Int. J. Adv. Res. Sci. Technol. Volume 15, Issue 7, 2026, pp. 2329-2335.

IJARST

International Journal of Advanced Research in Science and Technology

https://doi.org/10.62226/ijarst20262743

ISSN 2319 – 1783 (Print)

ISSN 2320 – 1126 (Online)

Development and Comparative Assessment of Four Plant-Derived Natural Indicators for Sustainable Acid–Base Titrations

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ARTICLEINFO

Article history:

Received 12 July 2026
Accepted 21 July 2026
Available online 29 July 2026

Keywords:

Natural indicators; Acid–base titration; Syzygium cumini; Rubus idaeus; Delonix regia; Bougainvillea glabra; Anthocyanins; Green chemistry.

Indexed in:

and in major libraries

ABSTRACT

The growing need for environmentally sustainable and economical methods of analysis has led to the search for natural indicators of plants as substitutes for artificial acid-base indicators. This project involves the exploration of four plant extracts, namely the Jamun (Syzygium cumini) fruit extract, Raspberry (Rubus idaeus) fruit extract, Gulmohar (Delonix regia) flower extract, and Bougainvillea (Bougainvillea glabra) flower extract for their use as natural acid-base indicators. The extracts were prepared through aqueous and ethanolic extraction techniques, and the pH was measured by means of a digital pH meter, pH paper, and red-blue litmus paper. The indicator activity of the extracts was assessed on the basis of color change in the pH range of 1.12-13.02 through standard acids and bases. In addition to that, the practicality of use of the extracts was tested by means of strong acid-strong base (HCl vs. NaOH), strong acid-weak base (HCl vs. Na_2CO_3), weak acid-strong base (CH_3COOH vs. NaOH), and weak acid-weak base (CH_3COOH vs. Na_2CO_3) titrations, and these extracts were compared to the usual synthetic indicators – phenolphthalein and methyl orange. All four extracts showed clear, repeatable and pH-sensitive color change reactions that made it possible to determine the endpoints of acid-base titrations efficiently. Of all the tested extracts, the one from Jamun fruit had the most similar behavior to the synthetic indicators, while Raspberry, Gulmohar, and Bougainvillea extracts gave satisfactory results as well. It can be stated that the natural extracts, especially Jamun fruit extract, have high prospects of being used as inexpensive, biodegradable and environmentally friendly alternatives to synthetic indicators.

Introduction:

Acid-base titration is one of the most commonly used methods of quantitative chemical analysis of acids and bases. Its use is justified by simplicity, high accuracy and precision, low cost, and wide application in pharmaceutics, environmental monitoring, food industry, industries, and education [1–3]. The accuracy of acid-base titration mostly depends on the accurate determination of the endpoint, which can be determined through the use of acid-base indicators, characterized by color change at certain pH levels [2,3]. In accordance with Ostwald's concept, indicators for acid-base titration are weak organic acids or bases, having two differently colored forms due to varying concentrations of hydrogen ions during the neutralization reaction [4].

The traditional synthetic indicators like phenolphthalein, methyl orange, methyl red, bromothymol blue, and bromophenol blue were extensively employed due to their clear-cut pH change range and reliable analytical properties [2,5]. The issues related to the toxicity, environmental effects, and waste management of synthetic chemicals, however, motivated the development of less harmful and more sustainable alternatives [6]. Such issues are well correlated with the concept of GAC that stresses the use of minimum hazardous chemicals, generation of no wastage, and environmentally-friendly analytical techniques without any compromise on analytical performances [7,8].

Plant-based natural indicators have come up as potential substitutes due to their being eco-friendly, easily obtainable, cost-effective, abundant, and generally harmless substances [8,9]. The change in color exhibited by these materials is mainly due to the reversible structural changes caused by pH variations in the presence of natural dyes such as anthocyanins, flavonoids, betalains, and other phenolics compounds [9-13].

