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Original Article

Extract of *Euphorbia milii* Flower: A Natural Indicator in Acid-Base Titration

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ABSTRACT

Introduction: Synthetic indicators had continuously been the first choice for all types of acid-base titrations since long time. However, due to certain disadvantages like high cost, availability problems and environmental pollution, an attempt is required to replace synthetic indicators with natural indicators.

Methods: Methanolic extract of *Euphorbia milii* flowers from family *Euphorbiaceae* were evaluated in different types of titrations like strong acid (SA) *v/s* strong base (SB), weak base (WB) *v/s* strong acid (SA), and weak acid (WA) *v/s* strong base (SB).

Results: Natural indicators gives sharp and intense colour change at the neutralization point and shows promising results when tested against available synthetic acid-base indicators such as methyl orange and phenolphthalein.

Conclusion: This natural indicators could be an excellent replacement for synthetic indicators since they are economical, easily available, non-toxic, easy to extract, ecofriendly and accurate in all types of acid-base titrations.

Keywords: Flower extract; *Euphorbia milii*; Natural indicator; Acid-base titration.

INTRODUCTION

Euphorbia milii, native to Madagascar, is a class of flowering shrub belongs to spurge family *Euphorbiaceae* (Figure 1). The plant is upright branched shrub rising up to 5 to 6 feet height, with cylinder-shaped or indistinctly angled branches lined with rigid, thin, diversified spines. Leaves, pale green in color having size up to 5 cm long, are limited in numbers, alternative, oblong-obviate, or short acuminate. The flowers, variably red, pink or white having size up to 12 mm broad, are small, subtended by a pair of conspicuous petal-like bracts.[1] Inflorescences arise from the upper leaf axils and is peduncle with 2-4 involucres, each involves with two spreading red kidney-shaped lobes.

Euphorbia milii is widely used as medicinal plant. The various biological activities have been thoroughly reviewed.[2] The various extract of the plant and its parts have been found to possess antimicrobial,[3,4] molluscicidal,[5] antioxidant and antitumor,[6] and antinociceptive[7] activity. The available literature also confirms the presence of terpenoids, tannins and flavonoids in crude extracts of aerial parts of plant.[8] Phytochemical investigation of diffrent extracts of *E. milii* red

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flowers shows presence of triterpenoids like taraxerol and 28-hydroxyfriedelan-1,3-dione-29-oic acid, flavone (quercetin-3-*O*-(2"-*O*-galloyl)-α-L-arabinofuranoside) and phenolic compounds like 7,7'-dihydroxy-8,6'-bicoumarin and 9-acetyl-3',4'-dimethoxydehydroconiferyl-3-ol.[9]

Indicators are the organic substances mainly of synthetic origins which show sharp and intense color changes in certain intervals of pH and widely used to determine the end point in acid-base titration. They are usually weak acid or bases which generally exist in two tautomeric forms in which at least one is colored. Alternatively, they are dyes or pigments that are isolated from a variety of sources, including plants, flower petals, fungi and algae.[10] For the first time in year 1964, Sir Robert Boyle reported the use of natural dyes as acid-base indicators in his collection of essays Experimental History of Colors.[11]

Figure 1: *Euphorbia milii* plant and its flower

Synthetic indicators are very costly and many of them cause environmental pollution and have toxic effects.[10,12,13] Therefore, there has been an urgent need to identify alternative sources of indicators from natural origins. The intention for using these natural indicators as alternative to synthetic available indicators is its easy availability, eco-friendly and cost effectiveness.

Plant pigments in general are highly colored substances known as flavonoids. These include flavone, flavonol, isoflavanol, anthocyanin, anthocyanidin etc. Flavonoids together with anthocyanins confer a wide spectrum of color to flowers and fruits. Extraction of anthocyanin from red cabbage leaves to form an acid-base indicator is a popular chemistry experiment. Curcumin extracted from *Curcuma longa* is an example of natural indicator. Many researchers have reported the effectiveness of natural indicators extracted from various plants in acid-base titrations.[12-17] Other than these few reported cases, it seems that no work has been done on the suitability of methanolic floral extract of *Euphoria milii* as indicator in acid-base titration. Therefore, the rationale of this undertaken work was to examine the acid-base indicator activity of methanolic floral extract of *Euphoria milii*.

METHODOLOGY

Chemicals and reagents

Analytical grade chemicals like methanol, hydrochloric acid, sodium hydroxide, acetic acid, ammonia, methyl orange and phenolphthalein were obtained commercially from Loba Company and used without further purification. All the reagents and volumetric solutions were prepared according to the Indian Pharmacopoeia 1996.

