss before pounding in to fine powder at room temperature. The dried flower petals were ground into fine powder with coffee grinding machine and the resulting powders were sieved and stored in a polyethylene bag before use [5, 12, 13]. Figure 3 above shows picture of the *Acanthus sennii chiovenda* flower, dried and powdered samples of the plant.

Extraction of coloring matter/ pH indicator

30 grams of dried powder of *Acanthus sennii chiovenda* flower was taken and mixed with 150 milliliters 97% ethanol in 250 milliliter Erlenmeyer flask and then the mixture was stirred by using a magnetic stirrer for 2 hours to disperse the powder completely. The mixture was kept at room temperature for 24 hours and then triturated in mortal and pestle and the resulting solution were filtered by using Whatman filter paper to remove the remaining plant matter. Similar procedures were applied by taking the same amount of powder and solvent for all the rest extracting solutions. The resulted 97% ethanol and 0.1% HCl in ethanol extract were further used

as natural pH indicator for acidimetry and alkalimetry. The extract was preserved in light closed container and stored away from direct sunlight to prevent photolysis and decomposition [5, 12, 13]. Figure 4 below shows colour of ethanol and acidified ethanol extracts of the *Acanthus sennii chiovenda* flower.

Figure 4: Colors of extracted solutions of Acanthus sennii chiovenda flower in ethanol (A) and acidified ethanol (B)

UV-Vis spectroscopy analysis

The UV-Vis absorption spectra of ethanol and acidified ethanol extracts of *Acanthus sennii chiovenda* flower were determined using UV-Vis spectrophotometer (6705, Jenway) in the wavelength range of 200 nm to 800 nm. The extracted solutions were diluted 10 times with the same solvent and 5 mL of the extract was measured and placed in the quartz cuvette. The wavelength of maximum absorption of each extract was measured and the compounds present in the extracts were interpreted.

Phytochemical screening of the extracts

The extracts were phytochemically screened in order to determine the presence of flavonoids and anthocyanins in the flowers of the *Acanthus sennii chiovenda* plant extracts.

Alkaline reagent test, Shinoda's test, hydrochloric acid test, Zinc test [24] and Ferric chloride test [25] were used for the presence of flavonoids. On the other hand, the presence of anthocyanins were tested by adding 2 mL of the plant extract in 2 mL of 2 M HCl. The appearance of a pink-red colour that turns purplish blue after addition of ammonia indicates the presence of anthocyanins [26].

Titration using Acanthus sennii chiovenda flower extract, methyl red, bromothymol blue and phenolphthalein indicators

Obtaining equivalence point in different types of titration of the *Acanthus sennii chiovenda* flower extracts in comparison with synthetic indicators was tested. Four titrations were performed i.e. strong acid versus strong base, strong acid versus weak base, weak acid versus strong base and weak acid versus weak base. The titrations were conducted in the order HCl and NaOH; HCl and NH_4OH; CH_3COOH and NaOH and CH_3COOH and NH_4OH. A volume of 10 mL of 1 M HCl was placed in an Erlenmeyer flask and four drops of *Acanthus sennii chiovenda* flower extract indicator were added. 1M NaOH was placed in a burette. The titrant (NaOH) was added to titrate (HCl) until a colour change was observed. Titrations were conducted in three replicate analyses. The procedure was repeated for all titrations i.e. HCl versus NH_4OH, CH_3COOH versus NaOH and CH_3COOH versus NH_4OH.

Equimolar titrations were performed using 10 mL of titrant with four drops of indicator. A set of three experiments each for all the types of acid base titrations was carried out by using 1M solution of acid and base. The mean and standard deviation for each type of acid base titrations were calculated and recorded as mean ± standard deviation.