However, recent studies have shown that plant-based indicators may serve as reliable substitutes for synthetic indicators during acid-base titrations and give satisfying results at endpoint [9,14-16]. At the same time, their efficiency greatly depends on the pigment composition, extraction procedure, solvent mixture, stability, and other conditions of experiments [15-18]. Moreover, most of the works have studied one particular type of plant or limited pH range, and highlighting the need for comparative evaluation of multiple plant extracts [15-18].

In this research, Jamun (Syzygium cumini), Raspberry (Rubus idaeus), Gulmohar flower (Delonix regia), and Booganvel flower (Bougainvillea glabra) have been chosen due to their high content of anthocyanin, flavonoid, and other phenolic compounds that show pH-dependent color changes [19–22]. The pH dependence of the color of the extracts has been determined for pH values ranging from 1 to 13 and compared with the data obtained by means of a digital pH meter and universal pH test paper. Their suitability as natural indicators has been checked in various acid-base titration experiments and compared with traditional synthetic indicators. This comparative study aims to develop economical, environmentally friendly, and sustainable alternatives to synthetic acid–base indicators while supporting the principles of Green Analytical Chemistry [7–9,23–24].

Materials and Methods:

Fresh Jamun (*Syzygium cumini*) fruits, Raspberry (*Rubus idaeus*) fruits, Gulmohar (*Delonix regia*) flowers, and Bougainvillea (*Bougainvillea glabra*) flowers were collected from the Mhasala region, Raigad, Maharashtra, India, and shown in Figure 1. The botanical identity of the plant materials was authenticated by the Department of Biology, V.N. College of Arts, Commerce and Barrister A.R. Antulay Science College, Mhasala, Raigad**.** Analytical-grade hydrochloric acid (HCl), sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), acetic acid (CH_3COOH), sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), phenolphthalein, methyl orange, ethanol, Whatman No. 1 filter paper, pH paper, and litmus paper were procured from Merck (India), SD Fine Chemicals, and Loba Chemie**.** All reagents used in the study were of analytical grade.

Fig 1. **Jamun (*Syzygium cumini*), Raspberry (*Rubus idaeus*), Gulmohar (*Delonix regia*), and Bougainvillea (*Bougainvillea glabra*)**

The experimental work was carried out using standard laboratory glassware and equipment, including test tubes, pipettes, burettes, conical flasks, volumetric flasks, funnels, droppers, a digital pH meter, heating mantle, hot air oven, laboratory thermometer, electronic analytical balance, wash bottle, spatula, glass stirrer, desiccator, mechanical blender, and a mortar and pestle**.**

Preparation of Fruit Extract (Jamun/Java Plum and Raspberry)

The freshly obtained jamun (java plum) and raspberry fruits were rinsed well with distilled water to get rid of any possible surface contamination. A certain amount of jamun pulp or entire raspberry fruit was taken in a clean beaker and mixed with 30 mL of distilled water. The solution was then crushed with the help of a glass rod to extract anthocyanins and other coloring agents from the fruits. This solution was then warmed up to a temperature range of 50-60°C for 10 minutes with intermittent mixing. After being cooled down to room temperature, the solution was filtered with the help of Whatman's No. 1 filter paper. The filtrate was collected in amber colored container and stored at 4 °C protected from direct sun light until further use [25-27].

Preparation of Flower Extract

The Fresh flower petals were washed with distilled water to remove adhering debris and then dried by gentle pressing with blotting paper. A specific amount of flower petals were then minced and transferred into a mortar with 30 mL of absolute ethanol and thoroughly ground to a homogeneous paste. The paste was kept macerated for 25-30 min at room temperature with occasional stirring to obtain a deep colored extract of anthocyanins and other colored ingredients of flower. The color extract was filtered by means of Whatman No.1 filter paper and filtrate was collected and stored in amber bottle at 4°C in the dark for further studies [25-27].

Determination of pH of plant extracts using pH and red–blue litmus papers

The pH value of each plant extract was established using pH paper along with blue and red litmus paper. The few drops of the extracts were dropped on different paper strips of pH and litmus paper. The color obtained from the pH paper was matched with the color chart of the pH paper.

