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STUDY OF FLORAL ETHANOLIC EXTRACT OF *THUNBERGIA LAURIFOLIA* AS NATURAL ACID-BASE INDICATOR IN TITRIMETRIC ANALYSIS

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ABSTRACT

An indicator is an important component in any titrimetric analysis because it gives a colour change at the end of the reaction, thus helping out to determine the end point. Most indicators used in laboratory are synthetic one i.e. they need to be synthesized by chemical reaction. Here we have simply used ethanolic floral extract of *Thunbergia laurifolia* i.e. no synthesis required. The filtered extract was used as alternative for acid-base indicators such as phenolphthalein. When burette reading of phenolphthalein was compared with floral extract graphically, it was similar. Thus the alcoholic floral extract of *Thunbergia laurifolia* can be used as a replacement for phenolphthalein indicator because it is cost effective and does not require chemical synthesis.

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INTRODUCTION

The basic aim of this study was to use flowers i.e. natural source as alternative acid-base indicator. Anthocyanins present in flowers are the basic chemical moieties responsible for colour change during change in pH and thus behaving as acid-base indicator.[6] Most indicators used in laboratory are synthetic one i.e. they need to be synthesized by chemical reaction. Consider example of phenolphthalein indicator which is prepared by heating pthalic anhydride and phenol. To synthesize phenolphthalein, pthalic anhydride with two equivalent of phenol is heated at 115-120 ^{0}C for about 40 hours. Heating at such temperature for 40 hours will obviously require a good amount of energy.[16] In this research we have used floral extract which is very easy to prepare and does not require any chemical synthesis. So, Instead of doing chemical synthesis, using floral extract as indicator is a good option. The study will be incomplete without understanding of flavonoid and anthocyanins because anthocyanins (class of flavonoids) are pH sensitive components present in flowers which are responsible for colour change when pH changes.[4,5]

Polyphenol constitute largest group of phyto-chemical distributed amongst plant kingdom. Polyphenols covers flavonoids, non-flavonoid and phenolic acids. Non-flavonoid polyphenols are hydrolysable tannins while phenolic acids are hydroxyl cinnamates and hydroxyl benzoate. [4]

Polyphenolic compounds are present in number of plants. Chemically they contain benzene with various hydroxyl groups. Due to the presence of more polar hydroxyl groups (-OH) they possess some water solubility. Many phenolic compounds in plants often glycosylated i.e. they exist as glycosides which causes good water solubility. The chemical classes which fall under the polyphenols include phenylpropanoids, flavonoid, Anthocyanin's, xanthones, tannins, etc. [5]

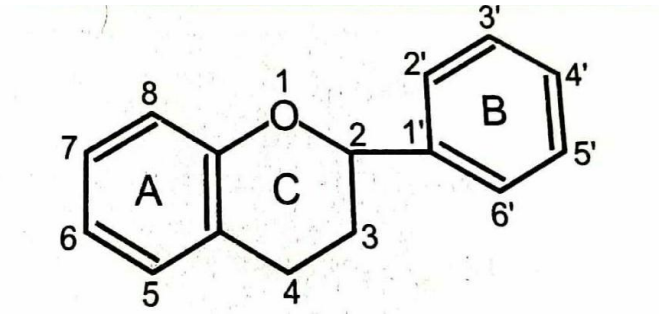


Fig-1: Flavonoid (basic backbone).[4]

Flavonoids:

Chemically, flavonoid a backbone of 15 carbon skeleton compound, which C6-C3-C6. Two phenyl rings are present out of which one is fused with a heterocyclic ring. The variation in the oxidation state of fused heterocyclic ring C decides the basic property and class into which the flavonoid belongs. The above structure is also known as flavonoid di-phenyl propane skeleton. Different compounds contain varying amount of hydroxyl (OH) groups in any rings. Flavonoids normally exist in plants as glycosides where one or more phenolic groups are combine with sugar residues. [4,5]

