



The Use of Stem and Root Barks Extracts from *Synadenium glaucescens* (Euphorbiaceae) as Acid Base Indicators

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ABSTRACT

Currently, the conduction of acid-base chemical reactions involves the use of industrial made indicators which are associated with environment pollutions. This situation necessitates the search for more acid-base indicators from the natural sources. The aim of this work was to study the acid-base indicating capacity of the extracts from *Synadenium glaucescens*. To study the indicating capacity from *S. glaucescens*, the extracts from leaves, stem and root barks were studied for their colour change, reversibility, pH range and effectiveness during titration by titration using strong and weak acids and bases. The results indicated that, only the indicators from stem and root barks extracts had indicating capacity as they were capable to change colour due to pH change. The pH range of the two indicators was from 2.9 to 12.7 which make them to be better universal indicators. Both indicators could be reversed clearly while in acidity and alkalinity conditions. Titration showed sharp colour change at the end points. The mean titre of the two indicators were ranging from 24.3 ± 0.31 to 25.4 ± 0.75 and 18.9 ± 0.17 to 24.1 ± 0.05 , respectively with their colour change from brick red to colourless and orange to colourless, respectively. The end points obtained by stem and root barks indicators correspond to the end points obtained by standard indicators, phenolphthalein and methyl orange. Thus, the stem and root barks extracts are suitable to serve as acid-base indicator. Further studies could be done aiming to develop paper indicators and isolate pure compound which is responsible for indicating capacity of *S. glaucescens*.

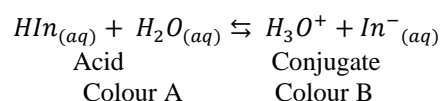
Keywords: Acid-base indicator; *Synadenium glaucescens*; Natural indicator; Titration

INTRODUCTION

Synadenium glaucescens (Euphorbiaceae), commonly known as ‘Mvunja-kongwa in “Kiswahili” or “Liyugi in Bena” is an indigenous to East Africa and commonly found growing in several regions in Tanzania [1,2]. The species has been reported to be of great importance to mankind, for indigenous use for treatment of both animal and human ailments such as excessive menstruation, skin conditions, sores and wounds [3-5]. While working with different extracts from *S. glaucescens* during the conduction of various experiments, it was accidentally observed that some of the extracts were changing colour when placed in different media of acids and bases (Faith P. Mabiki,

Direct communication, 10 November, 2011). Due to this observation, there was an increased curiosity to investigate the indicating properties of *S. glaucescens* and possible use of the extracts as acid base indicator to serve as the alternative to the synthetic indicators. Furthermore, on the best of the reviewed literatures, there was no any study that report on the use of the extracts from the *S. glaucescens* parts as acid base indicator.

Theoretically, acid-base (pH) indicator is a halochromic chemical compound that is added in small amounts (dropwise) to a solution so that the pH of the solution can be determined visually and change colour with variation in pH, hence a pH indicator is a chemical detector for hydronium ions (H_3O^+) or hydrogen ions (H^+) [6]. Acid-base indicators are generally weak acids or weak bases which form ions by dissociating slightly when dissolved in water [7]. An indicator which is a weak acid with the formula HIn and its conjugate base have different colours at equilibrium as can be best represented by the equilibrium equation below:



Thus, colour A in the solution is formed due to the presence of high concentration of H_3O^+ which causes the equilibrium to shift the left. This colour occurred at low pH values. On the other hand, colour B in the solution is formed at high pH due to the presence of low concentration of H_3O^+ and consequently causing the equilibrium to shift the right [7]. In acid-base titration, indicators are 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 [8]. Currently, synthetic indicators such as methyl orange, methyl red and phenolphthalein are used for acid-base titrations [7,8]. These indicators are not only that are expensive but proved to cause environment hazardous and harmful to human being due to carcinogenicity nature [8]. Following these synthetic indicators limitations, the search for natural indicators as acid-base indicator was highly emphasized in order to obtain alternative against the stated limitation [9]. This study aimed at investigating the potentiality of extracts from *S. glaucescens* as acid base indicator during titration. The study focused on the preparation and testing the indicating capacity, determination of the colour changes and their reversibility in different medium, examination of the transition range values, establishment of the colour scales, demonstration of the indicator using a titration reaction and finally, development of the titration curve of the extracts from *S. glaucescens*.

