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Original Article

Green Chemistry: A study on acid-base indicator property of various flower pigments

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ABSTRACT: Green Chemistry is considered as a significant tool to mitigate the use of hazardous chemicals. It encourages innovation and promotes the creation of products that are environmentally and economically sustainable. Synthetic chemicals which are used as internal indicators in acid- base titrations being hazardous can be substituted by using the natural indicators which gives results with the same accuracy. The accuracy of the observed results has been examined by performing titration between different acids and bases. Results supported this by less variation in the mean value and titre value from that of synthetic indicators. Thus, the use of natural indicators in the acid base titrations is statistically proved. The natural indicator prepared from flower petals is neither harmful to the environment nor it causes any health hazard. Therefore, in this work the use of natural indicator like petal extract is concluded more economical, simple, harmless, pollution free and inert.

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INTRODUCTION

Biological pigments, simply known as pigments or biochromes are produced by living organisms which include plant pigments and flower pigments [1]. The primary function of pigments in plants is photosynthesis, which uses the green pigment chlorophyll along with several red and yellow pigments that help to capture as much light energy as possible. Other functions of pigments include attracting insects to flowers to encourage pollination [2-4].

Suffering from the rapid depletion of the natural resources, the present world scenario calls for the need of sustainable development so as to obtain eco-friendly environment. Chemicals are one among the various things that are extremely dangerous but still their use cannot be avoided. Green Chemistry thus emerges as a significant tool to mitigate the use of hazardous chemicals. It encourages innovation and promotes the creation of products that are environmentally and economically sustainable [5].

Synthetic chemicals which are used as internal indicators in acid-base titrations being hazardous can be substituted by using the natural indicators which gives results with the same accuracy. Natural indicators are easy to prepare and are easily available [6]. Volumetric titrations between acid and base show sharp colour change at the equivalence point. Such natural indicators also satisfy the principles laid down by Green Chemistry.

Flower petals are the substitute for such hazardous internal indicators. Flowers are the miracle of the nature, by God [7]. Biochemists have developed a variety of methods for the purification and analysis of bio molecules. Several of these techniques will be used in this laboratory exercise in order to isolate and study the photosynthetic pigments, chlorophyll a, chlorophyll b, anthocyanins and carotenoids [8-10]. These include paper chromatography, TLC, spectrophotometry and other analytical techniques. Chromatography is an analytical or preparative technique used to separate, identify and determine the composition of the test substance or mixture of chemicals.

The undissociated molecules of phenolphthalein are colourless while Ph- ions are pink in colour. In presence of an acid, the ionisation of HPh is practically negligible as the equilibrium shifts to left hand side due to high concentration of H+ ions. Thus, the solution would remain colourless [11]. The undissociated molecules of phenolphthalein are colourless while Ph- ions are pink in colour. In presence of an acid the ionisation of HPh is practically negligible as the equilibrium shifts to left hand side due to high concentration of H+ ions. Thus, the solution would remain colourless [12].

An indicator is a substance which is used to determine the end point in a titration. In acid-base titrations, organic substances (weak acids or weak bases) are generally used as indicators. A pH indicator is a halo chromic chemical compound that is added in small amounts to a solution so that the pH (acidity or alkalinity) of the solution can be determined easily. Commercial/synthetic indicators are expensive and some of them have toxic effects on users and can also cause environmental pollution. For these reasons there has been an increasing interest in searching for alternative sources of indicators from natural origins in green chemistry [13]. These alternatives would be cheaper, more available, simple to extract, less toxic to users and environmentally friendly. Almost any flowers such as blue, purple or red in colour contain a class of organic pigment called 'Anthocyanins' that change colour with pH [14]. In this present study, we studied the effectiveness of indicators extracted from natural sources. In this work the separation of flower pigments and identification by using chromatographic techniques has been proposed. By means of titrimetric analysis the equivalence point can be determined by the change in PH from which the acid base indicator property of the flower extracts can be elucidated. The main objective is to observe the indicator property of various same colored flower petals of different species from which natural indicators can be synthesized which are more effective, ecofriendly, economical& less toxic when compared to synthetic indicators.

