Journal of Food Measurement and Characterization (2025) 19:3768–3777
https://doi.org/10.1007/s11694-025-03226-5

ORIGINAL PAPER

Natural plant extracts for quality assessment in milk by replacing conventional color reduction methods

Mizaj Shabil Sha[1,2] · Farsana Bin Faisal[1] · Thasneem Khalid Ottakath[1] · Nihal Maliekkal Poovadichalil[1] · Kishor Kumar Sadasivuni[1,2]

Received: 5 December 2024 / Accepted: 20 March 2025 / Published online: 1 April 2025
© The Author(s) 2025

Abstract

Our study proposes a novel approach using aqueous extracts of plant material *Ruellia simplex* to screen milk for microbiological quality rapidly. We tested plant extracts for colour changes at various pH levels, with different dyes as the control. The differential pH method was used to assess milk freshness using plant extracts. The *Ruellia simplex* flower extract was tested with its pH-dependent colour shift (6; purple to 1; dark pink). The *Ruellia simplex* extract has an anthocyanin content of 0.609 g/ml. The approach demonstrated great linearity ($R^2 = 0.978$), 100% accuracy, and reproducibility. The limits of detection and quantification were 0.027 and 0.082 g/mL, respectively. This technology has the potential to revolutionise milk quality testing by providing a cost-effective and efficient alternative to traditional dye-based procedures, especially in rural and remote locations. Its application in intelligent food packaging has the potential to alter dairy sector practices internationally by allowing for accessible and real-time quality monitoring.

Keywords Plant aqueous extracts · Milk quality · pH sensing · Colourimetry

Introduction

Nowadays, food packaging does more than enhance commodity shelf life; it also incorporates intelligent characteristics [1]. Intelligent or active packaging can detect, sense, trace, record, and communicate attributes to offer customers information on the state of food and its surroundings. Examples include time–temperature indicators, fluorescence indicators, radio frequency identification, and freshness sensors. All of these smart gadgets might be integrated into packaging materials [2, 3].

Because of their efficiency and simplicity, pH indicators have been used to monitor and signal the freshness of food in storage. A pH change usually occurs with deterioration. Consumers can distinguish between fresh and bad food based on visual colour changes without breaking the package [4]. Chemical pigments, including bromocresol green, purple, blue, and red, are widely utilised as sensitive pH-sensing dyes. However, achieving consumers' expectations for food safety is challenging due to their potential toxicity. Because artificial dyes are cytotoxic or mutant, they can affect both aquatic life and people [5]. As a result, natural pigments generated from plants are potential options for biodegradable packaging [6].

Many countries have developed milk quality standards and requirements for milk purchase [7]. Small-scale dairy producers may minimise on-farm milk cooling, leaving it around ambient temperature until it is delivered to regional chilling facilities or collected by tank drives or milk collectors [8]. The delay between milking and chilling could influence the microbiological quality of dairy products, which are fragile and prone to degradation if not rapidly chilled [9].

Dairy farmers are incentivised to generate milk of superior quality based on somatic cell and microbial counts and producer-imposed quality control rules. Subsistent dairy farms may require more control and knowledge to evaluate milk's microbiological content, making it an important economic factor [10]. Milk processing factories utilise platform testing to assure its safety and processability. The resazurin dye degradation method is a well-known platform test for

✉ Kishor Kumar Sadasivuni
kishor_kumars@yahoo.com

[1] Center for Advanced Materials, Qatar University, PO Box 2713, Doha, Qatar

[2] Department of Mechanical and Industrial Engineering, Qatar University, PO Box 2713, Doha, Qatar

assessing milk microbiological purity that has been in use for many years [11].

However, the high cost of this approach limits its application in fields and milk chilling facilities. This emphasises the need for cost-effective management options for milk collecting centres and small-scale dairy farmers [12].

Ruellia simplex is often called *Mexican petunia, Mexican bluebell,* or *Britton's petunia. Synonyms* include *Ruellia brittoniana* and *Ruellia malacosperma,* while *Ruellia* species include *tweediana, coerulea, microphylla,* and *ignorant* [13]. It does have certain conventional medical uses. While not as well investigated as other medicinal plants, it is known to have anti-inflammatory and antioxidant properties. Some traditional remedies use Mexican petunia to reduce inflammation and neutralise free radicals in the body [13].

Furthermore, there is evidence that plant extracts may have antimicrobial properties, which could make them useful in treating minor ailments [14]. The plant may also have modest diuretic properties that help treat water retention and related disorders [15]. *Mexican petunia* has long been used to heal wounds and treat various skin conditions [16].

Our study presents a novel approach to evaluating milk freshness using aqueous *Ruellia simplex* extracts as a natural, cost-effective pH indicator. Unlike traditional approaches, this methodology takes advantage of the plant's pH-sensitive anthocyanins, resulting in a biodegradable and non-toxic solution. This technology meets the specific needs of small-scale dairy farmers by allowing for rapid and reliable milk quality monitoring in the field. Furthermore, its potential incorporation into intelligent food packaging could change how milk quality is monitored worldwide, promoting improved accessibility and sustainability in the dairy industry.

