



WALLAGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

**PREPARATION OF NATURAL INDICATORS FROM
PLANT FLOWERS COLLECTED FROM LIMU GELILA,
ETHIOPIA, AND ITS APPLICATION IN ACID-BASE
TITRATION**

M.Sc. Thesis

By:

Enkosa Mijena

December 2024

Nekemte, Ethiopia



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**PREPARATION OF NATURAL INDICATORS FROM PLANT
FLOWERS COLLECTED FROM LIMU GELILA, ETHIOPIA, AND
ITS APPLICATION IN ACID-BASE TITRATION**

**A Thesis Submitted to the College of Natural and Computational
Sciences in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Analytical chemistry**

By:
Enkosa Mijena

Advisor: - Shibiru Yadeta (PhD)

December 2025

Nekemte, Ethiopia



WALLAGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
P.O. Box: 395, Nekemte, Ethiopia.

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STATEMENT OF THE AUTHOR

I, Mr. **Enkosa Mijena**, hereby declare and affirm that the thesis entitled "**Preparation of Natural Indicators from Plant Flowers Collected from Limu Gelila, Ethiopia and its Application in Acid-Base Titration**" is my own work conducted under the supervision of **Dr. Shibiru Yadeta** at Wallaga University. I have followed all the ethical principles of scholarship in the preparation, data collection, data analysis, and completion of this thesis. All the scholarly material that is included in the thesis has been given recognition through citation. I have adequately cited and referenced all the original sources. I also declare that I have adhered to all principles of academic honesty and integrity, and I have not misrepresented, fabricated, or falsified any idea, data, fact, or source in my submission. This thesis is submitted in partial fulfillment of the requirement for a degree from the Postgraduate Studies program at Wallaga University. I further declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Advisor: Shibiru YADETA (PhD): Signature



Date_____

BIOGRAPHY

The author was born on June 19, 1990 G.C. in Oromia regional state, East Wallaga Zone, Limu district, Warsu kebele. He attended his primary school education at Limu Primary School (1–8) from 1997 to 2003, and then he attended his secondary school Education in the East Wallaga Zone at Limu Galila Secondary School (9–10) from 2004 to 2005. Then he joined Nekemte Teacher Training College in 2009 and graduated with a Diploma in Natural Science in 2012 (G.C.). After that, he enrolled for the summer in Wallaga University's BSc Chemistry program from 2014 to 2018. In 2021, he finally enrolled at Wallaga University to pursue his M.Sc. in analytical chemistry.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
BTB	Bromothymol blue
CS	Cordia sebestena
GS	Guizotia scabra
MR	Methyl red
PH	Phenolphthalein
pH	power of Hydrogen
Rf	Retention factor
RH	Rose hybrida
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
ST	Solanum tuberosum
TLC	Thin layer Chromatography

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ABSTRACT

Today, a number of commonly used acid-base indicators in laboratories, such as methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, bromophenol blue, thymol blue, etc., are toxic and cause environmental pollution. However, due to environmental pollution, availability, and cost, the search for natural compounds as an acid-base indicator was started. Many flowers contain a class of organic pigment called anthocyanins that can change color with changes in pH. The main objective of the present study was to prepare *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* flower extracts in comparison to synthetic indicators for acid-base titration. Flower extracts of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida*, respectively, were collected from Limu Gelila district, Oromia regional state, Ethiopia. Then it was washed, rinsed with deionized water repeatedly, dried at room temperature for 15 days under shade, crushed, and the powders were extracted by using deionized water as a solvent. The pH of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* were 2.96, 2.91, 3.72, and 3.73, respectively. The colors of the prepared *Guizotia scabra* and *Cordia sebestena* extracts were yellow at pH 4 to pH 6 and orange at pH 8 and pH 10. The color of *Solanum tuberosum* was orange at pH 4–8 and yellow at pH 10. The color of *Rose hybrida* was yellow at pH 4 and 5, but orange at pH > 5. The flower extracts were characterized by thin layer chromatography, and the R_f values of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum* (spot 1 and 2), and *Rosa hybrida* were 0.6, 0.4, 0.5, 0.2, and 0.74, respectively. The end point results of HCl versus NaOH by using both synthetic and all flower extracts were close to each other except *Solanum tuberosum* and *Cordia sebestena*, whose end points were not close to the methyl red indicator. For titration of NH_3 vs. HCl, *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* can replace phenolphthalein, and *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* can replace methyl red. But all flower extract end points are not in agreement with the bromothymol indicator except *Cordia sebestena*. All flower extracts can replace phenolphthalein and methyl red in CH_3COOH vs. NaOH titration, except *Rosa hybrida*, whose end point is not close to the methyl red end point. Bromothymol blue can be substituted by *Guizotia scabra* and *Solanum tuberosum* flower extracts in a weak acid versus strong base (CH_3COOH vs. NaOH) titration.

Keywords: indicators; flowers; titration; *Guizotia scabra*; *Cordia sebestena*; *Solanum tuberosum*; *Rosa hybrida*

1. INTRODUCTION

1.1 Background the Study

Synthetic dyes are widely used in industries like textile, leather and, commercial indicators. Acid-base indicators are among synthesis dyes applied to the environment and pose pollution problem. They are chemicals employed to measure the alkalinity or acidity level through titration reaction by showing distinctive color changes when shift from acid to base or viscera. Synthetic indicators are man-made chemical substance in the laboratory which are used to determine pH of a substance that may include litmus paper. They are substances that indicate pH levels, but the item as a whole is made in the laboratory such as methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, pentamethoxy red, bromophenol blue, and thymol blue. Such synthetic indicators have certain disadvantages such as high cost, availability, and chemical pollution. Furthermore, some of these synthetic indicators can create environmental pollution and induce hazardous side effects in users, including diarrhea, pulmonary edema, hypoglycemia, and pancreatitis. They can also cause stomach pains, skin rashes, eruptions, erythema, and epidermal necrosis.

Therefore, these researches focus on the investigations of an alternative way for producing non-toxic, eco-friendly, and less expensive indicators. Since green chemistry requires natural acid-base indicators to solve difficulties with regard to cost, human health, and environmental pollution it is an alternatives to synthetic ones. This is because plants possess anthocyanin's molecules, organic pigment are responsible for the color variations of many flowers, including those that are red, purple, or blue in hue. Anthocyanin are molecules in plant which are polar and can be extracted by employing polar solvents including methanol, ethanol, and hot water. Therefore, it is crucial to compare the response of floral extract under various pH levels to commercial indicators using pH measurements in order to make use of this natural indicator.

The use of natural indicator made from plant extract as an acid-base indicator has the benefits of being inexpensive, readily accessible locally, non-toxic, and ecologically beneficial because it breaks down quickly in the environment. Long before chemists created artificial acid-base indicators, natural indicators have been utilized for hundreds of years (Suteerapataranon *et al.*, 2024). Titration is a widely used laboratory technique for quantitative chemical analysis that is used to ascertain analyte concentration. Some chemical analysts routinely used highly polluting, dangerous, costly, and highly polluting synthetic indicators that may damage environment and biological systems. For various titrimetric analysis a variety of synthetic chemical indicators are available. According to, the end point of a classical titrimetric assay is often indicated by a change in the indicator's color in the analyte solution. Methyl red, methyl orange, phenolphthalein, phenol red, methyl yellow, bromophenol blue, and thymol blue are a few of the indicators that are frequently employed as indicators in titrimetric analysis

The preparation of natural indicators for acid-base titrations is the subject of several publications written by various authors. *Phyllanthus muellerianus*, Butterfly pea (*Clitoria ternatea*), *Syzygium cumini* and *Euphorbia milii* flower are a few of these natural plants that have been identified as natural plant indicators. Additionally, reported employing *Allium cepa*, *Centrosema pubescens*, and *Dissotis rotundifolia* extracts in methanol and acetone as an acid-base indicator. Furthermore, natural indicators from *Tagetes Erecta*, *Impatiens Balsamina*, *Tecoma Stans*, and *Rosa rugosa var* were sensitized for acid-base titration in another work. These indicators gave positive findings at neutralization and may be utilized in place of synthetic indicators. also extracted *Hibiscus rosa-Sinensis*, red cabbage, Capsicum fruit, Pistacia *atlantica*, tomatoes, eggplant, Beetroot red beets, and Red Acacia for acid base titration. As they studied *Hibiscus* flower and cabbage leaf extracts are superior indicators for a strong-acid and strong-base titration to the synthesizer's indicators.

To this end, no study was conducted on preparation of natural indicators from *Guizotia scabra* (Vis) Chiov, *Cordia sebestena*, *Solanum tuberosum* and *Rose hybrida* flowers for acid-base titration in Western Oromia (Ethiopia). Consequently, this study focused to fill a research gap on preparation natural indicators from the aforementioned plant flowers collected from Limu (Gelila) district.

1.2 Statement of the problem

Commercial indicators are expensive, and some of them have toxic effects on users and can also cause environmental pollution (Witkowska-Dąbrowska & Świdyńska, 2024). They are carcinogenic and mutagenic to living organisms. In addition, the commercial indicator has weaknesses such as lack of availability. The most common synthetic indicators, phenolphthalein is an organic pigment that may bioaccumulate over time, decreasing water oxygen content and light penetration which is problem for humans. It also reported in aggravating cancer-causing dyes and the digestive system. Similarly, methyl red is permanent indicator used in acid-base titrations can cause a variety of negative effects in tumors, hives, angioedema, nasal congestion, and itchy skin. Additionally, these colors alter aquatic ecosystem composition and have an impact on aquatic creature survival. In another case, bromothymol blue cause skin, eyes, nose, and respiratory tract irritation contact or inhalation of. Furthermore, there has been evidence linking extended exposure to bromothymol blue to possible risks to the kidneys, liver, and heart.

For these reasons, there has been an increasing interest in searching for alternative sources of indicators from natural origins. These alternatives would be cheaper, more available, simpler to extract, less toxic to users, and more environmentally friendly. Thus, there has been a lot of research to find an alternative to substitute commercial indicators with natural indicators derived from natural products. But plant sources may vary in ecology and area of cultivation. Similarly, different plant flower extracts should be optimized for different acid-base indicators so that they are applicable for real titration experiments in elementary and secondary education chemistry.

1.3 Objectives of the study

1.3.1. Generally objective of the study

- The objective of this study was to investigate indicators from selected plant flowers collected in Limmu Gelila District, Oromia Regional State, Ethiopia, for use in acid-base titrations.

1.3.2 Specific Objectives

- To extract *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* flowers
- To investigate colour changes of plant extracts with pH change
- To compare indicator end point of plant extracts with standard synthetic indicators for acid-base titration.

1.4. Significance of the study

Synthetic indicators, such as methyl red, bromothymol blue, and phenolphthalein available for acid-base indicators might cause hazardous dangers due to their high cost and limited availability. However, natural indicators are cheap, environmentally friendly, and easily available. This study provides important information about the preparation and use of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* flowers as natural acid-base indicators in acid-base titration as an alternative to the use of toxic synthetic indicators. The findings of this research could be used to provide further awareness for researchers about the preparation of natural indicators for acid-base titration. Furthermore, the newly prepared plant flowers can serve as a baseline for future research development by other researchers using other plants and will be used as start for high school chemistry.

2. LITERATURE REVIEW

2.1 Acid-base and pH indicators

Substances that alter the color in acid or base solutions are known as indicators. An organic molecule whose color may vary in response to pH changes is called an acid-base indicator. By offering various acid or basic solutions, it is typically utilized to distinguish between acid and base solutions. An acid-base indicator is complex compound and can react with acid or base with color changes based on the hydrogen ion concentration with titration process. The titration processes utilize indicators to show the color changes and used to level pH interval. Indicators are dyes or pigments that can be isolated from a variety of sources, including plants, fungi, and algae. Nearly all flowers that are red, blue, or purple in color contain a class of organic pigments called anthocyanins that change color with pH.

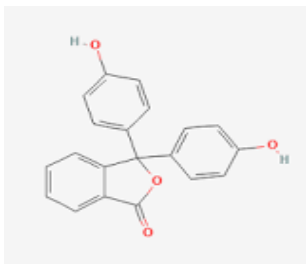
Mostly acid-base indicators used in a chemical laboratory are synthesized indicators. Each synthesized indicator has specific characteristics in the forms of pH trajectories. They are shown by color changes within acid and base conditions along with the constant indicator value. Some commercially available indicators includes phenolphthalein, red phenol, red methyl, blue bromophenol, blue thymol, etc. The availability of synthesized indicators is limited and so do the implementations. Besides such indicators have several weaknesses—as expensiveness, toxicity, and polluting the environment. Thus, alternative indicators (natural indicators) that can be easily obtained and environmentally green can replace those indicators.

Thus, the intention to find an alternative natural indicator source should be realized. The natural indicators in the form of solutions are the most frequently used. Acid-base indicators change color over a range of pH, *e.g.* phenolphthalein from 8.3 to 10, methyl red pH range is from 4.2 to 6.2 and bromothymol blue 6-7.6. The ability of the indicators to donate or accept electrons results in change of color with change in pH (acidity or alkalinity).

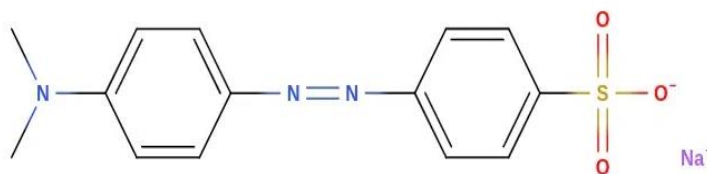
2.2. Synthetic acid-base indicators and their effect on environment

Most acid-base indicators and dyes used in the pigmentation and titration processes are derived from azo dyes (Falih *et al.*, 2024) (Figure 1). Azo dyes are xenobiotic substances with aromatic rings and one or more azo links. They make up the biggest category of dyes in

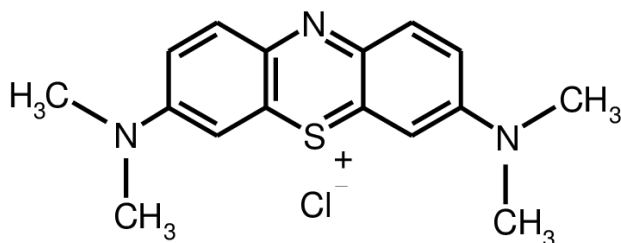
terms of color variety. Because of the azo linkage ($\text{N}=\text{N}$) and sulphonic (SO_3^-) groups, which are electron withdrawing groups and produce electron deficit inside the molecule, In the human body synthetic azo compounds are metabolized by enzyme azoreductase in the intestines and liver to aromatic amines. Aromatic amine, benzidine induces urinary bladder cancer in humans and tumors in some experimental animals. Table 1 summarizes some negative impacts of synthetic dyes used in acid base indicators. Under anaerobic conditions, azo dyes can be reduced to aromatic amines which are toxic, mutagenic and carcinogenic.



