Integrity Journal of Education and Training
Volume 4(4), pages 53-61, December 2020
Article Number: FE38A8641
ISSN: 2636-5995
https://doi.org/10.31248/IJET2020.087
https://integrityresjournals.org/journal/IJET

Full Length Research

The use of plant extracts as indicators in teaching acid-base titrations: Case of *Hibiscus sabdariffa (*Zobo*), Beta vulgaris* (Beet root)*, Hibiscus rosa sinensis* and *Acalypha wilkesiana*

Ayodele Olufunmilayo D*., Olabode Hawa O. and Bolarinwa Tawakalitu

Department of Chemical Sciences, Oduduwa University Ipetumodu, Ile-Ife, Osun-state, Nigeria.

*Corresponding author. Email: olufunmilayopapers@gmail.com

Received 7th September, 2020; Accepted 14th October, 2020

ABSTRACT: Acid-base titrations are the main part of volumetric analysis in the secondary school chemistry practical syllabus in Nigeria. Synthetic indicators are generally used in teaching students the procedure and skills required in acid-base titrations. However, some of these synthetic indicators are not readily available, some are toxic and there is a special procedure to be followed in preparing them for use as indicators. Hence there is need to consider the suitability of natural indicators which are more readily available, easy to prepare and ecofriendly compared. Therefore, this study aimed at investigating the suitability of *Hibiscuss rosa sinensis*, *Acalipha wilkesiana*, *Hibiscus sabdariffa* and *Beta vulgaris* L. extract as indicators in acid-base titration. The results showed that in strong-acid strong-base titration, all the plant extracts under study are suitable. All the plant extracts are fairly suitable for strong-acid and weak-base, except *Beta vulgaris* L. which is unsuitable for this titration. *H. rosa* sinensis is most suitable for weak-acid weak-base titrations similar to phenolphthalein, *Acalipha wilkesiana* is fairly suitable while *H. sabdariffa* and *Beta vulgaris* L. are unsuitable. For weak-acid strong-base titrations, all the plant extracts are fairly suitable especially *H. sabdariffa*. It was recommended that the use of these flower extracts as acid-base indicators should be incorporated in the teaching of acid-base titration especially in the secondary schools.

Keywords: Acid-base titration, plant indicators, synthetic indicators, *H. sabdariffa, H. rosa sinensis, A. wilkesiana*, *Beta vulgaris* L.

INTRODUCTION

In Nigeria, the major factors militating against science education are the increasing unavailability and high cost of conventional science teaching materials (Along, 1998; Garba, 2001). This has resulted in paucity of experimental activities in the schools which threatens the potential for increase in students' scientific knowledge. Acid base titration is one of such experimental activities required in the senior secondary school Chemistry practical syllabus. It involves the determination of the concentration of an acid or base by exactly neutralizing the acid or base with an acid or base of known concentration. Indicators are used to determine the equivalent points between reacting reagents (Nwosu et al., 2004). The choice of an indicator for a particular titration depends on the characteristics of the neutralization curve. In acid-base titration, an indicator is used to determine the end point of the titration at which the acid and base are in the exact proportions necessary to form salt and water only.

Majority of indicators in use today are synthetic. Synthetic indicators are man-made chemical substances produced in the laboratory which is used to determine pH of a substance, such as methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, bromophenol blue, and thymol blue. Synthetic indicators are slightly

expensive and some have toxic effects on users and can cause environmental pollution (Pathade et al., 2009). However, natural indicators from plant pigments have been found to be readily available (in the immediate surrounding or local market, easy to prepare (simple extraction)) and eco-friendly (Pathan and Farooqui, 2011; Vinayak et al., 2013; Eze and Ogbuefi, 2014; Bungale and Mali, 2014).

Hence, scientists have embarked on search for natural indicators which are less or even non-toxic and eco-friendly for acid-base titration. Natural indicators are dyes or pigments that are isolated from a variety of sources, including plants (plant parts like flowers, fruits, and leaves), fungi, and algae (Vinayak et al., 2013). The coloured pigments obtained from plants are found to exhibit colour changes with variation of pH (Abbas, 2012).

