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Fresh Versus Dehydrated Beetroot (Beta Vulgaris) Extract as a Natural Acid-Base Indicator: A Spectrophotometric and Titrimetric Comparison with Phenolphthalein

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Abstract: Phenolphthalein and its fellow synthetic dyes have dominated titrimetric end-point detection,, but growing concern about their toxicity and environmental persistence has renewed interest in plant-derived alternatives. Beetroot (Beta vulgaris) is a promising candidate because it's two betalain pigment classes -betacyanins (red-violet, $\lambda_{max} \approx$ 535-540 nm) and betaxanthins (yellow-orange, $\lambda max \approx$ 480 nm) are intensely coloured and pH-responsive. Most existing studies, however, have examined only fresh beetroot extract, leaving open whether dehydrated beetroot powders now sold as food supplements retain comparable indicator performance. This study compared fresh beetroot extract, a commercial dehydrated beetroot powder extract, and phenolphthalein side by side, using UV–Vis spectrophotometric scanning across a buffered pH range (1.3 - 11.5) and a weak acid-strong base titration (0.1 M CH_3COOH against 0.1 M NaOH). The fresh extract retained strong, stable absorbance at 480 nm and 535 nm across the acidic-to-neutral range, declining sharply only above pH 8-9, close to where phenolphthalein's absorbance rose, an intersection marking a shared transition zone. The powdered extract, by contrast, showed a consistently weak, largely pH-insensitive signal, with its apparent absorption maximum shifted to 300 nm. In the titration, the fresh extract's mean end point (24.3 ± 0.1 mL) deviated from the theoretical equivalence volume (20 mL) by only +21.3%, closely tracking phenolphthalein's own +17.5% deviation (23.5 ± 0.0 mL), while the powdered extract deviated by +61.7% (32.3 ± 0.6 mL). These results show fresh beetroot extract to be a chemically faithful, titrimetrically competitive natural indicator, whereas the powdered form needs a higher working concentration or prior calibration before quantitative use.

Keywords: *Beetroot; Beta Vulgaris; Betalains; Betacyanin; Betaxanthin; Natural pH Indicator; UV-Vis Spectrophotometry; Acid-Base Titration; Green Chemistry.*

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I. INTRODUCTION

Every acid-base titration comes down to one small decision: which indicator will signal that the reaction is complete. For most of the last century that decision has defaulted to the same handful of synthetic dyes - phenolphthalein, methyl orange, methyl red. mainly because they are cheap, sharp in their colour change, and easy to get hold of. That familiarity has a cost, though: it has let real questions about toxicity and environmental impact go largely unexamined in routine lab work. Methyl orange and other azo-based indicators, for instance, have been documented as carcinogenic and genotoxic, and they persist in wastewater long after they leave the flask [1]. Several of these dyes are manufactured through multi-step organic synthesis that generates its own hazardous waste, and phenolphthalein specifically has recently been flagged as an emerging environmental contaminant [2]. None of this is news to green analytical chemists, who have spent the last decade or so looking for plant-derived pigments that can do the same job - a sharp, repeatable colour change at a known pH without the toxicity or the disposal headache [3], evaluated against the

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same greenness metrics now used to benchmark such methods [4]. Phenolphthalein's own colour change, incidentally, is well understood at the molecular level: it flips from a colourless lactone to a deeply coloured, highly conjugated quinoid dianion once the solution turns alkaline, a transition mapped out in detail by earlier spectrophotometric work [5]. That lactone-to-quinoid switch turns out to be a handy reference point when reading the pH-dependent spectra of natural pigments later in this paper.

Betalains are one of the plant pigment families that keep coming up in this search. They are water-soluble, nitrogen-containing compounds behind the deep red-violet to yellow-orange colours found in beetroot, amaranth, and a scattering of other Caryophyllales species [6], and they come in two structurally related flavours: red-violet betacyanins, of which betanin is the best-known example, absorbing maximally around 535-540 nm; and yellow-orange betaxanthins, peaking near 480 nm [7-9]. Both classes are pH-sensitive, and beetroot in particular gets proposed again and again as a natural indicator candidate simply because it is cheap, easy to find, and visually dramatic when it changes colour. Beetroot is far from alone in this, though - researchers have been screening all sorts of flowers and vegetables for similar acid-base indicator behaviour [10]. Anthocyanins from red cabbage have drawn similar interest for pH-sensing [11], a reminder that what's being studied here is really a whole class of naturally pH-responsive pigments, not some quirk unique to beetroot.

