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Development of Natural Indicator and Ph Indicator Strips Using Beetroot*(Beta Vulgaris)*Extract

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Abstract:
Background*:Beetroot (Beta vulgaris) is fleshy, large root growing in the plant of the same name. Beetroot ranks in top 10 vegetables with respect to its antioxidant capacity. They possess antimicrobial and antiviral effects.Beetroot exhibits dark crimson red colour pulp which has a sweet taste.They impart this colour due to presence of specific chromophore group which is used in food and cosmetic industries. The present manuscript focuses on the extraction of Beta vulgaris pigment and it's use as an indicator and preparation of pHindicator strips. In this research endeavour, beetrootswere procured, dried, powdered and used as a natural indicator. This indicator can be a substitute forsynthetic indicators. Synthetic indicatorshave teratogenic and carcinogenic effects, allergic effect, hence they possess a great health hazard. In comparison to synthetic indicator, natural indicators are environment friendly, bio-degradable and have less health hazards.*
Methods and Materials: *The Beetroots (Beta vulgaris) were procured from Thane Vegetable Market, Mumbai. All the chemicals and reagents used were of analytical grade. Fresh Beetroot indicator and beetroot powder indicator was prepared and tested in Acid-Base titrations. Beetroot pH indicator strips were prepared using Whatman's Filter paper.*
Result:*The study was carried out in which theoptimum temperature at which the membrane ruptures was estimated. The beetroot powdered indicatordisplays stability for 2 months.Beetroot pH indicator strips displayes colour change in basic solutions more easily than that of acidic solutions.*
Conclusion: *Beetroot indicator can be a good natural alternative to synthetic indicators and it's pH indicator strips can be used for detecting pH of various solutions.*
Keywords:*Synthetic pigments, Natural dye, Chromophore, Beta vulgaris, Beetroot indicator, Beetroot pH indicator strips.*

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I. Introduction

Beetroot (*beta vulgaris*) is fleshy, large root growing in the plant of the same name. Its superficial, thin skin has a wide range of colours, from purple-pink and reddish-orange to a brownish tone. The dark crimson red colour pulp has a sweet taste. They are within the class *Dicotyledonae* and are included in the genus *Beta* and the species *vulgaris*. Beetroot (*Beta vulgaris*) was first used by the Greeks and the Romans**(1)**.There are total 122 various types of beetroots grown all over the globe.Beetroot ranks among first 10 most powerful vegetables with its antioxidant capacity**(2)**. They possess antimicrobial and antiviral effects**(3)**. Theyalso can inhibit the cell proliferation of human tumour cells**(4)**.The pigment in beetroot is betalain pigment. Betalains are water-soluble nitrogenous pigments and include two classes: red-violet betacyanin and yellow betaxanthin, which provides beetroot with their typical colour**(5,6)**. Betacyanins appear red to red violet in colour and they are absorbed in the 535-550nm range. Betaxanthins generally appear yellow in colour and they are absorbed in the 475-480nm range**(7)**.Betalains have no toxic effects on the human body and are seen as a natural and safe alternative to synthetic red dyes. Betalains are used as a natural additive for food, drugs and cosmetic products along with beet juice concentrate or beet powder**(8)**. In India beetroot is mainly cultivated in Haryana, Uttar Pradesh, Himachal Pradesh, West Bengal and Maharashtra**(9)**.

Synthetic pigments due to its teratogenic and carcinogenic effects, allergic reactions and also significant risk in brain tumours and thyroid tumours are becoming more noticeable.Natural pigments have several excellent properties including less side effects, high safety factor, biodegradable and are green environmental protective**(10)**.Present manuscript focuses on the extraction of *Beta vulgaris*pigment and it's use as indicator and preparation of pHindicator strips. Also beetrootswere dried, powdered and used as natural

indicator. The above indicator can be a substitute to synthetically prepared indicators and can be a safe alternative to be used at laboratory level.

II. Materials and Methods

The Beetroots (B*eta vulgaris*) were procured from Thane Vegetable Market, Mumbai. The peel of beetroot were discarded and the freshly cut pieces were used for sample preparations.

Study Design: Preparation of Natural Indicator

Study Location: The study was carried out in RamnarainRuia Autonomous College at Matunga, Mumbai, Maharashtra.