Researchers have worked on some plant extracts as indicators and have found them to be accurate and precise at equivalent points in acid-base titrations (Ayodele, 2019 b; Vinayak et al., 2013; Sharma et al., 2013; Jain, 2012; Pathan and Farooqui, 2011; Pathade et al., 2009; Izonfuo et al., 2006).

There is paucity of experimental activities in secondary school Chemistry laboratories due to lack of funds needed to get most of the chemicals. Some of these chemicals are expensive and are not available locally. Hence there is need to integrate available local materials as reagents in secondary school experimental chemistry (Ayodele, 2019 a). Acid-base titration is a major volumetric analysis in the secondary school Chemistry curriculum. It requires the use of pH indicator to show a colour change at the end point during titration. Synthetic indicators are slightly expensive, not readily available and some have toxic effect. The use of plant extracts would be a cheaper and a more sustainable alternative which would also promote green chemistry in experimental chemistry.

It is on this premise that this study sought to investigate the suitability of the aqueous and ethanolic extracts of *Hibiscus sabdariffa*, *Beta L vulgaris, Hibiscus rosa sinensis* and *Acalipha wilkesianna as* indicators in acid-base titrations.

LITERATURE REVIEW

Roselle (*Hibiscus sabdariffa L*)

Hibiscus sabdariffa L. is an annual, erect, bushy, herbaceous sub-shrub that may grow to 8 ft (2.4 m) tall, with smooth or nearly smooth, cylindrical, typically red stems (Figure 1). The leaves are alternate, 3 to 5 in (7.5-12.5 cm) long, green with reddish veins and long or short petioles. The capsule turns brown and splits open when mature and dry. The calyx stems and leaves are acidic and closely resemble the cranberry (Vaccinium spp.) in flavor. It is extensively cultivated in tropical Africa, Asia, Australia and Central America (Schippers, 2000). *H. sabdariffa* is native to West and East Africa and South-East Asia. It is used for making wine juice, jam, tea and syrup. *H. sabdariffa* has been found to possess antiseptic, diuretic, antioxidant and antimutagenic properties (Al-Hashimi, 2012; Olvera-Garcia et al., 2008)

Figure 1. *Hibiscus sabdariffa* plant.

Figure 2. Beetroot (*Beta vulgaris L.).*

Beetroot (*Beta vulgaris* L.)

This is crop belonging to the Quenopodiaceae family having bright crimson colour (Figure 2). It is famous for its juice value and medicinal properties; and known by several common names like beet, chard, spinach beet, sea beet, garden beet, white beet and Chukander (Yashwant, 2015). Beetroot contains different beneficial chemical compounds, for example betalains which have been reported to have high antioxidant activity (Kujala et al., 2000; Ravichandran et al., 2013), and also presence of anthocyanins in beetroot makes it a possible pH indicator. Beet root has been found to exhibit anti-inflammatory,

Figure 3. Tree of *Hibiscus rosa sinensis.*

hepatic protective and anti-cancer properties (Kapadia et al., 2003). It contains nutrients like vitamin E, magnesium, manganese, iron dietary fibres and minerals, carbohydrates, fats and protein (USDA database, 2014). It is used raw or boiled in salads, omelets and soups.

Hibiscus rosa sinensis

The plant is known generally as Chinese hibiscus, shoeblack plant, tropical hibiscus or China rose. It is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family Malvaceae, native to East Asia (Figure 3). It contains anthocyanins and comes in orange, pink, red white and yellow. it is a popular flowering plant used in the beautification of the outer gardens and surrounding (Arthur, 2000) such as in schools, workplaces and residential houses. It is also used as medicinal beverage (Manandhar, 2002).

Acalypha wilkesiana

This plant is commonly called Irish petticoat. It is native to the south pacific islands and belongs to the family Euphorbiaceae (Figure 4). The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables in the management of hypertension, being a diuretic plant (Ogundini, 2005; Oladunmoye,2006) It is a plant of great ornamental value due to its showily coloured foliage and is widely cultivated in the tropical and subtropical countries. *Acalypha wilkesiana* is frequently used in traditional medicine, exclusively or as a major constituent of many herbal preparations for the management or treatment of

Figure 4. *Acalypha wilkesiana* plant.

hypertension. This medicinal plant is of great importance to the health of individuals and communities (Omage and Azeke, 2014).