Where things get less clear is what happens once beetroot is dried and powdered - the form now sold everywhere as a food and nutraceutical supplement. Betalains don't just respond to pH; they're sensitive to heat, light, and whatever purification route was used to make the extract, and losses in both pigment content and purity have been reported even under carefully controlled processing [12]. Commercial drying methods in particular have been shown to reduce betalain retention and shift the resulting powder's colour coordinates relative to the fresh root, with the extent of loss depending strongly on the drying method and temperature used [13,14]. Separately, betalains are known to degrade sharply once the pH turns strongly alkaline [15]. Put those threads together and a fairly practical question emerges, one that the existing natural-indicator literature hasn't really answered: if you wanted the convenience of a shelf-stable, ready-to-use beetroot indicator instead of preparing a fresh batch every time, would a commercially dried powder actually hold up both in how it behaves spectrally and in how accurately it flags the end point of a real titration?

That is the question this study set out to answer directly. Fresh beetroot extract, a commercially available organic dehydrated beetroot powder extract, and standard phenolphthalein were run side by side under identical conditions, using two methods that complement each other: UV-Vis spectrophotometric scanning across a series of buffered pH values, to see how the characteristic betacyanin and betaxanthin absorption bands actually behave, and a weak acid-strong base titration, to check how closely each indicator's visible colour change lines up with the true equivalence point. The point wasn't just to confirm, again, that beetroot can work as a natural indicator - that much is already fairly well established - but to pin down how the fresh and powdered forms actually compare, both to each other and to the synthetic standard, so that anyone choosing between them has a clearer, more practically useful picture to go on.

II. MATERIALS AND METHODS

This section outlines the materials, sample preparation procedures, and analytical protocols used to generate the results reported later in the paper. In brief, three indicator solutions - fresh beetroot extract, commercially available dehydrated beetroot powder extract, and standard phenolphthalein were each prepared under controlled conditions and then carried through two parallel analytical procedures: UV-Vis spectrophotometric scanning at a series of fixed pH values, and a weak acid-strong base titration using acetic acid and sodium hydroxide. The same glassware, solvent, buffer set, and instrument settings were used for all three indicators throughout, so that any differences observed in the results could be attributed to the indicators themselves rather than to inconsistencies in handling. Materials and reagents used are summarised in Table 1.

- *Preparation of Fresh Beetroot Extract*

Fresh beetroot (Beta vulgaris) was procured from a local vegetable market in Waraseoni (Balaghat), washed thoroughly, and peeled to remove the outer skin. Ten grams of the peeled beetroot was cut into small pieces and crushed to obtain a coarse pulp. This pulp was soaked in 50% ethanol and allowed to stand for 6 h at room temperature to permit adequate diffusion of the pigment (betalains) into the solvent. At the end of this period, the mixture was filtered and centrifuged to separate the solid plant debris from the liquid phase, and the supernatant was subsequently filtered to obtain a clear, deep red-maroon extract. This filtrate was used as the fresh beetroot indicator solution in all further experiments (Fig 1a).

- *Preparation of Commercial Powdered Beetroot Extract*

For the powdered form, a commercially available organic dehydrated beetroot powder (Dry-Kart Organic Beetroot Powder, composed of 100% pure beetroot with no added preservatives, artificial colour, or additives) was used, so that the comparison would reflect a product that a consumer or a school/college laboratory could realistically obtain off the shelf (Fig 1b). Five grams of the beetroot powder was dissolved in 50% ethanol, thereby preparing a 1:10 (w/v) solution. The resulting mixture was centrifuged and then filtered, in the same manner as the fresh extract, to obtain a clear extract that could be used directly as an indicator solution (Fig 2).