Determination of pH of plant extracts using a pH meter

The prepared plant extracts were measured for their pH using calibrated digital pH meter. Calibration was performed on pH meter by standard buffer solutions (pH 4.0, 7.0, and 10.0). The electrode was dipped into plant extracts after rinsing by distilled water and pH values were noted after obtaining steady state.

Determination of color change of natural indicators at different pH

Preparations of acid, neutral, and alkaline standard solutions having a pH from 1 to 13 were made, and their actual pH values were determined using a calibrated digital pH meter. About 10 mL of each test solution was poured into individual, labeled test tubes, after which 1 mL of each plant extract prepared was added. The solutions were properly mixed, and the same procedure was done for all the plant extracts under the same experimental conditions to evaluate the color response at different pH values.

Acid–Base Titrimetry using natural and synthetic indicators

Acid base titrations were carried out for the following system combinations: strong acid-strong base (HCl vs. NaOH); weak acid-strong base (CH_3COOH vs. NaOH); strong acid-weak base (HCl vs. Na_2CO_3); and weak acid-weak base (CH_3COOH vs. Na_2CO_3). A standard base solution was delivered into a burette, whereas 10 mL of the appropriate acid solution was put into a conical flask with the help of a pipette. Then two to three drops of an appropriate indicator (phenolphthalein, methyl orange, or plant extracts prepared) were added to the acid solution, and the titration process was conducted while stirring until the permanent color change took place. The initial and final burette volumes were noted down, and the volume of titrant that reached the end point was calculated. Acid and base solutions used for all titrations were made with the concentration of 0.1 M.

Result and Discussion:

Mechanism of Color Change of Natural Indicators

The natural acid-base indicators are natural plant pigments that change their colour with the change in pH. The ability of the plant extract to behave as an indicator is mainly due to the anthocyanins present in the fruits of Jamun (Syzygium cumini), Raspberry (Rubus idaeus), and Bougainvillea (Bougainvillea glabra) while the flowers of Gulmohar (Delonix regia) contain specifically anthocyanins (a type of flavonoid) and carotenoids that can change their colour with the change in hydrogen ion concentration. The pigments behave as weak acid/base indicators and have reversible structural modifications with the change in hydrogen ions and this can be depicted by the following equilibrium reaction:

$$\underset{\text{(Color A)}}{HIn\ (aq)} + H_2O\ (l) \longleftrightarrow \underset{\text{(Color B)}}{In^-\ (aq)} + H_3O^+\ (aq)$$

where HIn is the protonated or acidic form of the indicator, while In^- is the deprotonated or basic form of the indicator. Since the two types have different molecular structures, they have different colors. With variation in pH in the solution, an equilibrium will be established between the two forms of the indicator to bring about different colors. Based on this concept, the extracted fruits and flowers were analyzed based on their acid-base indicator using pH values, different colors at varying pH levels, and acid-base titrations. The findings from the analysis are presented below.

Color of the prepared natural indicator extracts

The extracts of the natural indicators were obtained effectively from the fruits of Jamun (Syzygium cumini), the fruits of Raspberry (Rubus idaeus), the flowers of Gulmohar (Delonix regia), and the flowers of Bougainvillea (Bougainvillea glabra), as illustrated in Figure 2 below. The freshly obtained extracts had their characteristic colours. The extract of Jamun fruit had a deep violet colour, that of the Raspberry fruit had a deep red colour, that of Gulmohar flower had an orange-red colour, and that of the Bougainvillea flower had a deep pinkish-purple colour (magenta).

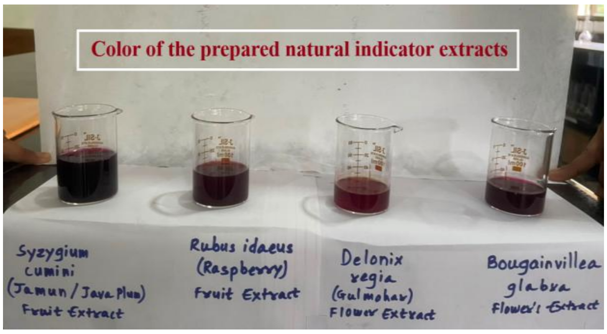

Fig 2. Extracts of Jamun, Raspberrt, Gulmohar and Bougainvillea

Acid–Base nature of plant extracts using pH and litmus papers

The acid–base nature of the prepared plant extracts was evaluated using pH paper and red and blue litmus papers, as shown in Figure 3. The pH paper indicated that all the extracts were slightly acidic, while the blue litmus paper changed to red and the red litmus paper showed no change, confirming the acidic nature of the extracts. These findings are in agreement with the pH values obtained using the digital pH meter (Table 1).

