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Research article

Study of *invitro* antioxidant, anti-inflammatory and acid-base indicator properties of flower extracts obtained from five traditional plants

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ABSTRACT

Anthocyanins are an important group of highly hydrosoluble pigments in most species of plants that may appear in red, purple, or blue depending on the pH. Anthocyanins are generally degraded at higher pH. The presence of this anthocyanin in the flower extract is responsible for the use as an indicator, antioxidant, and anti-inflammatory activity. The present study was done on the Flower extract of *Bougainvilea glabra*, *Butea monosperma*, *Calendulla officinalis*, *Ixora coccinea*, and *Hibiscus rosasinensis* to investigate the properties of the flower extract as an Antioxidant, Anti-inflammatory and Acid - Base Indicator.

Keywords: Anthocyanins, Antioxidant, Anti-inflammatory, Acid-Base Indicator.

INTRODUCTION

In recent years, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems [1]. Medicinal herbs are highly highlighted due to their wider use and lesser side effects [7]. The source of nutraceutical compounds in human diet is almost exclusively provided by fruits and vegetables. However, flowers are becoming important sources of several bioactive compounds that can be added in the diet as food. In the ancient time flowers were mainly eaten for their medicinal properties rather than their nutritional value. Nowadays, several metabolomics studies revealed the chemical compositions of wild and ornamental flowers, showing the presence of important bioactive molecules [2].

Free radicals are usually short-lived species but they possess a single unpaired electron, rendering them highly reactive against biologically important macromolecules including DNA, Proteins and membrane lipids. To counteract this threat to their integrity, cells have evolved a variety of defense systems based on both water-soluble and lipid-soluble antioxidant species and on antioxidant enzymes. A high proportion of the antioxidant systems of the human body are dependent on dietary constituents[4]. Inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [6]. However, studies have been continuing on

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inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use [3]. Therefore, the currently used analgesics and anti-inflammatory drugs are not useful in all cases; so, there arises the requirement for a medicinally active plant [7].

Due to environmental pollution, availability and cost, the search for natural compounds as an acid-base indicator was started. This natural indicator is easy to extract as well as easily available. Promising results were obtained when it was tested against standard synthetic indicators. Titration shows sharp colour change at the equivalence point. This indicator was found to be useful in all type of acid-base titrations except weak acid and weak base titration. It was found very useful, economical, simple and accurate indicator for said titrations [5].

Hence the objective of the present study is to investigate the property of the Flower extract of *Bougainvilea glabra*, *Butea monosperma*, *Calendulla officinalis*, *Ixora coccinea*, and *Hibiscus rosasinensis* as Antioxidant, Anti-inflammatory, and Acid-Base indicator.

MATERIALS AND METHODS

Plant collection

Flower petals of *Calendulla officinalis L.*, *Butea monosperma (Lam.)*, *Bougainvilea glabra Choisy*, *Hibiscus rosasinensis L.*, and *Ixora coccinea* L. were collected in the month of December to February from the campus of Jamal Mohamed College, Thiruchirapalli, Tamil Nadu, India.

Preparation of Flower Extract

About 2g of *Canlendulla officinalis L.*, *Butea monosperma (Lam.)*, *Bougainvilea glabra Choisy*, *Hibiscus rosasinensis L.*, *Ixora coccinea L.* flower petals were subjected to maceration with 90% ethanol in separate conical flasks for about 48 hours at room temperature. The flasks were closed tightly with cotton and were shaken periodically. Then after 48 hours the materials were filtered through the whatman filter paper without any residues. The extract was then poured carefully to a glass container through a funnel and stored aside separately without exposing to direct sunlight. For Phytochemical analysis aqueous extract of the five flowers under study was taken

Preliminary Phytochemical screening

The aqueous extracts of all flowers under study were subjected to preliminary phytochemical screening to detect for the presence of phytoconstituents using various qualitative reagents

Volumetric titrations

Volumetric titrations were performed by using the equimolar solution. A set of four titrations were carried out with standard indicators and flower extracts.

1. Hydrochloric acid, HCl, (strong acid) v/s sodium hydroxide, NaOH (strong base)
2. Ammonium hydroxide, NH_4OH (weak base) titrated with hydrochloric acid, HCl (strong acid)
3. Acetic acid, CH_3COOH, (weak acid) v/s sodium hydroxide, NaOH (strong base)
4. Acetic acid, CH_3COOH, (weak acid) v/s Ammonium hydroxide, NH_4OH, (weak base).

