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Research Article

Methanolic extracts of Flowers and Seeds: Natural Resource As Indicator In Acidimetry And Alkalimetry

Komal Tilekar, P.N.Jagtap, S.S.Kalaskar, R.S.Hake, A.P. Shewale, P.S.Patil and Dr.R.Y.Patil.

Pune District Education Association's

Shankarrao Ursal College of Pharmaceutical Sciences & Research Center, Kharadi, Pune Dt, Haveli, Maharashtra, India – 411014.

ABSTRACT

An acid-base indicator is a substance which changes colour according to how acidic or basic its environment is. There are numerous natural acid-base indicators that can be obtained from common flowers, fruits and vegetables. The plant pigments known as anthocyanins are responsible for many of the red, blue and violet colors seen in plants. The pink flowers of Gulbakshi,pink seeds of Pomegranate all owe their distinctive coloration to the presence of anthocyanins.The plants used in this experiment are *Mirabilis jalapa, Punica granatum, Bixa orellana* .Since they are water soluble, they are easily extracted for use in the laboratory. This experiment will examine the properties of various extracts made in the laboratory. The first part is to select a material to be tested. Natural indicator is easy to prepare as well as they are easily available. Promising results were obtained when it was tested against standard synthetic indicators. Titration shows sharp colour change at the equivalence point. The equivalence points obtained by the flower extract coincide with the equivalence points obtained by standard indicators.

Keywords : Anthocyanins, titration, equivalence point, natural indicator.

INTRODUCTION

An acid–base indicator is a substance that changes color as the pH of a solution changes. There are hundreds of different acid–base indicators, many of which can be extracted from common plants. Every indicator exhibits a different range of colors at different pH values. For example, the indicator phenolphthalein, which you have used in previous labs, is colorless in solutions with a pH less than 8 and pink in solutions with a pH greater than 8. Indicators work because they are weak acids which, when in solution, exist in equilibrium with their conjugate base. The acid and its conjugate base each have different colors, and as the equilibrium shifts from one direction to the other, the color of the indicator solution changes. Some indicators exhibit only two colors and some exhibit a wide range. Each indicator must be individually studied to determine its behavior as a function of pH. Almost any flower such as blue, purple or red in colour contain a class of organic pigment called anthocyanins that change colour with pH. Titration shows sharp colour change at the equivalence point. The equivalence points obtained by the flower extract coincide with the equivalence points obtained by standard indicators. In case of weak acid and weak base titration the results obtained by the flower's extract matched with the results obtained by mixed indicator. Thus natural indicator is found to be a very useful, economical, simple and accurate for the said titrations. The use of natural dyes as acid- base indicator was first reported in 1664 by Sir Robert Boyle in his collection of essays "Experimental History Of Colours."According

to Mendham (2004) the term titrimetric analysis refer to quantitative chemical analysis carried out by determining the volume of solution of accurately known concentration which is required to react quantitatively with a measured volume of a solution of the substance to be determined. The addition is made until reaction is
complete. This is called the end point or equivalence point of the reaction. The end point is detected by using a chemical compound known as an indicator. This indicator may be used internally or externally. Many acid base titration themselves indicate the end point of the reaction[1].

MIRABILIS JALAPA:

Fig No.1- mirabilis jalapa

Scientific classification:
Kingdom : Plantae
Sub kingdom : Trcheobionta
Division : Angiosperms
Class : Dicotyledons
Sub class : Caryophylidae
Order : Caryophyllales
Family : Nyctaginaceae
Genus : *Mirabilis*
Species : *M. Jalapa*