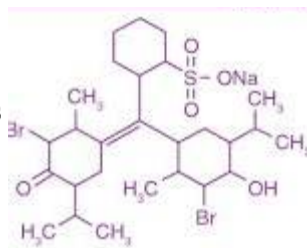
Phenolphthalein



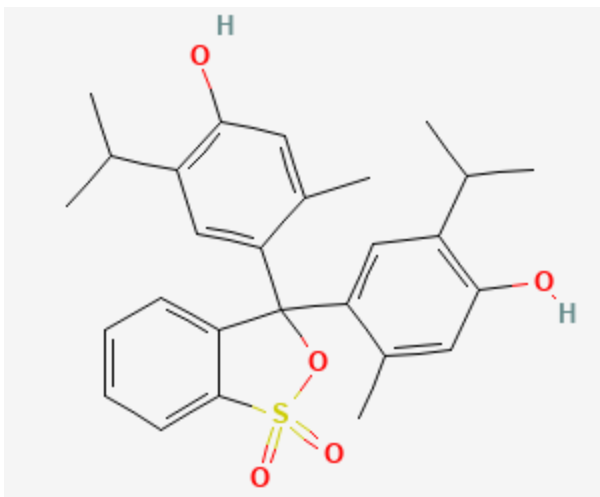
methyl orange



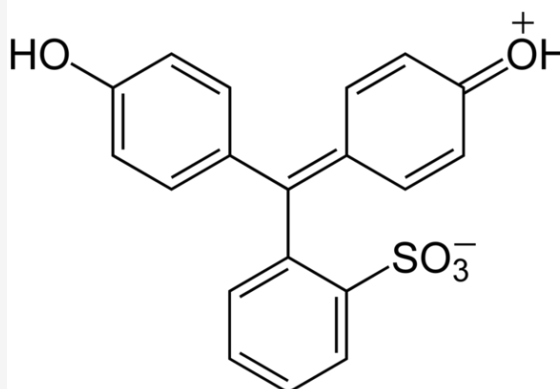
Methyl blue



bromothymol blue



Thymol blue



phenol red

Figure 1. Chemical structures of common synthetic dyes

2.3 Natural Indicators for acid base titration

Nature is colorful due to the presence of flowers and other colored material. In plants/flowers the color is due to the presence of naturally occurring organic substances like flavonoids, flavonol, xanthine, anthocyanins, azo compounds *etc.* (Bednarik & Grozea, 2022). Some of the naturally occurring flavonoids, flavonol, anthocyanins and other colored substances are pH sensitive—and exhibit different colours in acids and basic medium. These substances give sharp, distinct, and stable color change on a change of acid to alkaline medium. Thus, they may be used as acid base indicators in volumetric analysis.

The presence of pH sensitive anthocyanins and flavonoids has been linked to color variations in natural indicators at various pH levels. Anthocyanins are organic compounds that are usually found in the aqueous sap of the vacuole of the epidermal plant cells. These compounds have a complex structure consisting of an aromatic three-ring molecular region, one or more attached sugar molecules, and sometimes acyl groups attached to the sugar molecules. Anthocyanins are water soluble and are usually more stable in acidic media than in alkaline solutions. Therefore, almost any plant that have blue, violet, purple or red flowered colors contains such organic pigments (anthocyanins) that changes color with change in pH. The color stability of anthocyanins depends on structure of the anthocyanins, pH, temperature, oxygen, light, and water activity.

Natural indicators have been used for hundreds of years, long before chemists made synthetic acid-base indicators. The 17th century chemist Robert Boyle, described indicators extracted from roses and other plant materials in his book “The Experimental History of Colors” Boyle included the ability to turn plant juices red among the properties of acids. Natural acid-base indicators are green alternatives to synthetic ones as they demand of contemporary chemistry with regards to solving the problems of environmental pollution, cost and human health. There is an increasing need for the development of green indicators as effective substitutes for synthetic acid-base indicators used in laboratory. Plant pigments such as flavonoids with common types including anthocyanins, aurones, chalcones, flavonoids, and proanthocyanidins are commonly produced in many colors in flowers and plants such as red cabbage, berries, eggplant, turmeric, curry powder, cherries, tomato, and citrus fruits.

Table 1 Negative impacts of synthetic dyes used in acid base indicators

S/N	Dye	Negative impact	Reference
1	Phenolphthalein	irritate the digestive tract, promote cancer, affect the respiratory system, and raise blood pressure causes the terminal ileum's typical mucosal pattern to disappear, intestinal wall weakening, and persistent ulcerative colitis.	Chinchón-Payá <i>et al.</i> ,2016
2	Methyl red	Induce mutagenesis, teratogenesis, respiratory issues, gastrointestinal tract infections, skin and ocular irritation, and carcinogenesis.	Amari <i>et al.</i> ,2023
3	Methyl orange	toxicity and carcinogenic, cause eye irritation, skin irritation, cause gastrointestinal irritation with nausea,	Abdulsalam <i>et al.</i> ,2022
4	Bromothymol blue	Eye irritation, skin irritation, May cause gastrointestinal irritation with nausea, vomiting and diarrhea, Allergic reactions have occurred with similar compounds	Zaki <i>et al.</i> ,2024
5	Malachite green	Potentially mutagenic, carcinogenic, and teratogenic. Cause death, diminished reflexivity, lethargic lateral fins, heightened agitation, squirming, surface breathing, rolling, and other behaviors.	Etezzad <i>et al.</i> ,2021

Different studies revealed that various plants such as antirrhinum, cotton tree, marigold, dahlia, sunflower, China rose, beach morning glory, santan, fern tree, white mulberry, and pomegranate show good acid-base indicator activity against various synthetic pH indicators. For instance, prepared *Combretum indicum* ethanolic extract as an alternative for synthetic indicators in acid-base titrations to show sharp end points. The end *Combretum indicum* ethanolic extract found to be nearly closed with equivalence point by standard indicators for four types of titrations in equivalence using methyl red, methyl orange, phenolphthalein, and

mixed indicator. Similarly, *Impatiens balsamina*, *Tecoma stans*, white rose, and hybrid tea rose are presented by and are compared with methyl orange and phenolphthalein to show favorable results at neutralization. Additionally, the ethanolic extract of *Quisqualis indica* L., *Pentastlanceolate*, *Melastoma malabathricum* L., and *Impatiens acaulis* demonstrated a high degree of similarity with an equivalency point determined using standard phenolphthalein. also reported *Averrhoa bilimbi* L. floral extract for pH indicator in acid-base titration in another investigation.

2.3.1 Plants used to extract acid-base indicators

Guizotia scabra is a plant that grows in the highlands of Ethiopia, Nigeria, and Cameroon, among other east African nations. In some parts of Africa, a decoction made from leaves is used orally to treat syphilis, dyspepsia, gastritis, enteritis, diarrhea, constipation, gonorrhea, ulcers, and stomach aches. Similarly, *Cordia sebestena* is a perennial plant, it is known to produces an abundance of blooms in June and July in Ethiopia. It has ovate, 4.5–10 cm leaves, orange-red, 2–5 cm long blooms, and green or white fruit that may reach a height of 25–30 feet and a spread of 20–25 feet. *Solanum tuberosum* (Solanaceae), are currently the fourth most significant staple food crop in the world, after maize, wheat, and rice. This well-known vegetable is categorized globally not only by species and variation but also by color. The majority of potatoes come in various colored flowers yellow hues, blue, purple, and red skinned types that are utilized as a natural food coloring and employed to repair burn injuries. *Rose hybrida* is a major crop for the global economy and cultivated for landscape usage, essential oil, and cut flowers (Acimovic *et al.*, 2023). Known as the "Queen of Flowers" it is the most well-liked flower due to its exquisite beauty and delightful scent. A garden wouldn't be considered complete without roses because they are so crucial to landscaping. The enormous diversity of rose types may be attributed to variety hybridization, and the more times various kinds cross pollinate, the more variations of roses can be produced, increasing their potential applications. The unique characteristics of the various portions of the plant contribute their color. The color is caused by the quantity of phytochemicals, which include dihydropyrans, quinines, anthraquinonoids, naphthoquinones, flavonoids, acylated flavonoids, imines, indigoids, polymethines, diarylmethanes, and carotene. Among them, flavones are water and alcohol soluble yellow pigments present in plant

sources either in a free state or as glycosides or conjugated with tannins. Sometimes it is identified as anthoxanthins which is a chemically hydroxylated derivative of flavone. Many studies have been carried out on the use of plant extracts as substitute of synthetic acid-base indicators in titrations. Some of plant extract used for acid base indicators previously reported by researchers are compiled in Table 2. Raimundo *et al.* (2024) used root extract of *Euclea natalensis* for acid-base titration. In other study senna flower was used as natural acid-base indicator for acid base titration.

Table 2. Some of plant extract used as natural indicator for acid base titration

S. N	Plant name	Solvent used	colour in acid	Colour in base	Reference
1	<i>Hibiscus Sabdariff</i>	Ethanol	Wine	Greenish yellow	Genevieve <i>et al.</i> ,2020
2	<i>Celosia argentea</i>	Water	Green	colorless	Kawade <i>et al.</i> ,2014
3	<i>Clitoria ternatea</i> Linn	Ethanol, methanol	Pink	green	Saptarini <i>et al.</i> ,2015
4	<i>Bougainvillea glabra</i>	Distilled water	Red	yellow	(Kapilraj <i>et al.</i> , 2019)
5	<i>Lagerstroemia speciose</i> Extract	Methanol	Red	green	Gupta& Yadav,2020
6	Turmeric rhizome	Ethanol	Yellow	orange	Onuegbu, <i>et al.</i> ,2023
7	<i>Hibiscus rosa sinensis</i>	Water	Red	green	Mitarlis <i>et al.</i> ,2019
8	<i>Tagetes Erecta</i>	Ethanol	Yellow	brown	(Garg <i>et al.</i> , 2018)
9	<i>E. natalensis</i>	Water	Yellow	wine	Macuvele <i>et al.</i> ,2016
10	<i>Rosa's sp</i>	Ethanol	Faded red	Brownish green	Paristiowati <i>et al.</i> ,2019
11	<i>Plumbago zeylanica</i> Linn	Methanol	Yellow	pink	Adusei <i>et al.</i> ,2019
12	<i>Aster chinensis</i>	Distilled water	Yellow	colorless	(Nair <i>et al.</i> , 2018)

2.3.2 Botanical Description of *Guizotia scabra* and its Medicinal Values

Guizotia species, which are nearly abundant at intermediate and higher elevations, are the most significant broad-leaved weeds in Ethiopia. The plant growth as an erect, quickly growing up to 1-2 meters (up to 4 meters in certain places), however in poor soils, it can mature and start to seed widely at just 30 centimeters. The stems of the plant are usually

covered in short glandular hairs and the leaf of *G. scabra* was found to be active against *Erwinia carotovora* bacteria responsible for Irish potato soft rot. In Ethiopia, the leaf of *G. scabra* is squeezed and applied to wound to aid healing. Scientific classification of *G. scabra* (Figure 3a) is given on Table 3 (Chomo *et al.*, 2023). The *G. scabra* in Ethiopia is known locally as Hadaa (Afan Oromo), and Nehuka (Tigrigna) (MESERET, 2024)

Table 3 Scientific classification of *Guizotia scabra*

Kingdom	Plantae
Phylum	<u>Tracheophyta</u>
Class	<u>Magnoliopsida</u>
Order	<u>Asterales</u>
Family	Asteraceae
Genus	<i>Guizotia</i>
Species	<i>Scabra</i>

2.3.3 Botanical Description of *Cordia sebestena* and its Medicinal Values

Cordia sebestena plant (Figure 3b) is an evergreen that can grow up to 25 feet tall. It has been used as an ornamental plant due to its attractive flower (Abdel, 2023). The *C. sebestena* fruit is small and edible but not usually eaten by public. It is used in gastrointestinal disorders. It is locally named as gigi zaf by Amharic. Taxonomical, the classification of the plant is given on Table 4 (Abdel Kawy, 2023).

Table 4. Taxinomical classification of *Cordia sebestena*

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Boraginales
Family	Boraginaceae
Genus	<i>Cordia</i>
Species	<i>Sebestena</i>

2.3.4 Botanical Description of *Solanum tuberosum* and its Medicinal Values

Gnanasekaran and Basalingappa presented a botanical description of *Solanum tuberosum* in Table 5. They also highlighted the medical value of this plant, noting that it contains chemical compounds that protect human cells from free radical damage and prevent the oxidation of lipoprotein cholesterol. Additionally, the study found that the genetic materials of colored-skinned and -fleshed potatoes may help reduce the risk of certain cancers, neurodegenerative diseases, osteoporosis, and diabetes.

Solanum tuberosum (Figure 3c) have different vernacular name potato (by English), mosee (by Afan Oromo), and Dinich (by Amharic).

Table 5 Taxonomical classification of *Solanum tuberosum*

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Solanum
Species	Tuberosum

2.3.5 Botanical Description of *Rose hybrida* and Its Medicinal Values

Rose hybrida, a perennial woody shrub, is an important commercial crop cultivated in parks and gardens (Figure 3d). Its vibrant colors, strong fragrance, excellent nutritional content makes it a vital ingredient in functional meals and seasonings. Historically, roses have been used in herbal folk medicine to address blood circulation and menstrua issues. More recently, research has uncovered several potential applications for rose in controlling the growth of cancer cells. They are also used as an important ingredient in the production of spices and functional foods, owing to their brilliant color, rich aroma, and high nutritional value. Botanical description of *Rosa hybrida* was given on Table 6.