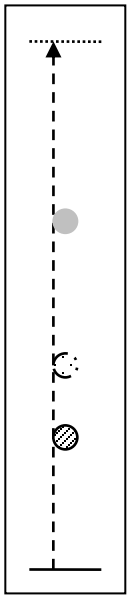
MATERIALS: Three different flowers like *Nerium indicum* L., *Ixora coccinea* Linn. *Rosa spps.*, were collected in the month of febraury from the area surrounding Tirupati, India. These flowers were washed thoroughly, shade dried, crushed to fine powder and stored in air tight bottles. The chemicals such as methanol, sodium carbonate, NaOH and silica gel are procured from Tiny Pharma Pvt. Ltd., acetic acid, methyl orange Indicator, phenolphthalein and ammonia are received from SD Fine chemicals Pvt. Ltd., 1M HCL is from Merck laboratories Pvt. Ltd., and butanol is procured from Hi media Laboratories Pvt. Ltd.,

METHODS:

PREPARATION OF EXTRACT: Extract is prepared by grinding the flower petals with pestle in a mortar by adding water and methanol if necessary.

PHYTOCHEMICAL ANALYSIS: The crude powder of different flowers was subjected to qualitative phytochemical analysis.

PAPER CHROMATOGRAPHY: Chromatography paper is made into strips following dimensions suggested by instructor. A fine pencil line across the strip about 2-3 cm is drawn from one end - this is the "origin". Papers are handled by the edges, taking care to touch them as little as possible - oils from fingertips can interfere with the migration of pigments up the paper [15].



the "solvent front" is the position of the liquid solvent on the chromatography paper at any given time. The solvent will gradually move from the bottom toward the top of the paper, carrying dissolved pigments with it. Stop the chromatogram **before** the solvent front reaches the top of the paper and mark the location. This

mark the location of each pigment **at the time** the chromatogram is stopped

origin (where pigment extract is applied)

Flower petals are added with a small amount of ethanol into a mortar and grinded completely with a pestle to release the pigments into solution. Continued adding ethanol (or flower petals) as necessary and got few millilitres of very dark concentrated liquid extract [16]. Dipped a capillary tube into the liquid portion of extract (Extract may contain fragments of flower petals which will clog the capillary tube. To minimize this, tilt the mortar slightly to allow the liquid fraction to run away from the solids). Allowed the extract to migrate up the capillary tube, which then moved out of the capillary tube onto paper fibers where it is absorbed. The extract is dried completely on the paper, and then repeated with another load of extract. In order to concentrate the pigments on paper, several loads of extract (probably 4 - 6) were applied. After each loading, paper is fully dried before applying the next load. Depending on the type of sample taken and the amount of water in it, it may take several minutes for the sample to be dried (2 - 10 minutes). Paper strip was placed into the solvent container provided with the origin end down. Level of the solvent is maintained below the origin on paper chromatogram and then, the chromatogram was frequently checked to observe the movement of solvent and pigment up the chromatography paper [17]. When the solvent "front" is within 2-3 cm from the top of the paper, remove the chromatogram and use a pencil to mark the location of the solvent front. The R_F value for each pigment in each chromatography solvent is calculated and the data is recorded,

$$Rf = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

THIN LAYER CHROMATOGRAPHY: A flower extract containing mixture of pigments was spotted onto a TLC plate at the "origin". The TLC plate was then placed into a container of solvent. As the solvent front moved up the plate, the pigments moved at different rates. The developed plates were dried in a hot air oven [18].

TITRATIONS: 0.1 ml of the extract was added as an indicator for each titration type:

- Strong acid-strong base (HCl versus NaOH),
- strong acid-weak base (HCl versus $NaHCO_3$),
- weak acid-strong base (CH_3COOH versus NaOH) and
- weak acid-weak base (CH_3COOH versus $NaHCO_3$) of 1M, 2M and 3M respectively.

The trials were repeated 3 times to check the precision. The titrations were again performed by using phenolphthalein indicator as standard and the results obtained were compared with the results of titrations using the flower extract indicator. The end points of the titrations using the extract were reached when colour changed from yellow to light pink [19-22].

RESULTS AND DISCUSSION: In the present work, the dried flower powders of three plants were evaluated for the presence of flavonoids, tannins, phlobatannins, saponins, steroids, cardiac glycosides, triterpenes and alkaloids.