METHODOLOGY

The experimental research design was adopted for the study. The raw materials used were *Hibiscus sabdariffa*, *Beta L. vulgaris*, *Hibiscus rosa sinensia* and *Acalypha wilkesiana*. The chemicals were ethanol, distilled water, ammonia hydroxide, hydrochloric acid, acetic acid, sodium hydroxide, methyl orange and phenolphthalein. The equipment used include, pH-meter, retort stand, burette, pipette, beaker, UV-Vis spectrometer, funnel, conical flask, dropper, blender, analytical balance, white Muslim cloth. All reagents were prepared according to the official method of preparation in AOAC (2019). The extraction and titration procedure were adapted from Vadivel and Chipkar (2016) and Pathade et al. (2009).

Sample collection

The fresh flower species of *Acalypha wilkesiana and Hibiscus rosa sinensis* were collected from the premises of Oduduwa University Ipetumodu, Ile-Ife, Osun State. The *Beta L vulgaris* (beetroot) and dried leaves of *Hibiscus sabdariffa* were purchased at Akinola market at Ipetumodu, Ile-Ife, Osun state. The samples were identified by a Botanist and colleague in Oduduwa University, Ile-Ife, Osun-state.

Extraction

The petals of *Hibiscus rosa sinensis* were rinsed to remove dirt and sundry. The dried *Hibiscus rosa sinensis* was blended into powdery form. 40 g of the powdered sample was dissolved in 400 ml of water and another 40 g was

Figure 5. Aqueous and ethanolic extract of *Hibiscus rosa sinensis.*

Figure 6. Aqueous and ethanolic extract of *Acalypha wilkesiana.*

dissolved in 400 ml of ethanol to extract the juice for 48 hours. After the extraction, the juice was filtered using a muslin cloth. The juice extracted from both water and ethanol extract of *Hibiscus rosa sinensis* were analysed using UV absorption spectrometer. The same was done for *Acalipha wilesiana* (Figures 5 and 6).

The dried zobo calyces (*Hibiscus sabdariffa*) were rinse and sundry to remove dirt. The dried zobo was blended into powdery form. 20 g of the powdered sample was dissolved in 200 ml of water and another 20 g was dissolved in 200 ml of ethanol to extract the juice. After the extraction, the juice was filtered using a white muslin cloth.

Figure 7. Aqueous and ethanolic extract of Hibiscus *sabdariffa (Z*obo).

Figure 8. Extract of *Beta L vulgaris* (Beetroot).

The juice obtained from both ethanol and water extract of *Hibiscus sabdariffa* were analyzed using UV absorption spectrometer (Figure 7).

The beetroot was peeled and chopped into small pieces. The chopped beetroot was blender with the addition of small volume of water and the mixture is sieved using white muslin cloth to extract the juice. The juice obtained was analyzed using UV absorption spectrometer (Figure 8).

Titrations

Strong acid-strong base titration (0.1M 0f hydrochloric, HCl and sodium hydroxide, NaOH)

25 ml of sodium hydroxide was pipette out into a clean conical flask and 3 to 5 drops of methyl was orange was added and titrated against 0.1M Hydrochloric acid in the burette. The titration was repeated for consisted values. Same titration procedure was followed for phenophtalein, *Acalypha wilkesiana*, *Hibiscus rosa sinensis, Hibiscus sabdariffa* and *Beta L. vulgaris* (Beetroot) (both ethanol and water extract). The pH of each indicator at both initial and at endpoint was taken.

Strong acid-weak base titration (0.1M of Hydrochloric acid, HCl and ammonium hydroxide, NH_4OH)

25 ml of ammonium hydroxide was pipette out into a clean conical flask and 3 to 5 drops of indicator was added and titrated against 0.1M Hydrochloric acid in the burette. The titration was repeated for consisted values. Same titration

Table 1. pH of indicators.

Indicators	pH	Colour change in Acid	Colour change in Base
Methyl orange	6.1	Reddish	Yellow
Phenolphthalein	6.3	Colourless	Pink
Aqueous *Acalypha wilkesiana*	5.9	Green	Light brown
Ethanolic *Acalypha wilkesiana*	6.2	Green	Brown
Aqueous *Hibiscus rosa sinensis*	3.9	Pink	Greenish
Ethanolic *Hibiscus rosa sinensis*	6.8	Pink	Pale green
Aqueous Hibiscus *sabdariffa*	2.8	Brown	Pale green
Ethanolic Hibiscus *sabdariffa*	3.2	Brown	Pale green
Aqueous Beetroot	6.1	Brown	Yellow

Table 2. Strong acid vs strong base titration.