Table 6 Botanical description of *Rosa hybrida*

Kingdom	Plantae
Division	Tracheophytes
Class	Angiosperms
Order	Rosales
Family	Rosaceae
Genus	<i>Rosa</i>
Species	<i>Hybrida</i>



Figure 2. *Guizotia scabra* (a) *Cordia sebestena* (b) *Solanum tuberosum* (c) *Rosa hybrida* (d) (picture by Enkosa M., July 2023, Limu, Ethiopia)

2.4 Extraction methods employed to extract the acid-base indicators

Anthocyanins, responsible for color changes in natural indicators, can be extracted from a variety of colorful plants or their components using water or other solvents. Many researchers utilize similar solvents for plant extraction; however, their primary distinction lies in the use of fresh versus dried flowers. For fresh flowers, the extraction method involves washing the flowers with distilled water, soaking them in an extracting solvent (such as ethanol, water or methanol), followed by filtration, with the resulting crude extract used as an indicator in titrimetric analysis. In cases where dried flowers are used, the method differ

primarily in that the flowers are ground into a fine powder using either a mechanical blender or a pestle and mortar. The subsequent steps include extraction with an appropriate solvent, filtration, utilization of the crude extract as an indicator.

2.5 Solvents used to extract anthocyanins (pH sensitive compounds)

Anthocyanins are primarily extracted using acidified solvents such as water, acetone, ethanol, methanol, or mixtures of aqueous solvents. The acid helps break down cell membranes, releasing anthocyanins; however, harsh chemical treatment such as the use of mineral acids like 1% HCl, can damage the native structure of the anthocyanin. In contrast, acidification with organic acids such as formic or acetic acid, does not compromise the anthocyanin structure. Various solvents have been employed for plant extraction, including water, ethanol methanol, ethyl acetate, and hexane (Tuslinah *et al.*, 2022). Numerous studies indicated that acidified solvents are generally more effective for extraction of anthocyanin compounds.

2.6 Application of Natural indicator in acid base Titration

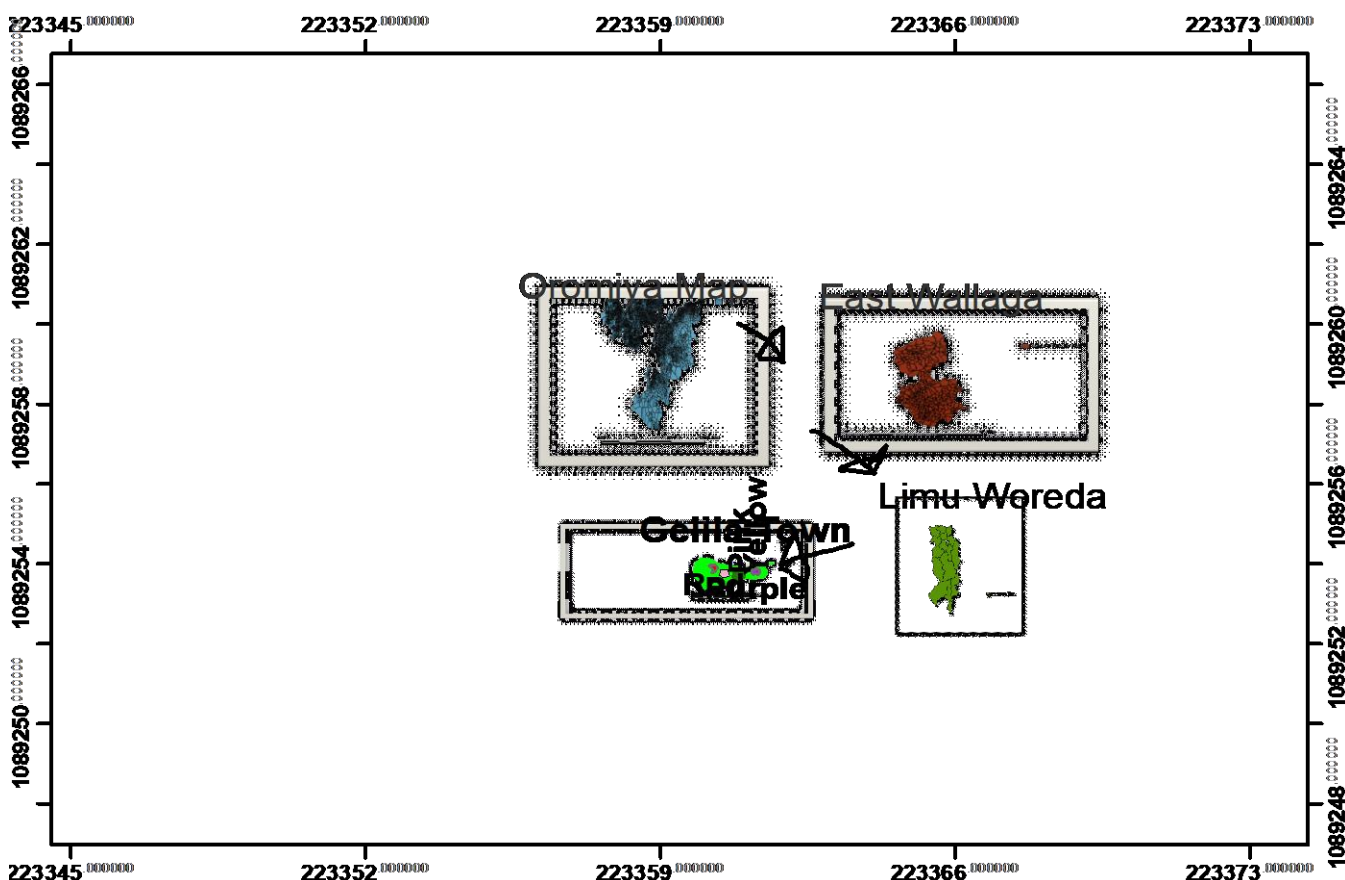
Titration is a fundamental chemical technique that utilizes standard solutions with known concentrations to quantitatively analyze the concentration of an unknown substance. The equivalent point in an acid-base titration is particularly difficult to observe, as the reaction between an acid and a base produces colorless salt and water. At this stage, the role of an indicator becomes essential in acid-base titrations. Acid-base titrations depend on the neutralization that occurs when an acid and a base are mixed in solution. Along with the sample, a suitable indicator is added to the titration chamber, to reflect the pH range of the equivalence point. The acid-base indicator signals the endpoint of the titration by changing color. Choosing the appropriate indicator carefully can minimize the indicator error. Many natural plants contain colored anthocyanins, which appear red in acidic solutions and green in basic solutions. Dyes extracted from these plants are commonly used as natural acid-base indicators.

Titration, also referred to as titrimetric analysis, is a widely used laboratory technique for quantitative chemical analysis. It involves measuring the volume of a liquid standard solution of known concentration as the titrant, to convert a component into a different form.

3. MATERIALS AND METHODS

3.1. Description of the study area

The scientific name of the plant flowers used in this study was identified and authenticated at the Department of Biology at Wallaga University. Different colored plant flowers, botanically differentiated and scientifically named, were collected from the Limu District in the East Wallaga Zone of the Oromia Regional State, Ethiopia. Limu District is one of the woredas in the Oromia Region, bordered to the south by the Benishangul-Gumuz Region, to the southwest by Sasiga, to the west again by the Benishangul-Gumuz Region, to the north by Ebantu, and to the east by Gida Kiremu. It is located 498 km from Addis Ababa and 165 km from the zonal capital city, Nekemte, with coordinates at latitude 9° 35' 60.0" N and longitude 37° 04' 60.0" E.



3.2 Chemicals and Reagents

All chemicals used in this study were of analytical grade used without further treatment. More specifically, sodium hydroxide (NaOH, 99.8%), hydrochloric acid (HCl, 37%), Butanol (C₄H₁₀O, 99 %), ammonia solution (NH₃, 25 %), phenolphthalein (C₂₀H₁₄O₄, Indicator grade), methyl red indicator (C₁₅H₁₅N₃O₂, Indicator grade) all from (Loba Chemie PVT LTD, India), glacial acetic acid (CH₃COOH, 99.5 %) (Abron Chemicals, India), bromothymol blue (C₂₇H₂₈Br₂O₅S, laboratory reagent) (Samir tech-chem PVT LTD, India), buffer solutions of pH 4 to 10, and deionized water were used throughout all experiment.

3.3 Instruments and apparatus

The apparatus and instruments used in this study were: different size beakers, conical flasks, volumetric flasks, burette, test tubes, measuring cylinders, electrical grinding machine, pipes, aluminum foils, droppers, mortar and pestles, refrigerator, glass rods, camber, washing bottle, Whatman filter paper number 1, ruler, pencil, funnel, capillary tube, electronic balance, pH meter (pH-013), analytical TLC pre-coated sheets (ALUGRAM® Xtra SIL G/UV254 (layer: 0.20 mm silica gel 60 with fluorescent indicator UVF254/365))

3.4 Sample collection and preservation

Different colored fresh flowers of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida*, were collected from Limu Gelila district, Oromia regional state, Ethiopia. The flower samples were washed and cleaned with tap water and then rinsed with distilled water repeatedly. Then the collected flower samples were dried at room temperature for 15 days under shade. The dried samples were crushed with a mortar and pestle. The powders were sieved through sieves and stored in a polyethylene bag before use.

3.5 Extractions of the pigments

The pigments of the flowers of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* were extracted using the same procedure.

3.5.1 Extraction of *Guizotia scabra* flower extract

A 1-gram sample of *Guizotia scabra* flowers was placed in a beaker, and 30 mL of deionized water was added. The mixture was boiled on a hotplate and stirred occasionally for 10 minutes. After boiling, it was allowed to cool, and the flower extract was obtained through filtration (Figure 4). The resulting filtrate was stored for further analysis.



Figure 3. Schematic representation of *Guizotia scambra* flower extract preparation

3.5.2. Extraction of *Cordia Sebestena* flower extract

A 1-gram sample of *Cordia sebestena* flowers was placed in a beaker, and 30 mL of deionized water was added. The mixture was boiled on a hotplate, stirring occasionally for 10 minutes. After boiling, it was allowed to cool. The flower extract (Figure 5) was then obtained through filtration, and the resulting filtrate was stored for further analysis.



Figure 4. Schematic representation of *Cordia Sebestena* flower extract preparation

3.5.3. Extraction of *Solanum tuberosum* flower extract

A 1-gram of *Solanum tuberosum* flower sample was placed in a beaker, and 30 mL of deionized water was added. The mixture was boiled on a hotplate, stirring occasionally for 10 minutes. After boiling, it was allowed to cool, and the flower extract (Figure 6) was obtained by filtration. The resulting filtrate was stored for further analysis.



Figure 5. Schematic representation of *Solanum tuberosum* flower extract preparation

3.5.4. Extraction of *Rose hybrida* flower extract

A 1-gram of the *Rose hybrida* flower sample was placed in a beaker, and 30 mL of deionized water was added. The mixture was then boiled on a hotplate while stirring occasionally for 10 minutes. After boiling, it was allowed to cool, and the flower extract (Figure 7) was obtained through filtration. The resulting filtrate was stored for further analysis.

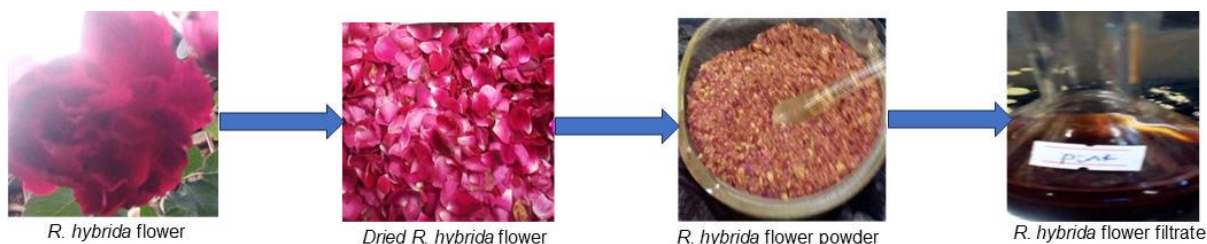


Figure 6. Schematic representation of *Rose hybrida* flower extract preparation

3.6 Preparation of Standard Solution

A 0.1 M sodium hydroxide (NaOH) solution was prepared by weighing 4 g of NaOH and dissolving it in a 1 L volumetric flask, then filling the flask with deionized water up to the mark. To prepare 1000 mL of a standard 0.1 M (mol/L) ammonia solution (NH₃), 8.3 mL of a 25% ammonia solution was added to a 1 L volumetric flask, which was then filled with deionized water to the mark. Similarly, a 0.1 M acetic acid (CH₃COOH) solution was made by measuring 5.7 mL of concentrated acetic acid (99.5%) in a 1000 mL volumetric flask and diluting it with deionized water to the mark. Finally, a 0.1 M hydrochloric acid (HCl) solution was prepared by transferring 8.36 mL of concentrated acid (37%) into a standard 1000 mL volumetric flask and adding deionized water to reach the mark. The concentration of the acids was confirmed to be accurately adjusted.

3.7 .Determination of color change of flower extract at different pH

Buffer solutions of pH 4 to 10 were prepared with the same procedure.with some modifications. A volume of 2 mL of each buffer solution was placed in a test tube, and a few drops (≈ 0.1 mL) of flower extracts were added to the buffer solution, then mixed by swirling, and the colors of the solutions were noted and recorded.

3.8. Application of the flowers extract and synthetic indicators in acid-base titration

Titration of a natural flower extract indicator was employed with the same procedure used by Riyanti *et al.* (2022) with some modifications. Accordingly, three titrations were performed, *i.e.*, strong acid vs. strong base, strong acid vs. weak base, and weak acid vs. strong base. The titrations were conducted in the following order: HCl and NaOH; HCl and NH_3 ; CH_3COOH and NaOH. A volume of 10 mL of analytical (unknown) solution was placed in a conical flask, and three drops of each flower extract indicator were added. Then titration was performed using a standard solution until color changes were observed. Titrations were conducted in three replicate analyses for reproducibility analysis. The procedure will be repeated for all titrations.

3.9. Data Analysis

All data obtained from this study was analyzed using Microsoft Excel 2010. All titrations were conducted in a three-replicate analysis; hence, the mean and standard deviation were calculated. Standard test statistics (t-test) were used to analyze the mean titer volume of the natural indicator and that of each synthetic indicator relative to the equivalence point. A one-way ANOVA was used to test the mean and variance of the extracted natural bio-indicator and synthetic indicators (phenolphthalein, methyl red, and bromothymol blue).

4. RESULTS AND DISCUSSION

4.1 Color of plant flowers extracts with pH

In the present study, yellow, red, purple, and pink colored pigment were collected and extracted from the *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida*, flowers respectively. The anthocyanin, which is composed of hydroxyl and methoxyl group, is highly soluble in water, hence, provides a promising high yield. The water extract of *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rose hybrida* have great thermal stability and so, it has the capability to withstand high temperature. After the preparation of flower extracts, their colour was investigated against the pH of these flower extracts (Table 7).

