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PRELIMINARY PHARMACEUTICAL CHARACTERIZATION OF SOME FLOWERS AS NATURAL INDICATOR

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ABSTRACT

Indicators used in titrations show well-marked changes of color in certain intervals of pH. Most of these indicators are organic dyes and are of synthetic origins. The present study was designated to evaluate the extracted flower petals of some easily available plants. A comparative study of *Delonix regia* Raf. and *Caesalpina pulcherrima* Swartz Obs. flower extracts with synthetic indicator viz. phenolphthalein and methyl red were carried out to evaluate the accuracy of flower extracts as acid-base indicator. The results indicated that flower extracts of these plants can be used as acid-base indicator in titration of strong acid with strong base because similar results were obtained by phenolphthalein and methyl red. In case of weak acid and weak base titration, the results obtained by the flower extract coincide with the results obtained by mixed indicator. The rationale behind using these natural indicators in preference to synthetic indicators is its easy availability, inertness, ease of preparation and cost effectiveness.

Keywords: Natural indicator, Anthocyanins, Titration, TLC.

INTRODUCTION

Indicators are the substances whose solutions change colour due to changes in pH. These are called acid-base indicators. They are usually weak acids are bases, but their conjugate base or acid forms have different colours due to differences in their absorption spectra. They are also known as neutralization indicator [1].

Delonix regia Raf. (Leguminosae) is also known as *Poinciana regia*. It is strikingly ornamental medium-sized tree, planted in avenues and gardens throughout India. It has a spreading crown of feathery foliage falls and the branches are nearly bare. The flowers are with panicles, varying in colour from deep crimson through scarlet orange to delicate salmon, appear in profusion in broad erect clusters along the branches, presenting a gorgeous appearance [2]. The seeds of plant are considered a source of low cost protein for use as food or feed. Flower extract showed high toxicity to larvae and pupae of the polyphagous pest, *Pericallia ricini*[3].

Caesalpinia pulcherrima Swartz Obs. (Caesalpiniaceae) is a shrub or small tree, obtained from America and cultivated throughout India. Flowers are arranged in racemes, which are very broad, the lower pedicels are 7.5-10 cm long. Calyx are 1.3-1.6 cm and glabrous. Petals are round, crisped, reddish yellow, with a very distinct claw, stamens are much exerted and filaments are bright red, 3-4 times than the length of the corolla. The leaves, flowers and seeds are largely used in Indian medicine. It is considered as tonic and stimulant. An infusion of the flowers is pectoral and febrifuge. It is usually prescribed in bronchitis, asthma and malarial fevers[4].

Presence of color pigments was investigated as per IP and the tests are given in Table 1. Anthocyanins are characterized by Band-275-280 nm (UV region). Actual color of extract is depending on number and position of hydroxyl and methoxy group. When these are fixed, the color then depends upon the pH and solvent [5].

Hence the present vocation was attempted to appraise the flowers as a natural indicator.

Materials and Methods

Materials

Fresh flowers were collected from the local market of Rajkot region, Gujarat, and they were authenticated from NISCAIR, New Delhi, Ref No: NISCAIR/RHMD/Consult/2010-11/1468/66. All other ingredients were of analytical grade and purchased from Loba Chemie Pvt Ltd, Mumbai.

Method

The flowers were cleaned by distilled water and cut into small pieces and macerated for two hours in 25ml of 90% ethanol. The

extract was preserved in tight closed container and stored away from direct sun light[6].

The experiment was carried by using the same set of glassware's for all types of titrations. As the same aliquots were used for both titrations i.e. titrations by using standard indicators and flowers extract, the reagents were not calibrated. The equimolar titrations were performed using 10 ml of titrant with three drops of indicator. All the parameters for experiment are given in Table 1. A set of five experiments each for all the types of acid base titrations were carried out. The mean and standard deviation for each type of acid base titrations were calculated from results obtained. The extract was also analyses for its λmax in Ultra Violet range on Systronics single beam spectrophotometer (Shimadzu UV 1800).

Thin Layer Chromatography

Ethanolic extracts of flowers of *Delonix regia* Raf. and *Caesalpinia pulcherrima* Swartz Obs. were subjected to thin layer chromatography studies, to find the presence of Anthocyanins on support of chemical test[7].