In this phyto-chemical screening of the flowers by performing various chemical tests it has been identified that the powdered sample of *Nerium indicum* shows the positive result for the presence of flavonoids, tannins, glycosides, alkaloids. In case of *Rosa centifolia*, it shows the presence of flavonoids, tannins, triterpenoids, whereas flavonoids, triterpenoids were present in *Ixora coccenia*.

Table 1: Qualitative phytochemical analysis of flower powder

TEST	*Neriun indicum*	*Rosa centifolia*	*Ixora coccinea*
Flavonoids	++	++	++
Tannins	++	++	-
Saponins	-	-	-
Cardiac glycosides	+	-	-
Triterpenoids	-	+	+ +
Alkaloids	++		
a) Mayer' reagent	+	-	-
b) Dragondorff's reagent	+	-	-
c) Wagner's reagent	+	_	_

CHROMATOGRAPHIC ANALYSIS:

***NERIUM INDICUM*:**

Paper: Whattmann filter paper 40

Mobile phase: Butanol, Acetic acid (15:85)

R_F VALUE CALCULATION:

$$R_F = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

ROSA CENTIFOLIA

Paper: whattmann filter paper 40

Mobile phase: Butanol, Acetic acid (15:85)

R_F VALUE CALCULATION:

$$R_F = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

IXORA COCCINEA

Paper: whattmann filter paper 40

Mobile phase: Butanol, Acetic acid (15:85)

R_F VALUE CALCULATION:

$$R_F = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

By this chromatographic study, the presence of flower pigments can be identified and the isolation of various pigments can be found out by comparing with the standard R_f values. In this present work, the experimental values obtained from paper and thin layer chromatogram for various flower extractions NPE, RPE, IPE are compared with standard RF values which coincides with the pigments namely anthocyanins. The Rf values of paper chromatography for NPE, RPE, IPE are 0.62, 0.58, 0.56 and the results of TLC are 0.62, 0.60, 0.61. The standard Rf values of anthocyanin ranges from 0.32-0.62. Here, the experimental values are near to the standard values. So, the presence of pigments anthocyanins is confirmed which are responsible for the colour in flowers and have the indicator property.

Table 2: Experimental R_F values of Nerium *Indicum*

FLOWER	DISTANCE TRAVELLED	TOTAL DISTANCE	TEST R_F VALUE
Nerium indicum			
a) Paper chromatography	3.8	6.1	0.62
b) TLC	3.6	5.8	0.62

Table 3: Experimental R_F values of *Rosa centifolia*

FLOWER	DISTANCE TRAVELLED	TOTAL DISTANCE	TEST R_F VALUE
Rosa centifolia			
a) Paper chromatography	3.4	6.0	0.58
b)TLC	3.5	5.8	0.60

Table 4: Experimental R_F values of *Ixora coccinea*

FLOWER	DISTANCE TRAVELLED	TOTAL DISTANCE	TEST R_F VALUE
Ixora coccinea			
a) Paper chromatography	3.7	6.3	0.58
b) TLC	3.2	5.2	0.61

Table 5: STANDARD Rf VALUES OF FLOWER PIGMENTS

PIGMENTS	STANDARD R_FVALUES
Carotene	0.98
Chlorophyll a	0.59
Chlorophyll b	0.42
Phophytin	0.81
Xanthophyll 1	0.28
Xanthophyll 2	0.15
Anthocyanins	0.32 – 0.62

Table 6: COMPARISION OF TEST R_F VALUES WITH STANDARD R_F VALUES

Name of the Flower	TEST R_F VALUES		Standard Rf value
	Paper Chromatography	TLC	
Nerium *indicum*	0.62	0.62	0.32-0.62
Rosa	0.58	0.60	
Ixora *coccenia*	0.56	0.61	

TITRIMETRIC ANALYSIS: 0.1ml of the extract was added as an indicator for each titration type. Strong acid-strong base (HCl versus NaOH), strong acid-weak base (HCl versus $NaHCO_3$), weak acid-strong base (CH_3COOH versus NaOH) of 1M. The trials were repeated 3 times to check the precision. The titrations were again performed using phenolphthalein indicator as standard and the results obtained were compared with the results of titrations using the flower extract indicator. The end points of the titrations using the extract were reached when colour changed from yellow to light pink.