As flavonoids occurs many times as glycosides, the analysis of the aglycone moiety should be given more important because a single parent aglycone moiety can form number of glycosylated compounds. The sugars be easily separated by treating with dilute acids such as HCl. The acid causes catalytic hydrolysis of glucosidal flavonoids to liberate aglycone (parent flavonoid moiety) and sugar. [5]

Anthocyanins:

The major pigments that cause color of flower are carotenoids, flavonoids (anthocyanins) and betalains. Othere pigments that can rarely produce flower color are chlorophyll, phenylpehnalenones and quinochacone(Davies 2004).Different types of anthocyanins are responsible for different colours of flowers such as pink, red, orange, scarlet, purple, blue, etc. The anthocyanins is derived from 2 Greek words antos: flower and kyanos: blue. [6]

Although anthocyanins are the major coloring component of petals, that are also present in other plant tissues such as roots, tubers, stems, bulbils and are found in various gymnosperms, ferns and some bryophytes. [7]

Chemistry of Anthocyanins:

Anthocyanins are glycoside which is composed of anthocyanidin (aglycone), sugar(s) and some cases acyl group. When anthanthocyanare dissolved in water, a series of secondary structures are formed from the flavyllium cation according to different acid-base reaction and tautomerism. More than 30 anthocyanidins have been discovered but around 90% of all the anthocyanins are based on only 6 anthocyanidins viz. pelargonidin (18%), cyaniding (30%), peonidin, delphinidin (2%) petunidin and malvidin. Among 539 anthocyanins or anthocyanidins that have been identified, 97% are glycosylated. The 3-deoxyanthocyanidins, sphagnorubins and rosacyanin B are the only anthocyanidins found in their non-glycosylated form in the plants. Nearly all the reports on anthocyanins specifying D or L configuration of the anthocyanin sugar moieties (monosaccharides), lack experimental evidence for this type.[7]

Plant profile

Fig-2: *Thunbergia laurifolia* flowers.

Common names:

Laurel Clockvine, Blue trumpet vine, Kar tuau(Malaysia), Rang jeud/chuet.[10] Laurel leaved clockvine, Laurel leaved Thunbergia, Purple Allamanda.[13] In Thailand, the plant is known as Rang jeud or Rang chuet, Yaw kaew, Kob Sha Nang, Gum Lung Chang Puak, Krua Nan Nae.[3]

Scientific name:

Thunbergia laurifolia belonging to family Acanthaceae (or Thunbergiaceae).

Description:

The plant is a climber as well as creeper.[11,12] The leaves of Thunbergia laurifolia are elliptical or oval shaped, dark green, opposite with a pointed tip and slightly serrated leaf margin.[1] The leaves are having the length of 7-18 cm, 2.5-6 cm wide. The leaves grows in opposite direction along the stem.[2]

The flowers are trumpet-shaped with a short broad tube. This tube is white from outside and yellow from inside.[18,28] The tube of flowers is about 3.5-4.5 cm long. Flowers can be upto 8 cm long and 6-8 cm wide. Thu number of petals are 5-7 with one larger than others. The coloured varieties of flowers are available viz. white, yellow, or purple.[19] The flowers are hermaphrodite (bisexual). The plant flowers in the morning and aborting in the evening, almost continuously throughout the year. The flowers do not have any fragrance. Carpenter bees visits the flower frequently, creeping for pollen and nectar. Black ants are also present as nectar scavengers. [1,9]

The capsules of the plant can be up to 3 cm long and 1.5 cm wide (Stone 1970).[12]

The plant should not be confused with its brother species *Thunbergia grandiflora* (Bengal clockvine). The flowers of both the species looks similar. The leaves of *Thunbergia laurifolia* are broadly elliptical to narrowly ovate. The leaves of *Thunbergia grandiflora* are not long but heart-shpaed. Thus, leaves can be used as primary marker to distinguish between both the species.[12]