It is a perennial herb or under shrub. An erect herb to 50-100 cm high, native of peru but now dispersed throughout tropics. It is a popular ornamental plant grows worldwide for the beauty of its flowers which can be white, red, pink, purple or multi colored[10].
Mirabilis Jalapa .L. is the herbaceous plant are erect and spreading, 2-3ft (0.6-0.9m) tall and just as wide. They have numerous branches and opposite, pointed leaves, coriaceous obovoid fruits and prominent tuberous roots, planted as an ornamental plant. The leaves are opposite measuring 3.5-7.5 cm wide, 2-4 in (5-10 cm) long, unequal, ovate to sub cordate. Flowers are tubular, cluster, funnel-shaped, simple or double, fragrant, white, yellow, pink or purple. .Flowers in group of three flowers with five green bracteoles, surrounding the perianth, usually yellow crimson, white or variegated and opening in the evening. Perianth lobes five, gamophyllus, stamens five with unequal filaments. Carpel one, unilocular, superior ovary with a single ovule, a nectariferous disc surrounds the ovary. Fruit achene surrounded by a leathery, ribbed, persistant perianth . The selfcompatible, perfect flowers each have 5-6 stamens and a single-ovulate ovary. An individual flower opens for one night in the early evening, the exact time depending on temperature and relative humidity, and closely the next morning. An individual plant produces between 25 and 75 flowers in one flowering seasons .The seeds are olive, brown or black in color. The root system of *Mirabilis Jalapa. L.* consists of a fairly thickened and tuberous up to 1mm high, stem swollen at nodes. Chemical analysis showed the presence of alkaloids, flavonoids, phenols, steroids , triterpenes, glycosides, tannins, saponins and lignins. Flowers mainly contain flavonoids and anthocyanins[2]. Chemical analysis showed the presences of alkaloids, flavonoids , phenols, steroids , triterpenes, glycosides, tannins, saponins and lignins. Better image analysis elucidation of compounds are visualized from TLC are alanine, arabinose, campesterol, daucosterol and dopamine, d-glucan, hexacon-1-ol, indicaxanthin, isobetanin, 6-methoxyboeravinone,C-methylabronisoflavones, miraxanthins, n-dotriacontane, nnonacosane, n-pentacosane, n-triacontane. Flowers mainly contain flavonoids, anthocyanins. A number of active compounds were extracted from different organs of MJ, including ribosome-in activating protein(RIP) associated with anti-viral activity, anti-fungal phenolic compounds, anti microbial peptides and rotenoids showing inhibition of HIV-1 reverse transcriptase, further isolation of active components is under progress[11].

POMEGRANATE:

The fruits of *Punica granatum* L. (Punicaceae), the pomegranate, are used to treat acidosis, dysentery, microbial infections, diarrhoea, helminthiasis, haemorrhage and respiratory pathologies have also reported popular use of the pomegranate plant in the treatment of respiratory disease. *Punica granatum* would appear to have interesting anti-viral activity. Extracts have been shown to be effective against the herpes virus and hydroalcoholic extracts of whole fruits have exhibited high activity against the influenza virus. Phytochemically the plant has been attributed to contain punicalagin, punicalin, strictinin and
Granatin[9]. Two new ellagitannins, diellagic acid rhamnosyl glucopyranoside and 5-Ogalloylpunicacortein-D were isolated and characterized together with four known tannin metabolites, punicacortein D, punicalin, punicalagin and 2-O-galloylpunicalin[8]. It also contains flavonoids, antioxidants and anthocyanins as well. The intention behind this study is simply to bring in market the use of plant pigments and to increase the

wealth of traditional medicinal system of India which is mostly plant based and to help farmers regarding cultivation, collection of plants as well as to industry regarding preparation of above indicators. Titrate and Titrant with indicator shows sharp and intense color changes at the equivalence point that is at neutralization[7]. Therefore the objective of this work was to explore the indicator activity of methanolic fruit extract of *Punica granatum*[3].