Preparation of flower extract

Fresh flowers of *Euphoria milii* were collected from the local nursery of Vadodara city, Gujarat region. The fresh petals of flowers were washed with distilled water and then cut into small fragments and macerated in methyl alcohol (10 mL) for 24 h. The extract then collected and transferred in tightly closed glass container and stored away from direct sunlight for future use.

Experimental procedure

The whole experimental work was done using the same volumetric set of glass wares for titrations. The calibration of apparatus like burettes,

pipettes, and other required glasswares were carried out as per Indian Pharmacopoeia 1996. As the same aliquots were used for all titrations using synthetic and natural indicators, the reagent were not calibrated.

Approximately 10 mL of equinormal quantities of HCl or CH_3COOH was titrated with NaOH at room temperature using the natural indicator *Euphoria milii* floral extract in the order of strong acid (SA) *v/s* strong base (SB) and weak acid (WA) *v/s* strong base (SB) respectively, and then 10 mL of equinormal quantity of NH_4OH was also titrated against the HCl in the order of weak base (WB) *v/s* strong acid (SA). 3-4 drops of the natural indicator were added to each volume of acid used for the titration. Each titration was carried out three times using different strengths of 0.1, 0.5 and 1N of acid and alkali respectively. For comparison, the same procedure as described above was repeated using synthetic indicators such as methyl orange and phenolphthalein.

RESULTS AND DISCUSSION

The preliminary phytochemical investigation and qualitative chemical tests of the methanolic extract of flower was performed using $FeCl_3$, lead acetate, Shinoda tests etc. which confirmed the presence of phenolic compounds, flavonoids and tannins as reported in Table 1. As flower extract is pH-sensitive and shows color change with variation of pH, it was hypothesized that plant pigment could be used as an acid-base indicator in acidimetry- alkalimetry titration. The flavonoids might be responsible for the indicator activity of the extract for acid-base titration.

Titrations of strong acid *v/s* strong base (HCl *v/s* NaOH), strong acid *v/s* weak base (HCl *v/s* NH_4OH) and weak acid *v/s* strong base (CH_3COOH *v/s* NaOH) were carried out using standard indicators and floral extract. Organic compounds that can be used as an indicator in the titration has characteristics of changing color when the pH of the solution changes. The methanolic floral extract of *E. milli* was also sensitive to change in pH and shows a sharp pink color change at alkaline pH and light yellow color at acidic pH as shown in Figure 2. The results are recorded as mean ± SD and are presented in Table 2. In all titrations, natural indicator gave a sharp end point identical to those reproduced with standard indicators, methyl orange and phenolphthalein.

Table 1: Preliminary phytochemical screening of methanolic extract

Phytoconstituents	Result
Flavanoids	+ve
Carbohydrates	-ve
Tannins	+ve
Alkaloids	-ve
Coumarin	+ve
Terpenoids	+ve
Glycoside	-ve
Steroids	-ve

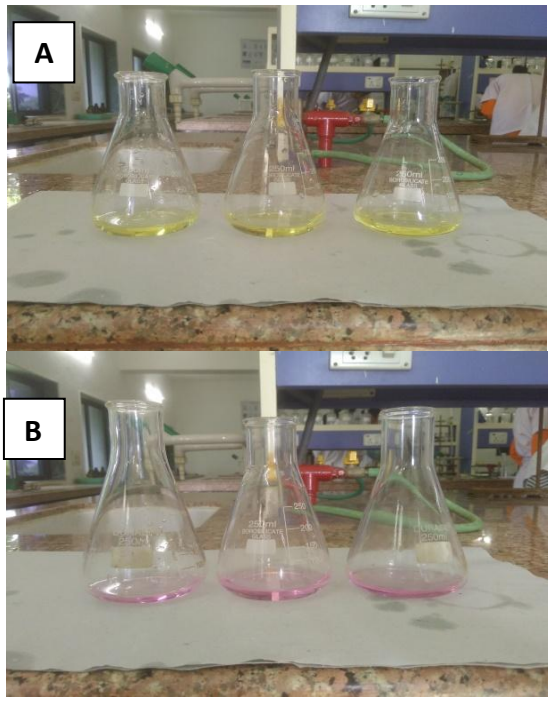

Figure 2: Color change at end point: (A) Strong or weak base *v/s* Strong acid; (B) Strong or weak acid *v/s* Strong base.

