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RESEARCH ARTICLE
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EXTRACTION AND EVALUATION OF SOME NATURAL FLOWER PIGMENTS: AN IMPROVISED NATURAL INDICATOR FOR ACID-BASE TITRATION

Suchitra Sharma[1]*, Amit Babu[1], Vikas Saxena[1]

[1]Rakshpal College of Pharmacy, Bareilly U.P. (243001), India.

***Corresponding Author:** Suchitra Sharma
*Assistant Professor at Rakshpal College of Pharmacy, Bareilly U.P. (243001), India.
Contact no.: +919528444412, Email: suchitrsharma4@gmail.com

Abstract:
Background: A member of the different families, these flowers are most widely cultivated ornamental bulbous plants for its intriguing blooms across the world.
Objective: The current study focuses on the extraction and assessment of an acid-base indicator from a Tabernaemontana *divaricata,* Brugmansia *suaveolens*, Hibiscus rosa-sinensis (White) and Campsis *grandiflora* species that is environmentally beneficial and native to the area.
Methods: In the current investigation, acid-base titrations of four distinct types—strong acid against a strong base, strong acid against a weak base, weak acid against a strong base, and weak acid against a weak base—were conducted. Two bases (NaOH and NH_4OH) and two acids (HCl and CH_3COOH) were chosen for the acid-base titration. These bases and acids were utilised at a concentration of 0.1N.
Result: Natural indicators are promising when compared to commercially available synthetic acid-base indicators like methyl orange and phenolphthalein because they exhibit a sharp and dramatic colourb change near the neutralizing point.
Conclusion: The use of Tabernaemontana *divaricata,* Brugmansia *suaveolens*, Hibiscus rosa-sinensis (White) and Campsis *grandiflora* flower extract as an indicator in all kinds of acid base titrations is also determined to be advantageous due to its affordability, eco-friendliness

Introduction

Tabernaemontana plants, belonging to the *Apocynaceae* family, have been documented as abundant reservoirs of diverse alkaloids. Tabernaemontana *divaricata* (TD) has been identified as a constituent of revitalizing and neurotonic treatments in Thai traditional medicine. Considerable evidence suggests that these interventions have the potential to mitigate forgetting and enhance memory function (1, 2). The initial findings from a Hippocratic screening indicate that the ethanolic extracts derived from different parts of T. pandacaqui (P.) and T. divaricata (L.) exhibit numerous pharmacological properties, including suppression of the central nervous system (CNS), reduction of pain, relaxation of skeletal muscles and hypotensive effects (3).

Fig. 1: Photograph of the selective plants i.e. **Tabernaemontana *divaricata***

Brugmansia suaveolens are about 300-catalogued species and 150 genera in the *Solanaceae* family. Tropical regions like Brazil host the majority of this family's species, which play a significant economic role for the entire world. It is ranked #1 among vegetables and the third-most significant plant family commercially. Among these species are the tomato (*Solanum lycopersicum*), which is crucial to agriculture, and Atropa belladonna, which is particularly notable for the pharmaceutical sector (4, 5). This family includes species that are easily found in homes and gardens, such as the "trumpet tree" (Brugmansia suaveolens), which is often grown as an ornamental plant because of its lovely flowers and distinctive scent. Within the purview of the study, tropane alkaloids—a class of frequently harmful secondary metabolites employed by plants as defense—are known to be produced by plants in the Solanaceae family (6).

Fig. 2: Photograph of the selective plants i.e. **Brugmansia *suaveolens***

Hibiscus rosa-sinensis (White), often known as shoe flower, is a perennial plant in the genus Hibiscus and family Malvaceae. About 275 species of hibiscus are found in the genus and are widely scattered throughout the world. They are native to tropical and south Asian countries. The majority of hibiscus species are grown for their aesthetic qualities (7). All year long, they yield stunning flowers with vivid colours. There are several cultivars with single or double flowers in hues of red, peach, white, pink, and orange (8).