Indicators	Experiment 1	Experiment 2	Mean value(cm^3)	Colour at end point
Methyl orange	19.40	19.20	19.30±0.1	Pale orange
Phenolphthalein	18.50	18.10	18.30±0.2	Pink
Aqueous *Acalypha wilkesiana*	19.00	18.60	18.80±0.2	Greenish brown
Ethanolic *Acalypha wilkesiana*	19.60	19.30	19.45±0.15	Greenish yellow
Aqueous *Hibiscus rosa sinensis*	19.90	20.30	20.1±0.20	Green
Ethanolic *Hibiscus rosa sinensis*	20.30	20.40	20.35±0.05	Pale green
Aqueous Hibiscus *sabdariffa*	17.00	17.20	17.10±0.10	Pale brown
Ethanolic Hibiscus *sabdariffa*	16.20	16.20	16.20±0,00	Pale brown
Aqueous Beta L *vulgaris*	20.3	20.0	20.15±0.15	Brown

procedure was followed for methtyl orange, phenophtalein, *Acalypha wilkesiana*, *Hibiscus rosa sinensis, Hibiscus sabdariffa* and *Beta L. vulgaris* (Beetroot) (both ethanol and water extract). The pH of each indicator at both initial and at endpoint was taken.

Weak acid-weak base titration (0.1M of aAcetic acid, CH_3COOH and ammonium hydroxide, NH_4OH)

25 ml of ammonium hydroxide was pipetted out into a clean conical flask and 3 to 5 drops of indicator was added and titrated against 0.1M acetic acid in the burette. The titration was repeated for consisted values. Same titration procedure was followed for methtyl orange, phenophtalein, *Acalypha wilkesiana*, *Hibiscus rosa sinensis, Hibiscus sabdariffa* and *Beta L*. *vulgaris* (Beetroot) (both ethanol and water extract). The pH of each indicator at both initial and at endpoint was taken.

Weak acid-strong base titration (0.1M of acetic acid, CH_3COOH and sodium hydroxide, NaOH)

25 ml of sodium hydroxide was pipetted out into a clean conical flask and 3 to 5 drops of indicator was added and titrated against 0.1M acetic acid in the burette. The titration was repeated for consisted values. Same titration procedure was followed for methtyl orange, phenophtalein, *Acalypha wilkesiana*, *Hibiscus rosa sinensis, Hibiscus sabdariffa* and *Beta L. vulgaris* (Beetroot) (both ethanolic and aqueous extract). The pH of each indicator at both initial and at endpoint was taken.

RESULTS AND DISCUSSION

The results of the experimental analyses are presented in the Tables 1 to 5. The results in Table 2 (Titration of strong acid/strong base) shows that the end points obtained for all the sample extracts (*Hibiscus rosa sinensis*, *Acalypha wilkesiana, H. sabdariffa and Beta L. vulgaris)* are very close to those obtained using synthetic indicator (phenolphthalein and methyl orange). This reveals that the plant extracts considered are suitable for strong acid vs strong base titration. Also, the results in Table 3 (strong acid/weak base) shows that the end points obtained with the plant extract in the titration of 0.1M solutions of hydrochloric acid and ammonium hydroxide are comparable to those obtained using synthetic indicators with the exception of *Beta L. vulgaris* and *Acalipha Wilkesiana*. Furthermore, results presented in Table 4 (weak acid-weak base) shows that *Hibiscuss sabdarriffa* and *Beta L vulgaris* like methyl orange are not suitable indicators for weak acid and weak base titrations. However, *Hibiscus rosa sinensis* is a suitable indicator

Table 3. Strong acid vs weak base.