Chemical components and Phytochemistry:

Table-1: Primary constituents of *Thunbergia laurifolia* [3]

Components	Percentage
Fibers	16.82 %
Ash	18.79 %
Protein	16.70 %
Fat	1.68 %
Carbohydrates	46.01 %

The leaves and floers of *Thunbergia laurifolia* were found to contain phenolic compounds like delphidin-3, 5-di-O-β-D-glucopyranoside, apigenin, apigenin-7-O-β-D-glucopyranoside, and chlorogenic acid. The aqueous extract of the leaves was found to containapigenin and apigenin glucoside, as well as phenolic acids like caffeic acid, gallic acid and protocatechuic acid. Earlier, leaves were found to contain 7 compounds viz. grandifloric acid, benzyl β-glucopyranoside, benzyl β-(2'-O-β-glucopyranosyl)-glucopyranoside, 6-C-glucopyranosyl apigenin, 6,8-di-C-glucopyranosyl apigenin, (E)-2-hexenyl-β-glucopyranoside, and hexanol-β-glucopyranoside. Now two new iridoid glycosides have been isolated i.e. 8-epi-grandifloric acid and 3'-O-β-glucopyranosyl-stilbericoside.[1] The leaves are also found to contain sterols like β-sitosterol, stigmasterol, alphaspinasterol. Carotenoids like lutein is also present.[3]

Therapeutic effects and uses:

In Thailand folk medicine, herbal tea prepared from T.laurifolia (mostly from leaves) is widely used. The tea is called 'Rang jeud' in local language. Till now various research papers have been published describing various therapeutic benefits. Antioxidant properties**,** Anti-inflammatory property**,** Anti-malarial property**,** Anti-diabetic property**,** Detoxifying property[1,2,3,8]

MATERIAL AND METHOD:

Material:

Apparatus: filter paper, funnel burette, stand, conical flask beaker, test tube, test-tube stand, tripod stand, aluminium foil, conical flask, beaker, etc.

Chemicals: Hydrochloric acid (HCl), Glacial Acetic acid (CH_3COOH), Sodium hydroxide (NaOH), Ammonia (NH_3), Ethanol (C_2H_5OH) Phenolphthalein, Distilled water, etc.

Flowers: *Thunbergia laurifolia.*

Method:

Extraction:

The flower *Thunbergia laurifolia was* collected from college campus. The flower was authenticated by a Botanist of 'Ramniranjan Jhunjhunwala College, Ghatkopar, Mumbai'. The flower was properly washed to remove any dirt. After washing, the flowers were spread on filter paper and were dried in the shade. After complete drying, the petals were taken in a clean mortat and were triturated. Once trituration is complete, the triturated mass is transferred in a conical flask and 30ml of ethanol is added. The mixture is mixed by slowly shaking the conical flask. After this, the mouth of conical flask is covered properly with aluminium foil. After above process, the mixture is kept for 24 hours for maceration. After 24 hours, the mixture was filtered with normal filter paper. The filtrate collected was immediately transferred into the clean conical flask and its mouth was covered.

Preliminary Test of floral extract:

It is just done to check that whether the extract changes the colour in different acidic and basic mediums. Five clean test tubes were taken and arranged on a test tube stand . In each test tube, 2ml distilled water, 1N HCl , 1N CH_3COOH , 1N NaOH and ammonia solution were added in each. After this 2-3 drops of floral extract was added in each test-tube and colour change was observed.

Titration of standard:

Clean burette was arranged on stand. It was filled with 1N NaOH. 10ml of 1N HCl was taken in conical flask and 2-3 drops of phenolphthalein indicator solution was added to it. This was titrated with 1 N NaOH till the end point (pink). It was repeated for 5 times and burette reading was recorded. Similarly 1N CH_3COOH was titrated with 1N NaOH.