Fig. 3: Photograph of the selective plants i.e. **Hibiscus *rosa-sinensis* (White)**

The Campsis Lour. genus has only two species: Campsis grandiflora (Thunb.) in East Asia and Campsis radicalis (L.) Bureau in North America. Due of its disjunction distribution, this genus was used to study evolutionary history. Both species were believed to have diverged around 24.4 million years ago (9). Campsis grandiflora's trumpet-shaped flowers have made it useful as a decoration. Furthermore, it was previously established that C. grandiflora has a number of beneficial phytocompounds, including triterpenoids, as well as anti-oxidative and anti-inflammatory properties. We finished the genome of the C. grandiflora chloroplast that was isolated in Korea in order to comprehend intraspecific variations of the genome in addition to the previously published genome obtained in China (10).

Fig. 4: Photograph of the selective plants i.e. **Campsis *grandiflora* (Thunb.)**

The plant **Ipomoea *carnea*** commonly referred to as Bush Morning Glory. This plant may be found all over the world, especially in Bolivia, Brazil, Argentina, and the American Tropics (11). It is widely dispersed throughout India, but is most common in Madhya Pradesh and Chhattisgarh (12). It has spread throughout India, taking up waste areas, roadside vegetation, canals, drain banks, marshes, and field edges. The plant grows quickly and may reproduce both vegetative through stems that root in a few days and sexually through seeds (13). I. carnea is utilized for both its decorative and therapeutic qualities. Because of the plant's anti-inflammatory properties, traditional medicine uses its latex as an antiseptic to cure sores (14). This plant's hot water extract lowers the teratogenic effects of cyclophosphamide and has anti-rheumatic properties (15). An important component of the nociceptive system is released by the plant extract, which inhibits the development of pain and relieves the symptoms of a variety of inflammations (16).

Fig. 5: Photograph of the selective plants i.e. **Ipomoea** ***carnea***

Natural acid-base indicators are safer for the environment than synthetic ones because they match the standards of modern chemistry in terms of addressing difficulties with cost, human health, and environmental contamination. As synthetic acid-base indicators become increasingly popular in school chemical labs, there is a growing demand for the invention of ecologically responsible alternatives (17). Synthetic and pricey indicators are routinely applied in these titrations. Additionally, some of them could contaminate the environment and have severe implications on the users. There have been growing issues about finding greener sources of acid-base indicators for these and other reasons. These greener options would be more economical, broadly accessible, easy to extract, less hazardous to users, and ecologically sound (18). In acid-base titrations, indicators are used to determine the end point (equivalency point). The indicator changes colour dramatically when the pH level changes. Synthetic indicators are often used for acid-base titrations. Variations in pH cause each indicator to show a different colour spectrum (19).

MATERIALS AND METHODS

Plant materials

Petals of different flower (Tabernaemontana *divaricata,* Brugmansia *suaveolens*, Hibiscus rosa-sinensis (White) and Campsis *grandiflora*) were collected from Garden of Rakshpal Bahadur College of Pharmacy, Bareilly (U.P.). The plant material was identified and authenticated by Dr. Alok Srivastava, Associate professor Department of Plant Science/ Botany, M.J.P. Rohilkhand University, Bareilly U.P. (Ref. No. RU/PS/Recognition/09, dated-17.07.2024). For future use, the Department received a voucher specimen of the plant sample that was gathered.

Chemicals and Reagents

We got our supplies from Central Drug House in New Delhi, India. We bought ethanol, sodium hydroxide, ammonium hydroxide, acetic acid, and hydrochloric acid. Analytical grade reagents were employed in the investigation, and the same set of glassware used for the extraction and titration process was used throughout the completely experimental process.

