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Extraction, Characterization, and Utilization of Different Plant Pigments as Ph Indicators in Titrimetric Analysis

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Abstract: ***Plant pigments are an alternative source for synthetic dyes which are generally toxic and costlier. Various plant parts contain different color pigments chemically either anthocyanins, xanthophylls, carotenoids etc. This chemical diversity exhibits different color reactions depends upon pH of the solution. In the present research work, an attempt has been made to understand the sensitivity of color solutions to acidic and alkaline environment and make use of such color reactions for titrimetric analysis.In present research work, we have selected carrot, beetroots, watermelon, sweet almond fruits, red sandal bark, hisbiscus, lantana and calendula flowers, pomegranate seeds. The fresh parts of all the selected plants were collected in their flowering seasons from the nearby area of Kolhapur and 5g each was macerated with ethanol: water (70:30) for 24h shaking frequently. The filtrate was evaporated to dryness at low temp (45^{0}C) and the residue obtained was subjected to pH sensitivity test of 1% w/v solutions at normal temp using digital pH meter. The different strength solutions of each plant extracts were tested for acid-base titrations as indicators along with commercial synthetic indicators. From the results, it was confirmed that watermelon, red sandal bark, pomegranate seeds show sharp end points in comparison with synthetic dyes.***

From the data obtained it is confirmed that natural plant pigments can serve as alternative indicators for various acid-base titrations at laboratory scale and is possible to develop pH indicator papers which are non-toxic, economical and biodegradable compare to synthetic dyes.

Keywords: Ph Indicators, Plant Pigments, Acid Base Titrations, Ph Sensitivity.

INTRODUCTION

Pigments produce the colors that we observe at each step of our lives because pigments are present in each one of the organisms in the world, and plants are the principal producers. They are in leaves, fruits, vegetables, and flowers; also, they are present in skin, eyes, and other animal structures; and in bacteria and fungi. Natural and synthetic pigments are used in medicines, foods, clothes, furniture, cosmetics, and in other products. However, natural pigments have important functions other than the imparted beauty, such as the following: we could not have photosynthesis or probably life all over the world without chlorophylls and carotenoids. In animals pigments like hemoglobin or myoglobin are responsible for oxygen and carbon dioxide transport. Under stress conditions plants show the synthesis of flavonoids; the quinones are very important in the conversion of light into chemical energy. The melanins act as a protective screen in humans and other vertebrates, and in some fungi, melanins are essential for their vital cycle; a lot of pigments have a well-known pharmacological activity in sicknesses such as cancer and cardiovascular diseases, and this has stressed pigment importance for human beings.[1,2,3,4,5,]

Additionally, since time immemorial human beings have associated product qualities with their colors, this is especially true for sweets, confectionary items and most meals. Historically, at the beginning of the food industry consumers did not take care of the kind of pigments used in food coloring (natural or synthetic), but recently people have shown their phobia to synthetic pigments when the concepts "synthetic pigments" and "illness" were associated, and when the attributed pharmacological benefits of natural pigments came into consideration. However, the natural pigments that are permitted for human foods are very limited, and the approval of new sources is difficult because the U.S. Food and Drug Administration (FDA) considers the pigments as additives, and consequently pigments are under strict regulations.[6,7,8]

Thus, an adequate understanding of the actual sources of pigments will contribute to their better use. At this time, it must be emphasized the ubiquity of pigments in living organisms (plants, fungi, bacteria, among others), the variety of chemical structures, and the large quantity of information generated for each pigment group. Consequently, we are focusing more on plant based

pigments which are more highly consumed as food products taking into account the biochemical and molecular biology information generated for their elucidation, and the processing and stability properties of these pigments as natural pigments.

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Produced color is due to a molecule-specific structure (chromophore); this structure captures the energy and the excitation of an electron from an external orbital to a higher orbital is produced; the nonabsorbed energy is reflected and/or refracted to be captured by the eye, and generated neural impulses are transmitted to the brain where they could be interpreted as a color.[9] Natural colors can be classified differently by different experts and are described briefly.

1. By Their Origin

Pigments can be classified by their origin as natural, synthetic, or inorganic. Natural pigments are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic pigments are obtained from laboratories. Natural and synthetic pigments are organic compounds. Inorganic pigments can be found in nature or reproduced by synthesis.[10]

2. By the Chemical Structure of the Chromophore

Also, pigments can be classified by taking into account the chromophore chemical structure
as: with conjugated systems: carotenoids, anthocyanins, betalains, caramel, synthetic pigments, and lakes.Metal-coordinated porphyrins: myoglobin, chlorophyll, and their derivatives.[11,12]

3. By the Structural Characteristics of the Natural Pigments

Moreover, natural pigments can be classified by their structural characteristics as Tetrapyrrole derivatives: chlorophylls and theme colors.Isoprenoid derivatives: carotenoids and iridoids.*N*-heterocyclic compounds different from tetrapyrroles: purines, pterins, flavins, phenazines, phenoxazines,and betalains.Benzopyran derivatives (oxygenated heterocyclic compounds): anthocyanins and other flavonoid pigments.Quinones: benzoquinone, naphthoquinone, anthraquinone.Melanins.