Table 7. Colour verses pH of flower extracts

S/N	Flower extract	pH
1	<i>Guizotia scabra</i>	2.96
2	<i>Cordia sebestena</i>	2.91
3	<i>Solanum tuberosum</i>	3.72
4	<i>Rose hybrida</i>	3.73

The flower extracts vary from pH 2.9–3.73 which seems slightly weak acidic property which is the property of acid-base indicators. *Guizotia scabra* and *Cordia sebestena* show yellow and red colors, respectively, which are associated with more strong acidic conditions. While *Solanum tuberosum* and *Rose hybrida* show purple and pink colors, respectively, which correspond to weak acidic pH. Therefore, these flower extracts can be used as natural pH indicators due to weak acidic properties.

4.2. Investigation of indicator colour change with different pH

In order to study the colour change of the plant pigments with pH, five different buffer solutions were taken and 3 drops of each extract were added and the colour change were recorded.

4.2.1 Color of flower extract at different pH

The color change of flower extract was determined with a pH range of 4 to 10 by adding three drops of flower extract to each test tube (Table 8, and Figure. 6)

Table 8 Color change observed for flower extract at different pH

S/N(1)	pH	Color
1	4	Yellow
2	5	Yellow
3	6	Slight yellow
4	8	slight orange
5	10	Orange
S/N(2)	pH	Color
1	4	Yellow
2	5	Yellow
3	6	Yellow
4	8	slight orange
5	10	slight orange
S/N(3)	pH	Color
1	4	Orange
2	5	Orange
3	6	Orange
4	8	deep orange
5	10	Yellow
S/N(4)	pH	Color
1	4	Orange
2	5	Orange
3	6	Yellow
4	8	yellow
5	10	Yellow

The color of *Guizotia scabra* flower extract changes from yellow at pH levels below 8 to orange at pH 10. This color shift is likely due to the significant impact of pH on the color of anthocyanin pigments. Additionally, the variation in color observed in the *Guizotia scabra* flower extract at different pH levels may result from the different pH can be due to the protonation or deprotonation of the pigments. Consequently, *Guizotia scabra* flower extract can function as a natural pH indicator transitioning from yellow in acidic conditions to orange in basic conditions, with intermediate of light yellow and light orange representing neutral to slightly basic pH levels. This extract effectively indicates a wide pH clear color transition, making it useful in applications that require a distinct visual change to signal the pH of a solution.

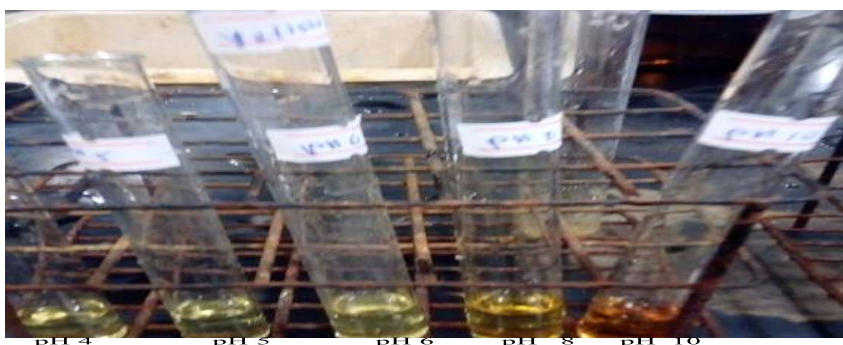


Figure 7.Colour change of *Guizotia scabra* flower extract at different pH

Utilized *Catharanthus rosea* as a pH indicator for acid-base titration observing that the color of the extracts changed from pink to yellow across buffer solutions ranging from pH 1 to 14. The discrepancies between their results and those of previous studies were attributed to variations in the plant extracts used, the extraction methods employed, and the components of the crude extract.

4.2.2. Color of *Cordia sebestena* flower extract at different pH

The color change (Figure 10) caused by *Cordia sebestena* flower extract at various pH levels was assessed by preparing solutions with pH values ranging from 4 to 10.

The flower extract of *Cordia sebestena* functions as a pH indicator, changing color from yellow in acidic and neutral conditions to a slight orange in basic conditions. Specifically, the extract shifts from yellow to orange at pH values below 8 and takes on slight orange to hue

at pH values above 8 (Figure 9). This color change is attributed to the flavonoids and anthocyanins presented in flower extract. As such, this extract serves as a natural pH indicator, effectively indicating transitions from yellow to slight orange across a range of pH levels, which can be useful for detecting pH changes in acidic or basic solutions. In study conducted by Riyanti *et al.* (2022), the extract's color changed to orange. This discrepancy is likely due to variations in the flower extracts and extraction methods employed in different studies.

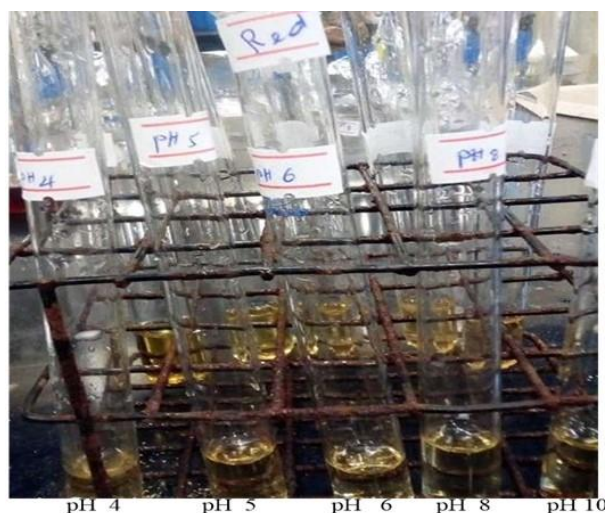


Figure 8. Colour change of *Cordia sebestena* flower extract at different pH

4.2.3. Colour of *Solanum tuberosum* flower extract at different pH

The color change of *Solanum tuberosum* flower extract at different pH was determined by preparing solutions with a pH range 4 to 10

Solanum tuberosum flower extract over a wide pH range. In acidic, conditions, it appears orange, while it shifts to yellow in more basic conditions. The extract displays a deeper orange hue in slightly basic conditions. Overall, *Solanum tuberosum* changes from orange to yellow at all tested pH levels (4–10).



Figure 9. Color of *Solanum tuberosum* flower extract at different pH

The change in color is attributed to the anthocyanin present in the flower extract across all pH levels, as well as the color transformation illustrated in Figure 11. This transformation is a result of intramolecular rearrangement, which the structure of the indicators and causes them to absorb light in different regions of the spectrum. This extract can serve as a natural pH indicator, demonstrating a clear color transition from orange to yellow across a range of pH levels. It is particularly effective for detecting changes in pH from acidic to neutral and basic conditions. The in this study closely align with those of Kanha *et al.* (2022), which cassava starch from pink to yellow in buffer solutions. The differing results in this study can be attributed to variations in plant materials.

4.2.4. Colour of *Rose hybrida* flower extract at different pH

The color change of the *Rose hybrida* flower extract at pH levels was assessed by preparing solutions with pH ranging from 4 to 10.

The color of *Rosa hybrida* changed from orange to yellow across all pH levels (4–10), This color transformation, illustrated in (Figure 12) is attributed to the presence of anthocyanin available in flower extract at all pH levels. The *Rose hybrida* flower extract functions as a pH indicator, exhibiting a color change around pH 6, transitioning from orange in acidic conditions to yellow in neutral to basic conditions. The extract maintains a stable yellow color throughout the neutral to basic pH range, indicating that yellow is the dominant color in less acid to more alkaline environment. Furthermore, the varying color changes observed in

the *Rosa hybrida* flower extracts at different pH levels may result from the protonation or deprotonation of the indicators present in the extract. The color shift observed in this study is comparable to the color change seen in *Echium amoenum* anthocyanins, where the color changes from red to yellow.

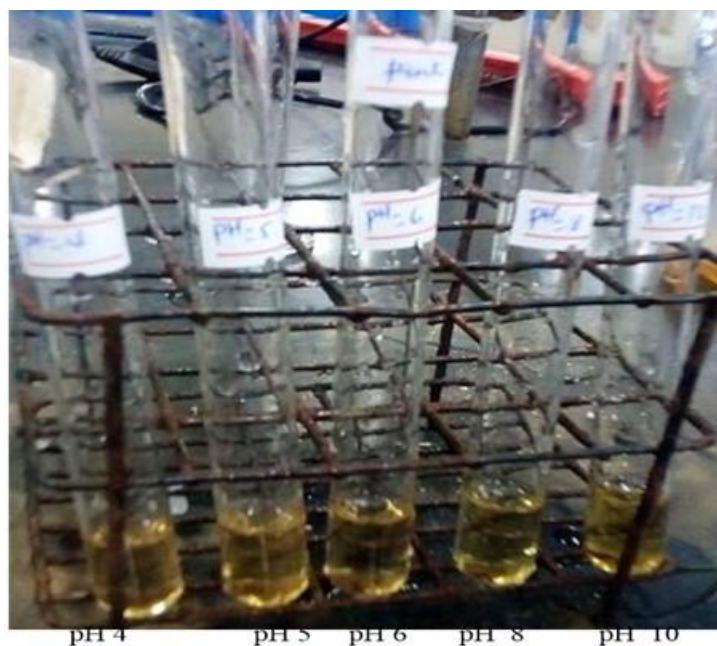


Figure 10. Color of *Rosa Hybrida* flower extract at different pH

4.3 Application of the flowers extract and Synthetic indicators in acid-base titration

The flower extracts were screened for their potential use as acid-base indicators in various titrations. The results of this screening were compared with those obtained using synthetic indicators: phenolphthalein, methyl red, and bromothymol blue, as presented in Table 12. A bar chart illustrating all three titrations strong acid versus strong base, strong acid versus weak base, and weak acid versus strong base can be found in Figure 14d. The findings in Table 13 indicated that four different flower extracts were utilized in the titration, demonstrating that some of the end points achieved with natural indicators were very close to those obtained with synthetic indicators.

Table 9.End point results in titrimetric analysis using synthetic and natural indicators

Titration (titrant vs titrand)	Indicator	Mean $\pm$ S.D in mL	colour change
HCl vs NaOH	Phenolphthalein	9.8 $\pm$ 0.62	colorless to pink
	methyl red	10.5 $\pm$ 0.36	red to yellow
	Bromothymol Blue	10.0 $\pm$ 1.21	yellow to blue
	<i>Guizotia scabra</i>	10.3 $\pm$ 1.13	yellow to clear orange
	<i>Cordia sebestena</i>	9.6 $\pm$ 0.26	yellow to dark brown
	<i>Solanum tuberosum</i>	9.0 $\pm$ 0.43	yellow to orange
	<i>Rose hybrida</i>	10.6 $\pm$ 0.17	pink to colorless
CH ₃ COOH vs NaOH	Phenolphthalein	11.0 $\pm$ 2.58	colorless to pink
	methyl red	10.0 $\pm$ 0.70	red to yellow
	Bromothymol Blue	9.2 $\pm$ 0.43	yellow to blue
	<i>Guizotia scambra</i>	10.0 $\pm$ 0.98	yellow to slight orange
	<i>Cordia sebestena</i>	10.1 $\pm$ 0.30	orange to slight yellow
	<i>Solanum tuberosum</i>	11.0 $\pm$ 0.55	yellow to orange
	<i>Rose hybrida</i>	12.4 $\pm$ 0.87	pink to yellow
NH ₃ vs HCl	Phenolphthalein	6.2 $\pm$ 0.4	pink to colorless
	methyl red	7.57 $\pm$ 0.9	yellow to red
	Bromothymol Blue	7.8 $\pm$ 0.36	blue to yellow
	<i>Guizotia scabra</i>	5 $\pm$ 0.7	slight yellow to colorless
	<i>Cordia sebestena</i>	7.2 $\pm$ 0.4	Brownish to clear orange
	<i>Solanum tuberosum</i>	6.0 $\pm$ 0.45	orange to colorless
	<i>Rose hybrida</i>	8.6 $\pm$ 0.45	yellow to brick red

Where SD = standard deviation

Figure 14: Titration curve of (a) strong acid vs strong base (b) weak bae vs strong acid (c) weak acid vs strong base by both synthetic and natural indicators and (d) bar chart of chart of titration of HCl vs NaOH, NH_3 vs HCl, and CH_3COOH vs NaOH.