RESULTS AND DISCUSSION

The extract was found to contain compound anthocyanins as it gives blue color to aqueous sodium hydroxide solution, yellow orange color to concentrated sulphuric acid while red color which fed out on standing with magnesium-hydrochloric acid solution Table 2.

TABLE 1: Standard chart for phytochemical identification

Phytochemicals	***Color with aq. NaOH***	***Color with Conc. H_2SO_4***	***Color with Mg-HCl***
Anthocyanins	Blue violet	Yellow to orange	Red (fades to pink)
Flavones	Yellow	Yellow to orange	Yellow to red
Flavonones	Yellow to orange (cold) Red to purple (hot)	Crimson Orange	Red, magenta, violet, blue
Isoflavones	Yellow	Yellow	Yellow
Leucoanthocyanins	Yellow	Crimson	Pink

TABLE 2: Technological characterization for analysis of chemical test

	Poly-Phenolic compound		***Flavonoid***	***Anthrocyanins***		
Sample	***Color with $FeCl_3$***	***Color with Lead acetate***	***Shinoda test***	***Color with aq. NaOH (Blue violet)***	***Color with Conc. H_2SO_4 (Yellow orange)***	***Color with Mg-HCl (Red)***
DRPI	+	+	+	+	+	+
CPPI	+	+	+	+	+	+

+; Presence of compound, DRPI: *Delonix regia*, CPPI: *Caesalpinia pulcherrima*

The flowers extract of *Delonix regia* Raf. and *Caesalpina pulcherrima* Swartz Obs. showed λmax in Ultra Violet region Table 3. The λmax suggested the presence of anthocyanins in the extract.

The flowers extract was screened for its use as an acid base indicator in various acid base titrations, and the results of this screening were compared with the results obtained by standard indicators methyl red, phenolphthalein and mixed indicator [methyl orange: bromocresol green (0.1:0.2) results are presented in Table 4[8].

TABLE 3: Determination of UV Visible absorption

Sample code	UV λmax
DRPI	319
CPPI	275

TABLE 4: Technological characterization for analysis and comparisons of color change.

Titrant	Titrate	Indicator Color Change		
		Standard (pH Range)	DRPI (pH Range)	CPPI (pH Range)
HCl	**NaOH**	Yellow- pink (12.55-4.87)	Green to colorless (13.03-0.70)	Yellow to colorless (12.66-1.04)
HCl	**NH_4OH**	Pink-Colorless (10.98-6.74)	Green to pink (11.77-0.76)	Yellow to colorless (11.09-1.61)
CH_3COOH	**NaOH**	Colorless-Pink (12.67-5.99)	Green to colorless (12.65-6.42)	Yellow to colorless (12.68-6.2)
CH_3COOH	**NH_4OH**	Orange-Green (4.73-2.86)	Green to pink (11.21-5.64)	Yellow to colorless (11.01-5.25)

HCl: Hydrochloric acid, CH_3COOH: Acetic Acid, NaOH: Sodium Hydroxide, NH_4OH: Ammonium Hydroxide, DRPI: *Delonix regia*, CPPI: *Cesaphenia pulcherima*

The titrations of strong acid with strong base (HCl & NaOH), strong acid with weak base (HCl & NH_4OH), weak acid with strong base (CH_3COOH & NaOH), and weak acid with weak base (CH_3COOH and NH_4OH) were carried out using standard indicators and flowers extract. The results of these titrations are given in Table 5, 6, 7 and 8. It could be due to these flavonoids, the sharp end point appeared in the above mentioned titrimetric analyses. The flowers extract of *Delonix regia* Raf. and *Caesalpina pulcherrima* Swartz Obs. were found to have Poly-Phenolic, flavonoids, anthocyanins and is pH sensitive. The end point determination of acid base titrations by the traditional indicators, compared with flowers extract indicator, it was observed that traditional indicators gave incorrect results due to addition of excess of titrant (base) after the neutralization reaction was completed, but flowers extract indicator has given sharp end

point because solutions give sharp color change at the equivalence points. Further an attempt has been made to separate the present compound by performing Thin layer chromatography. The solvent system was selected on the basic of the pigments i.e. Anthocyanins. The results are tabulated in the Table 8 and Figure 1 and 2.