RESULTS AND DISCUSSION

UV-Vis spectroscopy analysis of the Acanthus sennii chiovenda flower extracts

Ultraviolet-visible spectroscopy was carried out on extracts of *Acanthus sennii chiovenda* flower and the absorbance was plotted against wavelength. The wavelength regions of the spectral absorbance peaks could also be used to identify the pigments present in the plants. The UV-Vis absorption spectra for the dye solution extracted using different solvents are shown in Figure 5 below. As shown in Figure 5, the ethanolic extracted solutions have shown absorption peaks at 226 nm, 268 nm, 330 nm and 523 nm. The absorption peak of extracted dye is almost closely related to flavonoids and anthocyanin as reported earlier [27, 28, 29]. The UV-Vis absorption spectra of flavonoids consist of two distinctive bands in a broad range of 240 – 550 nm [27]. Anthocyanin can be readily distinguished from other flavonoid classes by performing colour test and UV-Vis analysis (λ_{max}). Anthocyanins generally have two absorption maxima, one in the UV-region of spectrum 260 – 280 nm (band II) and the second in the visible region of spectrum 490 – 550nm (band I) [30]. Almost all flavonoid classes give the same absorption at the region of band II, thus, anthocyanin can be distinguished with the other classes by observing the absorption region wavelength of band I [28].

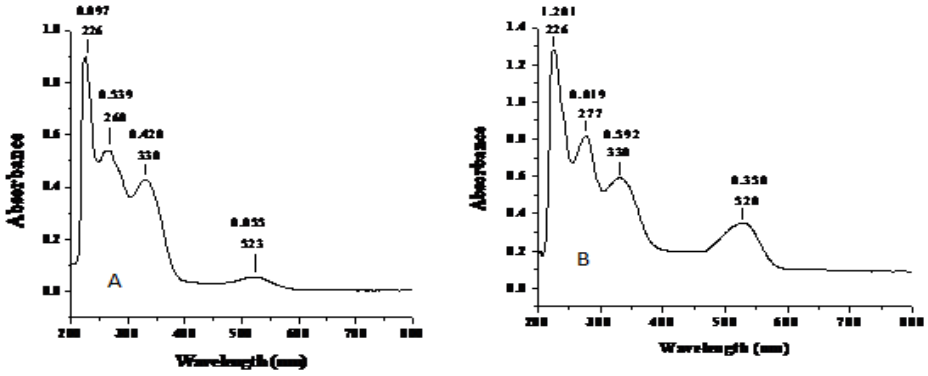

Figure 5: UV-Vis spectra of ethanolic (A) and acidified ethanol (B) extracts of *Acanthus senni chiovenda* flower

The acidified ethanol extracts of *Acanthus sennii chiovenda* flower showed UV- Vis absorption peaks at 226 nm, 277 nm, 330 nm and 528 nm as shown in Figure 5 above. These absorption peaks are closely related to the absorption peaks of flavonoid and anthocyanin [27, 30] which indicated flavonoids and anthocyanins are the major components of the observed pigments. The difference in the absorption peaks of *Acanthus sennii chiovenda* flower extracts at 523 nm and 528 nm respectively for the ethanol and acidified ethanol extracts in Figure 5 may be due to solvent polarity differences in the pure and acidified ethanol. As shown from Figure 5 above, solutions extracted by acidified ethanol have relatively higher absorbance than those by ethanol, due to an increase in the extraction of anthocyanin using an optimal acidification of extracting solvents which leads to a suitable protonation reaction. This indicates anthocyanin pigments are highly soluble in acidified ethanol for these studies. A similar finding was reported [31].

Phytochemical screening test of the Acanthus sennii chiovenda flower extracts

The preliminary phytochemical investigation and qualitative chemical tests of the ethanol and acidified ethanol extracts of *Acanthus sennii chiovenda* flower were performed using alkaline reagent test, hydrochloric acid test, ferric chloride test, zinc test, and Shinoda test which confirmed the presence of flavonoids and anthocyanin as reported in Table 1.

Table 1: Results of phytochemical screening of ethanol and acidified ethanol extracts of *Acanthus sennii chiovenda* flower

Phytochemicals	Chemical test	Observation	Ethanolic extract	Acidified ethanolic Extract
Flavonoids	Alkaline reagent test	Yellow	+	+
	Ferric chloride test	Greenish-black	+	+
	Hydrochloric acid test	Red	+	+
	Shinoda test	Red	+	+
	Zinc test	Red	+	+
Anthocyanins	Hydrochloric acid test	Purplish blue	+	+

+ indicates presence of active ingridients

The results obtained in this study were the same as results obtained by Etagegnehu *et al.* [1], Sikolia and Omondi [32] and Singh *et al* [33] when they conducted phytochemical test on *Acanthus sennii chiovenda* root, *Acanthus pubescens* leaves and *Delonix regia* and *Caesalpinia pulcherrima* flower respectively. These tests, combined with UV-Vis studies are confirmation of the presence of flavonoids and anthocyanins which are pH sensitive organic consituents.