EXPERIMENTAL PROCEDURES

Material

Fresh leaves stem and root barks of the *Synadenium glaucescens* were collected in Njombe region. The samples were treated to dryness under the shade followed by pulverization.

Reagents

Dichloromethane (DCM), Dimethylsulfoxide (DMSO), Ethanol (EtOH), Hydrochloric acid (HCl), Acetic acid (CH₃COOH), Sodium hydroxide (NaOH), Ammonium hydroxide (NH₄OH), buffer solution, distilled water, Ammonia 20% in a closed bottle, Baking soda, Distilled water, Methyl orange (M.O), and Phenolphthalein (P.O.P) were purchased from the suppliers by the Department of Chemistry and Physics, Solomon Mahlangu College of Science and Education, Sokoine University of Agriculture, Morogoro.

Extraction by Using Water (Total Extraction)

The pulverized leaves, stem and root barks of the *S. glaucescens* were obtained and, 10 g of each were measured separately by using digital chemical balance of which were placed in three different beakers. 150 mL of water were added to each beaker followed by gently heating of the content in beakers at 45°C for 25 minutes. The mixture was cooled. After cooling, the liquid was poured off separately followed by filtration to obtain the supernatant. Finally; the leaves, stem and root barks supernatant of the *S. glaucescens* were tested in acids and bases and the results were recorded in the tabular form. The plant part whose extracts showed positive result in changing colour was later considered for extraction by using soxhlet method.

Extraction Using Soxhlet Method

The extraction of the natural extracts using soxhlet involved the stem and root barks of *S. glaucescens*. 10 g of the pulverized stem and root barks of the *S. glaucescens* were measured by means of digital balance and placed into different thimbles (1 mm diameter, 33 mm diameter and 80 mm length) in the extraction chamber and extracted using a common Soxhlet apparatus consisting of a condenser, a Soxhlet chamber, and an extraction flask. Extraction time was 4 hours at a temperature of 30°C for dichloromethane and 60°C for Ethanol. The obtained supernatant were concentrated using rotary evaporator to obtain the crude extracts. The crude extracts separately were removed from the flask and used for preparation of the natural indicators by dissolving in Dimethylsulfoxide as solvent.

Preparation of Natural Indicators (RBSGI and SBSGI)

A 0.288 g and 0.286 g powders of root bark of *Synadenium glaucescens* extracts (RBSGI) and Stem Bark of *Synadenium glaucescens* extracts (SBSGI), respectively, were weighted using digital chemical balance and dissolved in 50 mL and 25 mL of DMSO, respectively to prepare the natural indicator. The prepared natural indicators were tested in acids and bases and the result were recorded in a tabular form. The experiments were carried out by using various graduated apparatus used for titration reactions, pH-meter, screw driver and Digital camera. Methyl orange (M.O) and Phenolphthalein (P.O.P) (Standard indicators) were prepared and used for control experiments.

Reversibility of RBSGI and SBSGI

A 30 mL of 0.1 M NaOH was measured into the beaker. 3-drops of (RBSGI) were added and the colour was recorded. Slowly the solution of 0.1 M HCl was added into the beaker containing the solution of 0.1 M NaOH and the natural extracts till the colour change. The colour change was recorded. Finally, the solution of 0.1 M NaOH was added slowly till the colour change. Results of the observation made were recorded. The same procedures were repeated by using SBSGI.

Transition Range Value of RBSGI and SBSGI

A 25 mL of 0.1 M of NaOH was pipetted into a titrating conical flask. 3-drops of RBSGI were added into the titrating flask. The pH-meter was immersed into the solution in the flask and the pH reading was made. The titration was allowed until the colour changed. The pH and the colour of the neutralized solution were taken and recorded in the tabular form. The experiment was repeated while using SBSGI. The pH transition range of the RBSGI and SBSGI were both evaluated by measuring the pH of the medium just before and after colour change has occurred, taking the two values as the pH range over which colour change occurred to indicate the equivalent point [10].