SHENDRI:

Bixa orellana, also known as achiote or annatto, is a plant belonging to the Bixaceae family. The dye extracted from the pericarp of *B.orellana* seeds is widely used in the food industry to replace synthetic dyes because of its lower cost and lack of toxicity. Phytochemical studies of extracts from different parts of *B. Orellana* showed the presence of steroidal compounds, flavonoids,carotenoids and isoprenoids such as geranylgeraniol, farnesylacetone, geranylgeranyl octadecanoate and tocotrienol[5]. As a dye Achiote (*Bixa orellana*) has been traditionally used for face and body painting[12]. In India also it is popular as Dhobi dye‘ used by some communities (washerman) to mark the clothes while washing. In the Philippines, the red pulp from the seeds is used in the polish for russet leather[13]. Annatto has remarkable affinity for both the protein fibres. Pre application of ferrous sulfate on wool and silk followed by dyeing with annatto produces most balanced improvements in colour uptake, light and wash fastness and colour retention on repeated washing of such protein fibres[14]. Annatto oil is an emollient, and its high carotenoid content provides antioxidant benefits on body care products, while adding a rich, sunny colour to creams, lotions, and shampoos. Similarly annatto paste filters out the ultraviolet rays of sunlight, thereby protecting the skin from excessive sunburn. Dyes for lipstick are also obtained from *Bixa orellana* L. hence, the name lipstick tree[15]. Annatto is one of the oldest safe dyes known to humans and is extensively used by food industries as a natural food grade colourant. Formulations of annatto extract are made to impart orange-yellow colour shades to processed foods[16]. Annatto is commonly used as a colouring agent for pharmaceutical ointments and plasters (Natural Standard Professional Monograph, 2011). It has been used in direct compression tablet coating and oral liquid drugs[17].

MATERIAL AND METHODS

Collection of Plant material

Fresh petals of **GULBAKSHI** flower & seeds of **POMEGRANATE** & **SHENDRI** were collected from local market. Natural indicator can be isolated from these plants.

Reagents:

1. Hydrochloric Acid (HCl)
2. Sodium Hydroxide (NaOH)
3. Acetic Acid(CH3COOH)
4. Ammonium hydroxide (NH4OH)
5. Phenolphthalein
6. Methyl Orange
7. Sodium Carbonate
8. Potassium Hydrogen Phthalate
9. Oxalic acid
10. Methanol

Preparation of extracts:

Flowers & seeds were cleaned by distilled water and petals & seeds of these plants were grinded into wet mass by triturating the mixture in the mortar & pestle. Add 50 ml of methanol & keep it for 48 hours.The resulting solution was filtered through muslin cloth. The resulted methanolic extract was further used as natural indicator for acidimetry and alkalimetry. The extract was preserved in light closed container and stored away from direct sunlight.

EXPERIMENTAL PROCUDURE:

To Observe Colour Intensity In An Increasing Order In Water:

1. 15 test tubes were taken and aranged them serially.
2. 0.1 , 0.2 , 0.3 ,0.4 ,0.5 gm triturated petals of GULBAKSHI & seeds of POMEGRANATE and SHENDRI was added into each of the test tubes containing 5ml of water.
3. The mixture was allowed to stand still for 30 min.
4. The colour of each test tube was observed visually.

To Observe Colour Change According To pH:

1. 7 test tubes were taken for each plant.
2. The pH of the solutions containing in each of the test tube was 2,4,6,7,8,10,12 respectively.
3. The first 3 test tubes contained acidic solution of 0.1N HCl, whereas the 4th test tube contained neutral solution while the last 3 test tubes contained alkaline solution of 0.1N NaOH
4. 0.1gm of the triturated petals of GULBAKSHI , seeds of POMEGRANATE & SHENDRI was added in each of the test tube.
5. The colour change was observed.