Study Duration: September 2019 – February 2020

Procedure Methodology:

a. Study the optimum temperature:

Determination of the optimum temperature at which the cell gets disrupted and all the pigments leaked was carried out.5mg beetroot slices were added to5ml distilled water in various test tubes. These tubes were incubated atvarious temperatures ranging from 0^oC-100^oC and were measuredcolorimetrically at 490nm.

b. Testing fresh beetroot indicator in Acid-Base titrations:

Fresh beetroot indicator was prepared using 100gm of beetroot sample with distilled water and ethanol and was tested with Acid-Base titrations and compared with phenolphthalein indicator.0.1N Hydrochloric Acid(HCl)/Acetic Acid(CH_3COOH)were the preferred acid while 0.1N Sodium Hydroxide(NaOH) was the preferred base.

c. Testing powdered beetroot indicator in Acid-Base titrations:

Beetroot powder was prepared by sun drying and it was used as indicator after adding hot distilled water and was tested in Acid-Base titrations i.e.0.1N HCl / CH_3COOH vs 0.1N NaOH titration.This powdered sample was preserved in air tight container and was tested for its stability for next 2 months with interval of 15 days was determined keeping all other experimental parameters same.

2.4 Preparation of Beetroot pH Indicator Strips and it's use as Indicator:

Whatman's Filter paper no. 42 strips were kept overnightin a beetroot solution. The beetroot pH indicator strips were air dried and tested against various pH solutions.

III. Results and Discussions

3.1 Beetroot Cell Rupture At Various Temperatures:

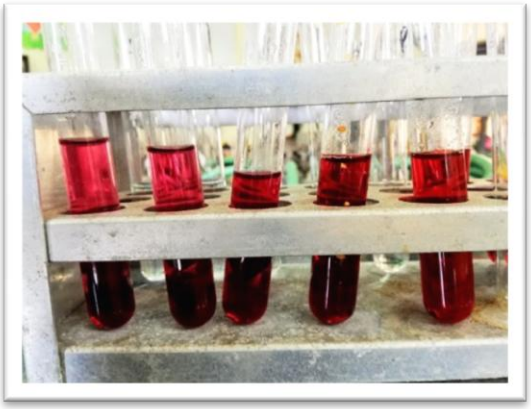

Figure 1: Tubes for Optimum Temperature (Key From Left to Right : 1: 0^OC, 2: Room Temperature, 3: 50^oC, 4: 75^oC, 5: 100^oC)

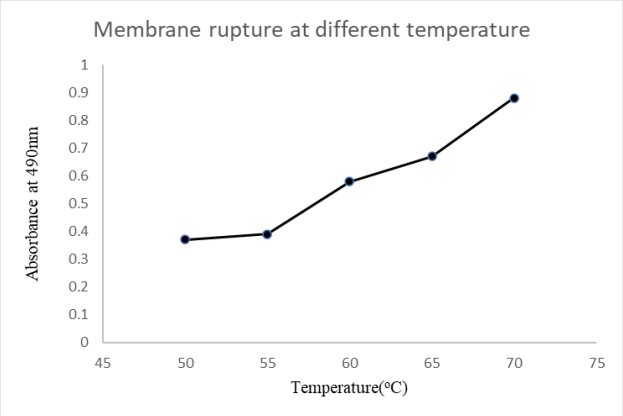

Figure 2: Optimum Temperature for Beetroot Cell Rupture

After plotting a standard graph of Temperature(^{o} C) vs Absorbance(at 490nm), it displayed an increase in slope between 60^{o}C to 65^{o}C.It can be concluded that the optimum temperature needed for disruption of beetroot was between 60^{o}C to 65^{o}C and all the anthocyanin inside the cell leaked out at this temperature.

3.2Testing Fresh Beetroot Indicator In Acid Vs Base Reaction:

Figure 3: Before titration **Figure 4:** After titration

	Fresh Beetroot Indicator(Constant Burette Reading)	Phenolphthalein Indicator(Constant Burette Reading)
Strong Acid vs Strong Base	10.5	10.3
Weak Acid vs Strong Base	11.3	11.1

Table 1: Comparison of Constant Burette Reading between Fresh Beetroot Indicator and Phenolphthalein Indicator

Comparative study between Phenolphthalein Indicator and Fresh Beetroot Indicator indicated that the beetroot indicator can also be a good natural alternative for Strong Acid vs Strong Base titrations and Weak Acid vs Strong Base titrations.

3.3 Testing The Fresh Beetroot Indicator And Beetroot Powder Indicator In Acid Vs Base Reaction :

	Fresh Beetroot Indicator(Constant Burette Reading)	Powdered Beetroot Indicator(Constant Burette Reading)
Strong Acid vs Strong Base	10.4	10.3
Weak Acid vs Strong Base	11.3	11.2

Table 2: Comparison of Constant Burette Reading between Fresh Beetroot Indicator and Powdered Beetroot Indicator

Comparative study between Fresh Beetroot Indicator and Beetroot Powder Indicatorindicated that the powdered indicator can also be good natural alternative for Strong Acid vs Strong Base titrations and Weak Acid vs Strong Base titrations.