Materials and methods

Chemicals used

Sigma-Aldrich provided chlorophenol red, cresol red, methylene blue, sodium hydroxide, and hydrochloric acid. All reagents used for analysis were analytical grade. The experiment used filtered water from a Millipore Milli-Q water system.

Sample preparation

Milk samples

The trial used pasteurized milk samples from the Baladna brand, accessible in Qatar. The milk samples were kept refrigerated until employed in the analysis. Using 0.1 N hydrochloric acid, the milk samples (pH 6.5) were changed to specific pH levels (5, 4, 3, 2, and 1). And to make basic solutions, 0.1 N sodium hydroxide solution was used.

Natural plant extract

Ruellia simplex flowers were collected from the premises of Qatar University. Approximately 10 g of flowers were weighed and washed thoroughly with DI water to remove dust and impurities. Afterwards, cleaned flowers were finely chopped and dried and then it was transferred to a conical flask and 100 mL DI water was added. The solution was then heated and boiled (100 ℃) for five minutes. The extract was filtered using Wattman filter paper. The weight of the flowers after drying was ~ 2 g. The pH of the fresh aqueous extract was ~ 6. Further, we adjusted the pH of the plant extract into both acidic (using HCl) and basic (using NaOH) ranges (1–13) and observed colour changes.

Dye solution preparation

Chlorophenol red and cresol red dye solutions were prepared by dissolving 0.013 g of dye powders in 20 ml DI water. 0.013 g of powder was dissolved in 250 ml DI water for the methylene blue dye solution. For the analysis, the stock solutions were used without further dilution.

Preliminary method developed

For each milk sample with varied pH values, 1 mL of each plant extract was mixed with 10 mL of milk in sterilised glassware, as indicated in Sect. 2.1.1. The visible colour changes were observed, and characterisation was carried out.

Periodic assessment of milk freshness

We further evaluated milk freshness periodically. Analysis was carried out on 1st day, 3rd day, 7th day and 14th day. For the analysis, a bottle of milk was kept at room temperature and on the analysis day, 10 mL of the milk was taken from this bottle, and 1 mL of plant extract was added to this sample. The colour change was observed and characterised using a UV–visible spectrometer.

Comparison of plant extract vs. control dyes

The dye degradation method served as a positive control for comparing plant colourants. The dye solution was then

promptly added to the beaker, which had already been filled with 10 mL of milk. And colour variations were noted.

Characterization

The UV–VIS spectroscopy was performed with a Biochrom UV spectrophotometer from Thermo Fisher Scientific, USA, which has a scanning range of 190–1100 nm. The dye samples were examined with a 300–750 nm scanning range and a medium scan speed. Characterisation was performed with a 1 nm step input and a 2 nm bandwidth.

Determination of anthocyanin concentration

The total anthocyanin concentration of *Ruellia simplex's* natural extract was evaluated using formula (1). The aid of a spectrophotometer (GENESYS®, G10s UV–Vis, Thermo Fisher Scientific, USA) at pH 1.0 and pH 4.5 [17, 18]. The anthocyanin concentration of *Ruellia simplex* was determined using a standard curve (X-axis: concentration (g/mL) and Y-axis: absorbance (520 nm)) created with a commercial anthocyanin standard (Biopurify Phytochemicals Ltd, Chengdu, China).

$$\text{The absorbance of the Anthocyanin} = \text{Absorbance at pH 1.0} - \text{Absorbance at pH 4.5} \quad (1)$$

Preparation of colour chart

A colour chart was created to assess milk pH for outdoor use quickly. To identify the hue of the natural plant extract, absorbance was measured using a UV spectrophotometer. A colour chart was created by comparing colours of varying pH values of milk and natural plant extract. The colours ranged from purple to pink.

Method validation

Linearity

The linearity of the *Ruellia simplex* flower water extract was evaluated using a calibration curve that plotted absorbance versus concentration. The proposed model's linearity was assessed by analysing the linear regression's correlation coefficient (R^2).

Precision

The method's precision was evaluated based on repeatability. The Relative Standard Deviation (RSD) determined the novel method's repeatability. The RSD was computed using pH from samples of *Ruellia simplex* flower water extract taken for five days. Over a short period, repeatability was assessed with consistent laboratory conditions and analysis.

Limit of quantification

LoQ was calculated by taking the standard deviation (σ) of the absorbance of *Ruellia simplex* flower water extract and the slope (m) of the calibration curve using an equation.

$$\text{LoQ} = 10(\sigma/\text{m}) \quad (2)$$

where σ represents the Standard Deviation, and m is the Slope of the calibration curve.

Limit of detection

LoD was computed by taking the Standard Deviation (SD) of the absorbance of *Ruellia simplex* flower water extract and calculating the slope of the calibration curve using Eq. (3).

$$\text{LoD} = 3.3(\sigma/\text{m}) \quad (3)$$

where σ represents the Standard Deviation, and m is the Slope of the calibration curve.