Estimation of Colour Scale of the RBSGI

By means of pH buffer solution and screw driver, the pH meter was set. A solution with pH=2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 were prepared by using hydrochloric acid and Ammonia solution. For the case of pH=7 tap water was used. 3 mL of the solutions of different pH were measured into different test tubes. 3-drops of RBSGI were added to each test tube. Pictures were taken showing the colour scale of the extract when exposed into different pH scale.

Effectiveness of RBSGI and (SBSGI) during Titration

The acid-base titration experiments used RBSGI, SBSGI, POP and MO. The reagents were not calibrated. The titrations were performed using 25 mL of titrate in the titrating flask with 3-drops of indicator against titrant from the burette. A set of four experiments each for all types of acid base titrations i.e. strong acid-strong base (HCl v/s NaOH), strong acid-weak base (HCl v/s NH₄OH), weak acid – strong base (CH₃COOH v/s NaOH), weak acid – weak base (CH₃COOH v/s NH₄OH) were carried out. The results in a tabular form were recorded. The mean and standard deviation for each of acid base titrations were calculated from results obtained.

Titration Curve of RBSGI and SBSGI

Titration curve of both natural indicator (RBSGI and SBSGI) and standard indicators (POP and MO) were obtained from all the four set of experiments for all types of acid base titration following the order of strong acid v/s strong base, weak acid v/s strong base, strong acid v/s weak base and weak acid v/s weak base. The results were plotted on graphs to estimate the titration curves.

RESULTS AND DISCUSSION

Results showed in Table 1 indicated that, the extracts from the Leaves of *Synadenium glaucescens* remain unaffected as there was no colour change while in different acid-base media. This indicated that the leaves of the *S. glaucescens* have no indicating capacity. On the other hand, the stem and root barks extracts from *S. glaucescens* behaved differently in different acidic and basic medium as the colour change was observed. Therefore, both stem and root barks of *S. glaucescens* were observed to have indicating capacity.

Table 1. Colour Observation Chart (0.1 M HCl and 0.1 M NaOH)

Plant part used	Solvent used to dissolve the extract	Extraction Method	Colour Observed	
			HCl	NaOH
Leaves	H ₂ O	Total extraction/heat	Yellow	yellow
Stem Barks	H ₂ O	Total extraction/heat	yellow	orange
Root Barks	H ₂ O	Total extraction/heat	yellow	Brick red
Stem Barks	DMSO	Soxhlet extraction/EtOH	colourless	orange
Root barks	DMSO	Soxhlet extraction/EtOH	Colourless	Brick red

The results for pH transition range were shown in Table 2. The relationship between the pH of an indicator, its dissociation constant, K_a and the concentrations of the conjugate base and acids forms of the indicator is mathematically expressed by the Hunderson-Hasselbalch equation which is reported by [10,11] as:

$$pH = pK_a + \log \frac{[In^-]}{[HIn]} \quad (1)$$

Where by In^- and HIn are the two forms of the indicator which are usually have different colours. At half the equivalence point; the concentration of the form In^- and HIn are equal and hence the equation 1 above is reduced to:

$$pH = pK_a \quad (2)$$

Therefore, basing on the pH range data provided on Table 2, the pK_a and K_a of the RBSGI are 8.3, 6.03, 8.0 and 7.05; and 5.0×10^{-9} , 9.3×10^{-7} , 1.0×10^{-8} and 8.9×10^{-7} , respectively. Likewise, the pK_a and K_a of SBSGI are 6.5, 6.4, 7.8 and 7.0; and 3.2×10^{-7} , 3.9×10^{-7} , 1.6×10^{-8} and 1.0×10^{-7} , respectively. The pH ranges of some common indicators used in acid base titration are reported as from 0.0 to 12.0 [12]. Most organic compounds of which, they are weak acids, their dissociation constants are reported range from 10^{-2} to 10^{-60} [13] Also, the pK_a values for most weak acids are reported to range from 4.7 to 15.7 [14]. RBSGI and SBSGI have the pH range that is within the common acid base indicator pH ranges. The pK_a and K_a values verify that both RBSGI and SBSGI are suitable to be used as acid base indicator. Furthermore, literatures reports on the pH ranges of both phenolphthalein and methyl oranges indicators to be 8.3 to 10.0 and 3.1 to 4.4 [11]. These pH ranges are narrow compared to the pH ranges of RBSGI and SBSGI (Table 2) together with the colour scale of the RBSGI (Figure 1). Therefore, this indicated that the extracted indicators have are wide pH ranges compared to synthetic indicators notably, phenolphthalein and methyl orange indicators and hence RBSGI and SBSGI can be widely used as universal indicator.