Indicators	Experiment 1	Experiment 2	Mean value(cm^3)	Colour at end point
Methyl orange	16.70	16.40	16.55±0.15	Yellow
Phenolphthalein	11.20	11.50	11.35±0.15	Colourless
Aqueous *Acalypha wilkesiana*	16.10	15.60	15.85±0.25	Pale Brown
Ethanolic *Acalypha wilkesiana*	3.40	3.50	3.45±0.05	Greenish yellow
Aqueous *Hibiscus rosa sinensis*	13.00	13.20	13.10±0.1	Green
Ethanolic *Hibiscus rosa sinensis*	14.70	14.50	14.60±0.1	Pale green
Aqueous Hibiscus *sabdariffa*	17.00	17.40	17.20	Dark brown
Ethanolic Hibiscus *sabdariffa*	15.70	15.20	15.45	Purple
Aqueous Beta L *vulgaris*	>50.00	>50.00		

Table 4. Weak acid vs weak base.

Indicators	Experiment 1	Experiment 2	Mean value(cm^3)	Colour at end point
Methyl orange	Not Suitable	Not Suitable		
Phenolphthalein	12.40	12.70	12.55±0.15	Colourless
Aqueous *Acalypha wilkesiana*	6.60	6.60	6.60±0	Brown
Ethanolic *Acalypha wilkesiana*	5.90	5.40	5.65±0.25	Pale brown
Aqueous *Hibiscus rosa sinensis*	12.70	12.40	12.55±0.15	Green
Ethanolic *Hibiscus rosa sinensis*	13.70	13.80	13.75±0.05	Green
Aqueous Hibiscus *sabdariffa*	>50.00	>50.00	Not suitable	
Ethanolic Hibiscus *sabdariffa*	>50.00	>50.00	Not suitable	
Aqueous Beta L *vulgaris*	>50.00	>50.00	Not suitable	

Table 5. Weak acid vs strong base.

Indicators	Experiment 1	Experiment 2	Mean value(cm^3)	Colour at end point
Methyl orange	23.20	23.20	23.20±0.00	Orange
Phenolphthalein	0.80	0.70	0.75±0.05	Colourless
Aqueous *Acalypha wilkesiana*	20.20	20.50	20.35±0.15	Yellowish green
Ethanolic *Acalypha wilkesiana*	20.20	20.40	20.30±0.10	Greenish yellow
Aqueous *Hibiscus rosa sinensis*	19.00	19.20	19.10±0.10	Green
Ethanolic *Hibiscus rosa sinensis*	18.50	18.60	18.55±0.05	Pale green
Aqueous Hibiscus *sabdariffa*	13.00	13.20	13.1±0.10	Pale brown
Ethanolic Hibiscus *sabdariffa*	8.80	8.80	8.80±0.00	Pale Brown
Crude extract of Beta L *vulgaris*	19.3	19.0	19.15±0.15	Brown

comparable to phenophtalein. Table 5 (weak acid-strong base) revealed that almost all the plant indicators gave titre values close to that of methyl orange.

The results obtained for suitability of *Hibiscus sabdarrifa* as acid base indicator comparable to methyl orange corroborate the reports of Izonfuo et al. (2006) and Nuryanti et al. (2013). Also, Bhuvaneshwari et al. (2015), Powar et al. (2013) reported findings similar for *Beta vulgari* as a suitable acid-base indicator. The result obtained for H. r*osa sinensis* corroborates the reports of Okoduwa et al. (2015) and Jain (2012) in its suitability as acid-base indicator comparable to phenophtalein. Moreover, Bhise et al. (2014) gave reports which also confirmed the suitability of Acalipha wilkesiana as acid-base indicator especially for strong-acid strong-base and strong-acid weak-base.

Conclusion

The suitability of the extracts of four plants as indicators in acid-base titration was investigated in this study. Based on the results obtained, it can be concluded that in strong acid-strong base titration, all the plant extracts (*Hibiscuss*

sabdariffa, Hibiscuss rosa sinensis, Acalipha wilkesiana and *Beta* L *vulgaris*) under study are suitable indicators. For strong acid and weak base, all the plant extracts are fairly suitable except *Beta L. vulgaris* which is unsuitable for this titration. *H. rosa sinensis* is most suitable, for weak acid-weak base titrations similar to phenolphthalein, *Acalipha wilkesiana* is fairly suitable while *H. sabdariffa and Beta L. vulgaris* are unsuitable. For weak acid-strong base titrations, all the plant extracts are fairly suitable especially *H. sabdariffa*.