Titration curve of strong acid vs strong base

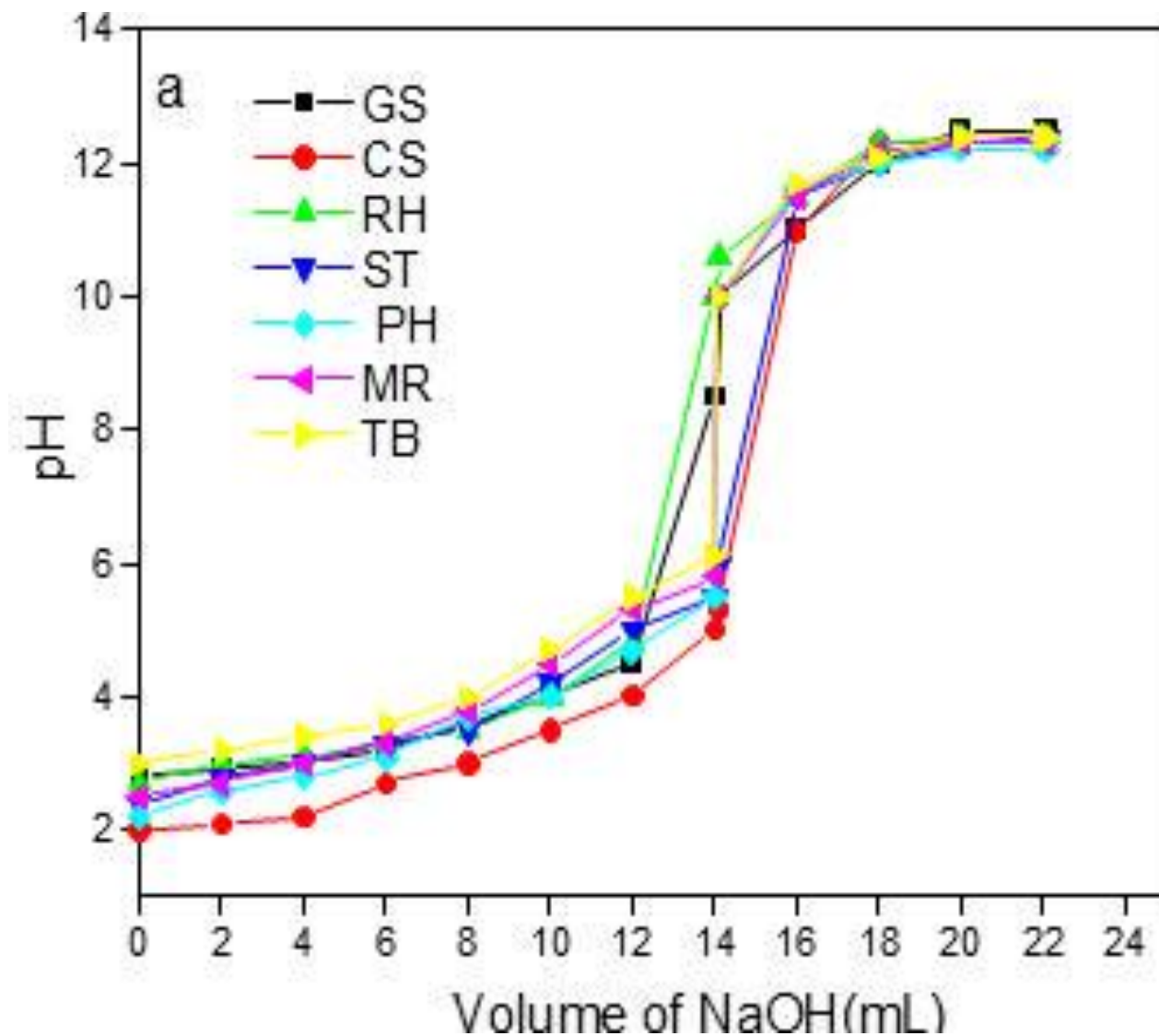


Figure 14: Titration curve of (a) strong acid vs strong base

Titration curve of weak base vs strong acid

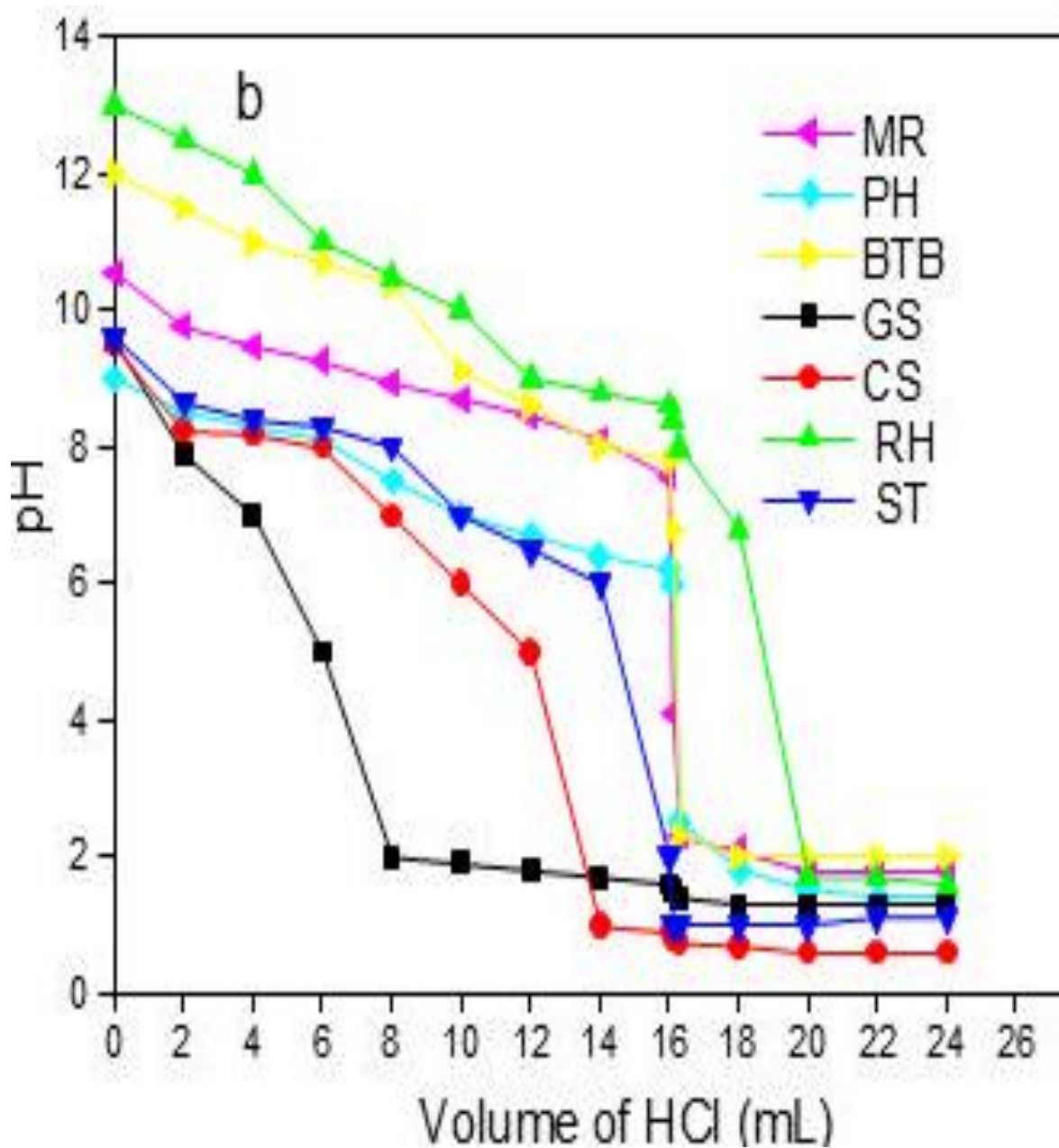
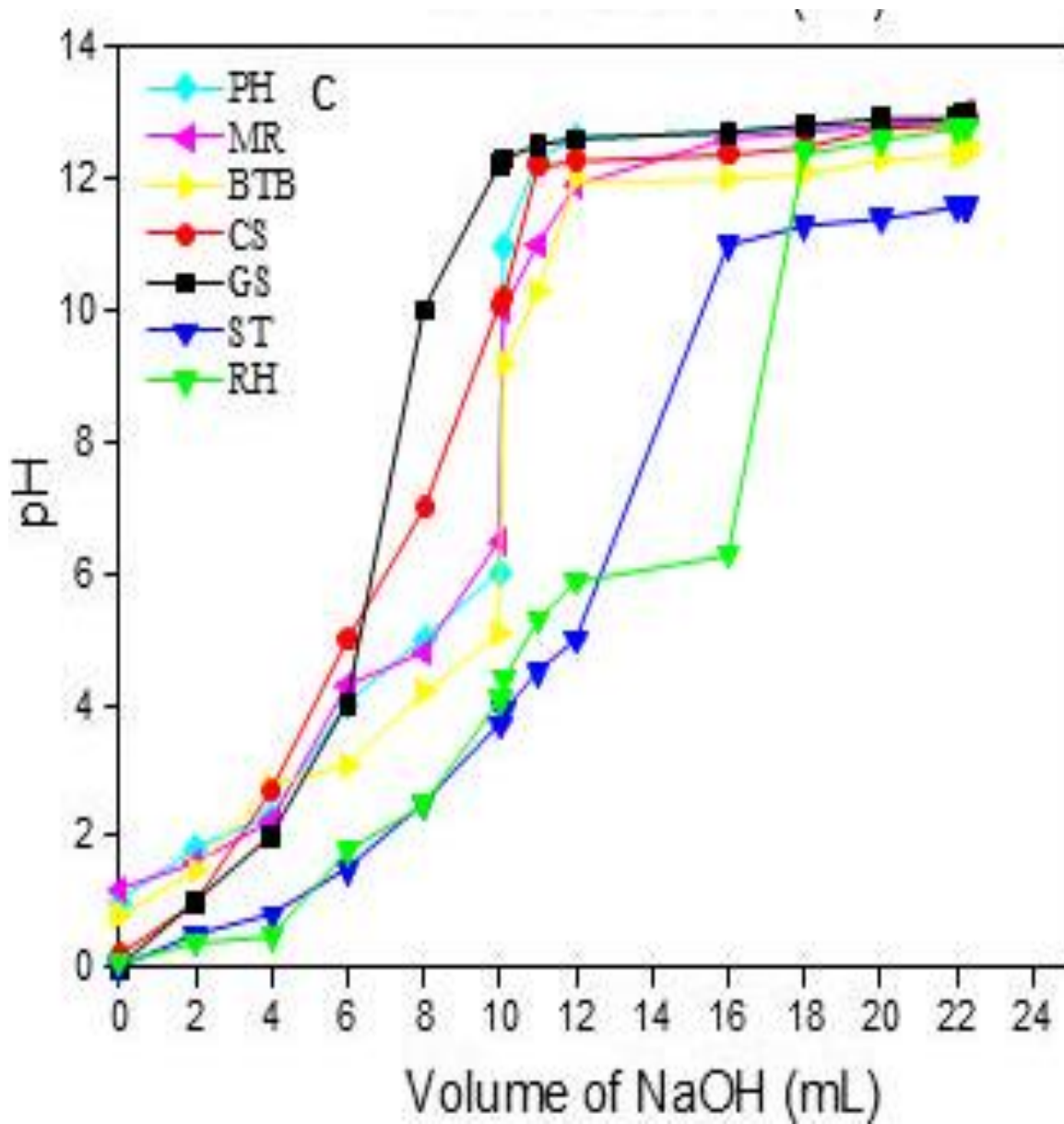
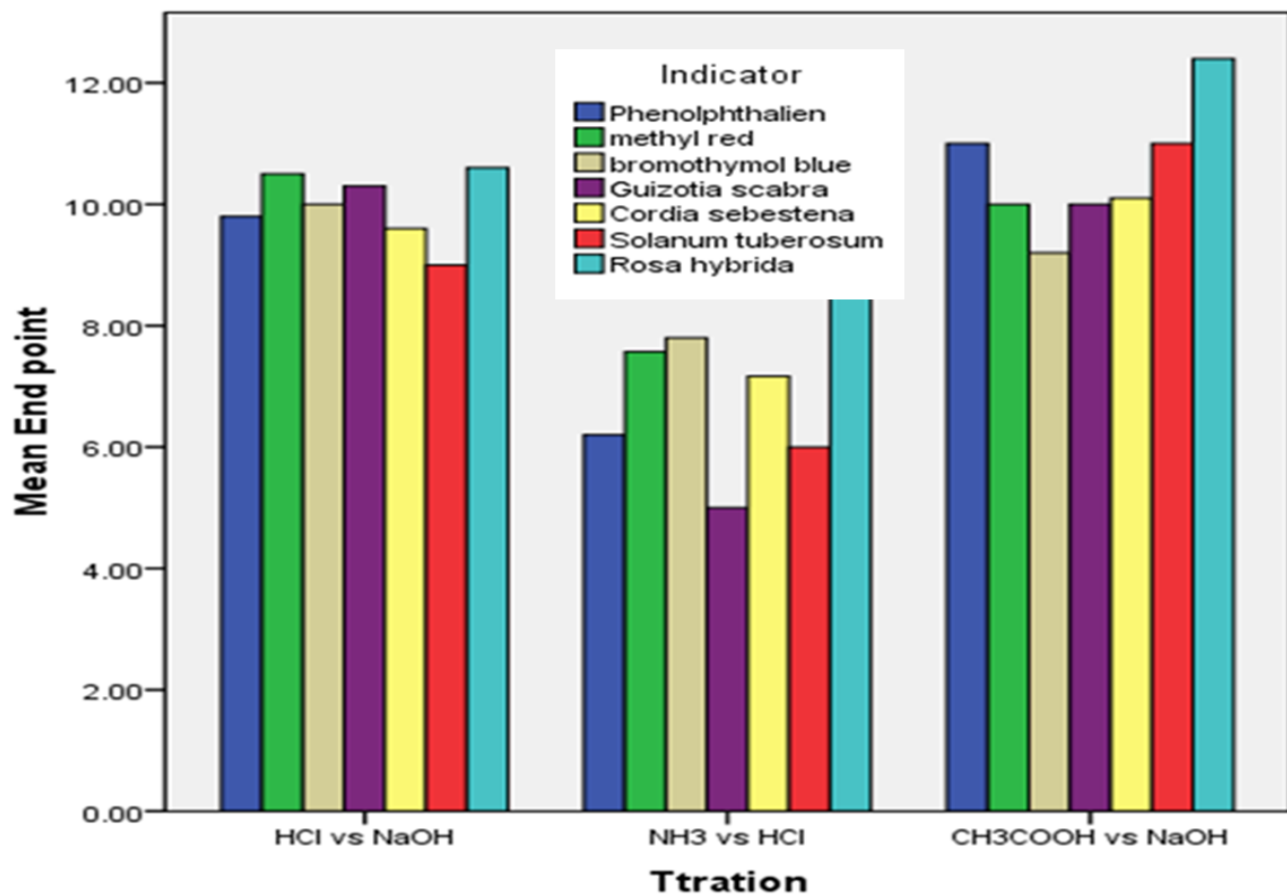


Figure 14(b) weak base vs strong acid

Titration curve of weak acid vs strong base



Figure(c) weak acid vs strong base



Figure(d) bar chart of chart of titration of HCl vs NaOH, NH₃ vs HCl, and CH₃COOH vs NaOH

The colors changed from yellow to clear orange, yellow to dark brown, yellow to orange, and pink to colorless for *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida*, respectively, during the titration of strong acid versus strong base (Figure 14a). This indicates that the volume of titrant used varies slightly among different indicators, but none exhibit extreme deviation. The diverse color changes suggest that the indicators are sensitive to slightly different pH ranges. For the strong acid versus strong base titration (HCl with NaOH), the end point volumes obtained for *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, *Rosa hybrida*, *phenolphthalein*, methyl red, and bromothymol blue were as follows: 10.3 ± 1.13 mL, 9.6 ± 0.26 mL, 9.0 ± 0.43 mL, 10.6 ± 0.17 mL, 9.8 ± 0.62 mL, 10.5 ± 0.36 mL, and 10.0 ± 1.21 mL, respectively. All flower extracts can replace

phenolphthalein, methyl red, and bromothymol blue except for methyl red, which cannot be substituted by *Cordia sebestena* and *Solanum tuberosum* (Table 13 and Figure 14a-c). This indicates that while most flower extracts can serve as alternatives to selected synthetic indicators *Cordia sebestena* and *Solanum tuberosum* are not suitable substitutes for methyl red. The endpoint of *G. scabra* in the present study is similar with the endpoint previously reported for *Catharanthus rosea*. Additionally, for *Cordia sebestena*, a similar endpoint result was reported for other plant extracts, where the end point titration for a strong acid versus a strong base was 9.6 mL