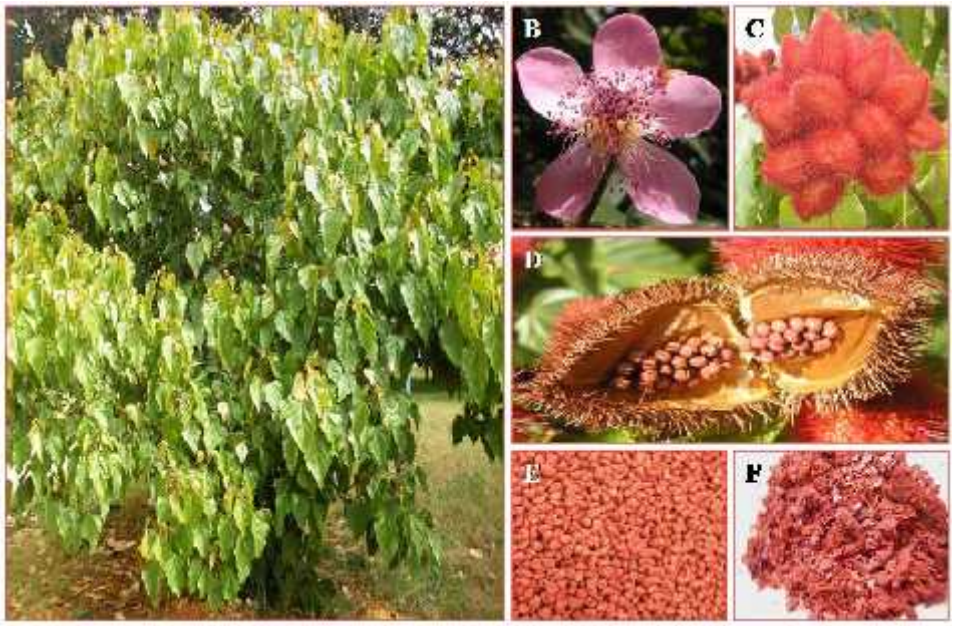

Fig 2

Annatto yielding *Bixa orellana* plant. **A:** Whole plant; **B:** Flower; **C:** Fruit bunch; **D:** Dehiscenced fruit; **E:** *Bixa* seeds and **F:** *Bixa* powder[4]

STANDARDISATION[6]:

1) 0.1N HCL

1. About 0.1 gm(100 mg) of Na_2CO_3 was accurately weighed by transfer method.
2 .Then it was transfered into conical flask and was dissolved in about 25 ml of distilled water.
3. 2-3 drops of methanolic extract of gulbakshi flower were added as an indicator instead of methyl orange.
4. The solution in the conical flask was titrated with 0.1N HCl until a permanent pink colour is produced.

2) 0.1N NaOH

1. About 0.2 gm of potassium hydrogen phthalate was weighed accurately by transfer method.
2. It was dissolved in 30ml of carbon dioxide free water in conical flask.
3.2-3 drops of methanolic extract of gulbakshi flower were added as an indicator instead of phenolphthalein and titrated with 0.1N NaOH solution until a permanent brown colour is produced.

RESULTS AND DISCUSSION

The flower seeds were screened for its use as an indicator in acid base titration and the results were compared with the results obtained by standard indicators phenolphthlein and methyl orange . The titrations of strong acid with strong base (HCl & NaOH), strong acid with weak base (HCl & NH4OH), weak acid with strong base (CH3COOH & NaOH), and weak acid with weak base(CH3COOH and NH4OH) were carried out using standard indicators and floral extract. These having coloring matter flavonoids, anthocyanins and these are pH sensitive. The pomegranet extract was screened for its use as an acidimetry indicator in acidimetry titration and the result of this screening compared with the result obtained by standard indicator methyl orange for strong acid v/s weak base (H2SO4 and NaHCO3). The methanolic or aqueous extract of Bixa orellana has been found to have diuretic, anti-diabetic activities etc. But does not show any specific colour change in various pH medium.

Figures 3,4,5,6 represents the colour intensity in an increasing order after addition of triturated flower petals as 0.1,0.2,0.3,0.4,0.5,0.6 gm in each of the test tubes containing 5 ml of water from left to right.

Figures 7,8,9 represents the colour change according to pH 2,4,6,7,8,10,12 from left to right of the methanolic extract of gulbakshi flower.

Figures 10,11 represent the colour intensity in an increasing order after addition of triturated flower petals as 0.1,0.2,0.3,0.4,0.5,0.6 gm in each of the test tubes containing 5 ml of water from right to left.

Figures 12 and 13 represents the colour change according to pH 2,4,6,7,8,10,12 from left to right of the methanolic extract of pomegranate seeds.