The experimental work was carried out by using the same set of glasswares for all type of titrations. As the same aliquots were used for both titrations i.e. titration by using standard indicators and flower extract, the reagent were not calibrated. The equimolar titrations were performed using 20 ml of titrant with three drops of indicator. A set of five experiments was carried out and mean and standard deviation were calculated from results.

Investigation of colour variation of flower extract with change in pH

The standard indicators and the flower extracts of all flower under study was taken. The acid and the base were added to the microtitre plate with gradually increase in pH. The pH ranges from pH1 – pH12. Then the indicators were added in drops.

DPPH Radical scavenging activity

For each flower a set of five test tubes were taken. In the first set of test tubes 1ml, 2ml, 3ml, 4ml and 5ml of standard were added. Similarly in all set of test tubes 1ml, 2ml, 3ml, 4ml and 5ml of ethanol extract of flower under study were added followed by 0.5ml of DPPH (1mM) in ethanol were added respectively. Then the tubes are mixed thoroughly and incubated at room temperature for 30 minutes. Then OD was taken at 540nm and the results were tabulated.

$$\% \text{ Antioxidant activity} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \times 100$$

In vitro Anti-inflammatory activity

Human Red Blood Cell (HRBC) Membrane stabilization method

Fresh blood was collected and centrifuged at 3,000 r/min. The Packed cells were washed with isosaline (0.90% NaCl) and a 10% suspension was made. The reaction mixture (4.5 ml) consists of 2ml of hyposaline (0.25% w/v NaCl), 1ml of 0.15M phosphate buffer (pH 7.4) and 1ml of test solution (100, 200, and 300 µg/ml) in isosaline, 0.5ml of 10% HRBC in isosaline was added. For test control, 1ml of distilled water was used instead of hyposaline (to produce 100% hemolysis), while product control lacked red blood cells. The mixture were incubated at 37ºC for 30 minutes and centrifuged at 3,000 r/min for 20 minutes. Diclofenac sodium was used as the reference drug. The haemoglobin content in the suspension was estimated using a spectrophotometer at 570 nm.

$$\% \text{ Membrane Stabilization} = 100 - \left\{ \frac{\text{OD of test sample}}{\text{OD of control}} \times 100 \right\}$$

RESULTS AND DISSCUSSION

Preliminary phytochemical screening

These results of the phytochemical screening shown below in table 1, showed that the aqueous extracts of Bougainvilea glabra Choisy, Butea monosperma (Lam.), Calendulla officinalis L., Ixora coccinea L., Hibiscus rosasinensis L. revealed the presence of Alkaloids, Flavanoids, Carbohydrates, Anthocyanins etc., which may have justified their use in ethnomedicine.

The Presence of phytoconstituents in the aqueous extract of *Bougainvilea glabra Choisy*, *Butea monosperma* (Lam.), *Calendulla officinalis L.*, *Ixora coccinea L.*, *Hibiscus rosasinensis L.* were shown in the Fig.-1 – Fig.-5.

Table 1: Phytochemical Screening of the aqueous extract of the Flowers

Phyoconstituents	***Bougainvilea glabra***	***Butea monosperma***	***Calendulla officinalis***	***Ixora coccinea***	***Hibiscus rosasinensis***
Alkaloids	Positive	Positive	Negative	Positive	Positive
Flavanoids	Positive	Positive	Positive	Positive	Positive
Carbohydrates	Positive	Negative	Positive	Positive	Positive
Glycosides	Positive	Positive	Negative	Negative	Positive
Saponins	Positive	Positive	Negative	Positive	Negative
Proteins & Aminoacids	Positive	Positive	Negative	Posiitve	Negative
Oils & Fats	Negative	Negative	Negative	Negative	Negative
Phenolic compounds and Tanins	Negative	Negative	Positive	Negative	Positive
Anthocyanin	Negative	Positive	Positive	Positive	Positive
Leucoanthocyanin	Positive	Negative	Negative	Positive	Negative

