ORIGINAL ARTICLE

Screening and implementation of *Bougainvillea spectabilis* Willd (Rosea, Alba and Flava) bract extracts as an indicator in acid–base titrations

Suresh G. Killedar[1], Sameer J. Nadaf[2], Anuja A. Pawar[1], Sachin A. Pishawikar[1]

[1]*Department of Pharmacognosy and Quality Assurance, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India,* [2]*Department of Pharmaceutics, Adarsh college of Pharmacy, Vita, Maharashtra, India*

Abstract

Background: Natural pigments present in different plants are highly colored substances and contributes to color changes with variation of pH. **Aims:** This study was deliberated to evaluate the flower pigment extracts obtained from *Bougainvillea spectabilis* bracts (Nyctaginaceae) as a titration indicator. **Settings and Design:** Three different varieties (Rosea, Alba, and Flava) of *B. spectabilis* were used for the isolation of extract. Acidified methanolic extracts were used as an indicator in different strong acid-strong base (HCl and NaOH), strong acid-weak base (HCl and NH_3), weak acid-strong base (CH_3COOH and NaOH), and weak acid-weak base (CH_3COOH and NH_3) titrations. A comparative study of *B. spectabilis* extract a natural indicator, with synthetic indicator phenolphthalein, methyl red, and phenol red was conducted to evaluate its accuracy as an acid-base indicator. **Statistical Analysis Used:** Further, the data were subjected to one-way analysis of variance analysis for analyzing the statistical difference. **Results and Conclusions:** Result showed that in all these titrations the *Bougainvillea* bract extracts were found to be accurate, useful and for indicating the end point (neutralization point). It gives sharp and intense color change, and results were found to be statistically significant ($P < 0.05$) as compared to phenolphthalein, methyl red, and phenol red. The presence of betalains in *B. spectabilis* bracts mainly contributes to the color change at different pH. From this, it can be concluded that *B. spectabilis* bract extracts of different varieties are emerging as a novel, very useful, economical, simple, accurate, environmentally and user-friendly indicator. This discovery can be a value addition to the plethora of numerous indicators available. From these investigations, new theories of indicators could be established.

Key words: Acid–base titrations, indicator, methanolic extract, methyl red, phenolphthalein

INTRODUCTION

Indicators are dyes or pigments that can be isolated from a multiplicity of sources, including plants, fungi, and algae whose solutions alter color due to changes in pH.[1] Indicators used in titrations show well-marked changes of color in certain intervals of pH. They are usually weak acids and bases, but their conjugate base or acid forms have different colors due to differences in their absorption spectra.[2] Although synthetic indicators are the choice of acid–base titrations in the recent era, the use of natural dyes as acid-base indicators dated back to 1664, first reported by Sir Robert Boyle.[3] Due to environmental pollution, availability cost and toxicity problem of commercial indicators, such as phenolphthalein and methyl orange,[4,5] the search for natural compounds as an acid-base indicator have been started. The use of flower pigments (extracts) from *Rosa indica,*[6] *Hibiscus rosa-sinensis,*[6] *Ipomoea biloba,*[7] and *Bombax ceiba*;[8] leaf extract from *Acalypha wilkesiana*;[9] fruit extract of *Bixa orellana*[1] and *Morus alba* Linn.[5] as natural indicator in acid–base titration has previously been reported.

Address for correspondence:
Dr. S. G. Killedar, Bharati Vidyapeeth College of Pharmacy, Kolhapur - 416 013, Maharashtra, India.
Phone: +91-231-2637286, Fax: 91-231-2638833.
E-mail: sureshgk_64@rediffmail.com

Received: 19-04-2014
Revised: 08-01-2015
Accepted: 02-02-2016

Bougainvilleas are very popular and attractive ornamental plants in most areas with warm climates.[10] The genus *Bougainvillea* from Nyctaginaceae family is a native of South America and commonly known as "paper flower" due to its bracts which are thin and papery.[11,12] It is about 1-12 m tall, thorny woody and utilizes their hooked thorns to scramble over the plants. They are evergreen where rainfall occurs all the year, or deciduous if there is a dry season. The leaves are alternate, simple, ovate, acuminate and 4-13 cm long. The actual flower of the plant is small and commonly white, but each cluster of three flowers is surrounded by three or six different colored bracts such as pink, purple, orange, white, red, magenta, or yellow.[13,14] The studies on *Bougainvillea spectabilis* leaves revealed that it has medicinal properties such as antidiabetic,[15-17] antifertility potential,[18] antibacterial,[19] anti-inflammatory,[20] antiviral,[21] and larvicidal.[22]

Literature survey reveals that ample of work has been carried out on *B. spectabilis* leaves, but still no enough work is reported on isolation of pigment from bracts. Hence, in this study an attempt has been made to isolate the pigment from bracts of three varieties of *B. spectabilis* (Rosea, Alba, and Flava) and its implementation in acid–base titration as an indicator.