Recommendation

It is therefore recommended that the extracts of *Acalypha wilkesiana, Hibiscus rosa sinensis*, *Hibiscus sabdariffa* and *Beta L. vulgaris* could be used as alternatives for synthetic indicators in the teaching of acid-base titration especially to secondary school students. The use of these plant extracts would:

1. ignite the awareness of students to chemistry and society.
2. promote green chemistry.
3. encourage use of indigenous or locally available materials in the teaching of experimental chemistry especially at the secondary school level.

It is also recommended that workshops should be organized for secondary school teachers on how to use locally available materials to conduct some qualitative and quantitative analyses. Also, advanced research could be conducted on the characterization of pigments found in these plant extract using instruments like Mass spec- Gas chromatography and HPLC.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

We would like to appreciate the Mr. Shaid Oseni (B.Sc. Botany (Hons), M.Sc. Botany), Department of Biological Sciences, Odudiwa University for his help in the identification of the plant samples.

REFRENCES

Abbas, S. K. (2012). Study of acid-base indicator property of flowers of ipomoea biloba. *International current Pharmaceutical Journal*, 1(12), 420-422.

Al-Hashimi, A. (2012). Antioxidant and antibacterial activities of Hibiscus sabdariffa L. extracts. *African Journal of Food Science*, 6(21), 506-511.

Along, E. I. (1981). Local materials for secondary school chemistry in Nigeria: Some crucial questions answered. *Journal of Science Teacher of Nigeria,* 20(1), 14-18.

AOAC International (2019). Official Methods of Analysis. 21st Edition.

Artur W. W. (2000). Tropical ornamental: A guide, Timber Press Inc., Oregon. ISBN:0-88192-448-2.

Ayodele, O. D. (2019b). Acid-base titration using the flower extract of *Ixora occinea* as Indicator. 42nd Annual International Conference of Chemical Society of Nigeria. 8th-13th September 2019.

Ayodele, O. D. (2019a). Teaching chemistry experiments using indigenous materials and processes in developing countries: From improvisation to Innovation. In: Opara, J. A. (ed.). *Outlook on human capacity building and development: A handbook of Research in Honor of Prof. Ibrahim Njodi*. University of Maiduguri Press. Pp. 131-138.

Bhise, S. H., Shinde, N. G., Surve, B. S., Pimpodkar, N. V., Shilkalgar, S. S. (2014). *Acalypha wilkesiana* as natural pH indicator. *International Journal of Natural Products Research*, 4(1), 33-35.

Bhuvaneshwari, B., Sivaelango, G., Parthiban, D., Arun, N., & Kumaravel, P. (2015). Natural dyes as acid-base indicators from beta vulgaris. *Research Journal of Pharmacognosy and Phytochemistry*, 7(2), 65-68.

Bungale, S. H., & Mali, A. V. (2014). Natural indicators as eco-friendly in acid-base titration. *Journal of Chemical and Pharmaceutical Research*, 6(5), 901-903.

Eze, S. O., & Ogbuefi, R. A., (2014). Analytica potentials of dye extract from *Aspiha africana* (oramujela) flowers. *Asian Journal of Natural and Applied Sciences*, 3(1), 54-60.

Garba, M. D. (2001). Enriching science instruction in secondary schools through improvisation. *African Journal of Material and Natural Sciences,* 1(3),18-22.

Izonfuo, W. A., Fekarurhobo, G. K., Obomanu, F. G., & Daworiye, L. T. (2006). Acid-base indicator properties of dyes from local plants I: dyes from Basella alba (Indian spinach) and *Hibiscus sabdariffa* (Zobo). *Journal of Applied Sciences and Environmental Management*, 10(1), 5-8.

Jain, P. (2012). Isolation of natural acid-base indicator from the flower sap of Hibiscus rosa sinensis. *Journal of Chemical and Pharmaceutical Research*, 4(12), 4957-4960.

Kapadia, G. J., Azuine, M. A., Sridhar, R., Okuda, Y., Tsuruta, A., Ichiisi, E., Mukainake, T., Tasaki, M., Konoshima, T., Nishimi, H., & Tokuda, H., (2003). Chemoprevention of DMB induced UN-B promoted NOR-1-induced TPA promoted skin carcinogenesis and DEN-induced phenobarbital promoted liver tumors in mice by extracts of beet root. *Pharmacological Research*, 47, 141-148.