OBSERVATION TABLE FOR GULBAKSHI PETALS

pH	COLOUR	NATURE
2	Slightly pink	Acidic
4	Light pink	Acidic
6	Dark pink	Acidic
7	No change	Neutral
8	Slightly brown	Alkaline
10	Light brown	Alkaline
12	Dark brown	Alkaline

OBSERVATION TABLE FOR POMEGRANATE SEEDS

pH	COLOUR	NATURE
2	Light Orange	Acidic
4	Dark Orange	Acidic
6	Dark Brown	Acidic
8	Light Brown	Alkaline
10	Light Brown	Alkaline
12	Light Brown	Alkaline

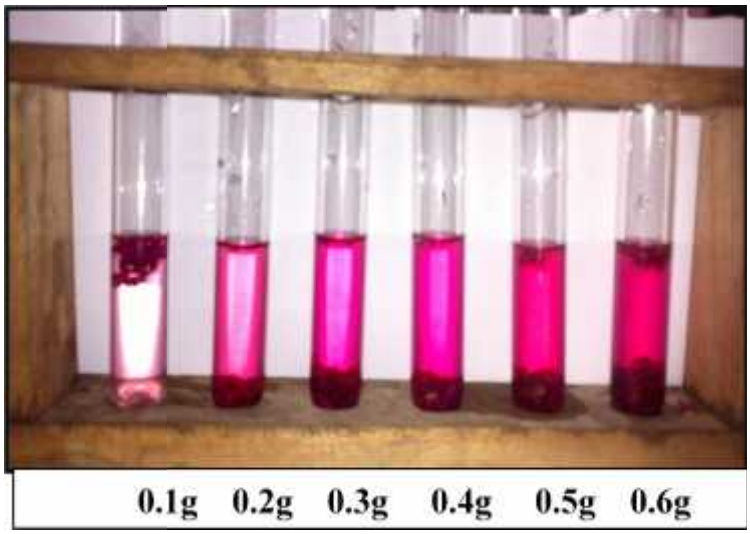

Fig 3
Change in colour intensity according to concentration

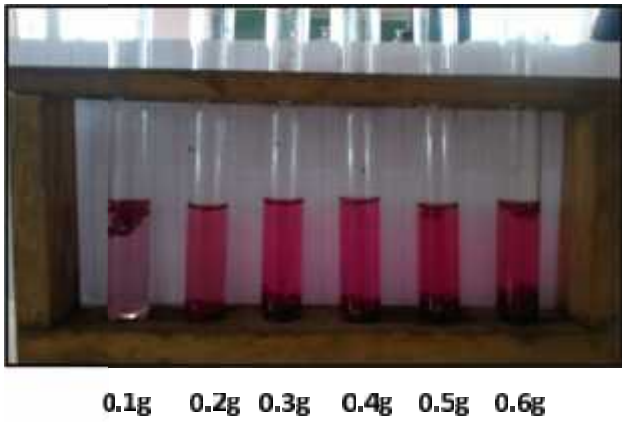

Fig 4
Change in colour intensity according to concentration

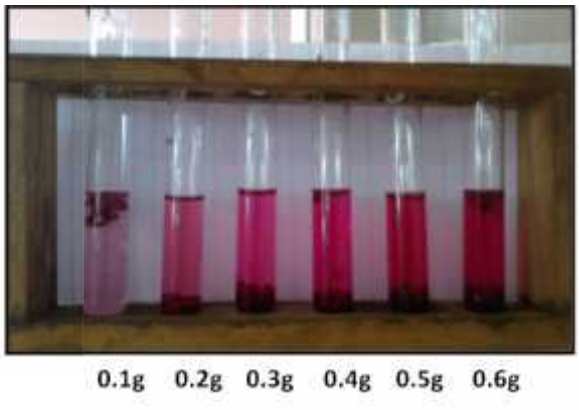

Fig 5
Change in colour intensity according to concentration

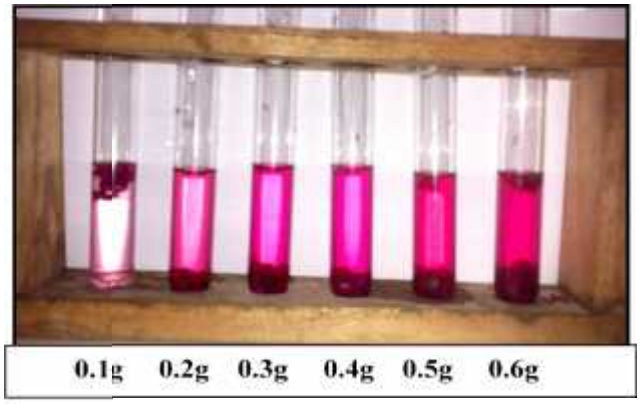

Fig 6
Change in colour intensity according to concentration

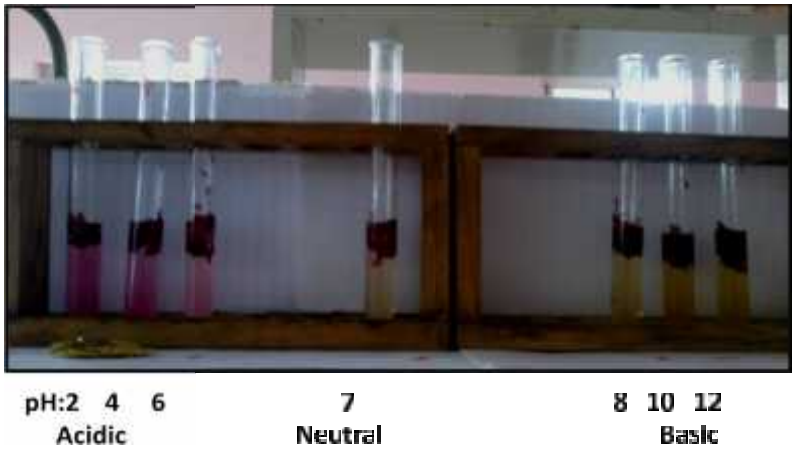

Fig 7