MATERIALS AND METHODS

Plant Material

B. spectabilis bracts were collected from the local area (NH4 highway between Kolhapur and Peth vadgaon) during the blooming season in the month of April to July. The collected material was air dried and packed in airtight bags. The plant material was taxonomically identified by Dr. M. Y. Cholekar-Bachulkar, Principal, Shri Vijaysinh Yadav Arts and Science College, Peth Vadgaon, Maharashtra, India. Voucher herbarium specimen (Pawar A. A. Specimen No. 2) sheet then submitted to the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India.

Chemicals

Sodium hydroxide, hydrochloric acid, acetic acid, ammonia solution, methanol, and buffer solutions of pH 1-12 were procured from local companies and dilutions were made as per requirement.

Experimental Method

Extraction

For the extraction purpose initially various solvents such as petroleum ether, acetone, ethanol, methanol, and methanolic HCl were used, but on the basis of percent yield and stability of extract in acidic pH (around pH 3), further methanolic HCl was optimized as the extraction solvent. Hence, the extracts were obtained by immersing Fresh bracts, dried bracts and dried bracts powder of *B. spectabilis* in 1% methanolic HCl for 24 h at room temperature. The extract was concentrated under reduced pressure. To the concentrated solution, a mixture of diethyl ether and light petrol (2:1) was added when the coloring matter separates. The supernatant liquid was decanted after 2 h. Finally, the deep blue-red colored viscous residue was dried in a vacuum desiccator over anhydrous calcium chloride. Percentage yield was found to be in the range of 18.3-22.5% w/win methanol.

Spectral and Infrared (IR) Characterization

The methanolic extract of all varieties of *B. spectabilis* bracts was scanned in the range of 400-800 nm using ultraviolet visible spectrophotometer (Jasco, V-630) and also subjected to IR characterization.

Color Change Over Different pH Range

B. spectabilis bracts extracts were evaluated at different pH value using buffer solution for their color change.

B. spectabilis Bracts (Rosea, Alba, and Flava) Extract as pH Indicator

The extracts of aforesaid three varieties of *B. spectabilis* bracts both in solution and powder form was screened for its use as an indicator in acid–base titration, i.e., strong acid-strong base (HCl and NaOH), strong acid-weak base (HCl and NH_3), weak acid-strong base (CH_3COOH and NaOH), and weak acid-weak base (CH_3COOH and NH_3). Moreover, results were compared with the results obtained by standard indicator phenolphthalein, methyl red, and phenol red.

For Solution

1. The air dried bracts were immersed in 1% methanolic HCl for 24 h. The extract obtained was concentrated under reduced pressure and it was preserved in a tight closed container and stored away from light.
2. The experiment was carried using the same set of glassware's for all types of titrations. Same aliquots were used for both titrations, i.e., titration using a standard indicator and bract extracts.
3. The equimolar titrations were performed using 10ml of titrant with two drops of indicator. All the parameters for experiment are given in Table 1.
4. A set of three experiments each for all types of acid–base titrations were carried out.
5. The mean standard deviation for each type of acid–base titrations was calculated from results obtained.

For Powder

1. The air dried bracts were immersed in 1% methanolic HCl for 24 h. The extract obtained was concentrated under reduced pressure and it was dried under vacuum desiccator over calcium chloride.
2. The different concentrations of powder such as 1, 1.5, 2, and 2.5% were prepared, and further titrations were carried out as per method is given as above, i.e., the indicator in solution form.

Statistical Analysis

Further, the data was subjected to one-way ANOVA (analysis of variance) for analyzing the statistical difference using the software Graphpad Prism (San Diego, CA, USA).

RESULT AND DISCUSSION

All the extracts were successfully isolated and evaluated for its application as pH indicator.