Titration with floral extract:

Clean burette was arranged on stand. It was filled with 1N NaOH. 10ml of 1N HCl was taken in conical flask and 5-6 drops of floral extract was added to it. This was titrated with 1 N NaOH till the end point . It was repeated for 5 times and burette rereading was recorded.

OBSERVATION*:*

Titration of standard: By using phenolphthalein as indicator

1N HCl was titrated with 1N NaOH

Table-2.

Sr. no.	Initial burette reading (ml)	Final burette reading (ml)
1	0	10.8
2	0	11
3	0	11
4	0	11.2
5	0	11

Average burewtte reading: 11 ml

1N CH_3COOH was titrated with 1N NaOH

Table-3.

Sr. no.	Initial burette reading (ml)	Final burette reading (ml)
1	0	11
2	0	10.9
3	0	10.9
4	0	11
5	0	10.8

Average burette reading: 10.9 m

Preliminary test of floral extract of *Thunbergia laurifolia*:

Table-4.

Solution	Observation
H_2O	Slightly yellow
HCl	Pink
CH_3COOH	Slightly yellow
NaOH	Dark yellow
NH_3	Light yellow

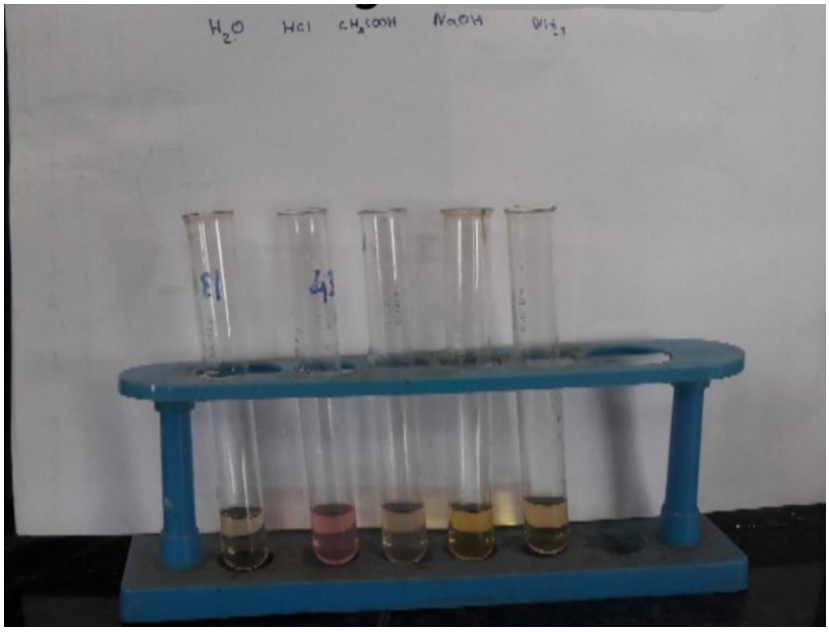


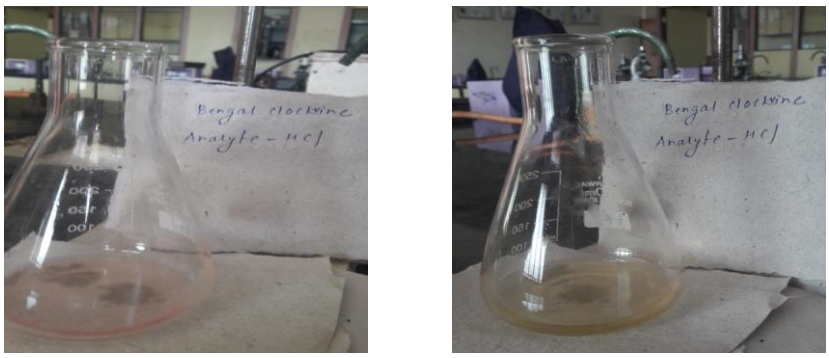
Fig-3: Preliminary test of floral extract of *Thunbergia laurifolia*.