Table 2. Transition Range of Syna-Indicators

Indicator	0.1 M Titrant v/s 0.1 M Titrate	Colour Change	pH-Range
RBSGI	HCl v/s NaOH	Brick red to Colourless	3.90 -12.7
RBSGI	HCl v/s NH ₄ OH	Brick red to Colourless	2.96-9.1
RBSGI	CH ₃ COOH v/s NaOH	Brick red to Colourless	4.8-11.2
RBSGI	CH ₃ COOH v/s NH ₄ OH	Brick red to Colourless	4.7-9.7
SBSGI	HCl v/s NaOH	Orange to colourless	2.9-10.1
SBSGI	HCl v/s NH ₄ OH	Orange to colourless	3.8-9.0
SBSGI	CH ₃ COOH v/s NaOH	Orange to colourless	5.6-10.0
SBSGI	CH ₃ COOH v/s NH ₄ OH	Orange to colourless	5.4-8.6



Figure 1. Colour scale for RBSGI estimated from solution of pH 2 to 11

The reversibility capacity of the extracts from both stem and root barks of *S. glaucescens* were shown on Figure 2. The reversibility property of any indicator is important in order to distinguish indicator dyes from other colour forming reagents [15]. Indicator dyes should be able to reverse their colours. The results in Figure 2 showed that regardless of the method used for their extraction, colours of both root and stem barks of *S. glaucescens* were reversed accordingly. This signifies the presence of the indicating molecule in both root and stems barks of *S. glaucescens* of and hence its qualification for providing indicating potentiality.

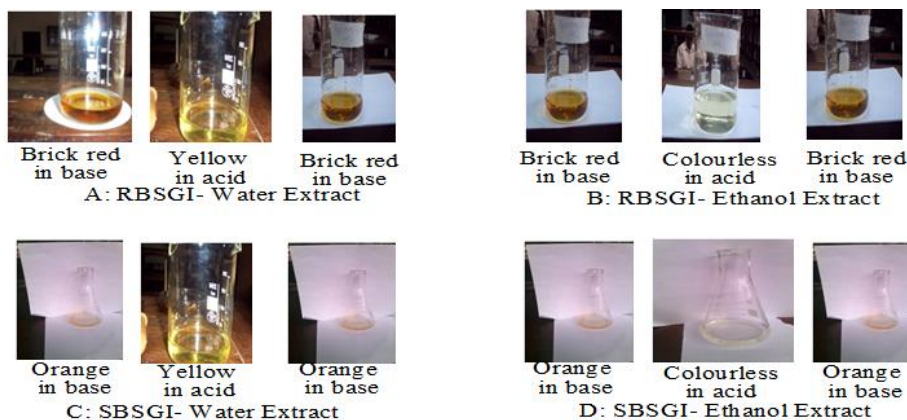


Figure 2. Reversibility of RBSGI and SBSGI

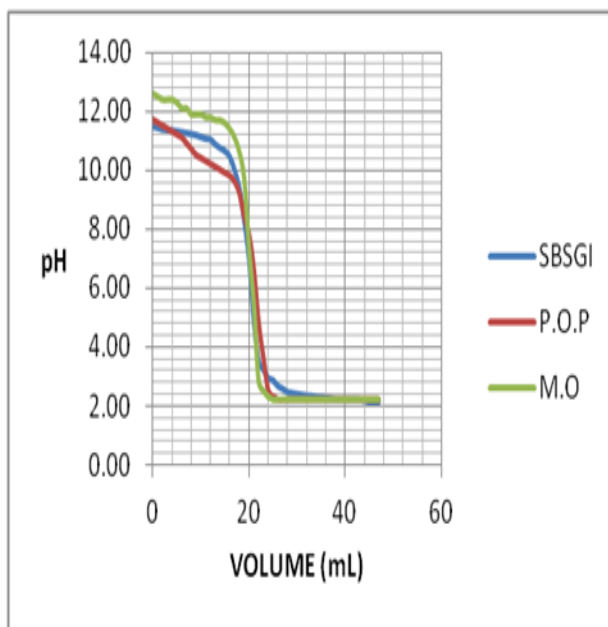
The results in Table 3 show the titration end points obtained with SBSGI, RBSGI, POP and MO. The results show that the end points of both RBSGI and SBSGI in the titration of 0.1 M HCl and NaOH are comparable to those obtained using POP and MO. The titration of 0.1 M of HCl and NH_4OH which is the strong acid against weak base and that of 0.1 M of CH_3COOH and NaOH which is weak acid and strong base, showed that, the end points of RBSGI and SBSGI are fairly comparable to both POP and MO. On the other hand, the end point of both RBSGI and SBSGI in the titration of 0.1 M of CH_3COOH and NH_4OH which are weak acid and weak base are not comparable to MO, though they are close related to the end point of POP. This indicated that, RBSGI and SBSGI is not good indicator for the titration of weak acid against weak base. The results for end points obtained in this work agree to