- Strong acid-strong base (HCl versus NaOH/ Na_2CO_3),
- strong acid-weak base (HCl versus $NaHCO_3$),
- weak acid-strong base (CH_3COOH versus NaOH) and of 1M.

The trials were repeated 3 times to check the precision. The titrations were again performed using phenolphthalein/methyl red indicator. The values obtained for each type of titration are noted and mean was calculated which can be used for the comparison of titre values obtained from titrations done by using natural extraction indicators. The titre values obtained for SA-SB were 5.22, for WA-SB the mean value is 4.80 and for SA-WB is about 4.42, these results are obtained by using synthetic indicator methyl red & phenopthalein.

Strong acid v/s strong base: 1N sodium carbonate is taken and titrated with a strong acid HCl by using a natural indicator. The equivalence point can be determined by the change in colour due to PH variation

Strong acid: Hydrochloric Acid (Hcl)

Strong base: Sodium Carbonate **(**Na_2CO_3)

Synthetic indicator: Methyl Red /Methyl Orange/Phenopthalein

Natural indicator: NIE, RPE, IPE indicators.

Weak acid v/s strong base: 1N CH_3COOH is taken and titrated with a strong base NaOH by using a natural indicator. The equivalence point can be determined by the change in colour due to PH variation

Weak acid: Acetic Acid

Strong base: Sodium Hydroxide

Synthetic indicator: Phenopthalein

Natural indicator: NPE, RPE, IPE.

Strong acid v/s weak base: An accurately weighed sample was dissolved in water then titrated with a weak base NH_3 by using a natural indicator. The equivalence point can be determined by the change in colour due to pH variation.

Strong acid: Hydrochloric acid

Weak base: Ammonia

Synthetic indicator: Methyl red/phenopthalein

Natural indicator: NIE, RPE, IPE

Table 7: Titration of SA-SB, WA-SB, SA-WB using synthetic indicator.

S. NO.	CONTENT OF THE FLASK	BURETTE READINGS		INDICATOR	END POINT	MEAN
		Initial (ml)	Final (ml)			
1	Strong acid Vs strong base	0 ml 0 ml 0 ml 0 ml	5.3 5.2 5.1 5.3	Methyl red/ Phenopthalein	Yellow to pink	5.22
2	Weak acid Vs strong base	0 ml 0 ml 0 ml 0 ml	4.4 4.5 4.5 4.3	Phenopthalein	Yellow to pink	4.80
3	Strong acid Vs weak base	0 ml 0 ml 0 ml 0 ml	4.9 4.8 4.8 4.7	Phenopthalein	Yellow to pink	4.42

Table 8: Titration of HCl against Na_2CO_3 using NIE, RPE, and IPE as indicator

S. NO.	CONTENT OF THE FLASK	BURETTE READINGS		INDICATOR	END POINT	MEAN
		Initial (ml)	Final (ml)			
1	0.25g Na_2CO_3 + HCl	0 ml 0 ml 0 ml 0 ml	5.5 5.3 5.2 5.2	*NERIUM INDICUM* EXTRACT (NIE)	Yellow to pink	5.3
2.	0.25g Na_2CO_3 + HCl	0 ml 0 ml 0 ml 0 ml	5.3 5.0 5.0 5.1	ROSE PETALS EXTRACT (RPE)	Greenish Yellow to pink	5.1
3.	0.25g Na_2CO_3 + HCl	0 ml 0 ml 0 ml 0 ml	5.3 5.2 5.0 5.4	*IXORA* PETALS EXTRACT (IPE)	Yellow to pale pink	5.2

Table 9: Titration of CH_3COOH against NaOH using NIE, RPE, and IPE as indicator

S. NO.	CONTENT OF THE FLASK	BURETTE READINGS		INDICATOR	END POINT	MEAN
		Initial (ml)	Final (ml)			
1	0.25g NaOH + CH_3COOH	0 ml 0 ml 0 ml 0 ml	4.7 4.6 4.6 4.5	*NERIUM INDICUM* EXTRACT (NIE)	yellow to faint pink	4.6
2	0.25g NaOH + CH_3COOH	0 ml 0 ml 0 ml 0 ml	4.4 4.6 4.5 4.6	ROSE PETALS EXTRACT (RPE)	Colour less to faint pink	4.5
3	0.25g NaOH + CH_3COOH	0 ml 0 ml 0 ml 0 ml	4.9 4.8 4.8 4.7	*IXORA* PETALS EXTRACT (IPE)	yellow to faint pink	4.80