Titration with floral extract of *Thunbergia laurifolia*

1N HCl was titrated with 1N NaOH (strong acid v/s strong base)

Table-5.

Sr. no.	Initial burette reading (ml)	Final burette reading (ml)
1	0	11
2	0	11
3	0	10.8
4	0	11
5	0	11



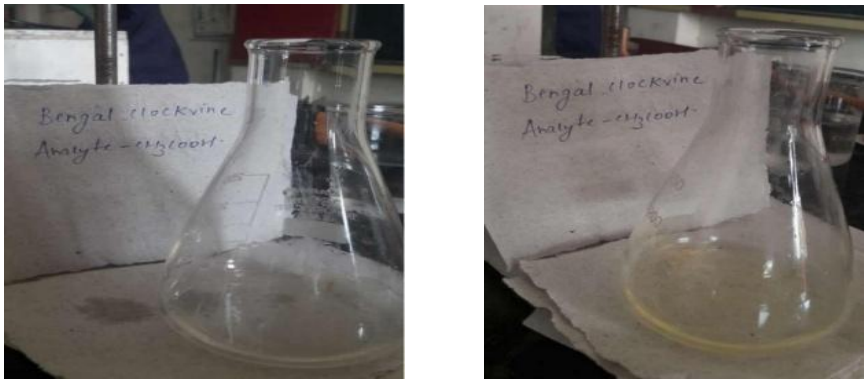
BEFORE **AFTER**

Fig-4: Titration result of HCl v/s NaOH using floral extract of *Thunbergia laurifolia as indicator.*

1N CH_3COOH was titrated with 1N NaOH (weak acid v/s strong base)

Table-6.

Sr. no.	Initial burette reading (ml)	Final burette reading (ml)
1	0	10.8
2	0	11
3	0	11
4	0	11.2
5	0	11



BEFORE **AFTER**

Fig-5: Titration result of HCl v/s NaOH using floral extract of *Thunbergia laurifolia* as *indicator.*

RESULT AND DISCUSSION

Result:

1N HCl was titrated with 1N NaOH (strong acid v/s strong base):

When we compare the burette reading of strong acid versus strong base of floral extract (Table-5) with average burette reading of phenolphthalein (Table-2) the burette reading obtained in both the cases is similar.

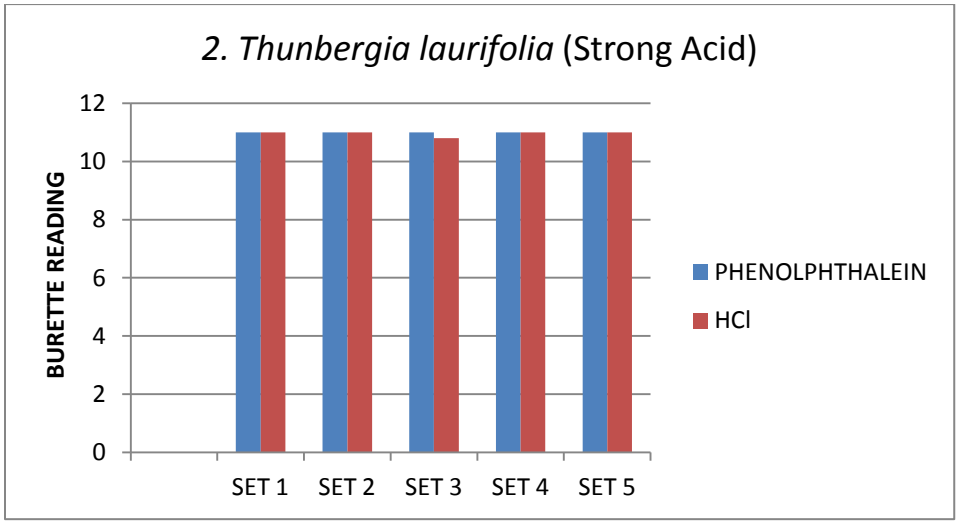


Fig-6: Strong acid versus strong base.