Colors of Acanthus sennii chiovenda flower extracts at pH of 1 – 14

The color change interval of *Acanthus sennii chiovenda* flower extracts were also determined by preparing solutions having pH in the range of 1 to 14 and adding four drops of *Acanthus sennii chiovenda* flower extracts to each test tube. It showed that from pH 1 to 7 ethanol and acidified ethanol *Acanthus sennii chiovenda* flower extract impart orange red color to the solution but from pH 8 to 11 it imparts colorless color in both extracts, at pH 12 it shows brown color in ethanol and acidified ethanol *Acanthus sennii chiovenda* flower extract also at pH 13 and 14, a greenish yellow color were observed in both extracts to the solution as shown in Table 2.

Table 2: Color change results after addition of ethanol and acidified ethanol *Acanthus sennii chiovenda* flower extract in a solutions of pH 1 – 14

pH	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Colour	Orange red	Orange red	Orange red	Orange red	Orange red	Orange red	Orange red	Colourless	Colourless	Colourless	Coloruless	Brown	Greenish yellow	Greenish yellow

The sensitivity of the extract to different pH may be attributed to the presence of flavonoids and anthocyanins. As described by Oswald's ionic theory of indicators [34, 35], different color change presented by the *Acanthus sennii chiovenda* flower extracts when subjected to different pH

may be due to protonation or deprotonation of the *Acanthus sennii chiovenda* flower extract indicators. The color change might be due to transformation shown in Figure 2 above due to the results of intramolecular rearrangement which changes the structure of the indicators, hence it absorbs in the different region of the spectrums [36]. The colors from literature changed from dark pink to yellow [37] whilst in this study they changed from orange red to greenish yellow in *Acanthus sennii chiovenda* flower extract. This might be attributed to different plant extracts being used in other studies and also the crude extract component matrix might be different between the plant extracts.

Acid-base titration using Acanthus sennii chiovenda flower extract, phenolphthalein, bromothymol blue and methyl red as indicators

In order to evaluate the potential for the use of the *Acanthus sennii chiovenda* flower extract as indicators in acid-base titrimetry, a number of demonstrated titrations were conducted. The end points of the titrations using four drops of the *Acanthus sennii chiovenda* flower extract are reported in Table 3 below. The end points of the acid-base titrations using commercially available indicators are also reported.

The results in the table showed that the end points obtained with the ethanol and acidified ethanol extracts of *Acanthus sennii chiovenda* flower in 1M solutions of hydrochloric acid and sodium hydroxide (i.e. strong acid versus strong base) gave similar end points 9.77 ± 0.115 and 9.83 ± 0.058 mL which were close to the end points obtained using phenolphthalein (9.80 ± 0.100 mL), bromothymol blue (9.77 ± 0.115 mL) and methyl red (9.70 ± 0.100 mL) and so the *Acanthus sennii chiovenda* flower extract can be used in place of phenolphthalein, bromothymol blue and methyl red in acid-base titrations involving a strong acid versus strong base.

A comparison of result of titration of 1M hydrochloric acid and 1M ammonium hydroxide solutions (i.e. strong acid versus weak base) using these plant extracts as indicators as presented in **Table 3** indicated that the average titre using ethanolic *Acanthus sennii chiovenda* flower extract is 11.30 ± 0.100 mL and acidified ethanol *Acanthus sennii chiovenda* flower extract is 11.27 ± 0.115 mL, while that of methyl red, bromothymol blue and phenolphthalein are 11.20 ± 0.200 mL, 11.30 ± 0.100 mL and 11.40 ± 0.173 mL respectively.