The end point of strong acid versus strong base for *Solanum tuberosum* was 9.0 ± 0.43 mL (Table 13 and Figure 14a). This is similar to study reported by Gupta & Yadav (2020) with *Lagerstroemia speciosa* plant extract in strong acid versus strong base. The color was changed from pink to colorless, yellow to red, blue to yellow, slight yellow to colorless, brownish to clear orange, orange to colorless, and yellow to brick red for titration of weak base-strong acid titration: phenolphthalein, methyl red, bromothymol blue, *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* flower extracts (Table 13). Figure 14b displays the titration curve for a weak base versus a strong acid. In the titration of the weak base (NH_3) with strong acid (HCl), the end points obtained using extract from *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida*, which correspond to phenolphthalein, methyl red, and bromothymol blue, were as follows: 5 ± 0.7 , 7.2 ± 0.4 , 6.0 ± 0.45 , 8.6 ± 0.45 , 6.2 ± 0.4 , 7.57 ± 0.9 , and 7.8 ± 0.36 mL, respectively. The results indicate that natural indicators *Guizotia scabra*, *Cordia sebestena*, and *Solanum tuberosum* can effectively replace phenolphthalein, while *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* can serve as a substitute for methyl red. However, all flower extracts, except for *Cordia sebestena*, did not align with end points of the bromothymol blue indicator. This suggests that these flower extracts are not suitable for substitute the bromothymol blue in weak base-strong acid titrations. The observed volumes are the lowest among the three titrations, likely due to ammonia being weak base that reacts more completely with the strong acid, HCl . The color changes observed reflect a broad range of pH sensitivity in the indicators.. The color change observed for weak acid versus strong base (CH_3COOH with NaOH) was changed from yellow to slight orange, orange to slight yellow, yellow to orange,

and pink to yellow for *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* flower extracts with end points of 10.0 ± 0.98 , 10.1 ± 0.30 , 11.0 ± 0.55 , and 12.4 ± 0.87 mL, respectively (Table 13 and Figure 14c). Based on the results of the present study, the phenolphthalein end point was determined to be 11.0 ± 2.58 mL, which can be substituted with any of the proposed flower extracts. The methyl red end point was found to be 10.0 ± 0.70 mL and can be substituted by *Guizotia scabra*, *Cordia sebestena*, and *Solanum tuberosum* in weak acid versus strong base titrations. However, *Rosa hybrida* is not a suitable substitute for an artificial indicator in weak acid-strong base titrations. The end point for *Guizotia scabra* closely aligns with previous findings reported by Trivedi *et al.* (2016) using *Euphorbia milii*. Additionally, the results for *Cordia sebestena* flower extract are consistent with findings for *Impatiens balsamina* (10.1 mL) reported by Aminah *et al.* (2019). Furthermore, the end point for bromothymol blue was measured at 7.8 ± 0.36 mL, which can also be replaced by *Guizotia scabra* flower extract.

4.4 Statistical Analysis of Data

The statistical analysis of the experimental data generated from titration was statistically analyzed using a one-way ANOVA with statistical significance at $p < 0.05$. The results of a one-way ANOVA are shown in Tables 14–16.

Table 10. **One-way ANOVA test of HCl vs NaOH using seven different indicators**

Source of variation	Sum of squares	Degree of freedom	Mean square	F	Significance
Between Groups	5.683	6	0.947	1.852	0.160
Within groups	7.160	14	0.511		
Total	12.843	20			

From the results obtained for titrations of strong acid versus strong base (HCl vs NaOH), there was no statistically significant evidence at a 95% confidence interval which shows that there were no significant differences ($p > 0.05$) between the mean titre volumes when using the seven indicators: phenolphthalein, methyl red, bromothymol blue, *Guizotia scabra*,

Cordia sebestena, *Solanum tuberosum*, and *Rosa hybrida* flower extracts (Table 14 and Appendix Table 4).

Table 11.One-way ANOVA test of NH₃ vs HCl using seven different indicators

Source of variation	Sum of squares	Degree of freedom	Mean square	F	significance
Between Groups	27.389	6	4.565	14.143	0.000
Within groups	4.519	14	0.323		
Total	31.908	20			

In the titration of a weak base (NH₃) against strong acid (HCl), significant differences ($p < 0.05$) were observed in the mean titrant volumes for the seven indicators (Table 16 and Appendix Table 5). This indicates that the choice of indicators substantially affects the measured volume of titrant, highlighting its importance in obtaining accurate titration results. There is no statistically significant difference ($p > 0.05$) in the mean volumes of titrant used across the seven different indicators for the titration of CH₃COOH vs NaOH (Table 17 and Appendix Table 6). T-test analyses are presented in Appendix Tables 7, 8, and 9. No Significant differences were only observed in the titration of HCl versus NaOH when comparing methyl red with *Cordia sebestena* and *Solanum tuberosum* ($p < 0.05$).

Table 12.One-way ANOVA test of CH₃COOH vs NaOH using seven different indicators

Source of variation	Sum of squares	Degree of freedom	Mean square	F	significance
Between Groups	19.363	6	3.227	2.378	0.085
Within groups	19.000	14	1.357		
Total	38.363	20			

For NH₃ versus HCl titration, no significant differences were found when comparing the titrant volumes of phenolphthalein with those of *Guizotia scabra*, *Cordia sebestena*, and *Solanum tuberosum* flower extracts. Additionally, there was no significant difference when comparing methyl red with *Cordia sebestena*, *Solanum tuberosum* and, *Rosa hybrida* ($p >$

0.05). No significant difference was observed when bromothymol blue was compared with *Cordia sebestena*. However, significance differences were noted when comparing phenolphthalein and methyl red with *Rosa hybrida* and *Guizotia scabra* respectively. Furthermore, a significant difference was observed when the bromothymol blue endpoint was compared with *Guizotia scabra*, *Solanum tuberosum*, and *Rosa hybrida* flower extracts.

For the titration of CH_3COOH versus NaOH , significant differences were found when comparing the titre volume of methyl red with *Rosa hybrida* and when comparing the titre volume of bromothymol blue with *Cordia sebestena* and *Rosa hybrida* ($p < 0.05$). However, no significant difference was observed when comparing the mean titre of phenolphthalein with all flower extracts. Similarly, there was no significant difference when comparing the titre volume of methyl red with *Guizotia scabra*, *Cordia sabestena*, and *Solanum tuberosum*. Additionally, no significant difference was found when comparing the mean titres of bromothymol blue with *Guizotia scabra* and *Solanum tuberosum* (Appendix Table 9). Therefore most of flower extract studied in the present study can substitute synthetic dye for acid –base titration because of their economic value, simplicity, eco-friendly nature, inertness, and accurate results.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In essence, a simple, economically viable, and green approach has been established for acid base titration by using *Guizotia scabra*, *Cordia sebestena*, *Solanum tuberosum*, and *Rosa hybrida* flower aquas. Extractions were done for all flower extracts using deionized water as a solvent. The pH of all the color pigments extracted was found to be acidic. Multiple color changes were observed in all flower extracts due to more than one proton donation or acceptance group in flower extracts. The results obtained in all the types of acid-base titrations lead to conclude that sharp color changes occurred at the end point of the titrations. The end point obtained by natural indicators was very close to the end point obtained by synthetic indicators (HCl versus NaOH titration). All flower extract end points were close to the phenolphthalein indicator except the end point obtained by *Rosa hybrida* in NH_3 versus HCl titration. In addition, *Cordia sebestena* flower extract can only substitute bromothymol blue indicator in a titration of NH_3 versus HCl. For titration of CH_3COOH vs. NaOH, all flower extract end points were close to the phenolphthalein end point, and the methyl red end point was close to *Guizotia scabra*, *Cordia sebestena*, and *Solanum tuberosum*, except *Rosa hybrida*. *Guizotia scabra* and *Solanum tuberosum* can replace bromothymol blue in a titration of CH_3COOH vs. NaOH. In NH_3 vs. HCl titration, no significant difference was observed when the phenolphthalein end point was compared with all flower extracts except *Rosa hybrida*. There was also no significant difference observed when methyl red was compared to all flowers except *Guizotia scabra* flower extract. There was no significant difference observed when the mean titre of bromothymol blue was compared with *Cordia sebestena* flower extract. For CH_3COOH versus NaOH titration, there was no significant difference when the titre volume of all flower extracts was compared with the phenolphthalein indicator. In addition, there was also no significance difference when the mean titre volume of all flower extracts except *Rosa hybrida* was comparable, and no significance difference was observed when the mean titre.

5.2. Recommendations

Based on the findings of this research, the following recommendations were made:

- We recommend the use of *Guizotia scabra*, *Cordia sabastena*, *Solanum tuberosum*, and *Rosa hybrida* flower extracts as indicators for acid-base titration as a substitute for hazardous synthetic indicators like phenolphthalein, methyl red, and bromothymol blue, thereby protecting and preserving the environment from pollution and contamination.
- Further research is needed on the isolation and identification of active compounds contained in all flower extracts and the comparison of those selected flower extracts with other synthetic indicators not used in the current study.
- It is advised to investigate the viability of applying these colored plant extracts to various additional titrations, such as complex formation titrations, precipitation titrations, or other titrations.

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7. APPENDIX

7.1. Appendix Tables

Appendix Table 1. Descriptive statistics of HCl vs NaOH

	N	minimum	maximum	mean	Standard deviation
phenolphthalein	3	9.30	10.50	9.80	0.62
Methyl red	3	10.10	10.80	10.50	0.36
Bromothymol blue	3	8.60	10.80	10.00	1.21
<i>Guizotia scabra</i>	3	9.50	11.60	10.30	1.13
<i>Cordia sebestena</i>	3	9.30	9.80	9.60	0.26
<i>Solanum tuberosum</i>	3	8.50	9.30	9.00	0.43
<i>Rosa hybrida</i>	3	10.40	10.70	10.60	0.17
Valid N(listwise)	3				

Appendix Table 2. Descriptive statistics of NH₃ vs HCl

	N	minimum	maximum	mean	Standard deviation
phenolphthalien	3	5.8	6.6	6.2	0.4
Methyl red	3	6.98	8.61	7.57	0.90338
Bromothymol blue	3	7.4	8.1	7.8	0.36056
<i>Guizotia scabra</i>	3	4.5	5.8	5	0.7
<i>Cordia sebestena</i>	3	6.6	7.5	7.1667	0.49329
<i>Solanum tuberosum</i>	3	5.6	6.5	6	0.45826
<i>Rosa hybrida</i>	3	8.2	9.1	8.6	0.45826
Valid N(listwise)	3				

Appendix Table 3.Descriptive statistics of CH₃COOH vs NaOH

	N	minimum	Maximum	mean	Standard deviation
phenolphthalein	3	8.20	13.30	11.0000	2.58650
Methyl red	3	9.50	10.80	10.0000	0.70000
Bromothymol blue	3	8.70	9.50	9.2000	0.43589
<i>Guizotia scabra</i>	3	9.20	11.10	10.0000	0.98489
<i>Cordia sebestena</i>	3	9.80	10.40	10.1000	0.30000
<i>Solanum tuberosum</i>	3	10.50	11.60	11.0000	0.55678
<i>Rosa hybrida</i>	3	11.80	13.40	12.4000	0.87178
Valid N(listwise)	3				

Appendix Table 4. One way ANOVA and Post Hoc Tests of HCl vs NaOH

ANOVA						
HCl vs NaOH						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	5.683	6	0.947	1.852	0.160	
Within Groups	7.160	14	0.511			
Total	12.843	20				
Multiple Comparisons						
Dependent Variable:						
Tukey HSD						
(I) result		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
phenolphthalein	methyl red	-0.70000	0.58391	0.883	-2.6938	1.2938
	Bromothymol blue	-0.20000	0.58391	1.000	-2.1938	1.7938
	<i>Guizotia scabra</i>	-0.50000	0.58391	0.974	-2.4938	1.4938
	<i>Cordia sebestena</i>	0.20000	0.58391	1.000	-1.7938	2.1938
	<i>Solanum tuberosum</i>	0.80000	0.58391	0.808	-1.1938	2.7938
	<i>Rosa Hybrida</i>	-0.80000	0.58391	0.808	-2.7938	1.1938
methyl red	phenolphthalein	0.70000	0.58391	0.883	-1.2938	2.6938
	Bromothymol blue	0.50000	0.58391	0.974	-1.4938	2.4938
	<i>Guizotia scabra</i>	0.20000	0.58391	1.000	-1.7938	2.1938
	<i>Cordia sebestena</i>	0.90000	0.58391	0.718	-1.0938	2.8938
	<i>Solanum tuberosum</i>	1.50000	0.58391	0.208	-0.4938	3.4938
	<i>Rosa Hybrida</i>	-0.10000	0.58391	1.000	-2.0938	1.8938

Bromothymol blue	phenolphthalein	0.20000	0.58391	1.000	-1.7938	2.1938
	methyl red	-0.50000	0.58391	0.974	-2.4938	1.4938
	<i>Guizotia scabra</i>	-0.30000	0.58391	0.998	-2.2938	1.6938
	<i>Cordia sebestena</i>	0.40000	0.58391	0.991	-1.5938	2.3938
	<i>Solanum tuberosum</i>	1.00000	0.58391	0.619	-0.9938	2.9938
	<i>Rosa Hybrida</i>	-0.60000	0.58391	0.939	-2.5938	1.3938
<i>Guizotia scabra</i>	phenolphthalein	0.50000	0.58391	0.974	-1.4938	2.4938
	methyl red	-0.20000	0.58391	1.000	-2.1938	1.7938
	Bromothymol blue	0.30000	0.58391	0.998	-1.6938	2.2938
	<i>Cordia sebestena</i>	0.70000	0.58391	0.883	-1.2938	2.6938
	<i>Solanum tuberosum</i>	1.30000	0.58391	0.341	-0.6938	3.2938
	<i>Rosa Hybrida</i>	-0.30000	0.58391	0.998	-2.2938	1.6938
<i>Cordia sebestena</i>	phenolphthalein	-0.20000	0.58391	1.000	-2.1938	1.7938
	methyl red	-0.90000	0.58391	0.718	-2.8938	1.0938
	Bromothymol blue	-0.40000	0.58391	0.991	-2.3938	1.5938
	<i>Guizotia scabra</i>	-0.70000	0.58391	0.883	-2.6938	1.2938
	<i>Solanum tuberosum</i>	0.60000	0.58391	0.939	-1.3938	2.5938
	<i>Rosa Hybrida</i>	-1.00000	0.58391	0.619	-2.9938	0.9938
<i>Solanum tuberosum</i>	phenolphthalein	-0.80000	0.58391	0.808	-2.7938	1.1938
	methyl red	-1.50000	0.58391	0.208	-3.4938	0.4938
	Bromothymol blue	-1.00000	0.58391	0.619	-2.9938	0.9938
	<i>Guizotia scabra</i>	-1.30000	0.58391	0.341	-3.2938	0.6938
	<i>Cordia sebestena</i>	-0.60000	0.58391	0.939	-2.5938	1.3938
	<i>Rosa Hybrida</i>	-1.60000	0.58391	0.158	-3.5938	0.3938
<i>Rosa Hybrida</i>	phenolphthalein	0.80000	0.58391	0.808	-1.1938	2.7938
	methyl red	0.10000	0.58391	1.000	-1.8938	2.0938
	Bromothymol blue	0.60000	0.58391	0.939	-1.3938	2.5938
	<i>Guizotia scabra</i>	0.30000	0.58391	0.998	-1.6938	2.2938
	<i>Cordia sebestena</i>	1.00000	0.58391	0.619	-0.9938	2.9938
	<i>Solanum tuberosum</i>	1.60000	0.58391	0.158	-0.3938	3.5938