4. As Food Additives

By considering the pigments as food additives, their classification by the FDA is**Certifiable**[13] These are manmade and subdivided synthetic pigments and lakes.

Functions[14,15]

Up to 1898, the increased interest on color in organisms was due to three main reasons:

(1)The color[16] phenomena are conspicuous for the survival of animals and plants; (2) the relation between color and evolution theories; and (3) their importance in comparative physiology. Thus, the studies on pigments were greatly impulsed by their multiple functions.[17] In agreement with the distribution and abundance, the most important pigments in higher Plants were chlorophylls, carotenoids, and anthocyanins when considering their capacity to impart colors.

The end point in traditional titrimetry is more often than not indicated by some substances added into the analyte solution, which changes color right away after the equivalence point has been attained. These substances are known as indicators [18,19,20]. Indicators are pigments or dyes that can be isolated from a variety of sources, including plants, fungi, and algae [21,22]. The majority of indicators in use today are synthetic substance in the laboratory which is used to determine pH of a substance, such as litmus paper[23,24]. Synthetic indicators have certain disadvantages such as high cost, availability, and chemical pollution; hence natural indicators obtained from various plant parts like flowers, fruits, and leaves will be more advantageous. In addition, some of these synthetic indicators have toxic effects on users such as diarrhea, pulmonary edema, hypoglycemia, and pancreatitis and they can result in abdominal cramps, skin rash, eruptions, erythema, and epidermal necrosis and cause environmental pollution.On the basis of these rationales of the hazardous effects of synthetic indicators, there has been an increasing interest in the search for alternative sources of indicators from natural sources of plant origin. several studies by various investigators have reported the effectiveness of natural indicators in acid-base titrations[25,26]. A natural indicator is a natural substance usually from plant origin that can be used to determine the pH of another substance. Hence in this research, we aim to evaluate the properties of some natural substances in order to ascertain their analytical potentials as indicators.

MATERIALS AND METHODS

Different parts of the plants such as watermelon, fruit, red sandal bark, pomegranate seeds, hibiscus, calendula, lantana flowers, beet and carrot roots were collected as per the availability from the local area. Extraction was carried out using orbital shaker (Lab time India Ltd) and pH of the color extracts was determined using digital pH meter (Systronics). Extracts were dried on thermostatic electric water bath (Sai Enterprises- Mumbai) and titrations were completed using calibrated burettes and pipettes. All the chemicals and reagents used were of analytical grade.

EXPERIMENTAL

a) Extraction of color pigments/dye

All the parts collected were weighed accurately (50g) and homogenized with a small quantity of water. Then extraction was carried out using 100ml ethanol: water (70:30) in an orbital shaker (at 25^0C and 60rpm) for 24h. All the extracts were filtered through muslin cloth and preserved in refrigerator until further use.[27]

b) Characterization of extracts:

i) Observation of color and pH: the extracts obtained were observed carefully in light and color produced was noted for all the extracts[28]. Also, the pH of extracts obtained was determined using digital pH meter at room temp (27.5^{0}C) as shown in the table-1

Table no.I: Natural colors are shown by the different parts of the plants used

Sr. no.	Name of the plant	Color produced	Ph
1	*Citrus lanatus* (Water melon fruit)	Red	4
2	*Beeta vulgaris* (Beet root)	Dark red	4
3	*Daucus carota* (Carrot root)	Reddish yellow	4
4	*Pterocarpus santalinus* (Raktchandan bark)	Red	3
5	*Calendula officinalis* (Zendu pivala flower)	yellow	6
6	*Hibiscus rosa-sinensis* (Jaswand lal flower)	Red	7
7	*Punica granatum* (Dalimb seeds)	Faint red	6
8	*Prunus dulcis* (Sweet almond fruits)	Yellow	4
9	*Lantena camara* (Ghaneri flowers)	Purple	6

ii) pH sensitivity test: All the extracts were tested for pH sensitivity test using 0.1N HCl and NaOH solutions to understand the color changes in alkaline and acidic medium respectively which will help to know the suitability of extracts for food colorants and titrimetric analysis. The results are given in table-2