Preparation of flower extract
The flowers petals (Tabernaemontana *divaricata,* Brugmansia *suaveolens*, Hibiscus rosa-sinensis (White) and Campsis *grandiflora*) were thoroughly washed with distilled water, cut in small pieces and crushing with the help of mortar-pestle and dissolved in 100 ml of ethanol and macerated for 24 hours and then extract was filtered. The extract was preserved in tightly closed container and stored away from direct sun light.

Preparation of solutions
0.1N HCl, 0.1N NaOH, 0.1N NH4OH and 0.1N CH_3COOH solution were prepared according to Indian Pharmacopoeia (20). Phenolphthalein and methyl orange indicator were also prepared according to Indian Pharmacopoeia.

Experimental procedure
By using several chemical tests, a qualitative phyto-chemical examination of the floral extract of Tabernaemontana *divaricata,* Brugmansia *suaveolens*, Hibiscus rosa-sinensis (White) and Campsis *grandiflora* was carried out. A strong acid (HCI) was used in the acid-base titrations against a strong base (NaOH), a weak base (NH4OH), a strong acid (HCI) against a weak base (NaOH), and a weak acid (CH_3COOH) against a weak base (NH4OH). Before usage, every piece of equipment and tool needed for the current study endeavor was calibrated.
Before use, every piece of equipment and tool needed for the current research endeavor was calibrated (21).
To facilitate the titrations, a volume of 10 ml of the acid was introduced into a conical flask, while the base was simultaneously introduced into a burette. In this experiment, two drops of phenolphthalein and methyl orange indicators were employed as indicators. Additionally, 1 ml of the extracted natural indicator transfer from Tabernaemontana *divaricata*, Brugmansia *suaveolens*, Hibiscus rosa-*sinensis* (White), and Campsis *grandiflora* was introduced into the conical flask prior to the titration analysis. Four acid-base titrations were conducted for each titration, utilizing three distinct sets of indicators: phenolphthalein, methyl orange, and extracts derived from Tabernaemontana *divaricata*, Brugmansia *suaveolens*, Hibiscus rosa-*sinensis* (White), and Campsis *grandiflora*. These extracts employed an environmentally sustainable acid-base indicator. The average volume for each data point was calculated and presented (22).

RESULT AND DISCUSSION:
The outcomes of qualitative phyto-chemical analysis of the flower extract of Tabernaemontana *divaricata*, Brugmansia *suaveolens*, Hibiscus rosa-*sinensis* (White), and Campsis *grandiflora* was shown in Table 1. The floral extract exhibited the presence of many bioactive compounds, including flavonoids, tannins, terpenoids, alkaloids, phenolic compounds, lignin, and steroids.

Table 1: Qualitative phyto-chemical analysis of flower extract.

S.No.	**TEST**	**Tabernae-montana *divaricata***	**Brugmansia *suaveolens***	**Hibiscus rosa-*sinensis* (White)**	**Campsis *grandiflora***	**Ipomoea Carnea**
1.	Flavonoid	++	+	+	+	++
2.	Tannin	++	++	+	++	++
3.	Terpenoid	++	++	++	++	++
4.	Alkaloid	++	++	++	++	++
a)	Mayer' reagent	+	+	+	+	+
b)	Dragondorff's reagent	+	+	-	+	+

c)	Wagner's reagent	+	+	+	+	+
5.	Phenolic compound	++	+	+	+	+
6.	Lignin	+	++	+	+	-
7.	Steroid	+	+	+	+	+

(++) Present (+) slightly present (-) absent

The colours of the indicators in the acidic and basic media, as well as their colour at the end point, were provided in Table 2, which displayed contrast colour and was extremely simple to visualize.

Table 2: Colour of indicators in solutions.