- *Use of Prepared Synthetic Indicator (Phenolphthalein)*

Phenolphthalein, a well-established synthetic acid-base indicator with a documented transition range of approximately pH 8.2-10.0, was included in the study as the well-characterised synthetic comparator, run alongside the two natural extracts (Fig 1c). A standard phenolphthalein solution (procured from Qualigens, Thermo Fisher Scientific India Pvt. Ltd.) was used both for spectrophotometric scanning and for

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the titrimetric trials, following the same procedure applied to the beetroot-based indicators, so that all three indicators could be compared under identical experimental conditions.

➢ *pH Profiling and UV-Vis Spectrophotometric Analysis*

To evaluate how each indicator responds across the pH scale, buffer solutions of pH 1.3, 3.3, 4.0, 7.0, 9.0 and 11.5 were prepared, and a fixed volume of each indicator was added to each buffer. Absorbance and UV-visible spectra of each pH-adjusted solution were recorded using a double-beam UV-Vis spectrophotometer (Labtronics, Model LT-2203) over a wavelength range of 190-600 nm, and absorbance values were recorded at fixed intervals. For the beetroot extracts, particular attention was paid to absorbance at 480 nm and 535-540 nm, since these wavelengths correspond respectively to the characteristic absorption maxima of betaxanthins and betacyanins, the two principal pigment classes that make up beetroot betalains [8,9] .

➢ *Titrimetric Analysis: Weak Acid-Strong Base Titration*

The titrimetric performance of the three indicators was evaluated using a weak acid-strong base titration system, in line with the approach commonly reported in the literature for testing natural pH indicators [3,10]. A fixed volume of 20 mL of 0.1 M acetic acid (CH3COOH) was taken in a conical flask, and 0.1 M sodium hydroxide (NaOH) was loaded into a 50 mL burette as the titrant. NaOH was added dropwise with continuous swirling, and the volume at which a distinct and stable colour change was observed was recorded as the end point. The titration was repeated three times for each indicator to obtain concordant readings (Table 5).

III. RESULTS AND DISCUSSION

➢ *Visual pH-Dependent Colour Profiling*

The visual colour change of each indicator across the pH range is worth noting first, since this is ultimately what an end user would rely on. Phenolphthalein behaved exactly as expected: it remained essentially colourless from pH 1 through pH 7, stayed faint at pH 8, and then developed a clear pink-to-magenta colour from around pH 9 onward(Fig 4a). The fresh beetroot extract retained a deep red-maroon colour across almost the entire pH range tested, turned yellowish pink at pH 9, and shifted dramatically to yellow only at the most strongly alkaline pH(Fig 4b). The commercial powdered extract showed a comparatively weak, pale amber-to-orange colour across pH 1-7 before turning yellow at pH 9 onward, without the sharp red-to-yellow shift seen in the fresh extract (Fig 4c).

➢ *UV-Vis Spectral Behaviour of Phenolphthalein*

The spectrophotometric scan of phenolphthalein (Table 2) confirmed the visual observation. At acidic and neutral pH values, absorbance near 480 nm and 535 nm remained very low, consistent with the colourless lactone form of the molecule [5]. Once the pH was raised to 9.0 and further to 11.5, absorbance at 535 nm rose sharply, from 1.178 to 1.984, mirroring the emergence of the quinoid dianion responsible for the pink colour(Fig 3).

➢ *UV-Vis Spectral Behaviour of Fresh Beetroot Extract*

The fresh beetroot extract showed a markedly different pattern (Table 3). Across the acidic-to-neutral range, absorbance at both 480 nm and 535 nm remained high and stable (1.7-1.9). From pH 7.0 to pH 9.0, absorbance declined moderately, and this decline became far more pronounced at pH 11.5, where absorbance at 535 nm fell to 0.14 and at 480 nm to 0.25 - a drop of nearly 90%. This pattern is consistent with reported betalain stability in strongly alkaline media, where betacyanins undergo hydrolytic degradation [15].

➢ *UV-Vis Spectral Behaviour of Commercial Powdered Beetroot Extract*

The powdered extract told a different story (Table 4). Absorbance at 480 nm and 535 nm was consistently low throughout the pH range - roughly a quarter to a third of the fresh extract's values. The powdered extract's highest absorbance (0.871) occurred at 300 nm rather than in the betacyanin region, pointing toward reduced intact betalain content following dehydration, possibly combined with other UV-absorbing compounds formed during drying.