Table 1: Parameters for titration

Titrant	Titrate	Indicator color change	
		Standard	Bract extract
HCl	NaOH	Colorless to pink	Colorless to yellow
HCl	NH_3	Red to yellow	Colorless to yellow
CH_3COOH	NaOH	Colorless to pink	Colorless to yellow
CH_3COOH	NH_3	Yellow to red	Colorless to yellow

Spectral and IR Characterization

Fourier transform IR spectra of color solution of *B. spectabilis* extract shows three characteristics peaks attributed to phenols, -OH stretching, aromatic C-H stretching, and Aromatic -C=C- stretching at 3375.89/cm, 2945.15/cm, and 1409.36/cm, respectively [Figure 1a]. The presence of broad peak in the range of 3200-3500/cm (3375.89/cm) indicates the presence of phenolic compounds. However, the presence of a peak at near about 3350/cm is also attributed to the indolic N-H stretching.[23] So from IR spectra of color solution of *B. spectabilis* it is conform that there is presence of red and yellow indole-derived pigment, i.e., betalains as it shows the presence of both -OH stretching and indolic N-H stretching. Visible spectra of methanolic extract of *B. spectabilis* Willd showed λ_{max} at 418 and 516 nm [Figure 1b].

Color Change Over Different pH Range

Methanolic extract of *B. spectabilis* bracts shows pink color up to pH 10 further the color changes to yellow [Figure 2].

B. spectabilis Bracts Extract in Solution form as pH Indicator

The study reveals that extract from all three varieties showed color change from colorless to yellow at the end point of the all type acid–base titrations. Mean volume (ml) required for titration at different strength of extracts (moles) has been reported in Tables 2-4 and presented graphically in Figures 3a, 4a and 5a. For all type titrations, methanolic extract of *B. spectabilis* willd in solution form gave either exactly coincided or much-closed estimates

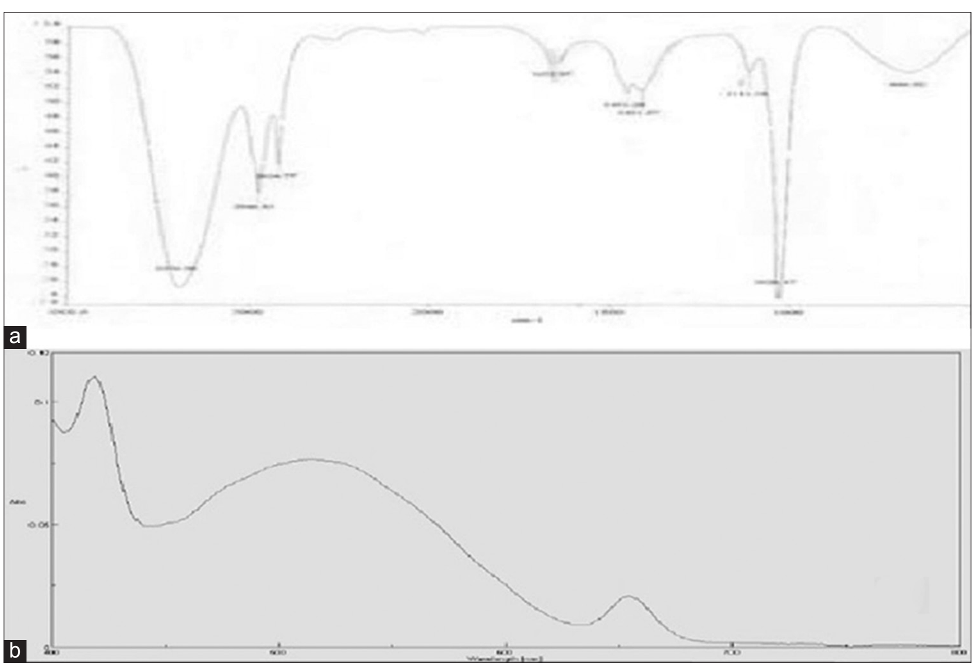

Figure 1: (a) Fourier transform-infrared spectroscopy spectra (b) and ultraviolet spectra of color solution of *Bougainvillea spectabilis* bracts in methanol

of the endpoint as compare to standard indicators phenolphthalein, methyl red or phenol red. For different varieties of *B. spectabilis;* at 1 mole of the concentration of an indicator, higher mean volume of indicator (ml) was required for neutralization to occur compared to 0.1 and 0.5 moles of concentration. Mean volume (ml) required for all type titrations was in order, 1 mole >0.5 mole >0.1 mole. Means indicator is useful at lower concentration as it requires less volume (ml) of indicator for titration to occur.