1N CH_3COOH was titrated with 1N NaOH (weak acid v/s strong base):

In case of weak acid versus strong base also, the burette reading of floral extract (Table-6) is matching with the average burette reading of phenolphthalein (Table-3).

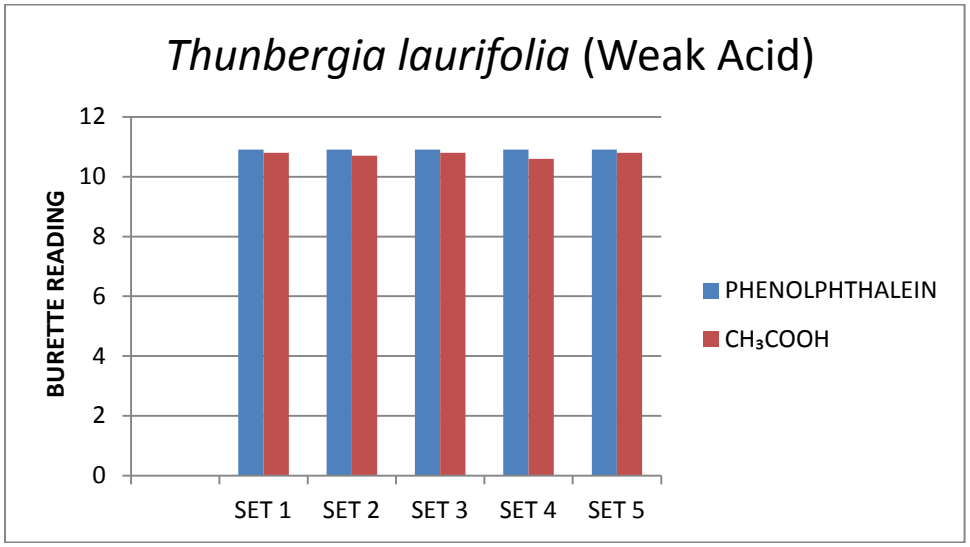


Fig-7: Weak acid versus strong base.

DISCUSSION

Probable theory behind the floral extract behaving ad pH indicator:

As previously mentioned anthocyanins are the constituents responsible for colour change in the experiment. Considering our preliminary test, the colour of all the solutions viz. H_2O, HCl, CH_3COOH, NaOH, NH_3 was different. It means that chemical changes in the anthocyanin structure occurs in both mediums i.e. acidic as well as alkaline.

As already discussed previously, anthocyanins are glycosides, so when they come in contact with aqueous acids, hydrolysis occurs which liberate the sugar and aglycone moiety called as anthocyanidin.

$$\underset{\text{(Anthocyanin)}}{\textbf{Anthocyanidin-O-Sugar}} + \textbf{HCl}_{(aq)} \longrightarrow \textbf{Anthocyanidin-OH} + \textbf{Sugar}$$

The anthocyanidin formed is an polyphenolic compound. As phenols are weakly acidic, they react with base to form the respective phenoxide salts. The salt ionizes in the medium and the phenoxide ion gives the probable colour change. In our titrimetric test, the anthocyanidins formed, react after all the HCl has been neutralized by NaOH, because anthocyanidins (polyphenolic compound) are weakly acidic as compared to HCl.[15]

Once phenoxides of anthocyanidins are formed and then if any amount of HCl is added to it then the colour again goes away. This might be because the phenoxide ion formed will be basic (because it is salt of strong base & weak acid) and HCl is acidic, thus the phenoxide is converted back to phenol.[15]

$$\underset{\text{(Anthocyanidin)}}{\textbf{Ar-OH}} + \textbf{NaOH} \longrightarrow \underset{\text{(Respective phenoxide)}}{\textbf{Ar - O}^{-}\textbf{Na}^{+}} + \textbf{H}_2\textbf{O}$$

$$\xrightarrow{\textbf{HCl}}$$

$$\underset{\text{(Phenol reformed)}}{\text{Ar-OH}} + \text{NaCl}$$

Where, Ar is basic backbone of aromatic ring of anthocyanidin.