➢ *Comparative Spectral Discussion*

Taken together, the spectral data show fresh beetroot extract preserving both principal pigments at high concentration until strongly alkaline pH, consistent with existing literature on betalain pH stability [9,15], while the dried powder retains only a fraction of this intensity, likely due to degradation and reduced extractability during processing [12,13,14] (Fig 5).

➢ *Titrimetric Evaluation*

For this weak acid-strong base system, the theoretical equivalence volume is 20 mL. Phenolphthalein's mean titre (23.5 mL) deviated by +17.5% from this value, expected given its pH 8.2-10.0 transition range lying slightly above the true equivalence point. Fresh beetroot extract gave a mean end point of 24.3 mL (+21.3%), remarkably close to phenolphthalein, while the powdered extract gave 32.3 mL (+61.7%), a far larger overshoot (Table 5). This agrees closely with the spectrophotometric findings and represents a reassuring cross-check between the two analytical approaches used in this study.

➢ *Overall Discussion*

Against the theoretical equivalence volume, phenolphthalein deviated least (+17.5%), fresh beetroot extract followed closely (+21.3%), and the powdered extract deviated far more (+61.7%) - a ranking that matches the spectral data. Fresh beetroot extract is a chemically faithful indicator that performs competitively in a real titration, whereas the powdered extract, though more convenient and shelf-stable, needs a higher working concentration or prior calibration for quantitative work. Fresh and powdered forms are not interchangeable; each suits a different context.

A few constraints of the present study are worth noting. UV-Vis readings at each pH were single measurements rather than replicates, unlike the triplicate titrations; the comparison relied on a single fresh source and a single commercial brand; and the deviations reported are descriptive rather than the

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result of formal statistical testing. Replicate spectral measurements, multiple sample sources, and statistical testing would strengthen any follow-up study.

IV. CONCLUSION

This study tested whether a commercially dehydrated beetroot powder could stand in for freshly prepared beetroot extract as a natural acid-base indicator. Fresh extract acts as a genuinely competent substitute for a synthetic indicator, while the powdered form is closer to a convenient but less precise alternative. Fresh extract prepared close to the time of use is recommended where accuracy matters; the powdered extract remains useful where convenience and shelf life are the priority, provided its weaker signal is compensated with a higher concentration or prior calibration. The results support beetroot betalains as a viable, low-cost, eco-friendly alternative to synthetic indicators, with future work extending the comparison to other titration systems, powder brands, and drying methods.

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Table 1 Materials and Reagents Used in the Study

Material	Source / Specification
Fresh beetroot (Beta vulgaris)	Local vegetable market; peeled, used same day
Commercial dehydrated beetroot powder	Dry-Kart Organic Beetroot Powder, 100% pure, FSSAI licensed
Ethanol (50% v/v)	Extraction solvent
Acetic acid (CH3COOH)	0.1 M, weak acid (titrand)
Sodium hydroxide (NaOH)	0.1 M, strong base (titrant)
Phenolphthalein	Qualigens, Thermo Fisher Scientific India Pvt. Ltd.
Buffer solutions	pH 1.3, 3.3, 4.0, 7.0, 9.0, 11.5

Table 2 Absorbance of Phenolphthalein at Selected Wavelengths Across pH

pH	Region (nm)	A_{480}	A_{535}	A_{600}
1.3	190-250	0.015	0.010	0.003
3.3	190-250	0.023	0.017	0.007
4.0	190-250	0.022	0.015	0.004
7.0	190-250	0.018	0.013	0.006
9.0	535 (rising)	0.288	1.178	0.080
11.5	535 (strong)	0.715	1.984	0.220

Table 3 Absorbance of Fresh Beetroot Extract Across pH

pH	Region (nm)	A_{480}	A_{535}	A_{600}
1.3	480 & 535	1.76	1.89	0.70
3.3	480 & 535	1.75	1.88	0.72
4.0	480 & 535	1.73	1.89	0.94
7.0	480 & 535	1.71	1.80	0.80
9.0	declining	1.42	1.40	0.76
11.5	sharply declined	0.25	0.14	0.07