Fig. 1 **a** *Ruellia simplex* flower and **b** aqueous flower extractions

Results and discussion

After preparing the plant extract, we further evaluated the pH dependency of *Ruellia simplex* flower water extract. The pH of the prepared plant extract was pH 6 (Fig. 1). For the pH dependency analysis, 5 ml of the plant extract was taken in each vial, and the pH of the flower extract was adjusted from pH 1 to 13 using 0.1 N HCl and 0.1 N NaOH. Corresponding colour differences were noted (At pH 13, the extract got saturated).

pH sensing

The pH dependency studies assessed the possibility of using the plant extract for practical applications. In the preliminary study, we used aqueous extracts of plant materials to examine colour variations with different pH levels (Fig. 2). The plant extract exhibited different colours at different pH. And in an acidic solution, it was observed that the colour varies from pink to grape wine colour. In the neutral solution, the colour is dark wine colour. Also, the colour varied from emerald green to pale yellow in the basic solution. Anthocyanin pigments are generally positively charged (flavylium cation) on the C-ring of the molecule, resulting in hue change depending on pH. Furthermore, if the C-ring contains a positive charge, the molecule becomes coloured. Hydrate of the C-ring eliminates the positive charge, leading to dominant colouration [19, 20].

Evaluation of milk freshness

After observing the pH dependency of anthocyanin pigment in aqueous media, we explored potential colour changes in milk (Fig. 3). The colour change of aurelia simplex extract at acidic pH suggests the potential for further improvement in detecting pH changes in milk samples. For the analysis, we used both fresh milk (pH 6.5) and spoiled milk (pH 4.5). 1 ml of plant extract is added to fresh and pH-adjusted milk. No notable change in pH was observed after adding plant extract (Fig. 4).

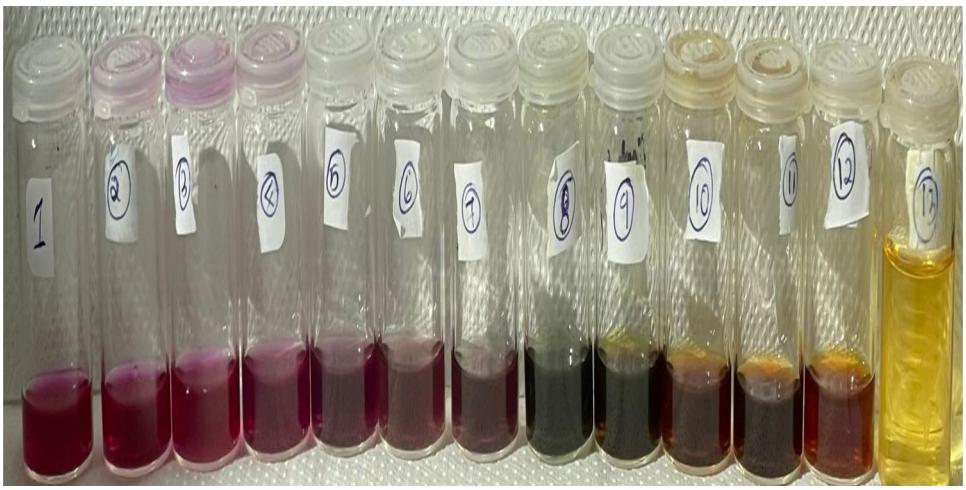


Fig. 2 Colour changes in plant water extracts as the pH shifts from acidic (pH 1) to alkaline (pH 13) circumstances

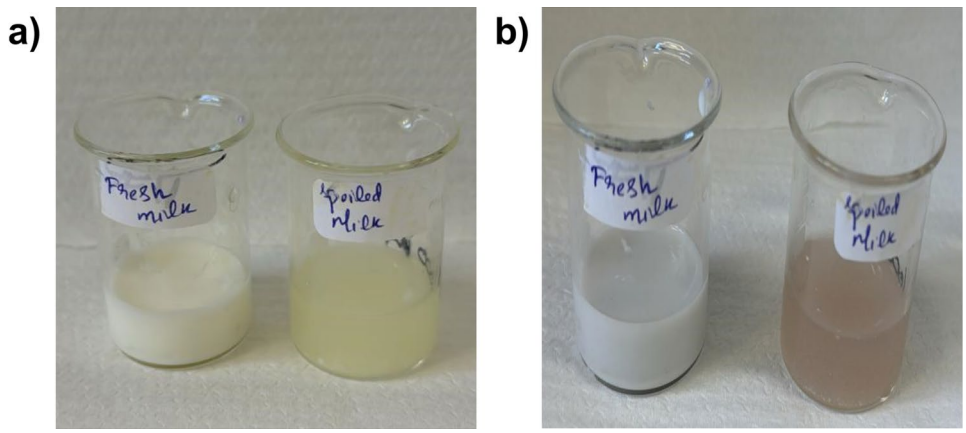


Fig. 3 **a** Fresh and spoiled milk before adding plant extract. **b** Fresh and spoiled milk after adding plant extract