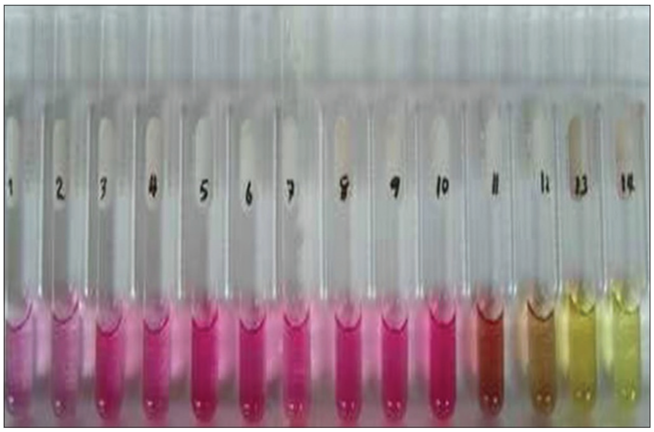

Figure 2: Color shown by extract at pH 1-14

The phytochemical investigation shows the presence two different forms of betalains. First one is cyanine having purple-red color and present in a high concentration. Whilst second is xanthine having a yellow color and obtained in low concentration. Betalains mainly contribute to the sharp color change over a range of pH.

B. spectabilis Bracts Extract in Powder form as pH Indicator

For all type titrations, methanolic extract of *B. spectabilis* willd in powder form gave either exactly coincided or much-closed estimates of the endpoint as compare to standard indicators phenolphthalein, methyl red or phenol red. Mean volume (ml) required for titration at different strength of extracts (moles) has been reported in Tables 5-7 and presented graphically in Figures 3b, 4b and 5b. In addition, to this results obtained by solution and powder form of different varieties are also close to each other. So for long term purpose indicator can be stored in powder form.

Statistical Analysis

Results of the volume of titrant in ml required for all type of titrations using indicator isolated from all varieties of

Table 2: Mean volume (ml)* at the equivalence point for titrations (Rosea bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.6±0.15	7.7±0.19	7.4±0.25	8.1±0.28	12.3±0.28	12.4±0.20	15.0±0.21	14.9±0.20
0.5	8.1±0.20	8.1±0.22	7.8±0.37	10.5±0.45	13.3±0.34	13.4±0.36	15.5±0.17	15.4±0.22
1.0	9.3±0.20	9.5±0.24	9.4±0.30	11.5±0.28	14.5±0.25	14.6±0.24	16.1±0.34	16.2±0.27

*Indicates±SD (*n*=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

Table 3: Mean volume (ml)* at the equivalence point for titrations (Alba bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.4±0.17	7.5±0.23	8.0±0.25	8.3±0.21	12.3±0.28	12.2±0.24	15.0±0.21	15.2±0.45
0.5	8.1±0.20	7.9±0.25	10.0±0.37	10.2±0.19	13.3±0.34	13.5±0.28	15.5±0.17	15.5±0.19
1.0	9.3±0.20	9.5±0.22	11.4±0.30	11.1±0.27	14.5±0.25	14.5±0.17	16.1±0.34	16.4±0.34

*Indicates±SD (*n*=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

Table 4: Mean volume (ml)* at the equivalence point for titrations (Flava bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.6±0.15	7.8±0.22	8.0±0.25	8.1±0.45	12.3±0.28	12.1±0.25	15.0±0.21	15.4±0.28
0.5	8.1±0.20	8.0±0.28	10.0±0.37	10.1±0.17	13.3±0.34	13.3±0.17	15.5±0.17	15.3±0.30
1.0	9.3±0.20	9.4±0.15	11.4±0.30	11.3±0.20	14.5±0.25	14.3±0.22	16.1±0.34	15.9±0.15