Table 4 Absorbance of Commercial Powdered Beetroot Extract Across pH

pH	Region (nm)	A_{480}	A_{535}	A_{600}
1.3	300	0.405	0.168	0.063
3.3	300	0.461	0.180	0.048
4.0	300	0.478	0.186	0.046
7.0	300 (max)	0.486	0.186	0.044
9.0	300	0.353	0.154	0.060
11.5	300 (weaker)	0.239	0.156	0.077

Table 5 Titre Values (mL of 0.1 M NaOH) vs Theoretical Volume (20 mL)

Indicator	T1	T2	T3	Mean±SD	Dev (%)
Fresh beetroot extract	24.2	24.2	24.4	24.3±0.1	+21.3
Powdered extract	32	32	33	32.3±0.6	+61.7
Phenolphthalein	23.5	23.5	23.5	23.5±0.0	+17.5

Fig 1A. Fresh Beetroot Soaked in 50% Ethanol.

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Fig 1B Commercially Available Dehydrated Beetroot Powder.

Fig 1C Standard Synthetic Indicator, Phenolphthalein.

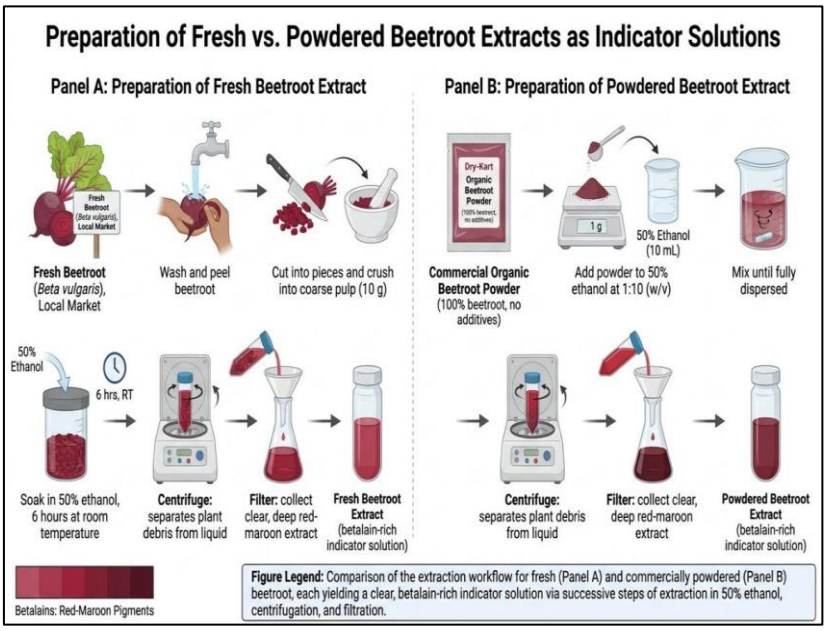

Fig 2 Preparation of Fresh vs. Powdered Beetroot Extract as Indicator Solutions.

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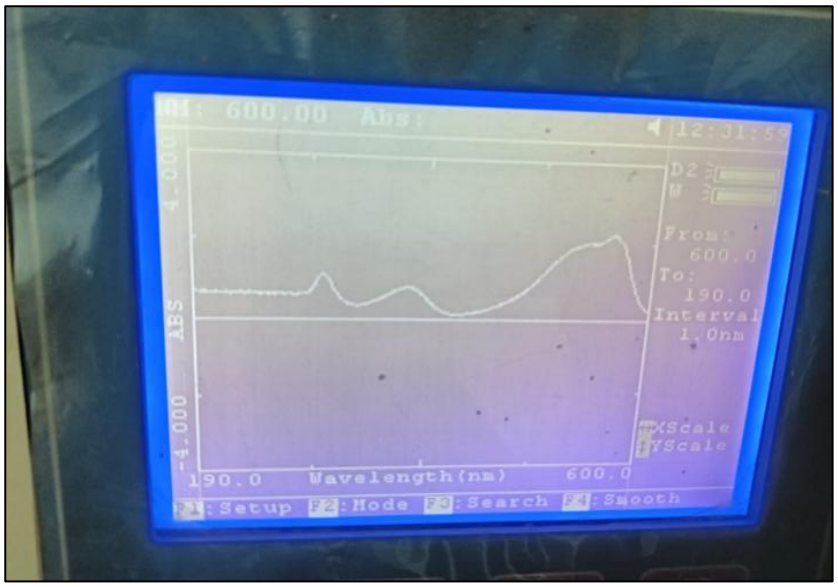

Fig 3A UV-Visible Spectra of Phenolphthalein, Acidic-to-Neutral Range (pH 1-7).