results obtained when *Hibiscus subdariffa* was used as an indicator, of which the end points were comparable to those obtained using POP and MO in the titration of 0.1 M HCl and NaOH [10].

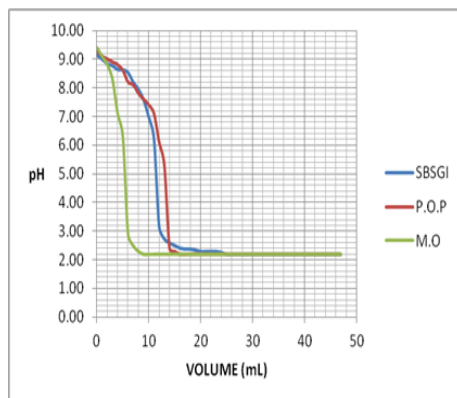
Table 3. Titration End Points (0.1 M of Titrant and Titrate)

Titrant v/s Titrate	Indicators	Mean \pm SD	Colour Change
HCl v/s NaOH	P.O.P	24.4 \pm 0.23	Pink to colourless
	M.O	24.3 \pm 0.21	Yellow to pink
	RBSGI	24.3 \pm 0.31	Brick red to colourless
	SBSGI	24.1 \pm 0.05	Orange to colourless
HCl v/s NH ₄ OH	P.O.P	22.1 \pm 0.82	Pink to colourless
	M.O	25.1 \pm 0.66	Yellow to pink
	RBSGI	25.4 \pm 0.75	Brick red to colourless
	SBSGI	18.9 \pm 0.17	Orange to colourless
CH ₃ COOH v/s NaOH	P.O.P	24.3 \pm 0.76	Pink to colourless
	M.O	25.2 \pm 0.25	Yellow to pink
	RBSGI	25.1 \pm 0.20	Brick red to colourless
	SBSGI	22.0 \pm 0.40	Orange to colourless
CH ₃ COOH v/s NH ₄ OH	P.O.P	24.8 \pm 0.25	Pink to colourless
	M.O	30.5 \pm 0.92	Yellow to pink
	RBSGI	24.3 \pm 1.27	Brick red to colourless
	SBSGI	24.0 \pm 0.04	Orange to colourless

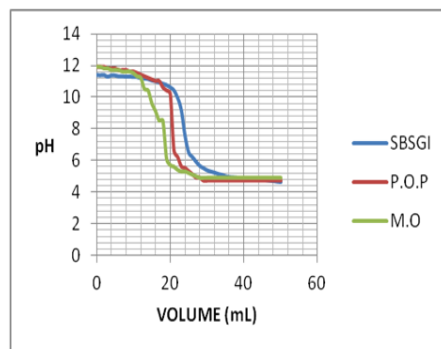
Moreover, the titration curves shown in Figures 3a-3d and 4a-4d below showed the potentiality of using SBSGI and RBSGI during the titrations of strong acid against strong base, strong acid against weak base and weak acid against strong base due to steep bit on the graphs which provide easy detection of the end point. However, the graphs for both SBSGI and RBSGI in the titration of CH₃COOH and NH₄OH, that is weak acid and weak base showed points of inflexion rather than a steep bit. Lack of steep bit causes difficultness in the detection of end points during the conduction of titrations of weak acid against weak base.