3.4 Stability Of Beetroot Powder Indicator:

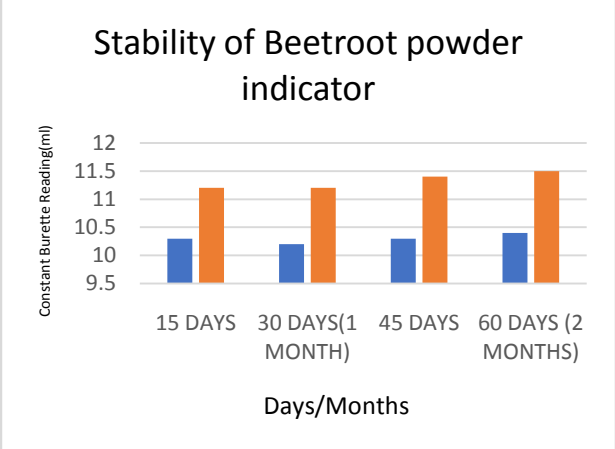

Figure 5: Stability of Beetroot Powdered Beetroot Indicator
(Key: Blue = Strong acid vs Strong Base Titration ; Orange = Weak Acid vs Strong Base Titration)

Comparative study indicated that the powdered beetroot indicator displayed stability till 2 months in Strong Acid vs Strong Base and Weak Acid vs Strong Base titrations keeping the experimental parameters constant.

3.5 Preparation Of Beetroot pH Indicator Strips And Its Use As An Indicator:

Figure 6: Beetroot pH Indicator Paper

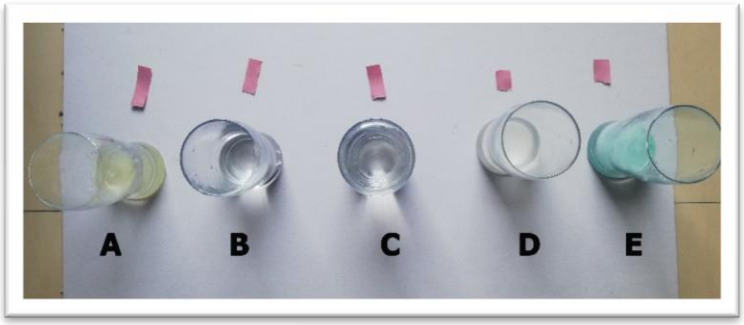

Figure 7: Beetroot pH Indicator Strips with solution A) Lemon juice B) Vinegar C) Water D) Baking Soda E) Detergent

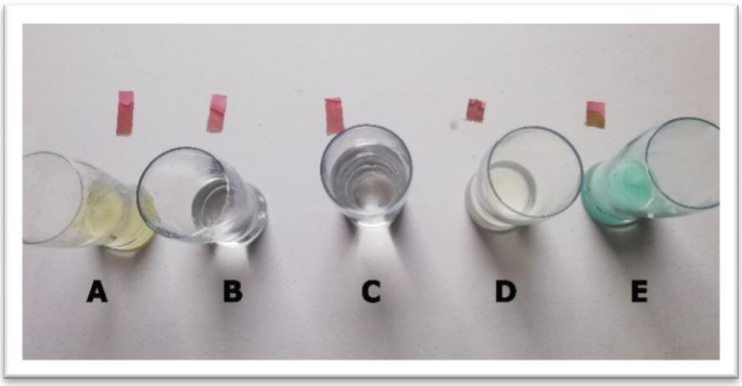

Figure 8: Change in beetroot pH Indicator Strips with solution at various ph solutions A) Lemon juice B) Vinegar C) Water D) Baking Soda E) Detergent

IV. Conclusion

The beetroot cells were disrupted at an optimum temperature, at this temperature all the pigments inside leaked out which were measured colorimetrically. This indicates that the optimum temperature is necessary for further pigment studies. Comparative study between fresh beetroot indicator and syntheticindicator suggested thatthe beetroot indicator can be a good natural indicator. As the fresh beetroot indicator developed microbial growth within few days, beetroot powder was extracted from beetroot and was stored. This powder can beused later as beetroot indicator by adding it to distilled water as the pigment is soluble in distilled water. The indicator prepared from powder displayed stability in reading for Acid vs Base titrations for 2 months. In general, the use of beetroot powder indicator resulted in good stability of the titration readings. Hence it can be a good natural alternative to synthetic indicators. The beetroot pH indicator strips were prepared from Whatman filter paper No. 42. As there was no use of synthetic indicators thus these indicator strips are found to be eco-friendly.Change in beetroot pH indicator strips was seen when tested with Baking Soda(Colour changes from pink to light yellow) and with Detergent (color changes from pink to dark yellow).Which cleary indicates that the beetroot pH indicator strips displayed colour change more prominiently in basic solutions as compared to acidic solutions.Also the process involved in making of such indicator strips is very convenient and reproducible.

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