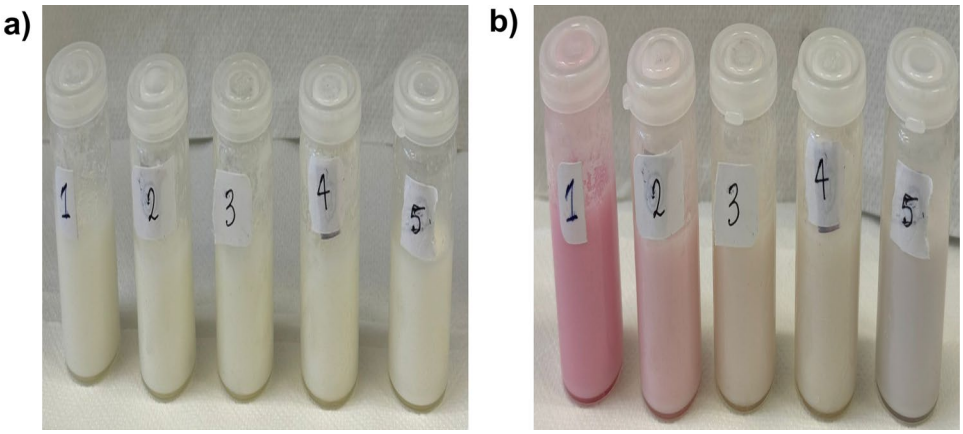


Fig. 4 **a** pH-adjusted milk solution before adding plant extract. **b** pH-adjusted milk solution after adding plant extract

Table 1 Summary of periodic evaluation of milk freshness

Day of analysis	pH before adding flower extract	pH after adding flower extract
Day 1	6.4	6.5
Day 3	6.2	6.3
Day 7	4.5	4.6
Day 14	4.1	4

pH dependency

Further, we adjusted the pH of the milk to (1–5) and added 1 ml of plant extract, and colour changes were observed when the pH of the milk was more acidic. Acidic pH indicates milk spoilage. Anthocyanin molecules change colour based on the pH of the medium. When pH rises, the flavyium cation's hydration process competes thermodynamically and kinetically. Yellow retro-chalcones are created at low pH and convert to quinonoid bases through deprotonation, resulting in a more stable quinonoid anion at higher pH [21, 22].

Effect of time on quality evaluation

Further, periodic evaluation of milk freshness was carried out. Analysis was conducted on the first, third, seventh and fourteenth days. For the analysis, a bottle of milk was kept at room temperature. On the analysis day, 10 mL of the milk was taken from this bottle, and 1 mL of plant extract was added to this sample (Fig. 5). Analysis was conducted on the first, third, seventh and fourteenth days. The difference in pH before and after adding flower extract is presented in Table 1.

The Data depicts milk's pH values before and after adding *Ruellia simplex* flower extract. On day one, the pH of fresh milk is 6.4, which rises slightly to 6.5 after adding the flower extract. By Day 3, the milk's pH had dropped to 6.2, with a tiny increase to 6.3 following adding the extract. These first pH results indicate that the milk is still relatively fresh, as fresh milk normally has a pH between 6.5 and 6.7. However, by Day 7, the pH had dropped dramatically to 4.5, indicating extensive bacterial growth and lactic acid generation, which are frequent indicators of spoiling. After adding the extract, the pH rises only slightly to 4.6. By Day 14, the pH had decreased to 4.1, with a minor decline to 4.0 after adding the

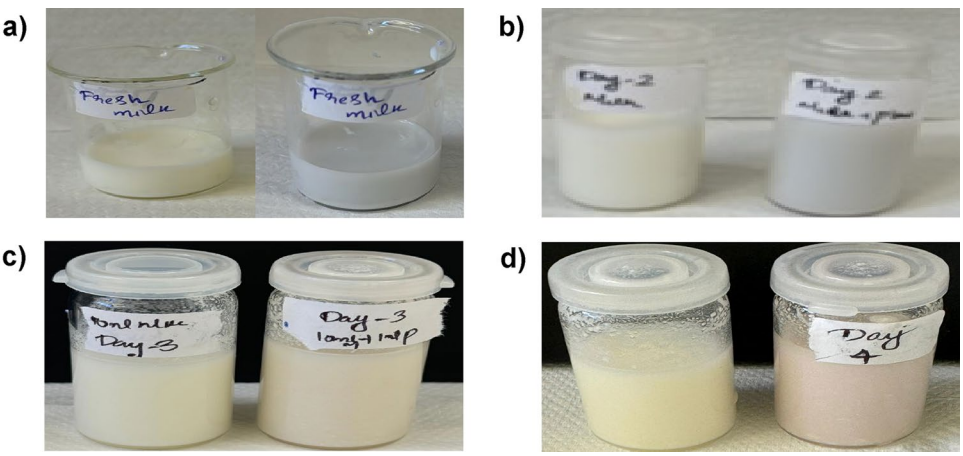


Fig. 5 Periodic evaluation of milk samples before(on the left of each image) and after adding flower extract (on the right of each image) **a** first, **b** third, **c** seventh and **d** fourteenth days

extract, indicating that the milk was highly acidic and spoilt. Adding *Ruellia simplex* extract slightly increases the pH of fresh milk, but milk itself is naturally close to neutral or slightly acidic (pH 6.5–6.7). However, as the milk degrades and becomes acidic, the extract has little effect in reversing or stopping the process. This observation is consistent with the normal deterioration process of milk, where bacterial activity causes increased acidity, indicating decomposition.