S.No.	**Indicator**	**pH of indicator**	**Colour of indicator**	**Colour of indicator in acidic media**	**Colour of indicator in basic media**	**Colour of indicator at end point**
1.	Tabernaemontana *divaricata*	8.2	Light Yellow	Colorless	Parrot green	Parrot green
2.	Brugmansia *suaveolens*	8.1	Light Pink	Light Orange	Yellow	Yellow
3.	Hibiscus rosa-*sinensis* (White)	4.1	Light Yellow	Colorless	Mustard Yellow	Mustard Yellow
4.	Campsis *grandiflora*	4.3	Orange	Light yellow	Turkish Green	Turkish Green
5.	Ipomoea Carnea	5.2	Green	Pink	Yellow	Green
6.	Phenolphthalein	8.5	Colorless	Colorless	Pink	Pink
7.	Methyl orange	3.9	Orange	Red	Yellow	Yellow

The results of the titration between strong acid and strong base were reported in Table 3, the natural flower extracts with average volume of base, 9.0, 11.0, 9.2, 9.5 and 9.3 ml competes with phenolphthalein with average volume of 9.3 ml and methyl orange with average volume of 10.5 ml.

Table 3: Titration results for strong acid (HCI) against strong base (NaOH)

Titration	**Titre value (ml)**						
	Tabernae-montana *divaricata*	**Brugmansia *suaveolens***	**Hibiscus rosa-*sinensis* (White)**	**Campsis *grandiflora***	**Ipomoea Carnea**	**Phenolphtha-lein**	**Methyl Orange**
1st	9.1	11.2	9.1	9.6	9.2	9.2	10.4
2nd	8.9	11.2	9.0	9.4	9.2	9.4	10.6
3rd	9.0	11.0	9.2	9.5	9.3	9.3	10.5
Average volume (ml)	9.0	11.1	9.1	9.5	9.2	9.3	10.5

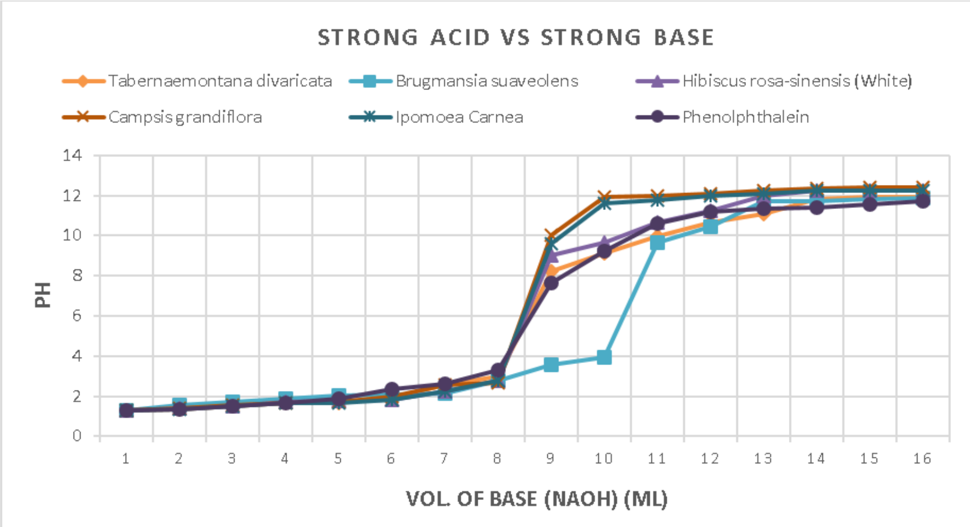

Fig.6 Titration results for weak acid (CH_3COOH) against strong base (NaOH)

The results of the titration between weak acid and strong base were reported in Table 4, the natural flower extracts with average volume of base, 11.9, 11.1, 12.0, 10.6 and 10.1 ml competes with phenolphthalein with average volume of 12.0 ml and methyl orange with average volume of 11.5 ml respectively.

Table 4: Titration results for weak acid (CH_3COOH) and strong base (NaOH).