Fig 3. pH and litmus paper tests of Jamun, Raspberry, Gulmohar, and Bougainvillea extracts

pH of the plant extracts

The pH of these pure extracts was determined by using a pH meter after the extraction process was done. Table 1 below shows the pH values of the extracted plants used in this experiment. All the extracts had pH between 3.42 and 5.02, showing that all the extracts were acidic in nature. Out of all the extracts, the extract of Jamun (Syzygium cumini) fruit extract had the least pH value (3.42), and on the other hand, the extract of Bougainvillea (Bougainvillea glabra) flower had the highest pH value (5.02). These extracts are acidic in nature due to the presence of naturally occurring organic acids and anthocyanin pigments, which contribute to their potential use as natural acid–base indicators.

Table 1. pH of the plant extract

Sr. No.	Plant Extract	pH
1	Jamun fruit extract (Syzygium cumini)	3.42
2	Raspberry fruit extract (Rubus idaeus)	3.50
3	Gulmohar flower extract (Delonix regia)	4.70
4	Booganvel flower extract (Bougainvillea glabra)	5.02

Color variation of plant extracts at different pH

The color responses of the prepared plant extracts were studied at various pH values ranging from 1.12 to 13.02 as tabulated in Table 2. The changes in colors noticed for each extract are presented in Table 3. All the prepared extracts showed characteristic color changes at different pH conditions. These findings indicate the pH dependence of the naturally occurring pigments present in these plants, as demonstrated in Figures 2, 4 and 5. Hence, change in colors within the pH range proves that the investigated fruits and flowers could be used as natural acid-base indicators.

Fig 4. Color response of Jamun and Raspberry (fruits) extracts to different pH

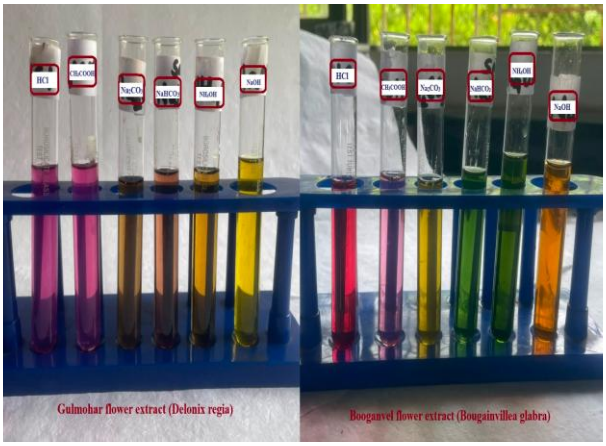

Fig 5. Change in color of Gulmohar extract and Bougainvillea extract with respect to pH

Table 3. Observed Color Changes of Plant Extracts at Different pH values

Sr. No.	Plant material (Fruit/ Flower)	pH / Color						
		1.12 HCl	2.88 CH_3COOH	7.0 water	8.32 $NaHCO_3$	10.94 NH_4OH	11.63 Na_2CO_3	13.02 NaOH
1	Jamun fruit extract (Syzygium cumini)	Intense ruby red	Orange-red	Purplish blue	Reddish-brown	Dark olive green	Olive brown	Lime green
2	Raspberry fruit extract (Rubus idaeus)	Crimson red	Pinkish red	Reddish pink	Brown	Grayish green	Khaki green	Yellowish green
3	Gulmohar flower extract (Delonix regia)	Magenta	Light Pink	Red orange	Amber Brown	Amber Yellow	Coffee Brown	Lemon Yellow
4	Booganvel flower extract (Bougainvillea glabra)	Ruby Red	Light Pink	Pink	Green	Dark green	Golden Yellow	Amber Orange