TABLE 5: Technological characterization of acid-base titration using standard indicator

Titration (Titrant v/s Titrate)	Strength in moles	Indicator	Mean ±S.D.*	Color	pH
NaOH v/s HCl	0.1	MR	12.3±0.12	Yellow to pink	12.32-5.77
	0.5	MR	11.2±0.16	Yellow to pink	12.55-4.87
	1.0	MR	11.2±0.15	Yellow to pink	12.63-3.30
HCl v/s NH_4OH	0.1	PT	05.9±0.01	Pink to colorless	10.50-6.74
	0.5	PT	06.6±0.08	Pink to colorless	10.61-8.28
	1.0	PT	06.5±0.16	Pink to colorless	10.98-8.29
CH_3COOH v/s NaOH	0.1	MR	12.0±0.11	Yellow to light red	12.33-6.01
	0.5	MR	11.9±0.14	Yellow to light red	12.56-5.96
	1.0	MR	12.0±0.09	Yellow to light red	12.67-5.99
CH_3COOH v/s NH_4OH	0.1	MI	05.0±0.05	Orange to green	03.25-4.52
	0.5	MI	05.6±0.19	Orange to green	02.81-4.68
	1.0	MI	06.1±0.17	Orange to green	02.86-4.73

*All values are mean ± S.D. for n=3

HCl: Hydrochloric acid, CH_3COOH: Acetic Acid, NaOH: Sodium Hydroxide, NH_4OH: Ammonium Hydroxide, MR; Methyl Red, MI: Mixed Indicator, PT: Phenolphthalein.

TABLE 6: Technological characterization of acid-base titration using *Delonix regia* Raf. Flower as indicator

Titration (Titrant v/s Titrand)	Strength in moles	Indicator	Mean ±S.D.	Color	pH
NaOH v/s HCl	0.1	DRPI	12.1±0.13	Green to colorless	12.78-5.80
	0.5	DRPI	11.5±0.17	Green to colorless	13.05-2.04
	1.0	DRPI	05.6±0.13	Green to colorless	13.03-0.70
HCl v/s NH_4OH	0.1	DRPI	04.2±0.05	Green to pink	11.02-6.38
	0.5	DRPI	00.5±0.15	Green to pink	10.23-1.66
	1.0	DRPI	06.5±0.08	Green to pink	11.77-0.76
CH_3COOH v/s NaOH	0.1	DRPI	16.4±0.16	Green to colorless	6.67-6.63
	0.5	DRPI	11.8±0.18	Green to colorless	12.56-6.42
	1.0	DRPI	10.2±0.12	Green to colorless	12.65-6.77
CH_3COOH v/s NH_4OH	0.1	DRPI	04.6±0.04	Green to pink	11.12-5.69
	0.5	DRPI	00.8±0.07	Green to pink	06.6-6.60
	1.0	DRPI	06.0±0.03	Green to pink	11.21-5.64

*All values are mean ± S.D. for n=3

HCl: Hydrochloric acid, CH_3COOH: Acetic Acid, NaOH: Sodium Hydroxide, NH_4OH: Ammonium Hydroxide, DRPI: *Delonix regia*

TABLE 7: Technological characterization of acid-base titration using *Caesalpina pulcherrima* Swartz Obs. Flower as indicator

Titration (Titrant v/s Titrand)	Strength in moles	Indicator	Mean ±S.D.	Color	pH
NaOH v/s HCl	0.1	CPPI	14.1±0.12	Yellow to colorless	12.49-3.40
	0.5	CPPI	11.0±0.19	Yellow to colorless	12.32-1.96
	1.0	CPPI	10.7±0.15	Yellow to colorless	12.66-1.04
HCl v/s NH_4OH	0.1	CPPI	20.0±0.08	Yellow to colorless	10.36-2.03
	0.5	CPPI	13.6±0.05	Yellow to colorless	10.83-1.58
	1.0	CPPI	07.0±0.03	Yellow to colorless	11.09-1.61
CH_3COOH v/s NaOH	0.1	CPPI	12.1±0.13	Yellow to colorless	12.33-6.22
	0.5	CPPI	11.6±0.17	Yellow to colorless	12.65-6.22
	1.0	CPPI	11.3±0.08	Yellow to colorless	12.68-6.47
CH_3COOH v/s NH_4OH	0.1	CPPI	05.4±0.16	Yellow to colorless	09.78-7.15
	0.5	CPPI	09.4±0.05	Yellow to colorless	10.68-5.25
	1.0	CPPI	09.9±0.06	Yellow to colorless	11.01-5.30

*All values are mean ± S.D. for n=3
HCl: Hydrochloric acid, CH_3COOH: Acetic Acid, NaOH: Sodium Hydroxide, NH_4OH: Ammonium Hydroxide, CPPI: *Cesaphenia pulcherima*

TABLE 8: Thin Layer Chromatography of ethanolic extracts of *Delonix regia* Raf. and *Caesalpinia pulcherrima* Swartz Obs.