Till now, we have discussed about chemical change of anthocyanidins in acidic medium but some chemical change occurs in purely alkaline medium also. Two possibilities are there. First concept is simple i.e. alkaline hydrolysis of anthocyanins. Here, anthocyanins are not first converted to anthocyanidins but are directly converted into respective phenoxide.

$$\underset{\text{(Anthocyanins)}}{\textbf{Ar – O – sugar}} + \textbf{NaOH} \longrightarrow \underset{\text{(Respective phenoxide)}}{\textbf{Ar - O}^{-}\textbf{Na}^{+}} + \textbf{HO – sugar}$$

Second possibility it that, in anthocyanins all hydroxyl (-OH) groups will not be hydroxylated. With these free hydroxyl groups NaOH will react and forms salt.

$$\textbf{HO-Anthocyanidin-O-Sugar} + \textbf{NaOH} \longrightarrow {}^{+}\textbf{Na}^{-}\textbf{O-Anthocyanidin-O-Sugar} + \textbf{H}_2\textbf{O}$$

Correlation of 'Ostwald's theory of acid-base indicator' with our natural indicator:

Ostwald's theory was the first theory of acid-base indicators Wilhelm Ostwald (1853-1932). According to Ostwald's theory, the undissociated indicator acid (HIn) or a base (HOIn) has a colour different than it's respective ion.[14]
Consider a acid indicator; for which equivalence can be written as,

$$HIn \rightleftharpoons H^+ + In^-$$

In acidic medium (i.e., analyte is acid) there is a depression in the ionization of 'HIn' because of the common ion effect. It means that initially concentration of HIn is more and 'In^-' is very less. As the reaction proceeds (i.e. alkali is added) the concentration of analyte acid decreases and after equivalence point the alkali reacts with acidic indicator and concentration of 'In^-' increases causing the colour change.[14]

In case of basic analyte, indicator will also be basic and other things will be same.[14

From the Ostwald's theory of acid-base indicators, two things can be concluded that acid-base indicators themselves are weak acids (for acid analyte) or weak bases (for basic analyte) and the colour change is due to the ionization of the indicator.
In the previous text, we have discussed that the colour change shown by the floral indicator is due to the formation of phenoxide ion of the respective anthocyanidin. As in this case also, colour change is due to the formation of ion, Ostwald's theory of acid-base indicators can be applied to our floral indicator.

CONCLUSION

Commonly used indicators for acid base titration are synthetic. This experiment was performed to find out eco-friendly and natural indicator from floral extracts of *Thunbergia laurifolia*. Thr floral extract of *Thunbergia laurifolia* was used to perform titration with Strong acid-Strong base and weak acid-Strong base. We have obtained sharp and clear colour change from the floral extract. The floral extract shows sharp colour change with acid and base making the floral extract suitable for use as acid-base indicator. As the floral extracts are very simple, cost effective, and give good results with sharp colour change at the end points of the titration so, it will be possible to replace the synthetic indicators such as phenolphthalein used in labs with natural floral indicator.

In this research article we have only hypothesized the chemistry of anthocyanin that how they change colour in acidic and basic medium based on general acid base behaviour but we have not done any confirmatory test. For confirmation of this theory detailed molecular study will be needed. So, the detailed molecular study can be a topic for future research of this theory.

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LIST OF ABBREVIATIONS:

(-OH)	: Hydroxyl group
i.e.	: Id est (that is)
v/s	: Versus
Ar-OH	: Anthocyanidin
Ar-O-sugar	: Anthocyanin
Ar-O-Na	: Anthocyanidin phenoxide
HIn	: Acidic indicator
HOIn	: Basic indicator

CONFLICT OF INTEREST

Yes

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