Titration	Titre value (ml)						
	Tabernae-montana *divaricata*	Brugmansia *suaveolens*	Hibiscus rosa-*sinensis* (White)	Campsis *grandiflora*	Ipomoea Carnea	Phenolphtha-lein	Methyl Orange
1st	11.8	11.2	11.9	10.5	10.1	12.2	11.4
2nd	11.8	11.2	12.1	10.7	10.2	11.9	11.6
3rd	12.0	11.0	12.1	10.6	10.0	12.0	11.5
Average volume (ml)	11.9	11.1	12.0	10.6	10.1	12.0	11.5

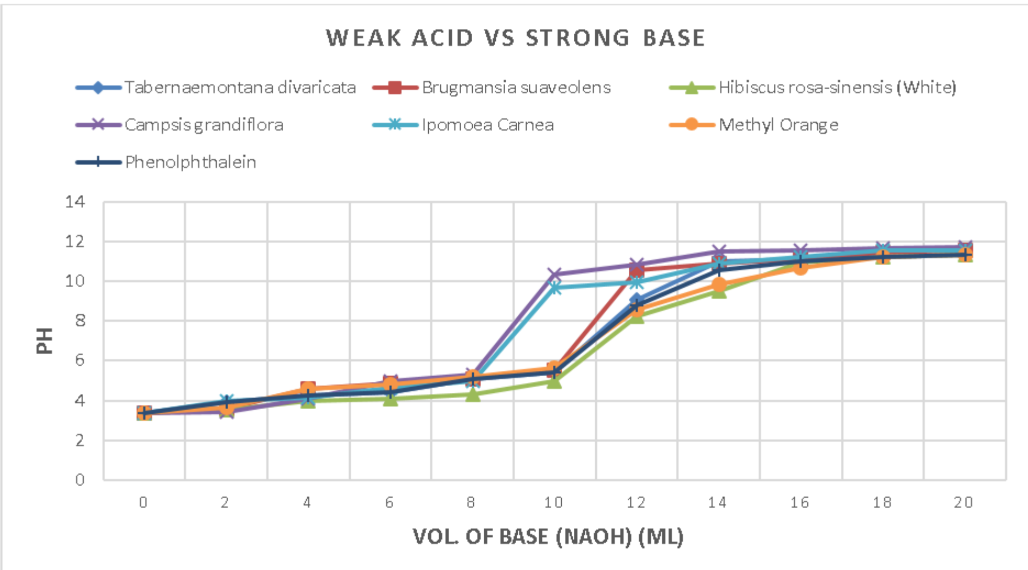

Fig.7 Titration results for strong acid (HCI) against strong base (NaOH)

The results of the titration between strong acid and weak base were reported in Table 5, the natural flower extracts with average volume of base, 5.1, 5.3, 5.1, 4.5 and 5.6 ml competes with phenolphthalein with average volume methyl orange with average volume of 5.5 ml respectively.

Table 5: Titration results for strong acid (HCI) and weak base (CH_3COOH).

Titration	Titre value (ml)					
	Tabernae-montana *divaricata*	Brugmansia *suaveolens*	Hibiscus rosa-*sinensis* (White)	Campsis *grandiflora*	Ipomoea Carnea	Methyl Orange
1st	5.1	5.5	5.1	4.5	5.5	5.5
2nd	5.0	5.3	5.1	4.5	5.7	5.8
3rd	5.1	5.3	5.2	4.6	5.4	5.5
Average volume (ml)	5.1	5.3	5.1	4.5	5.6	5.5