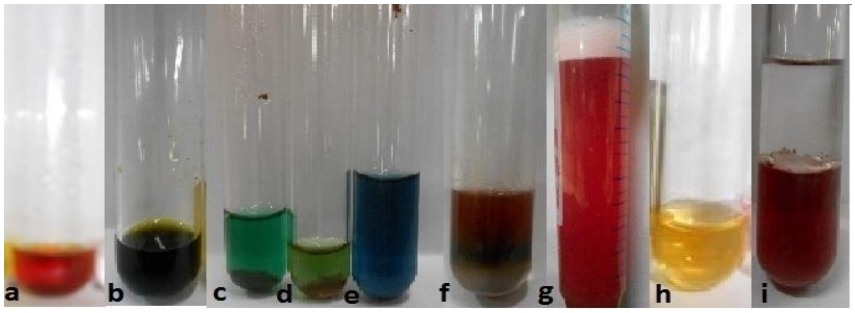

Fig.1: Phytochemical analysis of the aqueous extract of Bougainvilea glabra Choisy. a) Test for alkaloids (Wagner's Test); b) Test for Flavanoids ($FeCl_2$ Test) c) Test for Carbohydrates (Barford's Test) d) Test for Carbohydrates (Benedict's Test) e) Test for Carbohydrates (Fehling's Test) f) Test for Glycosides g) Saponins h) Test for proteins and aminoacids (Xanthoproteic Test) i) Test for Leucoanthocyanin

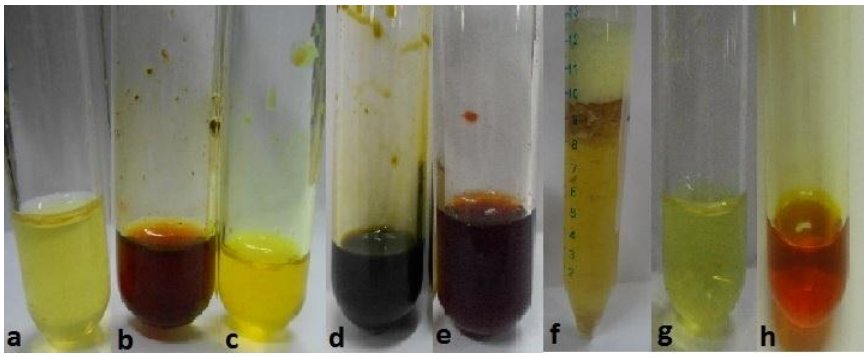

Fig.2: Phytochemical analysis shows the presence of Phytoconstituents in the aqueous extract of Butea monosperma (Lam.) a) Test for Alkaloids (Mayer's Test) b) Test for Alkaloids (Wagner's Test) c) Test for Alkaloids (Hager's Test) d) Test for Flavanoids ($FeCl_2$ Test) e) Test for Glycosides f) Saponin Test g) Test for Proteins and aminoacids h) Test for Anthocyanins

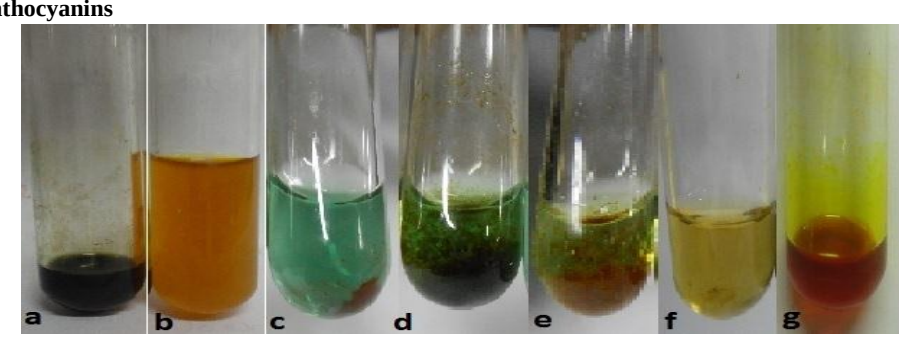

Fig.3: Phytochemical analysis shows the presence of Phytoconstituents in the aqueous extract of Calendulla officinalis L. a) Test for Flavanoids ($FeCl_2$ Test) b) Test for Flavanoids (NaOH Test) c) Test for Carbohydrates (Fehling Test) d) Test for Carbohydrates (Barford's Test) e) Test for Carbohydrates (Benedict's Test) f) Test fot Phenolic compounds & Tannins g) Anthocyanin Test