The natural buffering ability of milk, which prevents major pH changes from added chemicals, is probably the cause of the slight pH changes seen after adding *Ruellia simplex* extract. The extract's anthocyanins serve as passive indicators by changing structurally to reflect the milk's current pH without changing it chemically. Furthermore, interactions between anthocyanins and milk proteins (such as casein) may stabilize the colours and avoid significant pH changes. The extract's impact is further reduced by its low concentration, which enables it to function as a visual indicator of spoiling without upsetting the milk matrix [23].

Comparison with dye reduction test

Ruellia simplex flower extract's capacity to detect pH changes in milk led to comparative studies with the current standardised dye reduction test. Three dyes (methylene blue, chlorophenol red and cresol red) were chosen and added to the milk for the analysis. The aqueous solution of methylene blue (pH 5.7) was dark blue, but when added to fresh milk, the colour changed to sky blue at pH 6.7. cresol red dye (pH 3.2) changes colour from brick red to yellow at pH 6.6, whereas chlorophenol red (pH 2.9) changes from brick red to violet (pH 6.6), indicating the potential application of an aqueous extract from *Ruellia simplex* flowers (Fig. 6).

A previous study suggests the resazurin dye reduction approach works on the same premise. As microbial load in milk increases, oxygen levels fall, resulting in an oxidation–reduction reaction. Resazurin dye hue changes with milk's oxidation–reduction processes caused by microbial activities. The aqueous extract of *Ruellia simplex* flowers can replace dye in visualising pH differences and predicting microbiological quality in milk [24–26].

Analysis of anthocyanin content of the flower extract

The anthocyanin concentration in the extracted substances was determined using a spectrophotometer with a pH differential method. This procedure was chosen because it is simple and economical, with consistent results. The absorbance band (520–560 nm) examined the data. Hibiscus extract has an anthocyanin concentration of 0.609 g/mL, calculated using the pH differential method and Eq. 1.

$$\text{The absorbance of the anthocyanin} = \text{absorbance at pH 1.0} - \text{Absorbance at pH 4.5}$$

$$= 2.97 - 0.75 = 2.22$$

According to the anthocyanin standard curve (Fig. 7):

$$Y = 3.634 * X + 0.00672$$

$$X = (2.22 - 0.00672)/3.634$$

$$X = 0.609\ \text{g/ mL}$$

The comparison of anthocyanin content across various plant extracts (Table 2) highlights that *Ruellia simplex* (0.609 g/mL) and *Hibiscus rosa-sinensis* (0.59 g/mL) possess the highest concentrations, making them the most effective for pH-based color changes. In contrast, *Clitoria ternatea, Beta vulgaris, Opuntia dillenii,* and *Rosa damascena*

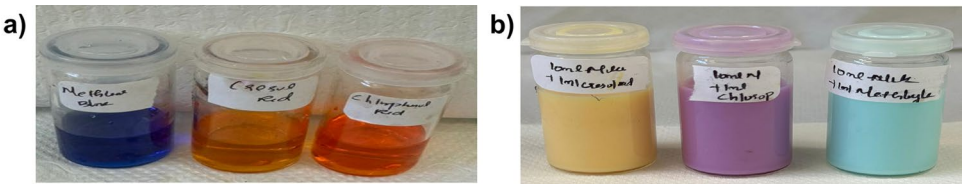


Fig. 6 Comparison of colour changes in milk **a** dye solutions, **b** colour changes when dyes are added into milk

Table 2 Anthocyanin content in different plants

Plant extract	Anthocyanin content	References
Hibiscus rosa-sinensis	0.59 g/mL	[17]
Clitoria ternatea (Butterfly Pea)	0.24–0.42 mg/g (dry weight)	[27]
Beta vulgaris (Beetroot, not a flower)	0.12–0.34 mg/g	[28]
Opuntia dillenii (Prickly Pear Cactus Flower)	0.15–0.30 mg/g	[29]
Ruellia simplex	0.609 g/mL	Present work

have lower anthocyanin levels, resulting in less pronounced pH-dependent shifts.

Preparation of colour chart

In addition to the dye reduction test, we created a colour chart to evaluate milk's pH and microbiological quality using a unique plant indicator, enabling quick detection of pH changes (Table 3). This will make it easier and more user-friendly to determine milk quality at outstation locations. The new method shows that changing pH from 6 to 1 causes a colour change from blue to pink (Fig. 4).