Table 2: Mean volume† at equivalent point for different type of titration

Strength	HCl *vs* NaOH		NH_4OH *vs* HCl		CH_3COOH *vs* NaOH	
	PP*	Floral extract	MO#	Floral extract	PP*	Floral extract
0.1 N	7.8 ± 0.20	7.7 ± 0.24	5.08 ± 0.08	5.14 ± 0.05	7.9 ± 0.20	7.8 ± 0.24
0.5 N	8.0 ± 0.40	7.9 ± 0.32	5.16 ± 0.11	5.04 ± 0.08	8.1 ± 0.24	8.2 ± 0.42
1.0 N	10.2 ± 0.3	10.0 ± 0.34	5.14 ± 0.11	5.08 ± 0.13	9.8 ± 0.30	9.9 ± 0.34

† Average of three reading along with standard deviation (SD); * PP = Phenolphthalein; # MO = Methyl Orange.

CONCLUSION

Euphoria milii flower extract shows sharp color changes at the end point due to the presence of flavonoids and could be used as an indicator in acid-base titration (strong acid *v/s* strong base, weak acid *v/s* strong base and weak base *v/s* strong acid). As the methanolic floral extract produces comparative results with standard synthetic indicators, it can be used with absolute accuracy and reliability for acid-base titration.

REFERENCES

1. Huxley A. The New RHS Dictionary of Gardening. 1 ed. London: Macmillan; 1992.
2. Shi QW, Su XH, Kiyota H. Chemical and pharmacological research of the plants in genus Euphorbia. Chemical reviews. 2008;108(10):4295-327.
3. Devanaboyina N, Mounisha A, Bhavani B, Sireesha G, Kamari V, Reddy MR. Antimicrobial studies on flowers of *Euphorbia Milii*. Der Pharmacia Lettre. 2015;3(7):196-204.
4. Murugan S, Anand R, Devi U. Efficacy of *Euphorbia milli* and *Euphorbia pulcherrima* on aflatoxin producing fungi (*Aspergillus flavus* and *Aspergillus parasiticus*). African Journal of Biotechnology. 2007;6(6):718-9.
5. Zani CL, Marston A, Hamburger M, Hostettmann K. Molluscicidal milliamines from *Euphorbia milii* var. *hislopii*. Phytochemistry. 1993;34(1):89-95.
6. Sreenika G, Naga SK, Lakshmi BVS, Thulja P, Sudhakar M. Antioxidant and antitumor activity of *Euphorbia milii* flower extract against in vivo breast cancer and colon cancer in mice. World Journal of Pharmacy and Pharmaceutical Science. 2015;4(6):912-34.
7. Rauf A, Muhammad N, Qaisar M, Uddin G, Hussain I. Preliminary antinociceptive studies of methanol extract of *Euphorbia milli*. Middle-East Journal of Medicinal Plants Research. 2012;1(3):68-70.
8. Pancorbo S, Hammer RH. Preliminary phytochemical investigation of *Euphorbia millii*. J Pharm Sci. 1972;61(6):954-7.
9. Kamurthy H, Dontha S. Phytochemical screening on *Euphorbia milii* red flowers - Isolation of terpenoids, flavone and phenols. American Journal of Ethnomedicine. 2015;2(6):322-32.
10. Okoduwa SIR, Mbora LO, Adu ME, Adeyi AA. 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. 2015;2015:1-6.
11. Boyle R. Experimental history of colours. The works of Robert Boyle. 1964;4:1-183.
12. Nwosu F, Adekola F, Ihedioha K. Simple titrimetric colour indicators from some natural flower petals. Centrepoint (Science Edition). 2004;12(1):74-89.
13. Agrawal S, Raj NR, Chouhan K, Raj CN, Jain S, Balasubramaniam A. Isolation of herbal acid-base indicator from the seeds of Punica granatum. Journal of Chemical and

Pharmaceutical Research. 2011;3(2):168-71.
14. Ekandem J, Owor A. Local flower extracts as simple acid-base indicators. J Chem Soc Nig. 1997;22:98-103.
15. Eze S, Okereafo A. Industrial and analytic potentials of dye extracts from *Telfaira occidentalis* (Fluted pumpkin). J Chem Soc Nig. 2002;27:143-6.
16. Bhagat VC, Patil RD, Channekar PR, Shetty SC, Akarte AS. Herbal indicators as a substituent to synthetic indicators. International Journal of Green Pharmacy. 2008;2(3).
17. Pimpodkar N, Shikalgar S, Shinde N, Bhise S, Surve B. *Rhoeo syathacea* and *Allamanda cathartic* extract as a natural indicator in acidometry-alkalimetry. Asian Journal of Pharmaceutical Analysis. 2014;4(2):82-4.