Table no.II: pH sensitivity test for extracts using acidic and alkaline medium

Name of the plant	0.1N HCl Vol. in (ml)	Color change	pH	0.1N NaOH Vol. in (ml)	Color change	pH
Water melon	1	Creamish	6	5	Faint blue	11
	5	Dark buff	5	6	Blue	12
	10	Dark buff	4	7	Dark blue	13
	15	Colorless	-	8	Dark blue	14
Beetroot	1	Red	4	1	Red	4
	2	Red	3.9	2	Red	4.2
	3	Red	3.6	3	Red	4.4
	4	Red	3.3	4	Red	4.8
	5	Red	2.9	5	Red	5.4
	5.5	Green	2.8	5.2	green	8
Carrot root	3	Yellow	4	3	Blue	10
	9	Yellow	4	8	Dark blue	11
	15	Yellow	4	15	Dark blue	11.5
Raktchandan	2	Green	7	2	YR	4
	3	R.Yellow	6	10	YR	4
	15	R.Yellow	5	12	FG	7
	20	Red	3	14	G	8
	30	Colorless	2	20	G	8
				47	DG	12
Zendu pivala	2	FR	3	1	Orange	4
	4	DR	1	2	GR	7
	11	DR	1	3	GR	8
	15	DR	1	4	GR	9
				6	BR	10
				7	B	

						11
Jaswand lal	1 2 3 25	Orange Red Red Red	4 3 2 1	1 2 3 25	GR DG DG DG	8 10 11 11
Dalimb seeds	1 2 3	Orange Orange Orange	3 3 3	1 2 3	B B B	12 13 14
Sweet almond	1 4 5	Orange Red Red	3 1 1	1 3 5 7 9 11	Y Y DG YB B DB	4 5 9 10 12 14
Ghaneri mixed	1 3 5 6 28	Y Orange Orange Orange Red	7 6 3 3 1	1 2 8 15	B B B B	14 14 14 14

iii) Effect of temperature: All the extracts were subjected to stability study by exposing to the different temperatures keeping in thermostatic hot air oven at different temperatures for 1h and changes were noted as given in table -3

Table no.III: Effect of temperature on extracts color

Name of the plant	**Oven temp in ^{0}C**				
	28	**37**	**45**	**65**	**100**
Watermelon	Stable	No change	Fading	Red	DB
Beetroot	Stable	No change	Red	Dark red	Brown
Carrot root	Stable	No change	Brownish	RY	Brown
Raktchandan	Stable	No change	Red	Maroon	Maroon
Zendu pivala	Stable	No change	Faint brown	YB	Orange
Jaswand lal	Stable	No change	RB	Brownish	Brown
Dalimb seeds	Stable	No change	Fading	Faint red	Dull red
Sweet almond	Stable	No change	YB	Yellow	Brownish
Ghaneri mixed	Stable	No change	Brown	Purple	Crimson

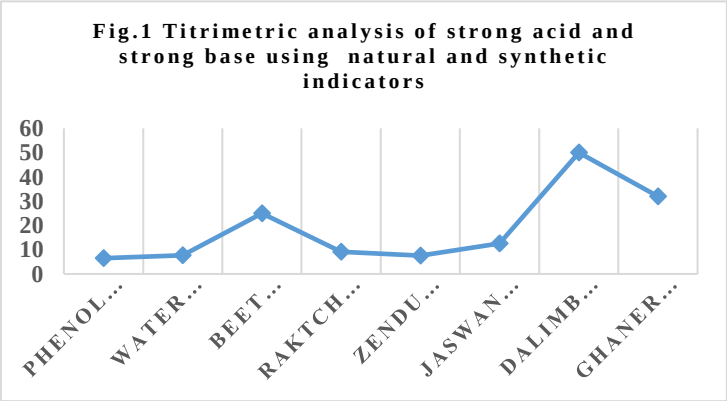
v) Titrimetric analysis[25,26]

a) **Strong acid strong base:** 10ml OF 0.1 N HCl was taken in a conical flask and 50 ml 0.1N NaOH was taken in the burette. Two to three drops of Natural indicators along synthetic indicators were added and titration was carried out till the change in color of the solution of the flask. The results obtained were given in table 4

Table no.IV: Titrimetric analysis of strong acid and strong base using natural and synthetic indicators

Name of the indicator	**Burette reading in ml**	**Endpoint**
Phenolpthelein	6.5	Colorless-pink
Watermelon	7.7	Colorless-pink
Beetroot	25	Brown- orange
Raktchandan	9.2	Colorless-pink