The link between pH, colour change, and milk quality allows a visual and measurable assessment of milk freshness. As the pH lowers, suggesting greater acidity and bacterial activity, the colour of the *Ruellia simplex* flower extract changes, showing that the milk's quality is diminishing. Dark purple in fresh milk denotes great quality. In contrast, light blue to dark pink indicates progressive spoiling and declining quality, while dark pink indicates severely terrible, spoilt milk.

Method validation

Validating an analytical procedure promotes scientific accuracy and reliability. Technique validation seeks to confirm the technique's fitness for its intended application. The approach was validated in various situations, including field testing and tiny businesses' milk chilling facilities. Validation demonstrates that this method is reliable for routine milk procurement analysis.

Linearity

As shown in Fig. 7, the proposed predictive approach produced a very excellent correlation between absorbance and concentration. The regression line's linearity can be evaluated using the correlation coefficient (R^2), which in this case is 0.978. Linearity in a procedure refers to the accuracy of the response vs. concentration calibration curve. An R^2 score of around one (1) indicates appropriate linearity, but an R^2 near zero (0) indicates a lack of proportionality. A correlation coefficient greater than 0.9 suggests a good fit between the data and the regression line. A high correlation coefficient often suggests that the procedure is linear [30].

Precision; repeatability

Repeatability is the ability to sustain precision under consistent operating conditions over a short period. It is the repeated measurement of the same sample, called intra-assay precision. The repeatability of analytes is often measured by standard deviation and is influenced by their concentration. Procedure validation evaluates repeatability by evaluating the variability of data produced from the analytical procedure. The current investigation confirms that an assay method requires an RSD of < 2% to be considered precise [31]. As a result, the unique procedure is highly repeatable (Table 4).

To measure resilience, repeatability tests were performed under various settings, including varying storage

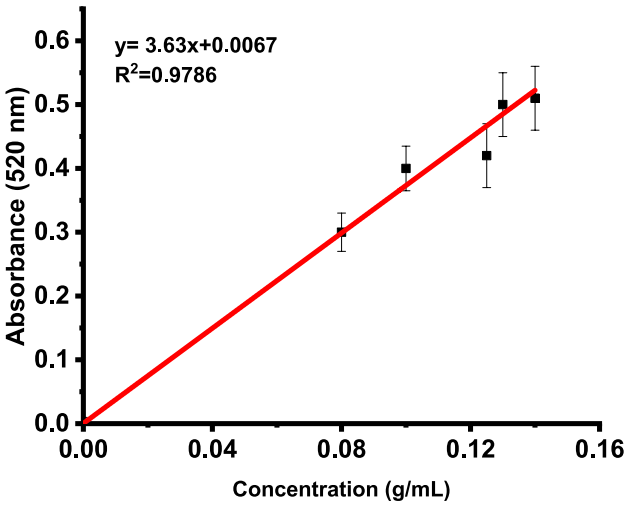


Fig. 7 Anthocyanin standard curve used to determine anthocyanin concentration in the *Ruellia simplex* flower water extract

Table 3 pH, colour of the plant extract in milk solution, and quality

pH of the milk	Colour of the plant extract in milk solution	Quality of the milk
6	Dark purple	Fresh
5	Light blue	Very good
4	Off white	Good
3	Pale yellow	Bad
2	Light pink	Very bad
1	Dark pink	Extremely bad

Table 4 Repeatability analysis of the novel method

Day of analysis	pH	RSD	Absorbance at 520 nm
Day 1	5.44 ± 0.02	0.13	0.6
Day 2	5.45 ± 0.02		0.6
Day 3	5.44 ± 0.01		0.6
Day 4	5.43 ± 0.03		0.6
Day 5	5.44 ± 0.01		0.6

temperatures (4 °C, 25 °C, and 35 °C), ambient and UV light exposure, and milk samples from various sources. Across all conditions, the extract maintained stable pH values and low fluctuations in absorbance at 520 nm (0.6 across several trials), indicating stability in various situations. This is especially useful in field applications where controlled laboratory conditions are unavailable.

The approach was tested for practical validation in various real-world contexts, including dairy collecting centres and small-scale milk processing plants where quick quality assessment is essential. Even in these conditions, the *Ruellia simplex*-based indicator produced consistent and repeatable findings, highlighting its promise as an alternative to traditional dye-based approaches.

Limit of quantification

LoQ is the smallest amount of an element in a sample that can be quantified accurately and precisely. LoQ is a performance statistic that assesses a statistical method's simplicity for quantification or analysis. LoQ was calculated by applying Eq. (2) to the standard deviation (0.03) and slope (3.63) of the plotted linearity curve. The lowest detectable amount of *Ruellia simplex* flower water extract was 0.082 g/mL while testing the microbiological purity of milk.

Limit of detection

LoD is the smallest amount of a component in a sample that can be reliably detected using a given analytical method. The innovative procedure produced a LoD value of 0.027 g/mL, demonstrating the lowest detectable concentration of *Ruellia simplex* extract for milk purity testing.