Appendix Table 5. Oneway ANOVA and Post Hoc Tests of NH₃ vs HCl

ANOVA						
NH ₃ vs HCl						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	27.389	6	4.565	14.143	0.000	
Within Groups	4.519	14	0.323			
Total	31.908	20				
Multiple Comparisons						
Dependent Variable:						
Tukey HSD						
(I) result		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
phenolphthalien	methyl red	-1.37000	0.46388	0.111	-2.9540	0.2140
	Bromothymol blue	-1.60000*	0.46388	0.047	-3.1840	-0.0160
	<i>Guizotia scabra</i>	1.20000	0.46388	0.202	-0.3840	2.7840
	<i>Cordia sebestena</i>	-0.96667	0.46388	0.411	-2.5506	0.6173
	<i>Solanum tuberosum</i>	0.20000	0.46388	0.999	-1.3840	1.7840
	<i>Rosa Hybrida</i>	-2.40000*	0.46388	0.002	-3.9840	-0.8160
methyl red	phenolphthalein	1.37000	0.46388	0.111	-0.2140	2.9540
	Bromothymol blue	-0.23000	0.46388	0.999	-1.8140	1.3540
	<i>Guizotia scabra</i>	2.57000*	0.46388	0.001	0.9860	4.1540
	<i>Cordia sebestena</i>	0.40333	0.46388	0.972	-1.1806	1.9873
	<i>Solanum tuberosum</i>	1.57000	0.46388	0.053	-0.0140	3.1540
	<i>Rosa Hybrida</i>	-1.03000	0.46388	0.344	-2.6140	0.5540
Bromothymol	phenolphthalein	1.60000*	0.46388	0.047	0.0160	3.1840

blue	methyl red	0.23000	0.46388	0.999	-1.3540	1.8140
	<i>Guizotia scabra</i>	2.80000*	0.46388	0.000	1.2160	4.3840
	<i>Cordia sebestena</i>	0.63333	0.46388	0.811	-0.9506	2.2173
	<i>Solanum tuberosum</i>	1.80000*	0.46388	0.022	0.2160	3.3840
	<i>Rosa Hybrida</i>	-0.80000	0.46388	0.612	-2.3840	0.7840
<i>Guizotia scabra</i>	phenolphthalein	-1.20000	0.46388	0.202	-2.7840	0.3840
	methyl red	-2.57000*	0.46388	0.001	-4.1540	-0.9860
	Bromothymol blue	-2.80000*	0.46388	0.000	-4.3840	-1.2160
	<i>Cordia sebestena</i>	-2.16667*	0.46388	0.005	-3.7506	-0.5827
	<i>Solanum tuberosum</i>	-1.00000	0.46388	0.375	-2.5840	0.5840
	<i>Rosa Hybrida</i>	-3.60000*	0.46388	0.000	-5.1840	-2.0160
<i>Cordia sebestena</i>	phenolphthalein	0.96667	0.46388	0.411	-0.6173	2.5506
	methyl red	-0.40333	0.46388	0.972	-1.9873	1.1806
	Bromothymol blue	-0.63333	0.46388	0.811	-2.2173	0.9506
	<i>Guizotia scabra</i>	2.16667*	0.46388	0.005	0.5827	3.7506
	<i>Solanum tuberosum</i>	1.16667	0.46388	0.225	-0.4173	2.7506
	<i>Rosa Hybrida</i>	-1.43333	0.46388	0.088	-3.0173	0.1506
<i>Solanum tuberosum</i>	phenolphthalien	-0.20000	0.46388	0.999	-1.7840	1.3840
	methyl red	-1.57000	0.46388	0.053	-3.1540	0.0140
	Bromothymol blue	-1.80000*	0.46388	0.022	-3.3840	-0.2160
	<i>Guizotia scabra</i>	1.00000	0.46388	0.375	-0.5840	2.5840
	<i>Cordia sebestena</i>	-1.16667	0.46388	0.225	-2.7506	0.4173
	<i>Rosa Hybrida</i>	-2.60000*	0.46388	0.001	-4.1840	-1.0160
<i>Rosa Hybrida</i>	phenolphthalien	2.40000*	0.46388	0.002	0.8160	3.9840
	methyl red	1.03000	0.46388	0.344	-0.5540	2.6140
	Bromothymol blue	0.80000	0.46388	0.612	-0.7840	2.3840
	<i>Guizotia scabra</i>	3.60000*	0.46388	0.000	2.0160	5.1840
	<i>Cordia sebestena</i>	1.43333	0.46388	0.088	-0.1506	3.0173
	<i>Solanum tuberosum</i>	2.60000*	0.46388	0.001	1.0160	4.1840
*. The mean difference is significant at the 0.05 level.						

Appendix Table 6.One way ANOVA and post hoc test of CH₃COOH vs NaOH

ANOVA						
CH ₃ COOH vs NaOH						
	Sum of Squares	df	Mean Square	F	Sig.	
Between Groups	19.363	6	3.227	2.378	0.085	
Within Groups	19.000	14	1.357			
Total	38.363	20				
Multiple Comparisons						
Dependent Variable:						
Tukey HSD						
(I) result		Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
phenolphthalein	methyl red	1.00000	0.95119	0.932	-2.2479	4.2479
	Bromothymol blue	1.80000	0.95119	0.515	-1.4479	5.0479
	<i>Guizotia scabra</i>	1.00000	0.95119	0.932	-2.2479	4.2479
	<i>Cordia sebestena</i>	0.90000	0.95119	0.958	-2.3479	4.1479
	<i>Solanum tuberosum</i>	0.00000	0.95119	1.000	-3.2479	3.2479
	<i>Rosa Hybrida</i>	-1.40000	0.95119	0.756	-4.6479	1.8479
methyl red	phenolphthalein	-1.00000	0.95119	0.932	-4.2479	2.2479
	Bromothymol blue	0.80000	0.95119	0.976	-2.4479	4.0479
	<i>Guizotia scabra</i>	0.00000	0.95119	1.000	-3.2479	3.2479
	<i>Cordia sebestena</i>	-0.10000	0.95119	1.000	-3.3479	3.1479
	<i>Solanum tuberosum</i>	-1.00000	0.95119	0.932	-4.2479	2.2479
	<i>Rosa Hybrida</i>	-2.40000	0.95119	0.223	-5.6479	0.8479
Bromothymol blue	phenolphthalein	-1.80000	0.95119	0.515	-5.0479	1.4479
	methyl red	-0.80000	0.95119	0.976	-4.0479	2.4479
	<i>Guizotia scabra</i>	-0.80000	0.95119	0.976	-4.0479	2.4479
	<i>Cordia sebestena</i>	-0.90000	0.95119	0.958	-4.1479	2.3479
	<i>Solanum tuberosum</i>	-1.80000	0.95119	0.515	-5.0479	1.4479
	<i>Rosa Hybrida</i>	-3.20000	0.95119	0.055	-6.4479	0.0479
<i>Guizotia scabra</i>	phenolphthalien	-1.00000	0.95119	0.932	-4.2479	2.2479
	methyl red	0.00000	0.95119	1.000	-3.2479	3.2479
	Bromothymol	0.80000	0.95119	0.976	-2.4479	4.0479

	blue					
	<i>Cordia sebestena</i>	-0.10000	0.95119	1.000	-3.3479	3.1479
	<i>Solanum tuberosum</i>	-1.00000	0.95119	0.932	-4.2479	2.2479
	<i>Rosa Hybrida</i>	-2.40000	0.95119	0.223	-5.6479	0.8479
<i>Cordia sebestena</i>	phenolphthalein	-0.90000	0.95119	0.958	-4.1479	2.3479
	methyl red	0.10000	0.95119	1.000	-3.1479	3.3479
	Bromothymol blue	0.90000	0.95119	0.958	-2.3479	4.1479
	<i>Guizotia scabra</i>	0.10000	0.95119	1.000	-3.1479	3.3479
	<i>Solanum tuberosum</i>	-0.90000	0.95119	0.958	-4.1479	2.3479
	<i>Rosa Hybrida</i>	-2.30000	0.95119	0.260	-5.5479	0.9479
<i>Solanum tuberosum</i>	phenolphthalein	0.00000	0.95119	1.000	-3.2479	3.2479
	methyl red	1.00000	0.95119	0.932	-2.2479	4.2479
	Bromothymol blue	1.80000	0.95119	0.515	-1.4479	5.0479
	<i>Guizotia scabra</i>	1.00000	0.95119	0.932	-2.2479	4.2479
	<i>Cordia sebestena</i>	0.90000	0.95119	0.958	-2.3479	4.1479
	<i>Rosa Hybrida</i>	-1.40000	0.95119	0.756	-4.6479	1.8479
<i>Rosa Hybrida</i>	phenolphthalein	1.40000	0.95119	0.756	-1.8479	4.6479
	methyl red	2.40000	0.95119	0.223	-0.8479	5.6479
	Bromothymol blue	3.20000	0.95119	0.055	-0.0479	6.4479
	<i>Guizotia scabra</i>	2.40000	0.95119	0.223	-0.8479	5.6479
	<i>Cordia sebestena</i>	2.30000	0.95119	0.260	-0.9479	5.5479
	<i>Solanum tuberosum</i>	1.40000	0.95119	0.756	-1.8479	4.6479

Appendix Table 7.T-test results of HCl vs NaOH using selected natural flower extract and synthetic indicators

Phenolphthalein vs <i>Guizotia scabra</i>				Phenolphthalein vs <i>Cordia sebestena</i>	
		Phenolphthalein	<i>Guizotia scabra</i>	Phenolphthalein(equal variance assumed)	<i>Cordia sebestena</i> (equal variance not assumed)
Equality of variance	F	2.118		2.909	
	Sig	0.219		0.163	
t-test for Equality of Means	T	-0.668	-0.668	0.511	0.511
	Df	4	3.108	4	2.696
	Sig.(2 tailed)	0.541	0.550	0.636	0.648
	Mean difference	-0.50000	-0.50000	0.20000	0.20000
Phenolphthalein vs <i>Solanum tuberosum</i>				Phenolphthalein vs <i>Rosa Hybrida</i>	
		Phenolphthalein	<i>Solanum tuberosum</i>	phenolphthalein	<i>Rosa Hybrida</i>
Equality of variance	F	0.615		5.000	
	Sig	0.477		0.089	

t-test for Equality of Means	T	1.819	1.819	-2.138	-2.138
	Df	4	3.575	4	2.306
	Sig.(2 tailed)	0.143	0.151	0.099	0.149
	Mean difference	0.80000	0.80000	-0.80000	-0.80000
Methyl red vs <i>Guizotia scabra</i>				Methyl red vs <i>Cordia sebestena</i>	
Equality of variance		Methyl red	<i>Guizotia scabra</i>	Methyl red	<i>Cordia sebestena</i>
	F	5.786		0.400	
	Sig	0.074		0.561	
t-test for Equality of Means	T	0.291	0.291	3.486	3.486
	Df	4	2.399	4	3.670
	Sig.(2 tailed)	0.786	0.795	0.025	0.029
	Mean difference	0.20000	0.20000	0.90000	0.90000
Methyl red vs <i>Solanum tuberosum</i>				Methyl red vs <i>Rosa hybrida</i>	
		Methyl red	<i>Solanum</i>	Methyl red	<i>Rosa hybrida</i>

			<i>tuberosum</i>		
Equality of variance	F	0.286		2.000	
	Sig	0.621		0.230	
t-test for Equality of Means	T	4.593	4.593	-0.433	-0.433
	Df	4	3.864	4	2.876
	Sig.(2 tailed)	0.010	0.011	0.687	0.695
	Mean difference	1.50000	1.50000	-0.10000	-0.10000
Bromothymol blue vs <i>Guizotia scabra</i>				Bromothymol blue vs <i>Cordia sebestena</i>	
		Bromothymol blue	<i>Guizotia scabra</i>	Bromothymol blue	<i>Cordia sebestena</i>
Equality of variance	F	0.040		8.800	
	Sig	0.852		0.041	
t-test for Equality of Means	T	-0.312	-0.312	0.556	0.556

	Df	4	3.981	4	2.189
	Sig.(2 tailed)	0.770	0.771	0.608	0.630
	Mean differenc e	-0.30000	-0.30000	0.40000	0.40000
Bromothymol blue vs <i>Solanum tuberosum</i>				Bromothymol blue vs <i>Rosa hybrida</i>	
		Bromothymol blue	<i>Solanum tuberosum</i>	Bromothymol blue	<i>Rosa hybrida</i>
Equality of variance	F	5.492		10.868	
	Sig	0.079		0.030	
t-test for Equality of Means	T	1.340	1.340	-0.846	-0.846
	Df	4	2.505	4	2.081
	Sig.(2 tailed)	0.251	0.289	0.445	0.484
	Mean differenc e	1.00000	1.00000	-0.60000	-0.60000

Appendix Table 8.T-test results of NH₃ vs HCl using selected natural flower extract and synthetic indicators