Kujala, T. S., Loponen, J. M., Klika, K. D., & Pihlaja, K. (2000). Phenolics and betacyanins in red beetroot (*Beta v ulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural and Food Chemistry*, 48(11), 5338-5342.

Manandhar, N. P. (2002). *Plants and people of Nepal*. Timber Press, Oregon. ISBN-0-88192-527-6

Nuryanti, S., Matsjeh, S., Anwar, C., Raharjo, T. J., & Hamzah, B. (2013). Corolla of roselle (Hibiscus sabdariffa L.) as acid-base indicator. *European journal of chemistry*, 4(1), 20-24.

Nwosu, F. O., Adekola, F. A., & Ihedioha, K. C. (2004). Simple titrimetric colour indicators from some natural flower petals. *Centrepoint (Science Edition)*, 12(1), 74-89.

Ogundini, A. O. (2005). From greens into medicine, taking a lead from nature. An inaugural lecture delivered at Oduduwa Hall, Obafemi Awolowo University, ile-ife, Nigeria, Inaugural lecture

series 176. O.A.U press limited, Ile-Ife, Nigeria. Pp. 12-15.
Okoduwa, S. I., Mbora, L. O., Adu, M. E., & Adeyi, A. A. (2015). Comparative analysis of the properties of acid-base indicator of Rose (Rosa setigera), Allamanda (Allamanda cathartica), and Hibiscus (Hibiscus rosa-sinensis) flowers. *Biochemistry Research International*, Volume 2015, Article ID 381721.
Oladunmoye, M. K. (2006). Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana*. *Trends in Applied Sciences Research,* 1, 538-541.
Olvera-Garcia, V., Castano-Tostado, E., Rezendiz-Lopez, R. I. (2008). *Hibiscus sabdariffa* L. extracts inhibits the mustagenic in micro suspension assay and the proliferation of Hela cells. *Journal of Food Science*, 73(5), 75-81.
Omage, K., & Azeke, M. A. (2014). Medicinal potential of *Acalypha wilkesiana*. Advances in research, 2(11), 655-665.
Pathade, S. K., Pattl, S. B. Konda-Wa N., & Magdum, C. S., (2009). Mons-alba fruit-herbal alternative to synthetic acid-base indicators. Int. Journal of Chemistry Technology Research, 3(1), 549-551.
Pathan, M. A. K., & Farooqui, M. (2011). Analytical application s of plant extracts as natural pH indicators: a review. *Journal of Advanced Scientific Research*, 2(4), 20-27.
Powar, P. V., Sharma, P. N., Onkar, P. R., Avari J. G., (2013). Beet root (*Beta vulgaris* l) herbal alternative to synthetic acid-base indicators used in titrimetric analysis. *International Conference on Pharmacology, Phytochemistry and Natural Products*. Radisson Blu plaza Hotel, Hyderabad, India. October 21-23, 2013.
Ravichandran, K., San, N. M. M. T., Mohdaly, A. A. A., Gabr, A. M. N., Kastell, A., Riedel, A., Cai, Z., Knorr, D., & Smentanska, I., (2013). Impact of processing of red beet onbtalain content and antioxidant activity. *Food Research International*, 50, 670-675.
Schippers, R. R. (2000). African Indigenous vegetables: An overview of cultivated vegetables *Science Journal,* 11, 1-6.
Sharma, P., Gupta, R., Roshen, S., Sahu, S., Tatuway, S., Shukla, A., & Garg, A. (2013). Plant extracts as acid-base indicator: An overview. Inventi Journals (P) ltd. Pp. 1-3.
United States Department of Agriculture, USDA National Nutrient Database (2014). Nutritional facts and analysis for chard per 100 grams.
Vadivel, E., & Chipkar, S. D. (2016). Eco-friedly natural acid-base indicator properties of four flowering plants from Western Ghats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(6), 250-252.
Vinayak, S. M., Shrkant, S. K. Suresh, G. K., & Dipak, P. M. (2013). (2013). *Boerhavia Erecta Linn*. stem bark extract: A natural acid-base indicator. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *3*(16), 10-13.
Yashwant, K. (2015). Beetroot: A super food. *International Journal of Engineering Studies and Technical Approach*, 1, 20-26.