Table 10: Titration of NH_3 against HCL using NIE, RPE, and IPE as indicator

S. NO.	CONTENT OF THE FLASK	BURETTE READINGS		INDICATOR	END POINT	MEAN
		Initial (ml)	Final (ml)			
1	0.25g NH_3 + Cl	0 ml 0 ml 0 ml 0 ml	4.0 4.3 4.3 4.2	*NERIUM INDICUM* EXTRACT (NIE)	Yellow to pink	4.2
2	0.25g NH_3 + HCl	0 ml 0 ml 0 ml 0 ml	4.2 4.4 4.5 4.4	ROSE PETALS EXTRACT (RPE)	Yellow to pink	4.3
3	0.25g NH_3 + HCl	0 ml 0 ml 0 ml 0 ml	4.4 4.3 4.3 4.2	IXORA PETALS EXTRACT (IPE)	Greenish Yellow to pink	4.3

Table 11: Comparison of Experimental Results with the Standard Titre Value

S. NO.	TYPE OF TITRATION	STD INDICATOR	*NERIUM INDICUM* EXTRACT (NIE)	ROSE PETALS EXTRACT (RPE)	*IXORA* PETALS EXTRACT (IPE)
1.	**SA Vs SB**	**5.2**	**5.3**	**5.1**	**5.2**
2	**WA Vs SB**	**4.8**	**4.6**	**4.6**	**4.8**
3	**SA Vs WB**	**4.4**	**4.2**	**4.3**	**4.3**

Figure 1: PAPER & THIN LAYER CHROMATOGRAM OF *NERIUM INDICUM*

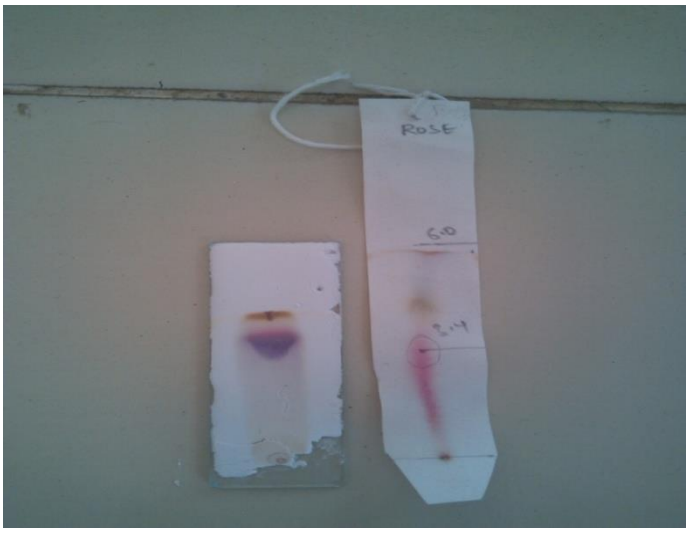


Figure 2: TLC & PAPER CHROMATOGRAM OF *ROSA CENTIFOLIA*

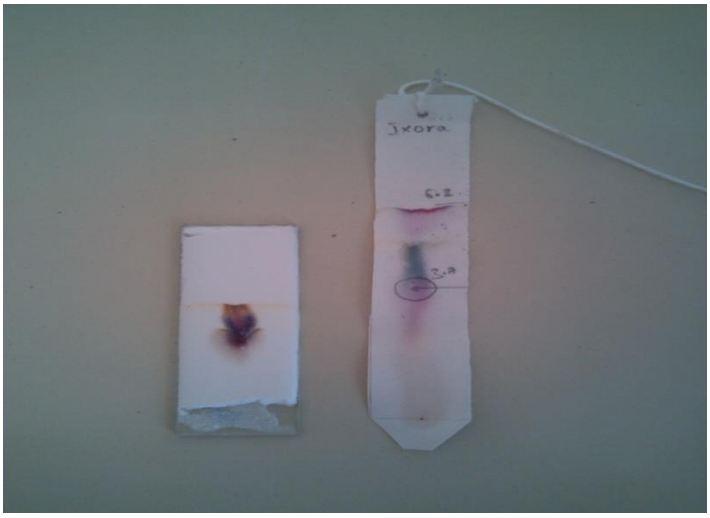


Figure 3: TLC & PAPER CHROMATOGRAM OF *IXORA COCCINEA*

(a) NIE

(b) RPE

(c) IPE

Figure 4: End Point Detection of S.A Vs S.B (HCl – Na_2CO_3)