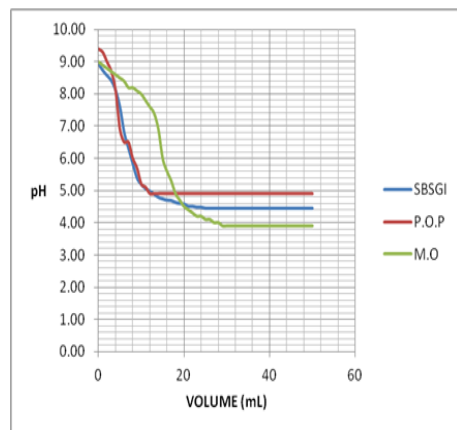
3a. Strong acid v/s strong base (HCl v/s NaOH)



3b. Strong acid v/s Weak base (HCl v/s NH₄OH)

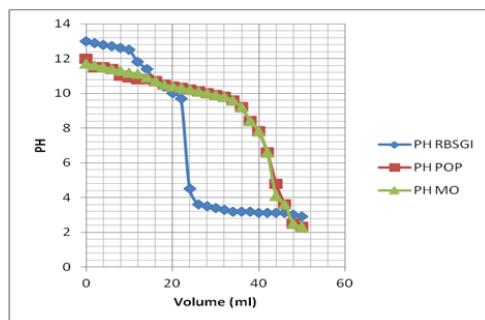


3c. Weak acid v/s strong base CH₃COOH v/s NaOH



3d. Weak acid v/s weak base CH₃COOH v/s NH₄OH

Figure 3a-d. Titration curves for SBSGI, POP and MO



4a. Strong acid v/s strong base HCl v/s NaOH

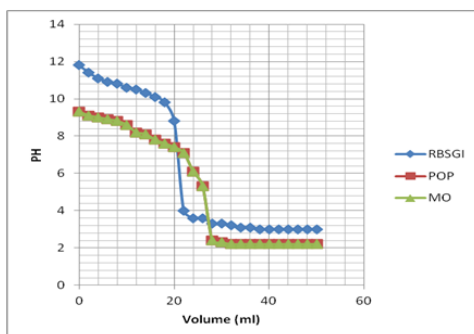
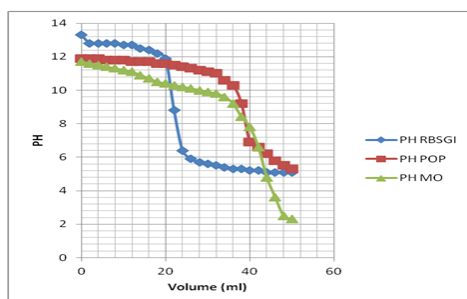
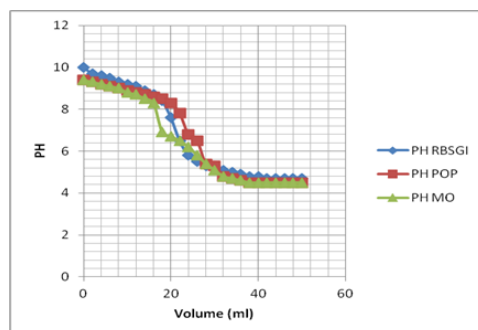
4b. Strong acid v/s weak base HCl v/s NH₄OH4c. Weak acid v/s strong base CH₃COOH v/s NaOH4d: Weak acid v/s weak base CH₃COOH v/s NH₄OH

Figure 4a-d. Titration curves for RBSGI, POP and MO

CONCLUSION

It can be concluded from the results obtained that, both extracts from the stem and root barks of *Synadenium glaucescens* can be used as suitable acid base indicators for the titration reactions of strong acid and strong base, strong acid and weak base and; weak acid and strong base. However, they are not suitable for the reaction that involves weak acids and weak bases. The obtained indicators can be used as universal indicator. This is due to their wide pH ranges. Following the suitability of the stem and root barks extracts to be used as acid base indicators, further studies could be done in order to develop the Syna-paper indicators from these extracts. In addition to that the isolation and characterization of the pure compound(s) which are responsible for indicating capacity of the *S. glaucescens* can be conducted.

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