Phenolphthalein vs <i>Guizotia scabra</i>				Phenolphthalein vs <i>Cordia sebestena</i>	
		Phenolphthal ein	<i>Guizotia scabra</i>	Phenolphthalein (equal variance assumed)	<i>Cordia sebestena</i> (equal variance not assumed)
Equality of variance	F	1.829		.448	
	Sig	.248		.540	
t-test for Equality of Means	T	2.578	2.578	-2.636	-2.636
	Df	4	3.180	4	3.836
	Sig.(2 tailed)	.061	.077	.058	.060
	Mean differenc e	1.20000	1.20000	-.96667	-.96667
Phenolphthalein vs <i>Solanum tuberosum</i>				Phenolphthalein vs <i>Rosa Hybrida</i>	
		Phenolphthali en	<i>Solanum tuberosum</i>	phenolphthalein	<i>Rosa Hybrida</i>
Equality of variance	F	.448		.138	
	Sig	.540		.729	
t-test for Equality of Means	T	-2.636	-2.636	-6.834	-6.834
	Df	4	3.836	4	3.928
	Sig.(2	.058	.060	.002	.003

	tailed)				
	Mean difference	-.96667	-.96667	-2.40000	-2.40000
Methyl red vs <i>Guizotia scabra</i>				Methyl red vs <i>Cordia sebestena</i>	
Equality of variance		Methyl red	<i>Guizotia scabra</i>	Methyl red	<i>Cordia sebestena</i>
	F	.485		2.403	
	Sig	.525		.196	
t-test for Equality of Means	T	3.895	3.895	.679	.679
	Df	4	3.765	4	3.095
	Sig.(2 tailed)	.018	.020	.535	.545
	Mean difference	2.57000	2.57000	.40333	.40333
Methyl red vs <i>Solanum tuberosum</i>				Methyl red vs <i>Rosa hybrida</i>	
		Methyl red	<i>Solanum tuberosum</i>	Methyl red	<i>Rosa hybrida</i>
Equality of variance	F	2.810		2.810	
	Sig	.169		.169	
t-test for Equality of Means	T	2.685	2.685	-1.761	-1.761

	Df	4	2.965	4	2.965
	Sig.(2 tailed)	.055	.076	.153	.177
	Mean differenc e	1.57000	1.57000	-1.03000	-1.03000
Bromothymol blue vs <i>Guizotia scabra</i>				Bromothymol blue vs <i>Cordia sebestena</i>	
		Bromothymol blue	<i>Guizotia scabra</i>	Bromothymol blue	<i>Cordia sebestena</i>
Equality of variance	F	2.462		.448	
	Sig	.192		.540	
t-test for Equality of Means	T	6.159	6.159	-2.636	-2.636
	Df	4	2.991	4	3.836
	Sig.(2 tailed)	.004	.009	.058	.060
	Mean differenc e	2.80000	2.80000	-.96667	-.96667
Bromothymol blue vs <i>Solanum tuberosum</i>				Bromothymol blue vs <i>Rosa hybrida</i>	
		Bromothymol	<i>Solanum</i>	Bromothymol	<i>Rosa hybrida</i>

		blue	<i>tuberosum</i>	blue	
Equality of variance	F	.200		.138	
	Sig	.678		.729	
t-test for Equality of Means	T	5.347	5.347	-6.834	-6.834
	Df	4	3.790	4	3.928
	Sig.(2 tailed)	.006	.007	.002	.003
	Mean difference	1.80000	1.80000	-2.40000	-2.40000

Appendix Table 9.T-test results of CH₃COOH vs NaOH using selected natural flower extract and synthetic indicators

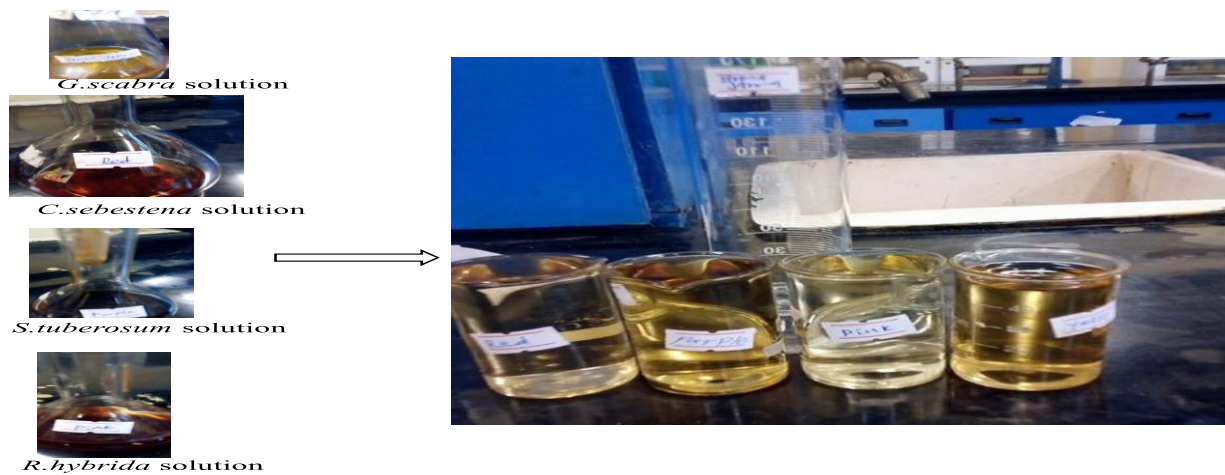
Phenolphthalein vs <i>Guizotia scabra</i>				Phenolphthalein vs <i>Cordia sebestena</i>	
		Phenolphthal ein	<i>Guizotia scabra</i>	Phenolphthalein (equal variance assumed	<i>Cordia sebestena</i> (equal variance not assumed)
Equality of variance	F	.529		5.041	
	Sig	.507		.088	
t-test for Equality of Means	T	.000	.000	.685	.685
	Df	4	3.610	4	2.086
	Sig.(2 tailed)	1.000	1.000	.531	.562
	Mean differenc e	.00000	.00000	1.03333	1.03333
Phenolphthalein vs <i>Solanum tuberosum</i>				Phenolphthalein vs <i>Rosa Hybrida</i>	
		Phenolphthal ein	<i>Solanum tuberosum</i>	phenolphthalein	<i>Rosa Hybrida</i>
Equality of variance	F	4.209		3.852	
	Sig	.110		.121	
t-test for	T	.000	.000	-.391	-.391

Equality of Means					
	Df	4	2.185	4	2.232
	Sig.(2 tailed)	1.000	1.000	.716	.730
	Mean difference	.00000	.00000	-.60000	-.60000
Methyl red vs <i>Guizotia scabra</i>				Methyl red vs <i>Cordia sebestena</i>	
Equality of variance		Methyl red	<i>Guizotia scabra</i>	Methyl red	<i>Cordia sebestena</i>
	F	.529		3.571	
	Sig	.507		.132	
t-test for Equality of Means	T	.000	.000	-.227	-.227
	Df	4	3.610	4	2.711
	Sig.(2 tailed)	1.000	1.000	.831	.836
	Mean difference	.00000	.00000	-.10000	-.10000
Methyl red vs <i>Solanum tuberosum</i>				Methyl red vs <i>Rosa hybrida</i>	
		Methyl red	<i>Solanum tuberosum</i>	Methyl red	<i>Rosa hybrida</i>

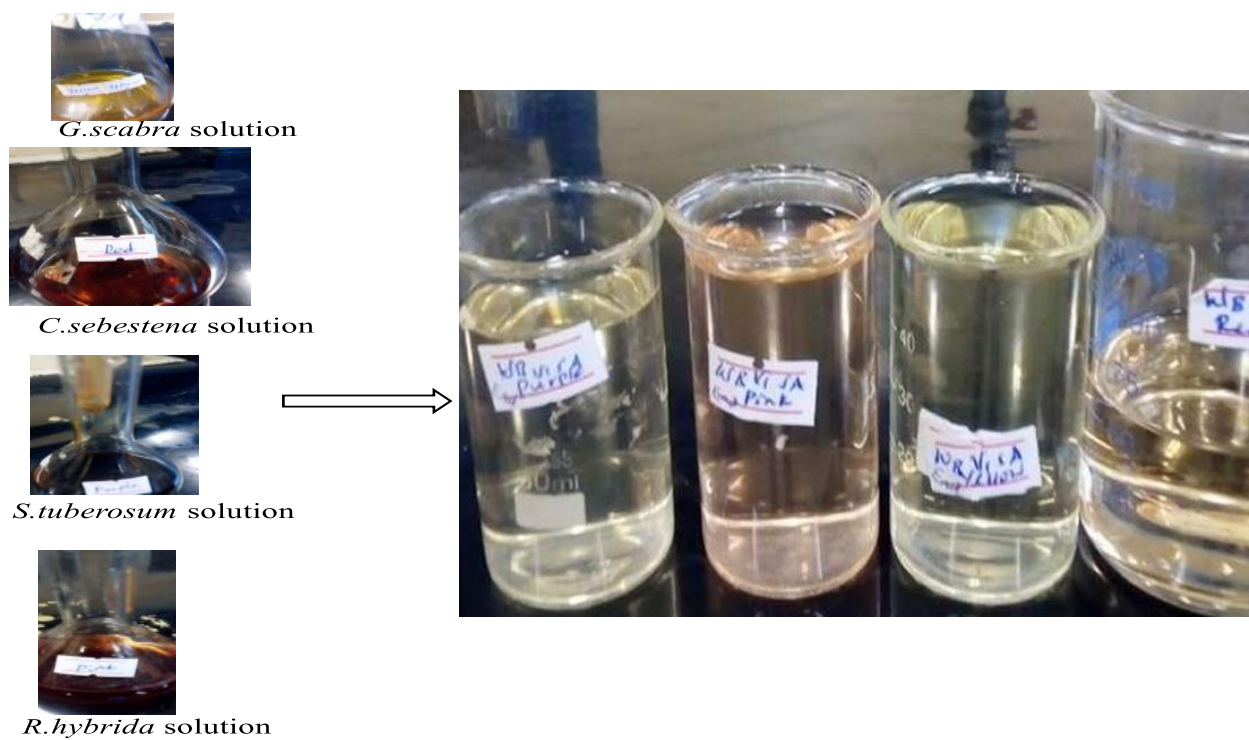
Equality of variance	F	.400		.226	
	Sig	.561		.659	
t-test for Equality of Means	T	-1.936	-1.936	-3.826	-3.826
	Df	4	5.041	4	3.857
	Sig.(2 tailed)	.125	.128	.019	.020
	Mean difference	-1.00000	-1.00000	-2.43333	-2.43333
Bromothymol blue vs <i>Guizotia scabra</i>				Bromothymol blue vs <i>Cordia sebestena</i>	
		Bromothymol blue	<i>Guizotia scabra</i>	Bromothymol blue	<i>Cordia sebestena</i>
Equality of variance	F	2.571		1.000	
	Sig	.184		.374	
t-test for Equality of Means	T	-1.287	-1.287	-2.946	-2.946

	Df	4	2.755	4	3.548
	Sig.(2 tailed)	.268	.296	.042	.049
	Mean differenc e	-.80000	-.80000	-.90000	-.90000
Bromothymol blue vs <i>Solanum tuberosum</i>				Bromothymol blue vs <i>Rosa hybrida</i>	
		Bromothymol blue	<i>Solanum tuberosum</i>	Bromothymol blue	<i>Rosa hybrida</i>
Equality of variance	F	1.424		2.857	
	Sig	.299		.166	
t-test for Equality of Means	T	-2.982	-2.982	-5.687	-5.687
	Df	4	3.146	4	2.941
	Sig.(2 tailed)	.041	.055	.005	.011
	Mean differenc e	-1.53333	-1.53333	-3.20000	-3.20000

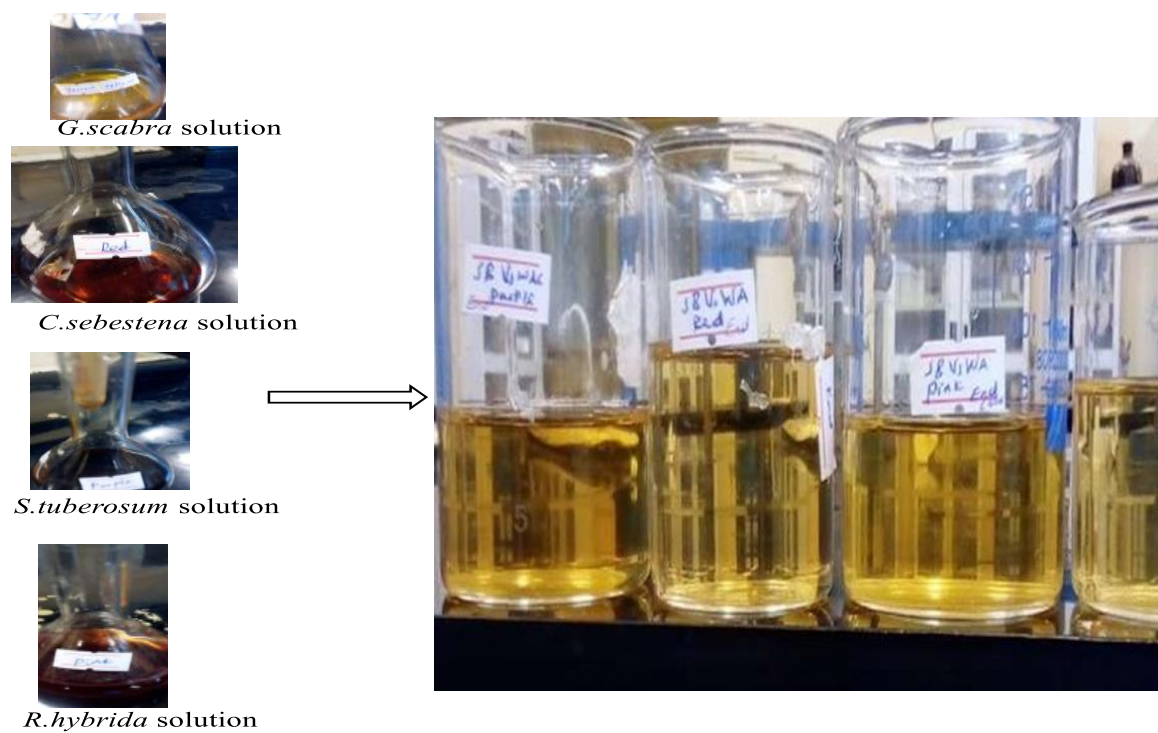
7.2. Appendix Figures



Appendix Figure 1. Colour change observed at end for titration of HCl vs NaOH



Appendix Figure 2. Colour change observed at end for titration of NH_3 vs HCl



Appendix Figure 3. Colour change observed at end for titration of CH_3COOH vs NaOH