Table 3: Mean volume of base used (in mL) at end points and color change for the four titrations using *Acanthus sennii chiovenda* flower extract, phenolphthalein, bromothymol blue and methyl red as indicators

Titration	Indicators	Mean ± S.D*	Color change
HCl vs NaOH	Methyl red	9.70 ± 0.100	Red to yellow
	Bromothymol blue	9.77 ± 0.115	Yellow to blue
	Phenolphthalein	9.80 ± 0.100	Colourless to pink
	Ethanol *Acanthus sennii chiovenda* flower extract	9.77 ± 0.115	Orange red to colourless
	Acidified ethanol *Acanthus sennii chiovenda* flower extract	9.83 ± 0.058	Orange red to colourless
HCl vs NH_4OH	Methyl red	11.20 ± 0.200	Red to yellow
	Bromothymol blue	11.30 ± 0.100	Yellow to blue
	Phenolphthalein	11.40 ± 0.173	Colourless to pink
	Ethanol *Acanthus sennii chiovenda* flower extract	11.30 ± 0.100	Orange red to colourless
	Acidified ethanol *Acanthus sennii chiovenda* flower extract	11.27 ± 0.115	Orange red to colourless
CH_3COOH vs NaOH	Methyl red	9.53 ± 0.058	Red to yellow
	Bromothymol blue	9.67 ± 0.058	Yellow to blue
	Phenolphthalein	10.40 ± 0.100	Colourless to pink
	Ethanol *Acanthus sennii chiovenda* flower extract	10.37 ± 0.115	Orange red to colourless
	Acidified ethanol *Acanthus sennii chiovenda* flower extract	10.33 ± 0.058	Orange red to colourless
CH_3COOH vs NH_4OH	Methyl red	8.07 ± 0.115	Red to yellow
	Bromothymol blue	8.77 ± 0.208	Yellow to blue
	Phenolphthalein	8.83 ± 0.058	Colorless to pink
	Ethanol *Acanthus sennii chiovenda* flower extract	8.13 ± 0.153	Orange red to colorless
	Acidified ethanol *Acanthus sennii chiovenda* flower extract	8.90 ± 0.100	Orange red to colorless

** Standard Deviation*

Hence the end points obtained using the extracts of *Acanthus sennii chiovenda* flower are fairly comparable to the end points obtained using the commercial indicators i.e. methyl red, bromothymol blue and phenolphthalein so the flower extract can be used as a substitute of methyl red, bromothymol blue and phenolphthalein for strong acid against weak base titrations.

For weak acid against strong base titration, the end point obtained using ethanol and acidified ethanol extracts of *Acanthus sennii chiovenda* flower did not show any significant difference 10.33 ± 0.058 mL and 10.37 ± 0.115 mL which is close to that obtained using phenolphthalein (10.40 ± 0.100 mL) but is obviously different from the end point obtained with methyl red (9.53 ± 0.058 mL) and bromothymol blue (9.67 ± 0.058 mL) in the titrations involving 1M acetic acid and 1M sodium hydroxide solutions. The natural indicator (*Acanthus sennii chiovenda* flower extract) can be a good substitute for phenolphthalein in this type of titration. For this combination of weak acid vs. strong base titration, the end point obtained with acidified ethanol extracts of *Acanthus sennii chiovenda* flower is 8.90 ± 0.100 mL; which deviates significantly from the result of ethanol *Acanthus sennii chiovenda* flower extract (8.13 ± 0.153 mL). The end points obtained using acidified ethanol extracts of *Acanthus sennii chiovenda* flower are comparable to the end points obtained using phenolphthalein (8.83 ±0.058 mL) and bromothymol blue (8.77 ± 0.208 mL) but statistically different from the end point obtained with methyl red (8.07 ± 0.115 mL) while the end points obtained using ethanol *Acanthus sennii chiovenda* flower extract is close to the end point obtained with methyl red but different from the end point obtained with phenolphthalein and bromothymol blue in this medium. Hence the acidified ethanol extracts can be used in place of phenolphthalein and bromothymol blue while the ethanol extracts can replace methyl red in weak acid versus weak base titrations.

The results of this study are similar to the observations reported on Jaspreet *et al*. [38], Thote *et al.* [3], Abbas [9] and Senathirajah *et al.* [37] that did related work on indicators using different parts of plant extracts, but there was a slight difference in the result as compared with that of Nhapi [5] and Pimpodkar *et al*. [8].