Fig 3B UV-Visible Spectra of Phenolphthalein, Basic Range (pH 8-12).

Fig 4A Colour Profile of Phenolphthalein Across pH 1.3-11.5.

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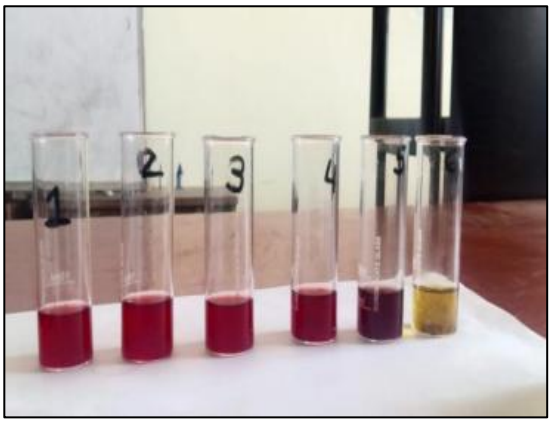

Fig 4B Colour Profile of Fresh Beetroot Extract Across pH 1.3-11.5.

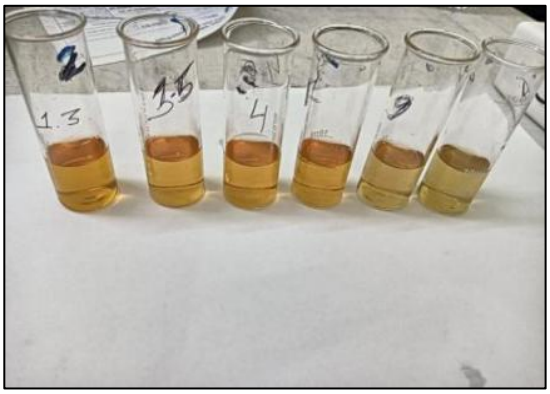

Fig 4C Colour Profile of Commercial Powdered Beetroot Extract Across pH 1.3-11.5.

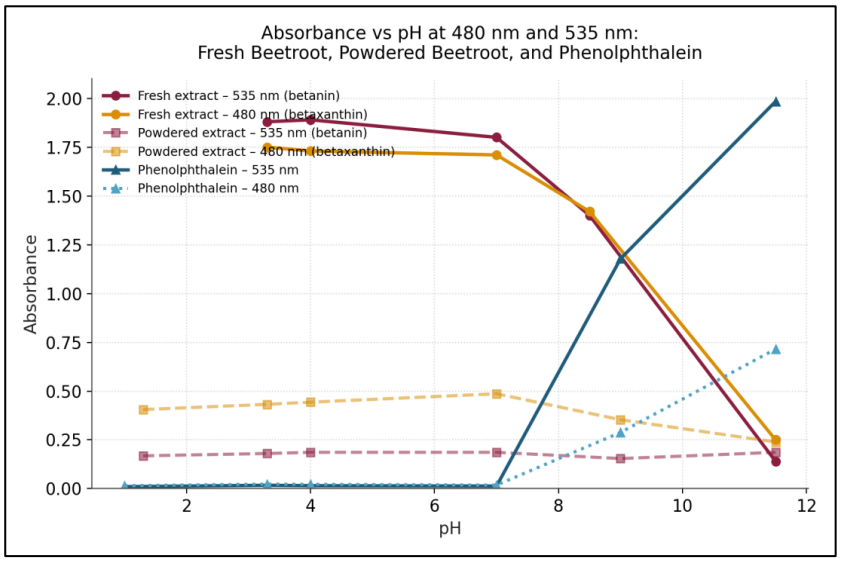

Fig 5 Comparative Absorbance at 535 nm and 480 nm Across pH for all three Indicators.