*Indicates±SD (*n*=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

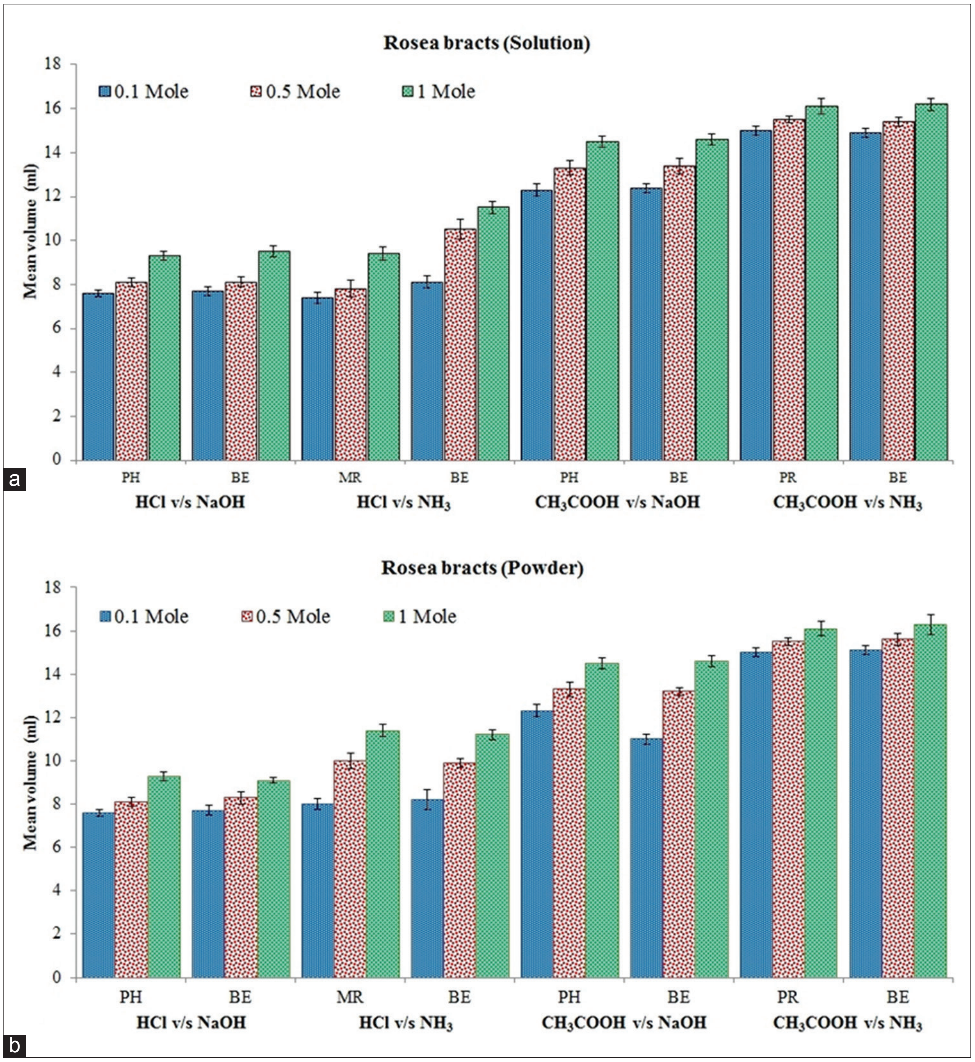

Figure 3: Mean volume (ml) required for all type of acid–base titration using *Bougainvillea spectabilis* Rosea bracts in (a) solution (b) powder form

Table 5: Mean volume (ml)* at the equivalence point for titrations (Rosea bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.6±0.15	7.7±0.23	8.0±0.25	8.2±0.45	12.3±0.28	11.0±0.24	15.0±0.21	15.1±0.19
0.5	8.1±0.20	8.3±0.29	10.0±0.37	9.9±0.20	13.3±0.34	13.2±0.17	15.5±0.17	15.6±0.30
1.0	9.3±0.20	9.1±0.15	11.4±0.30	11.2±0.25	14.5±0.25	14.6±0.27	16.1±0.34	16.3±0.45

*Indicates±SD (n=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

B. spectabilis bract (Rosea, Alba, and Flava) were found to be statistically significant ($P < 0.05$) with results of standard indicators, i.e., phenolphthalein, methyl red, and phenol red.

CONCLUSION

The results obtained in all types of titrations lead us to conclude that, it was due to the presence of betalains which leads to sharp color changes occurred at the end point of titration. Preparation of indicator from *B. spectabilis* bracts could be the source of economical gain. Henceforth, it is always beneficial to use *B. spectabilis* willd bracts extract as an indicator in all type of acid–base titrations because of its economy, simplicity, easy availability and cost-effectiveness compares to commercial indicators. Unlike the other natural extracts it can be effective and potent in powder form also, hence can be stored for future studies.