Table 4. Acid–Base Titrations using Natural and Synthetic Indicators

Sr. No.	Titration	Plant extract/ Indicator	End point in mL	Color change
1	Strong acid–Strong base (HCl vs. NaOH)	Phenolphthalein	10.3	Colorless to pink
2		Jamun fruit extract	10.3	Intense ruby red to Green
3		Raspberry fruit extract	10.5	Crimson red to greenish yellow
4		Gulmohar flower extract	10.7	Magenta to faint greyish green
5		Bougainvillea flower extract	10.7	Ruby Red to brownish yellowish
6	Strong acid–Weak base (HCl vs. Na_2CO_3)	Methyl orange	5.3	Red to yellow
7		Jamun fruit extract	5.5	Ruby red to blue
8		Raspberry fruit extract	5.9	Crimson red to light green

9		Gulmohar flower extract	5.7	Magenta to Greyish blue
10		Bougainvillea flower extract	6.0	Ruby Red to faint brownish yellow
11	Weak acid–Strong base (CH_3COOH vs. NaOH)	Phenolphthalein	9.7	Colorless to pale pink
12		Jamun fruit extract	9.8	Faint pink to lime green
13		Raspberry fruit extract	10.1	Faint pink to light greenish yellow
14		Gulmohar flower extract	10.2	Light pink to faint grayish green
15		Bougainvillea flower extract	10.3	Light pink to brownish yellow
16	Weak acid–Weak base (CH_3COOH vs. Na_2CO_3)	Phenolphthalein	5.6	Colorless to faint pink
17		Jamun fruit extract	5.8	Light pink to faint green
18		Raspberry fruit extract	6.1	Light pink to yellowish green
19		Gulmohar flower extract	6.0	Light pink to colorless
20		Bougainvillea flower extract	6.2	Light pink to colorless

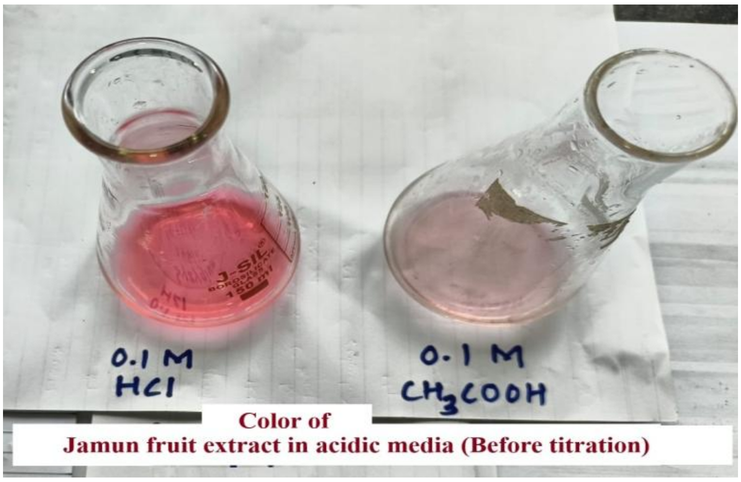

Fig 6. Color of Jamun fruit extract (before titration)

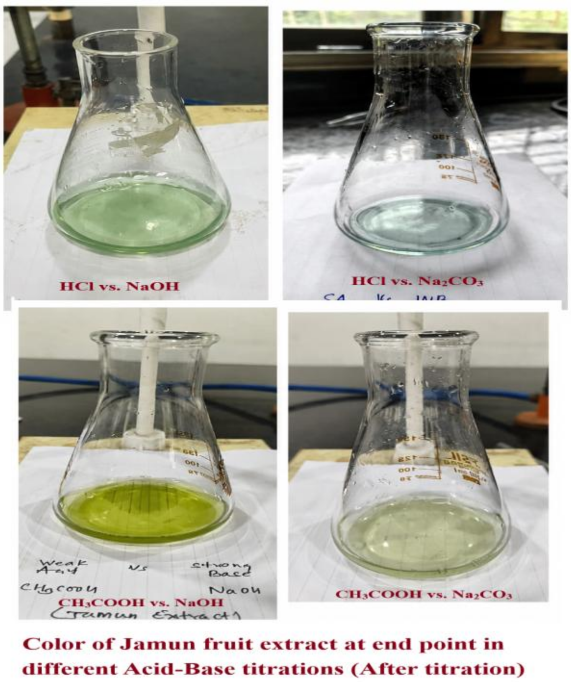

Fig 7. Color responses of Jamun fruit extract at the end point in acid-base titrations