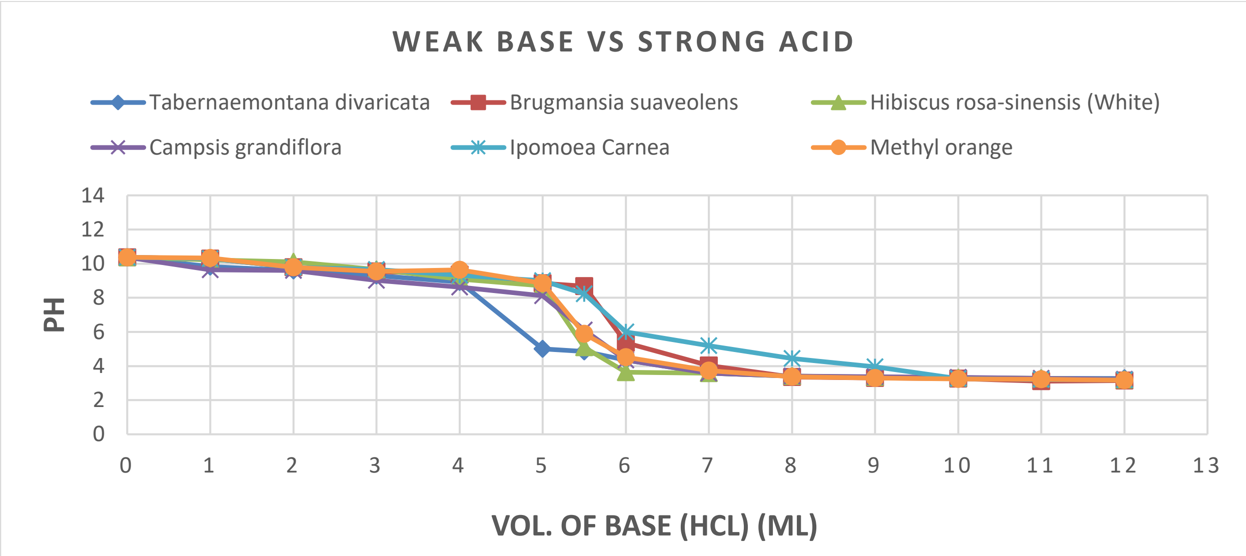

Fig.8 Titration results for strong acid (HCI) and weak base (CH_3COOH).

Gladiolus Red Cascade (red) and Gladiolus Red lime flower (Orange) are both gives three different colour at different pH during the acid base titration and that both will gives results as Thymol blue indicator. The further activity check in another future research.

Conclusion:

The current study recommends that natural indicators be used to reduce user toxicity and environmental damage. By carrying out several acid-base titrations and plotting their pH graphs, the accuracy of the data has been assessed. According to the findings, eco-friendly natural floral extract made from Tabernaemontana *divaricata*, Brugmansia *suaveolens*, Hibiscus rosa-*sinensis* (White), and Campsis *grandiflora* may be a quick and easy replacement for phenolphthalein and methyl orange. The results obtained sharp end point in [strong acid (HCI) against strong base (NaOH), weak acid (CH_3COOH) and strong base (NaOH) and weak base (NH_4OH) against strong acid (HCI)] acid base titrations and Do not give sharp end point in weak acid (CH_3COOH) and weak base(NH_4OH) titrations. Chemical analyses were performed to determine if anthocyanins and flavonoids were present in the plant extract as active ingredients. The authors came to the conclusion that because they are more readily available, less expensive, easier to use, and less harmful to the environment than synthetic indicators, natural acid-base indicators made from pure and acidified ethanol extracts of Tabernaemontana *divaricata*, Brugmansia *suaveolens*, Hibiscus rosa-*sinensis* (White), and Campsis *grandiflora* flowers might be a good alternative.

The accessibility as well as simple extraction process, together with superior performance and precise outcomes, render these natural flower indicators appropriate alternatives to synthetic indicators employed in numerous laboratories and research institutions. In summary, industries, research laboratories, educational institutions, and chemical companies that utilize indicators for assessing acidity, alkalinity, humidity, and reaction extent would find the preliminary results of this study valuable for developing efficient indicators derived from flowers as substitutes or potential replacements for conventional indicators. The standard technique used by the American Society for Testing and Materials (ASTM) to measure a lubricant's resistance to the harmful effects of acidic ingredients is colorimetric titration with synthetic indicators. In contemporary labs, natural floral indicators could take the place of these artificial ones.

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