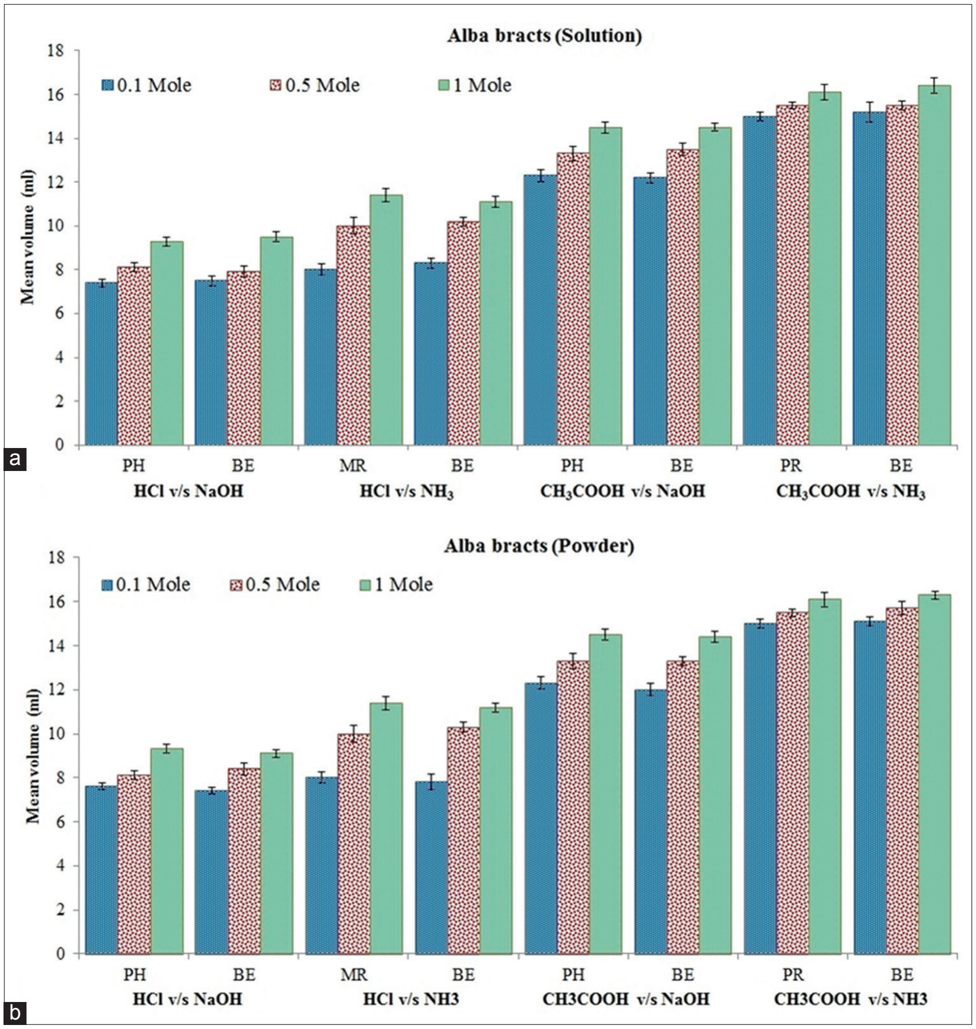

Figure 4: Mean volume (ml) required for all type of acid–base titration using *Bougainvillea spectabilis* Alba bracts in (a) solution (b) powder form

Table 6: Mean volume (ml)* at the equivalence point for titrations (Alba bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.6±0.15	7.4±0.15	8.0±0.25	7.8±0.34	12.3±0.28	12.0±0.27	15.0±0.21	15.1±0.19
0.5	8.1±0.20	8.4±0.28	10.0±0.37	10.3±0.24	13.3±0.34	13.3±0.22	15.5±0.17	15.7±0.30
1.0	9.3±0.20	9.1±0.17	11.4±0.30	11.2±0.20	14.5±0.25	14.4±0.25	16.1±0.34	16.3±0.19

*Indicates±SD (*n*=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

Table 7: Mean volume (ml)* at the equivalence point for titrations (Flava bracts)

Strength in moles	HCl versus NaOH		HCl versus NH_3		CH_3COOH versus NaOH		CH_3COOH versus NH_3	
	PH	BE	MR	BE	PH	BE	PR	BE
0.1	7.6±0.15	7.5±0.25	8.0±0.25	7.8±0.40	12.3±0.28	12.4±0.22	15.0±0.21	15.2±0.28
0.5	8.1±0.20	8.1±0.24	10.0±0.37	10.4±0.17	13.3±0.34	13.5±0.17	15.5±0.17	15.4±0.40
1.0	9.3±0.20	9.4±0.17	11.4±0.30	11.1±0.34	14.5±0.25	14.6±0.19	16.1±0.34	16.4±0.17