Effectiveness of the prepared plant extracts as natural acid-base indicators was checked by comparing the end points obtained for the prepared natural indicators with the end points obtained by using the commonly used synthetic indicators, namely phenolphthalein and methyl orange (Table 5). All the tested natural indicators had distinct end points with reproducible color change in case of acid-base reaction. In the case of strong acid-strong base (HCl/NaOH) titration, the end points of natural indicators were quite in agreement with the end points of synthetic indicator, phenolphthalein. The most accurate results were obtained by using Jamun fruit extract in comparison with other tested natural indicators. There was slight deviation in case of Raspberry, Gulmohar, and Bougainvillea extracts from the phenolphthalein endpoint. Distinct color changes observed in this case prove the suitability of these extracts as natural indicators for strong acid-strong base titration. In the case of strong acid-weak base (HCl/Na_2CO_3) titration, the end points of natural indicators were quite in agreement with each other but there was some deviation from the end point of methyl orange. These variations are expected because carbonate-based titrations involve the formation of carbonic acid and the evolution of carbon dioxide, resulting in a comparatively broader endpoint region. The titration of the weak acid (CH_3COOH) and strong base (NaOH) gave highly accurate results in terms of the colour transition of the natural extracts and phenolphthalein. It can be concluded that the colour transition range of the obtained extracts is suitable enough to determine the end point of titration of a weak acid and a strong base. The results of the tests proved that naturally occurring pigments react well to the sudden change of pH level at the end point. As far as the titration of the weak acid (CH_3COOH) and weak base (Na_2CO_3) was concerned, the results were relatively inconsistent because of the slow change in the pH level at the end point, which makes precise visual endpoint detection inherently more difficult. Nevertheless, all the investigated extracts exhibited observable and reproducible colour transitions, indicating their applicability for qualitative and educational titration experiments. Overall, the fruits and flowers extracts under examination provided quite good indicator characteristics in all four types of titrations. It is evident that the extract from jamun fruit was the most similar to conventional synthetic indicators, which means its higher sensitivity compared to other natural indicators under consideration. The extracts from raspberry, gulmohar flower, and bougainvillea also performed quite well in titration reactions, though their endpoints were more shifted. From this one can conclude that these extracts can serve as cheap, ecological, and sustainable substitutes for synthetic acid-base indicators.

Conclusion:

This current study is capable of developing and comparatively evaluating four extracts of plants, which are Jamun (Syzygium cumini) fruit, Raspberry (Rubus idaeus) fruit, Gulmohar (Delonix regia) flower and Bougainvillea (Bougainvillea glabra) flower, which serve as natural acid-base indicators. These natural indicators all show slightly acidic pH with a distinct colour change dependent on pH ranging from 1.12 to 13.02, thereby indicating the feasibility of using their inherent pigments for acid-base determination. These practical uses of the extracts were demonstrated using four acid-base titration experiments in comparison with two synthetic acid-base indicators, phenolphthalein and methyl orange. In all these tests, the natural indicators gave well-defined and repeatable end point, whereby Jamun fruit extract shows the closest similarity with the synthetic indicators while the other extracts such as Raspberry, Gulmohar and Bougainvillea also exhibit good results. As a result, the studied plant extracts, especially the Jamun fruit extract, are very good candidates as alternative low-cost, biodegradable, non-toxic and environmentally friendly acid-base indicators for routine lab work. Their usage is consistent with the tenets of green chemistry, allowing for a sustainable approach towards analysis. Additional work on the stability and standardization of these natural indicators is advised to broaden their usage.

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