Using Ruellia simplex extract as a milk freshness indicator offers considerable economic, accessibility, and environmental benefits. Unlike synthetic dyes, which need sophisticated chemical synthesis and can be expensive, this plant-based extract is low-cost and simple to manufacture, making it ideal for small-scale dairy producers and resource-limited places. Furthermore, because it is biodegradable and non-toxic, it eliminates the environmental risks connected with synthetic dyes. However, problems such as light sensitivity, temperature variations, and storage conditions may impact the extract's stability, necessitating additional research into stabilisation procedures. Future research should focus on scaling up this approach for commercial usage, such as creating freeze-dried formulations or incorporating the extract into smart packaging materials for real-time freshness monitoring. Furthermore, broadening its applicability to other perishable goods, including meat and seafood, and assessing regulatory requirements and customer approval would be critical for widespread use in the food business.

Conclusions

The aqueous extract of *Ruellia simplex* flowers includes anthocyanin (0.609 g/mL), which is very sensitive to pH changes compared to the other plant-based sources of anthocyanin used in this study. The study proposes that an aqueous extract of flowers could replace dye reduction in assessing the microbiological purity of milk at the field level, allowing for faster screening at milk collection facilities. When properly performed and evaluated, the suggested analytical method can help determine milk quality in rural areas while saving time and money. Additional lab quality evaluations should be performed to determine the field test results. Future studies should focus on creating a user-friendly application for *Ruellia simplex* flower water extracts, such as water-soluble pouches or pellets, after the freeze-dried anthocyanin extract. This study's findings can be utilised to rapidly assess the microbiological quality of milk in the field.

Funding Open Access funding provided by the Qatar National Library. Qatar National Research Fund supported this work under grant no. MME03-1226-210042. The statements made herein are solely the responsibility of the authors. Open access funding provided by the Qatar National Library.

Availability of data and materials Data will be made available on request.

Declarations

Competing interest All authors declare that they have no competing interest.

Ethical approval and consent to participate None.

Consent for publication None.