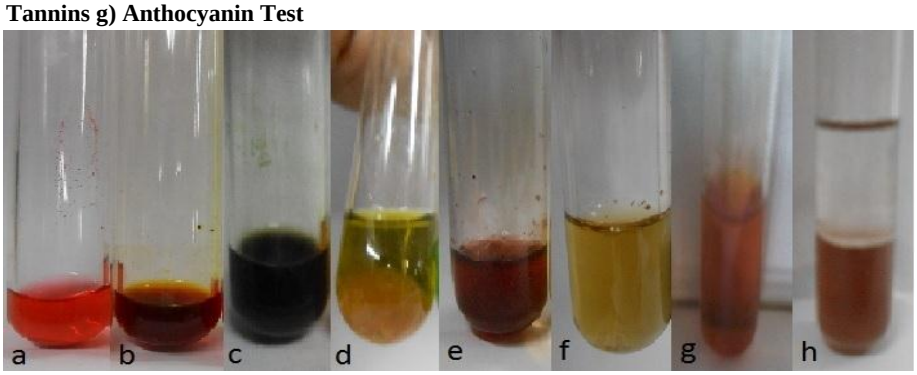

Fig.4: Phytochemical analysis shows the presence of Phytoconstituents in the aqueous extract of Hibiscus rosasinensis L. a) Test for Alkaloids (Mayer's Test) b) Test for slkaloids (Wagner's Test) c) Test for Falvanoids ($FeCl_2$ Test) d) Test for Carbohydrates (Benedict's Test) e) Test for Glycosides f) Test for Phenolic compounds and tannins g) Anthocyanin Test h) Leucoanthocyanin Test

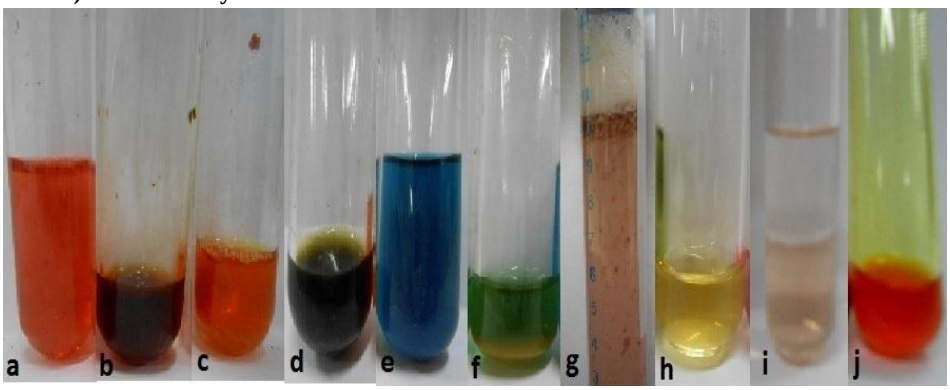

Fig.5: Phytochemical analysis shows the presence of Phytocinstituents in the aqueous extract of Ixora coccinea L. a) Test for alkaloids (Mayer's Test) b) Test for Alkaloids (Wagner's Test) c) Test for alkaloids (Hager's Test) d) Test for flavonoids ($FeCl_2$ Test) e) Test for Carbohydrates (Fehling Test) f) Test for Carbohydrates (Benedict's Test) g) Saponin test h) Test for Proteins and aminoacids i) Leucoanthocyanin Test j) Anthocyanin Test

Variation in colour of the extract with change in pH

The Ethanolic extract of the Bougainvilea glabra Choisy, Butea monosperma (Lam.), Calendulla officinalis L., Ixora coccinea L. and Hibiscus rosasinensis L. shows a gradual change in their colour with gradual increase in pH. The pH ranges from pH1 – pH12. (Fig.-6). The colour of the flower extracts in Acid and Basic condition was reported in the table 2.

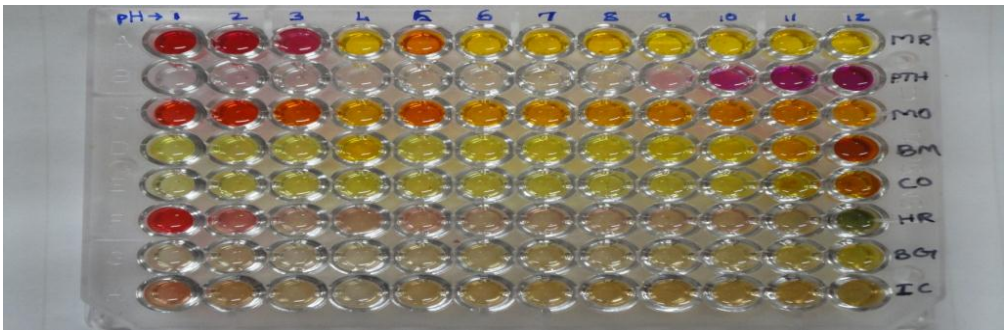

Fig.6: Variation in the colour of Standard indicators and the flower extract with gradual increase in pH ranges from pH1 – pH12.