*Indicates±SD (*n*=3), PH: Phenolphthalein, BE: *Bougainvillea spectabilis* extract, MR: Methyl red, PR: Phenol red, SD: Standard deviation

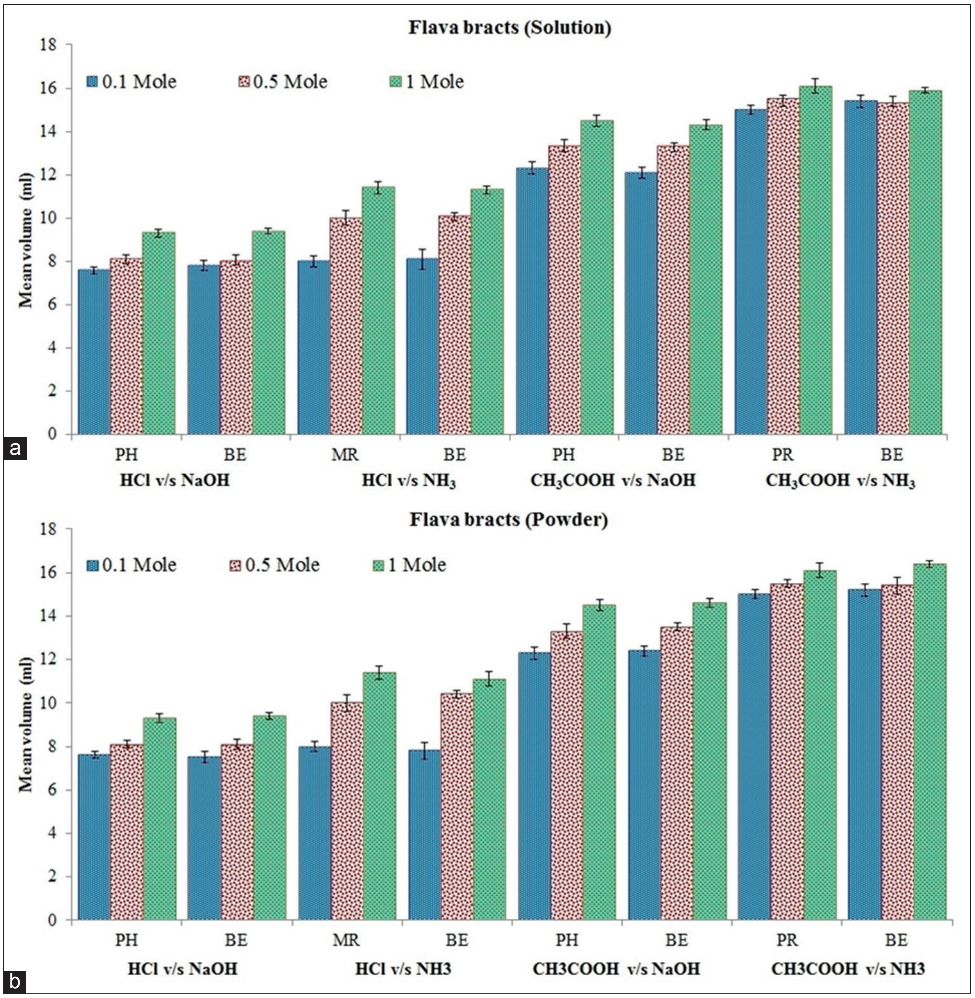

Figure 5: Mean volume (ml) required for all type of acid–base titration using *Bougainvillea spectabilis* Flava bracts in (a) solution (b) powder form