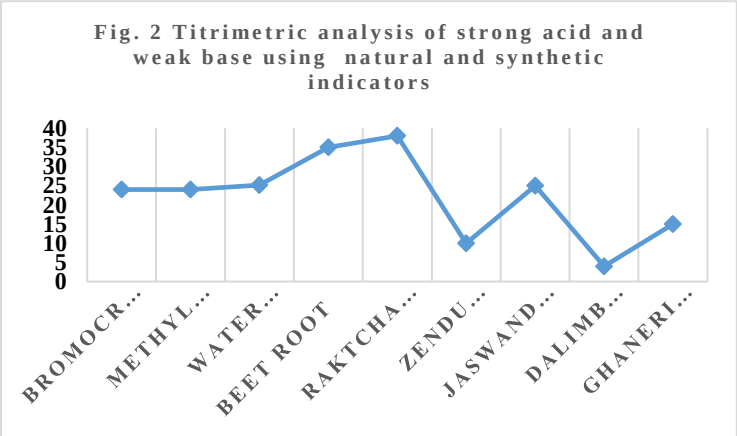
Zendu pivala	7.5	Yellow-red
Jaswand lal	12.6	Colorless-green
Dalimb seeds	50	Pink- orange
Ghaneri mixed	32	Faint pin- yellow



a) **Strong acid weak base:** 10ml OF 0.1 N HCl was taken in a conical flask and 50 ml of 0.1N ammonia was taken in the burette. Two to three drops of Natural indicators along synthetic indicators were added and titration was carried out till the change in color of the solution of the flask. The results obtained were given in table 5

Table no.V: Titrimetric analysis of strong acid and weak base using natural and synthetic indicators

Name of the indicator	Burette reading in ml	Endpoint
Bromocresol green	24	Orange to green
Methyl red	24	Red to green
Water melon	25.2	Red to colorless
Beetroot	35	Red to pink
Raktchandan	38	Colorless-yellow
Zendu pivala	10	Yellow-colorless
Jaswand lal	25	Red to Colorless
Dalimb seeds	4	Pink- green
Ghaneri mixed	15	Faint pin- colorless

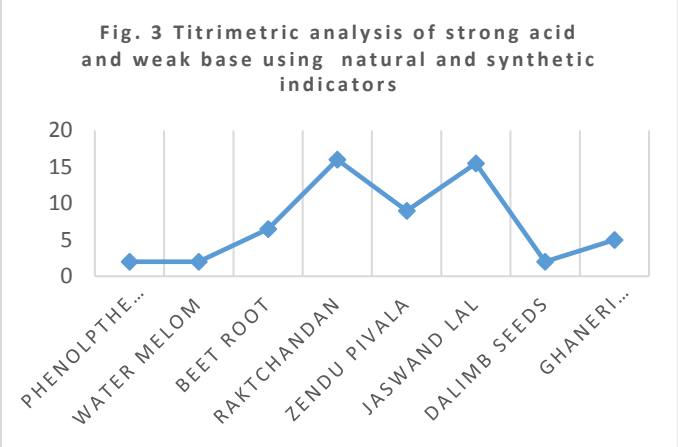


a) **Weak acid strong base:** 10ml of 0.1 N acetic acid was taken in a conical flask and 50 ml of 0.1N NaOH was taken in the burette. Two to three drops of Natural indicators along synthetic indicators were added and titration was carried out till the change in color of the solution of the flask. The results obtained were given in table VI.

Table no.V: Titrimetric analysis of weak acid and weak base using natural and synthetic indicators

Name of the indicator	Burette reading in ml	Endpoint

Phenolphthalein	2	Colorless to pink
Water melon	2	Colorless to pink
Beetroot	6.5	Brown to green
Raktchandan	16	Colorless-yellow
Zendu pivala	9	Colorless-yellow
Jaswand lal	15.5	Colorless to yellow
Dalimb seeds	2	Pink to orange
Sweet almond	4	Pink- green
Ghaneri mixed	5	Colorless to green



RESULT AND DISCUSSION

From the experimental data, it is observed that natural plant pigments/ dyes are easy to extract and are quite stable to heat and normal temperature. The pH of most of the color pigments extracted was found too acidic except Jaswand which has shown neutral pH. All the color pigments obtained were found suitable to be used as pH indicators since they have shown sensitivity to change in pH by producing different colors which are suitable to be used as end point determinations. From the plant colors used Pomegranate seed, Ghaneri and Raktachandan have shown significant sensitivity to pH change compare to other plant extracts screened. In titrimetric analysis, most of the plant colorants found suitable for end-point determinations with a variable amount of acid or base consumption.

CONCLUSION

From the results obtained it is confirmed that natural color pigments can be an alternative source of the synthetic dyes which are toxic and problems for cleanings in titrimetric analysis. It is also confirmed that these natural pigments can be used economically as compare to synthetic dyes. Further investigation is on for the development of indicator paper and development of an analytical method for some API using the pigments.

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