Sr. no	Extracts	Solvent system	Spraying agent	Number of spot	R_f Value	Color
1	Ethanolic of *Delonix regia* Raf.	Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)	Anisaldehyde-sulphuric acid	1	0.23	Pink
2	Ethanolic of *Delonix regia* Raf.	n-Butanol: glacial acetic acid: water (40:10:20)	Anisaldehyde-sulphuric acid	1	0.35	Blue-Violet
3	Ethanolic of *Caesalpinia pulcherrima* Swartz Obs.	Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)	Anisaldehyde-sulphuric acid	1	0.22	Pink
4	Ethanolic of *Caesalpinia pulcherrima* Swartz Obs.	n-Butanol: glacial acetic acid: water (40:10:20)	Anisaldehyde-sulphuric acid	1	0.32	Blue-Violet

Figure 1: TLC of ethanolic extract of *D. regia* and *C. pulcherrima* in Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26)

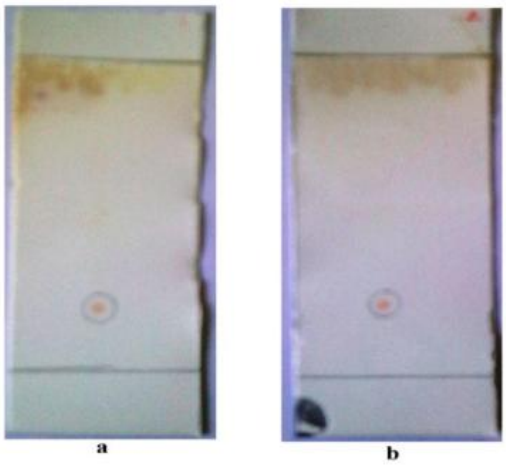
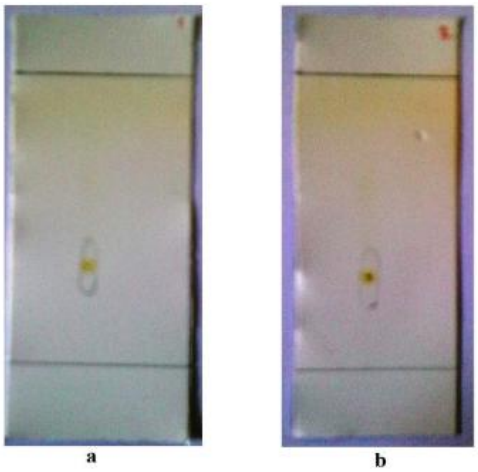


Figure 2: TLC of ethanolic extract of *D. regia* and *C. pulcherrima* in n-Butanol: glacial acetic acid: water (40:10:20)



The ethanolic extract of *Delonix regia* Raf in both the solvent system [Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26) and n-Butanol: glacial acetic acid: water (40:10:20)] showed single spot at R_f at 0.23 and 0.35, *Caesalpina pulcherrima* Swartz Obs. also showed single spot at R_f 0.22 and 0.32.

Thus natural indicator employed in the acid base titrations was found economic, safe and an efficient alternative for traditional indicators. In comparison to this, chemical indicators were found more expensive and hazardous, which proves that flowers extract of *Delonix regia* Raf. and *Caesalpina pulcherrima* Swartz Obs. as a natural indicator is more worthy.

CONCLUSION

The results obtained in all the types of acid-base titrations lead us to conclude that, it was due to the presence of flavonoids sharp color changes occurred at end point of the titrations. We can also conclude that, it is always beneficial to use *Delonix regia* Raf. and *Caesalpina pulcherrima* Swartz Obs. flowers extract as an indicator in all types of acid base titrations because of its economy, simplicity and wild availability.

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