REFERENCES

1. Pimpodkar NV, Surve BS, Bhise SH. Use of *Bixa orellana* fruit extract as a natural indicator in acid base titration. Int J Pharm Res Sch 2014;3:156-9.
2. Kasture AV, Mahadik KR. Pharmaceutical Analysis. 17th ed. Nagpur, India: Nirali Prakashan, Reprint; 2012.
3. Igidi JO, Nwabue FI, Omaka ON. Physicochemical studies of extracts from napoleonavogelii grown in ebonyi state as a source of new acid - Base indicators. Res J Eng Appl Sci 2012;1:96-101.
4. National Toxicology Program. NTP Toxicology and carcinogenesis studies of phenolphthalein (CAS No 77-09-8) in F344/N rats and B6C3F1 mice (feed studies). Natl Toxicol Program Tech Rep Ser 1996;465:1-354.
5. Pathade KS, Patil SB, Kondawar MS, Naikwade NS, Magdum CS. *Morus alba* fruit-herbal alternative to synthetic acid base indicators. Int J Chemtech Res 2009;1:549-51.
6. Bhagat VC, Patil RD, Channekar PR, Shetty SC, Akarte AS. Herbal indicators as a substituent to synthetic indicators. Int J Green Pharm 2008;2:162-3.
7. Abbas SK. Study of acid-base indicator property of flowers of *Ipomoea biloba*. Int Curr Pharm J 2012;1:420-2.
8. Khan PM, Hussain S, Mohsin M, Farooqui M, Zaheer A. *Bombax ceiba* flower extract: Biological screening and application as pH indicator. J Chem Biol Phys Sci 2012;2:43-8.
9. Bhise SH. Use of *Acalypha wilkesiana* Leaf Extract as Natural Indicator in Acid Base Titration. B Pharm Thesis; 2012. p. 21-2.
10. Adebayo IG, Alabi TO, Owoyele VB, Soladoye OA. Anti-diabetic properties of the aqueous leaf extract of *Bougainvillea glabra* on alloxan-induced diabetic rats. Rec Nat Prod 2009;3:187-92.
11. Chew S. Anatomical features of *Bougainvillea* (*Nyctaginaceae*). Stud Undergrad Res Guelph 2010;4:71-8.
12. Saifuddin M, Hossain AB, Normaniza O. Impacts of shading on flower formation and longevity, leaf chlorophyll and growth of *Bougainvillea glabra*. Asian J Plant Sci 2010;9:20-7.
13. El-Quesni F, Kandil M, Mahgoub M. Some studies on the effect of putrescine and paclobutrazol on the growth and chemical composition of *Bougainvillea glabra* L. at

Nubaria. Am Eur J Agric Environ Sci 2007;2:552-8.
14. Moneruzzaman KM, Hossain AB, Amru NB, Saifudin M, Imdadul H, Wirakarnain S. Effect of sucrose and kinetin on the quality and vase life of *Bougainvillea glabra* var. Elizabeth angus bracts at different temperatures. Aust J Crop Sci 2010;4:474-9.
15. Saikia H, Das S. Antidiabetic action of *Bougainvillea spectabilis* (leaves) in normal and alloxan induced diabetic albino rats. Indian Drugs 2009;46:391-7.
16. Narayanan CR, Joshi DD, Mujumdar AM, Dhekne VV. Pinitol - A new antidiabetic compound from the leaves of *Bougainvillea spectabilis*. Curr Sci 1987;56:139-41.
17. Narayanan CR, Joshi DD, Mujumdar AM. Hypoglycemic action of *Bougainvillea spectabilis* leaves. Curr Sci 1984;53:579-81.
18. Mishra N, Joshi S, Tandon VL, Munjal A. Evaluation of antifertility potential of aqueous extract of *Bougainvillea spectabilis* leaves in swiss albino mice. Int J Pharm Sci Drug Res 2009;1:19-23.
19. Umamaheswari A, Shreevidya R, Nuni A. *In vitro* antibacterial activity of *Bougainvillea spectabilis* leaves extracts. Adv Biol Res 2008;2:1-5.
20. Joshi DD, Mujumdar AM, Narayanan CR. Anti-inflammatory activity of *Bougainvillea spectabilis* leaves. Indian J Pharm Sci 1984;46:187-8.
21. Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battelli MG, *et al.* New ribosome-inactivating proteins with polynucleotide: Adenosine glycosidase and antiviral activities from *Basella rubra* L. and *Bougainvillea spectabilis* Willd. Planta 1997;203:422-9.
22. Ali MS, Ibrahim SA, Ahmed F, Pervez MK. Color versus bioactivity in the flowers of *Bougainvillea spectabilis* (*Nyctaginaceae*). Nat Prod Res 2005;19:1-5.
23. Morzyk-Ociepa B, Michalska D, Pietraszko A. Structures and vibrational spectra of indole carboxylic acids. Part I. Indole-2-carboxylic acid. J Mol Struct 2004;688:79-86.

Source of Support: Nil. **Conflict of Interest:** None declared.