Table 2: Colour of the Flower extract at acid and base condition

S. No.	Flower Extract	Colour of the indicator	
		Acid	Base
01.	*Butea monosperma (Lam.)*	Yellow	Reddish orange
02.	*Calendulla officinalis L.*	Yellow	Orange
03.	*Hibiscus rosasinensis L.*	Pink	Green
04.	*Bougainvilea glabra Choisy*	Colourless	Yellow
05.	*Ixora coccinea L.*	Pink	Light Green

Antioxidant Activity

Total antioxidant capacity of the ethanolic extract of *Bougainvilea glabra Choisy, Butea monosperma (Lam.), Calendulla officinalis L., Ixora coccinea L. and Hibiscus rosasinensis L.* were evaluated by the DPPH method. % Inhibition was represented in Table 2. The comparative analysis of Total antioxidant activity between the ethanolic extract of *Bougainvilea glabra Choisy, Butea monosperma (Lam.), Calendulla officinalis L., Ixora coccinea L. and Hibiscus rosasinensis L.* were represented in the Fig.-7

Table 3: % inhibition of DPPH free radical by Bougainvilea glabra Choisy, Butea monosperma (Lam.), Calendulla officinalis L., Hibiscus rosasinensis L., Ixora coccinea L. extracts/ascorbic acid at 517nm

S.No.	Concentration (mg/ml) Flower extract/ Ascorbic acid	% Inhibition					
		Bougainvilea glabra	*Butea monosperma*	*Calendulla officinalis*	*Ixora coccinea*	*Hibiscus rosasinensis*	Ascorbic acid
01.	100	77.647	76.470	71.764	52.941	60	50.588
02.	200	68.235	62.352	62.352	40	45.882	42.352
03.	300	51.764	44.705	56.470	22.359	41.176	37.647
04.	400	28.235	38.823	42.352	9.411	28.235	24.705
05.	500	4.705	36.470	24.705	4.705	10.588	7.058

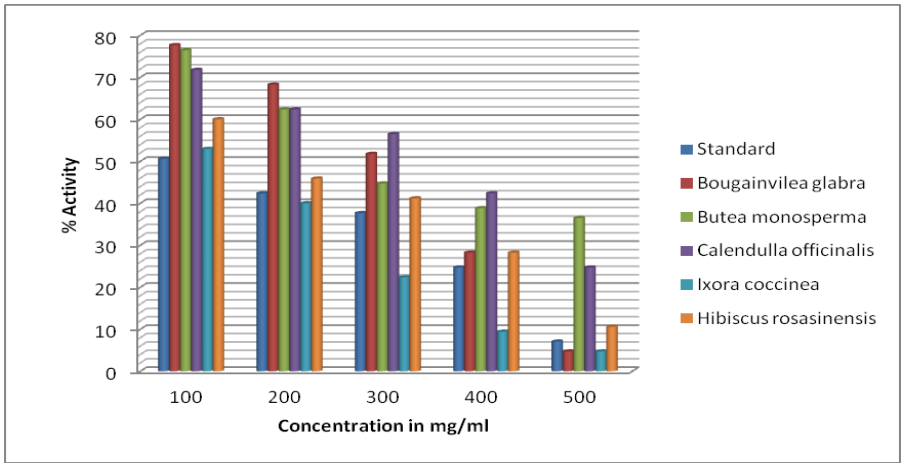

Fig.7: DPPH Radical scavenging activity of Extracts and Ascorbic acid

In vitro Anti-inflammatory activity

HRBC Membrane stabilization method

The ethanolic extract of *Bougainvilea glabra* Choisy, *Butea monosperma* (Lam.), *Calendulla officinalis* L., Ixora coccinea L. and Hibiscus rosasinensis L. were studied for *invitro* anti-inflammatory activity by HRBC membrane stabilization method and the results were represented in the Table 4.