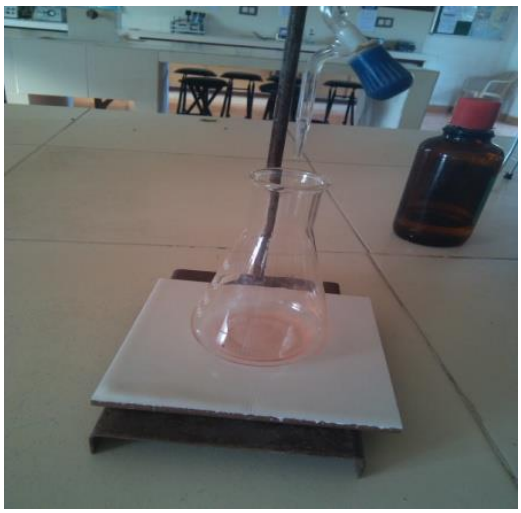
(A) NIE

(A) NPE

(B) RPE

(B) RPE

(C) IPE



(C) IPE

Figure 5: End Point Detection of W.A Vs S.B (CH_3COOH – NaOH)

Figure 6: End Point Detection of S.A Vs W.B (HCl – NH_3)

The extract from *Nerium indicum, rosa centifolia, ixora coccenia* flower was tested for its use as an acid-base indicator and the results was compared with that obtained using phenolphthalein for strong acid-strong base (HCl and NaOH), strong acid-weak base (HCl and $NaHCO_3$), weak acid-strong base (CH_3COOH and NaOH) titrations. The equivalence point of the titrations using flower extract is almost close or coincides with that of synthetic indicators for all the titrations .The titer values obtained by using natural indicator are for Ner NIE were SA Vs SB-5.3, WA Vs SB-4.6, SA Vs WB-4.2, for RPE SA Vs SB-5.3, WA Vs SB-4.6, SA Vs WB-4.2, for IPE SA Vs SB-5.2, WA Vs SB-4.8, SA Vs WB-4.3. By comparing the standard titre value mean ranges with the experimental mean values of the natural indicators. The equivalence point of the titrations using the flower extract either coincided or almost reached close to the equivalence point using the standard indicator, phenolphthalein/methyl red for all the titrations. In several cases it proved to be more reliable than the standard indicator and gave sharp colour change at equivalence point, however there was slight difference in the result as compared with the one obtained in. This show usefulness of flower extracts as an indicator in acid- base titration and IPE use in weak acid-strong base and strong acid-strong base was found to be more significant over standard indicator as it gives sharp color change. When compared to the standard indicator the NPE& RPE indicator are showing slight variation in the mean value which can be negligible, whereas IPE indicator coincides with the standard titre value. By this present study it can be proved that the natural indicators NPE, RPE, IPE can be used in place of standard synthetic indicators phenolpthalein and methyl red for acid base titration in both alkali metric and acidimetric titration.

CONCLUSION: The synthetic indicators like phenolphthalein, methyl orange and phenol red are not only hazardous to health but are also prominent pollutants. The fundamentals of Green Chemistry prove that these unsafe chemicals can be substituted by the petal extract as an indicator for acid base titration [23]. The accuracy of the observed results has been examined by performing titration between different acids and bases. The results are also supported by less variation in the mean value and titre value from that of synthetic indicators. Thus, the use of natural indicators in the acid base titrations is statistically proved. The natural indicator prepared from *Nerium indicum, Rosa centifolia, Ixora coccinea* flower petal is neither harmful to the environment nor it causes any health hazard. This methanolic extract is equally effective as the other synthetic indicator and provides reliable and accurate results. Therefore, the use of natural indicator like petal extract is more economical, simple, harmless, pollution free and inert. So by this work it can be concluded that the proposed herbal indicators can be used as a substitute to synthetic indicators.

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