Colour change according to pH

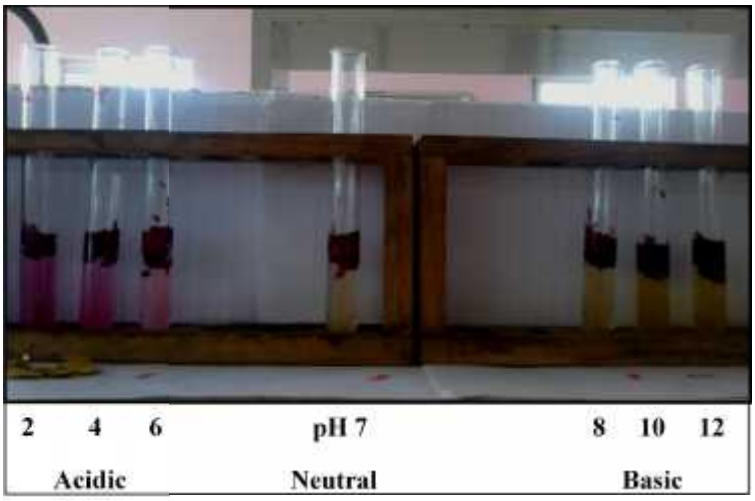

Fig 8
Colour change according to pH

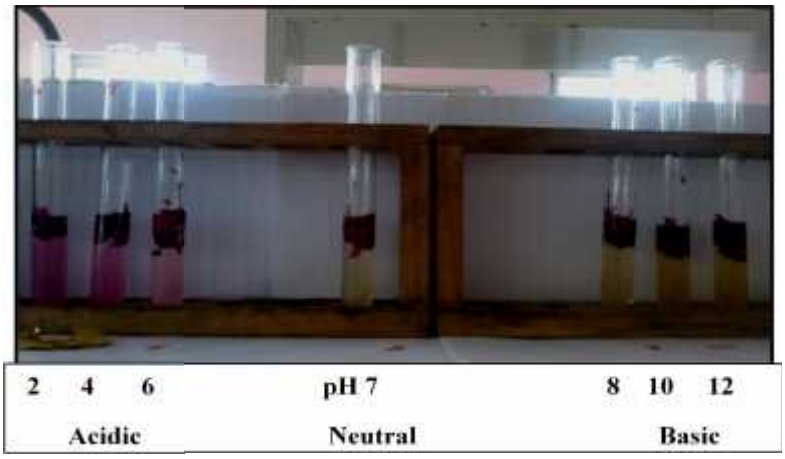

Fig 9
Colour change according to pH

Fig 10
Change in colour intensity according to concentration

Fig 11
Change in colour intensity according to concentration

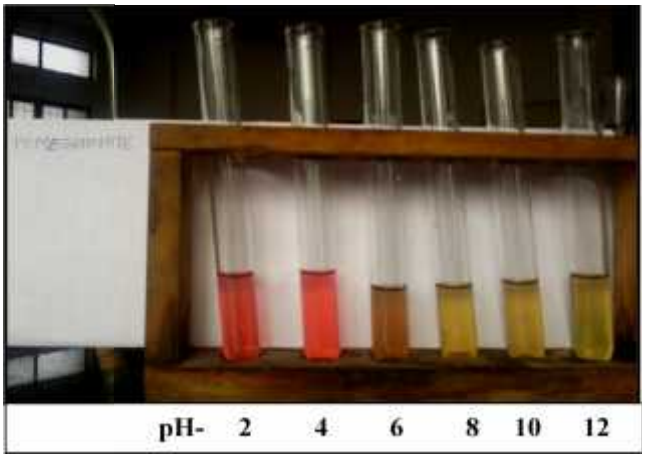

Fig 12
Colour change according to pH

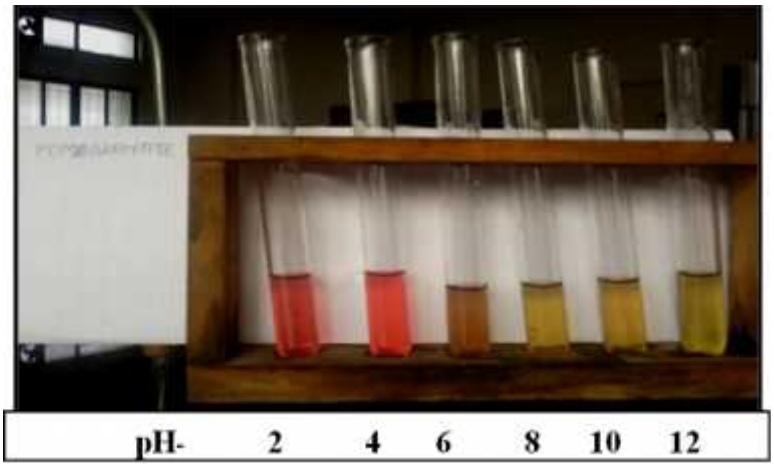

Fig 13
Colour change according to pH

Fig 14
Change in colour intensity according to concentration

Fig 15
Change in colour intensity according to concentration

Fig 16
Change in colour intensity according to concentration

OBSERVATION FOR SHENDRI SEEDS

Figures 14,15,16 are representing that the colour intensity was not found in an increasing order after addition of triturated seeds of shendri as 0.1,0.2,0.3,0.4,0.5,0.6 gm in each of the test tubes containing 5 ml of water from right to left.Further this methanolic extract was added dropwise in acid as well as base in two separate test-tubes.But there was no significant colour change according to pH.Therefore it was not used for titration as an indicator.

CONCLUSION

The results obtained in all the types of acid base titrations lead us to conclude that it may be due to the presence of anthocyanins sharp colour changes, which occurred at end point of titrations. At the end point it is observed that *Mirabilis Jalapa*,*Punica granatum* methanolic extract can be used as an indicator in all types of acid base titrations because of its economic, simple, accurate and precise. But the extract of Bixa orellana seeds cannot be used as an indicator, as it does not show any colour change according to the pH.

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