The comparative analysis of Anti-inflammatory activity between the ethanolic extract of *Bougainvilea glabra* Choisy, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Ixora coccinea* L.and *Hibiscus rosasinensis* L. were represented in the Fig.-7.

Table 4: % Protection of heamolysis from HRBC membrane stabilization method by using *Bougainvilea glabra Choisy*, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Hibiscus rosasinensis* L., Ixora coccinea L. flower extract

S.No.	Concentration (µg/ml) Flower extract/ Diclofenac Sodium	% Activity					
		Bougainvilea glabra	*Butea monosperma*	*Calendulla officinalis*	*Ixora coccinea*	*Hibiscus rosasinensis*	Diclofenac Sodium
01.	100	40.83	32.5	20.83	40.83	24.16	76.667
02.	200	34.16	30.83	12.5	30	20.83	72.5
03.	300	25	18.33	8.33	23.33	12.5	60

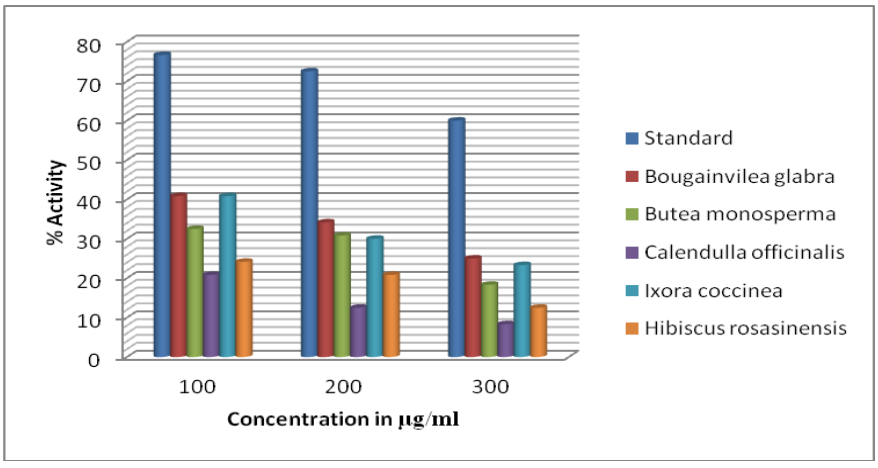

Fig.7: Anti-inflammatory activity of the ethanolic extract of *Bougainvilea glabra* Choisy, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Ixora coccinea* L. and *Hibiscus rosasinensis* L.

Titration

The flower extract (Ethanolic extract) of *Bougainvilea glabra* Choisy, Butea monosperma (Lam.), *Calendulla officinalis L*., *Ixora coccinea* L. , *Hibiscus rosasinensis* L. was screened for its use as an acid base indicator in various acid base titrations, and the results of this screening compared with the results obtained by standard indicators methyl red, phenolphthalein for strong acid v/s strong base (HCl and NaOH), Strong acid v/s weak base (HCl and NH_4OH), weak acid v/s strong base (Acetic acid and NaOH), and weak acid v/s weak base (Acetic acid and NH_4OH) titrations respectively.

The pH range and the results obtained for the titrations were shown in the Table 5. For all titrations the equivalence points obtained by the flower extract were more or less coincide with the equivalence points obtained by standard indicators.

All flower extract show accurate colour change at the equivalent point in all titrations. The results obtained in all the types of acid base titrations lead us to conclude that it was due to the presence of flavonoids and anthocyanins sharp colour changes occurred at the end point of the titrations.

Table 5: Characterization of Acid-Base Titration using Standard indicator/ Flower extract