Statistical analysis of data

The statistical analysis of the experimental data generated from titration was statistically analyzed by using one-way ANOVA with statistical significance at p (significance) < 0.05. The results of one-way ANOVA are shown in Table 4 a-d. From the results obtained for titrations of strong acid versus strong base (HCl vs NaOH) and strong acid versus weak base (HCl vs NH_4OH), there was no statistically significant evidence at 5 % confidence interval to show that there were no significant differences between the mean titre volumes when using the five indicators: Methyl Red (MR), Bromothymol Blue (BB), Phenolphthalein (Hph), Ethanol *Acanthus Sennii Chiovenda* Flower Extract, Acidified Ethanol *Acanthus Sennii Chiovenda* Flower Extract. For the titrations of weak acid versus strong base (CH_3COOH vs NaOH) and weak acid versus weak base (CH_3COOH vs NH_4OH), it was found that there were significant differences between the mean titre volumes of the five indicators.

Table 4a: One-way ANOVA tests of HCl vs NaOH titration using the five indicators

Source of variation	Sum of squares	Degree of freedom	Mean squares	F	Significance
Between groups	0.029	4	0.007	0.733	0.590
Within groups	0.100	10	0.010		
Total	0.129	14			

Table 4b: One-way ANOVA results of HCl vs NH_4OH titration using the five indicators

Source of variation	Sum of squares	Degree of freedom	Mean squares	F	Significance
Between groups	0.063	4	0.016	0.758	0.575
Within groups	0.207	10	0.021		
Total	0.269	14			

Table 4c: One-way ANOVA tests of CH_3COOH vs NaOH titration using the five indicators

Source of variation	Sum of squares	Degree of freedom	Mean squares	F	Significance
Between groups	2.149	4	0.537	80.600	0.000
Within groups	0.067	10	0.007		
Total	2.216	14			

Table 4d: One-way ANOVA tests of CH_3COOH vs NH_4OH titration using the five indicators

Source of variation	Sum of squares	Degree of freedom	Mean squares	F	Significance
Between groups	1.969	4	0.492	26.375	0.000
Within groups	0.187	10	0.019		
Total	2.156	14			

CONCLUSION

Phytochemical screening tests and UV-Vis spectra results lead us to the conclusion that, it was due to the presence of anthocyanins and flavonoids that cause sharp colour changes at the end point of the titrations. Even though, there are some differences in color and end point titre values during titration of acids and bases of all combinations, the use of *Acanthus senni chiovenda* flower extracts as acid-base indicators is experimentally confirmed. In this study, it is indicated that ethanolic and acidified ethanol extracts of *Acanthus sennii chiovenda* flower are good replacements to phenolphthalein, bromothymol blue and methyl red in acid-base titrations involving a strong acid versus strong base and a strong acid versus weak base. They can also be used as effective substitutes to phenolphthalein in a weak acid versus strong base titrations. And also the acidified ethanol extracts can be used in place of phenolphthalein and bromothymol blue while the ethanol extracts can replace methyl red in weak acid versus weak base titrations. We also concluded that it is beneficial to use *Acanthus sennii chiovenda* flower extract as an indicator in all types of acid base titration because of its economy, eco-friendly nature, ease of preparation, easy availability, simplicity, non-carcinogenicity, precise and accurate results.

IMPLICATION FOR CHEMISTRY EDUCATION

Volumetric analysis is still at the center of high school, college and even University chemistry education. At the top of volumetric analysis, we almost always experience titrations that

employ acid-base indicators which contain weak organic acids characterized by distinct colors in their molecular and ionized forms.

The acid –base indicators used in the chemistry laboratory are very often synthetic which are expensive for the schools to afford for their large number of laboratory sessions, toxic to students and teachers especially when they produce vapors in the laboratory classes, pollutants to the school surrounding affecting animal and human health.

The preparation and introduction of natural acid-base indicators like that obtained from *Acanthus senni chiovenda*, in this study, is of practical importance from many perspectives. It is easy to extract, store, and apply under normal school environments. It is cheaper when compared to the synthesized ones, environmentally benign and has no health risk to humans and animals.

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