Synthetic Indicator/ Flower Extract	Molarity	Mean ± SD			
Methyl Red,	0.1	8.854±3.195	5.38±3.04	9.29±2.85	7.03±1.63
Phenolphthalein, Mixed	0.5	8.285±3.215	5.7±3.7	9.185±2.655	6.93±2.43
Indicator	1.0	7.67±3.97	7.63±1.91	9.04±2.75	7.22±2.4
Calendulla officinalis	0.1	7.445±4.69	5.43±2.97	8.92±8.1	6.59±1.83
	0.5	6.885±5.115	5.69±3.67	8.565±3.235	6.65±2.7
	1.0	6.51±5.23	5.8±3.75	8.475±3.275	6.85±2.74
Butea monosperma	0.1	7.33±4.58	6.135±3.85	8.605±3.59	7.09±1.79
	0.5	7.855±3.84	5.835±3.59	8.73±3.17	7.01±2.39
	1.0	7.065±4.56	5.865±3.81	8.635±3.07	6.88±2.66
Ixora coccinea	0.1	5.275±4.52	5.57±3.87	11.01 – 5.22	6.345±3.075
	0.5	5.215±4.54	5.595±3.69	11.25 – 5.56	6.6±2.34
	1.0	5.77±5.23	5.03±3.1	10.95 – 5.70	6.67±1.68
Hibiscus rosasinensis	0.1	5.275±4.22	6.31±3.71	8.895±2.88	6.81±1.71
	0.5	5.635±5.13	5.59±3.65	8.85±2.83	6.705±2.61
	1.0	5.88±5.15	5.775±3.75	8.79±2.77	6.89±2.65
Bougainvilea glabra	0.1	9.1±3.02	6.11±3.52	8.88±3.17	6.96±2.48
	0.5	8.9±3.95	6.22±3.09	8.745±3.31	6.69±2.57
	1.0	8.245±4.52	5.795±3.83	8.68±3.09	7.02±1.5

CONCLUSION

To check the presence of certain phytochemicals, the phytochemical analysis was done on the aqueous extract of *Bougainvilea glabra* Choisy, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Ixora coccinea* L. and *Hibiscus rosasinensis* L..All extracts shows the presence flavonoids and anthocyanins which is very important for the property of indicator, antioxidant and anti-inflammatory agent. The colour changing property was investigated using microtitre plate and all the ethanolic flower extract shows the variation in their colour to that of the pH which concludes that it can definitely be used as an indicator. The total antioxidant activity was investigated with the ethanolic extract of the flowers *Bougainvilea glabra Choisy*, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Ixora coccinea* L., and *Hibiscus rosasinensis* L.. The result of the study concludes that the activity was high in the extracts of *Butea monosperma* and *Bougainvilea glabra*. This is due to the presence of flavonoids and anthocyanins. The activity of the extract is equivalent to that of the standard ascorbic acid. The anti-inflammatory activity was investigated with the ethanolic extract of the flowers *Bougainvilea glabra* Choisy, *Butea monosperma* (Lam.), *Calendulla officinalis* L., *Ixora coccinea* L.,

Hibiscus rosasinensis L.. The result of the study concludes that the activity was high in the extract of *Bougainvilea glabra* and *ixora coccinea* and the extract is efficient than the standard. The ethanolic extract of the flowers *Bougainvilea glabra* Choisy, *Butea monosperma* L., *Calendulla officinalis* L., *Ixora coccinea* L., *Hibiscus rosasinensis* L.shows remarkable change in colour at the equivalent point. But *Ixora coccinea* does not show remarkable change in colour at the particular point and it is difficult to identify the end point. The indicator property is due to the presence of flavonoids and anthocyanins. This study was conducted as an initial step to elucidate the therapeutical, nutriceutical and cosmeceutical potential of these plant products until all the active components of these plants will be clearly established.

REFERENCES

[1] Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S. 1998. Antinociceptive and anti-inflammatory effects of Sambucas ebulus rhizome extract in rats. J of Ethnopharmacology; 61:229- 35.

[2] Cocetta, Cavaiuolo, and Ferrante. 2013. The Antioxidants Changes in Ornamental Flowers during Developmentand Senescence. Antioxidants. 2, 132-155.

[3] Kayaalp SO. 1998. Medical Pharmacology in terms of rational treatment. 3rd ed.New York: Hacettepe-Tas Publication; pp.946-48.

[4] Nehir, E.I.S and Karakaya S. 2004. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. Int. J. Food. Sci. Nutr., 55: 67-74.

[5] Patil, S.B., Kondawar, M.S., Ghodke, DS., Naikwade, NS., and Magdum CS. 2009. Use of Flower Extracts as an Indicator in Acid-Base Titrations. Research J. Pharm.and Tech. 2(2).

[6] Sosa S, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G. 2002. Screening of Anti- inflammatory activity of medicinal plants. J of Ethnopharmacology; 8:211-15.

[7] Tomar, V., Kannojia, P., Jain, K N., and Dubey, K S. 2010. Anti-Noceceptive and Anti -Inflammatory Activity of Leaves of Hibiscus rosasinensis. IJRAP. 1 (1) 201-205.

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