Ethical guidelines None.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. P.R. Salgado, L. Di Giorgio, Y.S. Musso, A.N. Mauri, Recent developments in smart food packaging focused on biobased and biodegradable polymers. Front. Sustain. Food Syst. (2021). https://doi.org/10.3389/fsufs.2021.630393
2. S. Kalpana, S.R. Priyadarshini, M. Maria Leena, J.A. Moses, C. Anandharamakrishnan, Intelligent packaging: trends and applications in food systems. Trends Food Sci. Technol. **93**, 145–157 (2019). https://doi.org/10.1016/j.tifs.2019.09.008
3. E. Osmólska, M. Stoma, A. Starek-Wójcicka, Application of biosensors, sensors, and tags in intelligent packaging used for food products—a review. Sensors **22**(24), 9956 (2022). https://doi.org/10.3390/s22249956
4. D. Liu, C. Zhang, Y. Pu, S. Chen, L. Liu, Z. Cui, Y. Zhong, Recent advances in pH-responsive freshness indicators using natural food colorants to monitor food freshness. Foods **11**(13), 1884 (2022). https://doi.org/10.3390/foods11131884
5. B.K. Ndwandwe, S.P. Malinga, E. Kayitesi, B.C. Dlamini, Recent developments in the application of natural pigments as pH-sensitive food freshness indicators in biopolymer-based smart packaging: challenges and opportunities. Int. J. Food Sci. Technol. **59**(4), 2148–2161 (2024). https://doi.org/10.1111/ijfs.16990
6. H. Wu, C. Jiao, S. Li, Q. Li, Z. Zhang, M. Zhou, X. Yuan, A facile strategy for development of pH-sensing indicator films based on red cabbage puree and polyvinyl alcohol for monitoring fish freshness. Foods **11**(21), 3371 (2022). https://doi.org/10.3390/foods11213371
7. N.C. Felipus, S.A.B. Bajaluk, A.T. Neto, D.A. Knob, D.R.M. Alessio, Quality of refrigerated raw milk according to the bulk transport conditions. Acta Scientiarum. Anim. Sci. **2023**, 45 (2023)
8. S. Kiambi, E.M. Fèvre, P. Alarcon, N. Gitahi, J. Masinde, E. Kang'ethe, G. Aboge, J. Rushton, J.O. Onono, Assessment of milk quality and food safety challenges in the complex nairobi dairy value Chain. Front. Vet. Sci. **9**, 892739 (2022). https://doi.org/10.3389/fvets.2022.892739
9. S.N. Nyokabi, I.J.M. de Boer, P.A. Luning, L. Korir, J. Lindahl, B. Bett, S.J. Oosting, Milk quality along dairy farming systems and associated value chains in Kenya: an analysis of composition, contamination and adulteration. Food Control **119**, 107482 (2021). https://doi.org/10.1016/j.foodcont.2020.107482
10. C.S. Cortinhas, C.E. Fidelis, N. Sharma, M.V. dos Santos, The association between socioeconomic profiles, attitudes, and knowledge of dairy farmers regarding somatic cell count and milk quality. Animals **13**(17), 2787 (2023). https://doi.org/10.3390/ani13172787
11. A. Fadillah, B.H.P. van den Borne, O.N. Poetri, H. Hogeveen, T. Slijper, H. Pisestyani, Y.H. Schukken, Evaluation of factors associated with bulk milk somatic cell count and total plate count in Indonesian smallholder dairy farms. Front. Vet. Sci. (2023). https://doi.org/10.3389/fvets.2023.1280264
12. B. Dijkink, E. Esveld, J. Broeze, M. Vollebregt, Evaluation of scenarios for improving the collection system for a milk factory in Ethiopia. Front. Sustain. Food Syst. (2021). https://doi.org/10.3389/fsufs.2021.645057
13. Km, A. Irsyam, A. Mountara, A. Dewi, M. Hariri, P. Peniwidiyanti, R. Irwanto, Z. Al Anshori, THE OCCURRENCE OF NATURALIZED Ruellia Simplex (ACANTHACEAE) IN JAVA ISLAND. **2022**. https://doi.org/10.15408/kauniyah.v15i1.18125.
14. M.M. Matotoka, G.T. Mashabela, P. Masoko, Phytochemical content, antibacterial activity, and antioxidant, anti-inflammatory, and cytotoxic effects of traditional medicinal plants against respiratory tract bacterial pathogens. Evid Based Complement Alternat Med **2023**, 1243438 (2023). https://doi.org/10.1155/2023/1243438
15. I. Vieitez, L. Maceiras, I. Jachmanián, S. Alborés, Antioxidant and antibacterial activity of different extracts from herbs obtained by maceration or supercritical technology. J. Supercrit. Fluids (2017). https://doi.org/10.1016/j.supflu.2017.09.025
16. A.M. Tache, L.D. Dinu, E. Vamanu, Novel insights on plant extracts to prevent and treat recurrent urinary tract infections. Appl. Sci. **12**(5), 2635 (2022). https://doi.org/10.3390/app12052635
17. R. Madushan, J.K. Vidanarachchi, P.H.P. Prasanna, S. Werellagama, H. Priyashantha, Use of natural plant extracts as a novel microbiological quality indicator in raw milk: an alternative for resazurin dye reduction method. LWT **144**, 111221 (2021). https://doi.org/10.1016/j.lwt.2021.111221
18. T.N. Pham, D.-V. Vo, T. Lam, V. Tran, T. Quoc Toan, H. Vu-Quang, N. Le, P. Anh, M. Le Bui, Preparation and determination of total anthocyanins extraction from the skin of vigna cylindrica skeels (Dolichos Catjang Burm. f). IOP Conf. Ser. Mater. Sci. Eng. **542**, 012037 (2019). https://doi.org/10.1088/1757-899X/542/1/012037
19. A. Pagan, J.I. Lee, J. Kang, Concentration-dependent association of flavylium chloride with differential hydroxy moieties in ethanol. Colorants **1**(1), 20–37 (2022). https://doi.org/10.3390/colorants1010004
20. B. Enaru, G. Drețcanu, T.D. Pop, A. Stănilă, Z. Diaconeasa, Anthocyanins: factors affecting their stability and degradation. Antioxidants **10**(12), 1967 (2021). https://doi.org/10.3390/antiox10121967
21. L. Cruz, N. Basílio, N. Mateus, V. de Freitas, F. Pina, Natural and synthetic flavylium-based dyes: the chemistry behind the color. Chem. Rev. **122**(1), 1416–1481 (2022). https://doi.org/10.1021/acs.chemrev.1c00399
22. E. Azman, Y. Nurhayati, A. Chatzifragkou, D. Charalampopoulos, Stability enhancement of anthocyanins from blackcurrant (Ribes Nigrum L.) pomace through intermolecular copigmentation. Molecules **27**, 5489 (2022). https://doi.org/10.3390/molecules27175489
23. T. Aydogdu, J.A. O'Mahony, N.A. McCarthy, pH, the fundamentals for milk and dairy processing: a review. Dairy **4**(3), 395–409 (2023). https://doi.org/10.3390/dairy4030026
24. J.-C. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in mycobacterium tuberculosis. Antimicrob. Agents Chemother. **46**(8), 2720–2722 (2002). https://doi.org/10.1128/AAC.46.8.2720-2722.2002
25. A.V. Sychev, A.I. Lavrova, M.Z. Dogonadze, E.B. Postnikov, Establishing compliance between spectral, colourimetric and photometric indicators in resazurin reduction test. Bioengineering **10**(8), 962 (2023). https://doi.org/10.3390/bioengineering10080962
26. B. Vieira-da-Silva, M.A.R.B. Castanho, Resazurin reduction-based assays revisited: guidelines for accurate reporting of relative differences on metabolic status. Molecules **28**(5), 2283 (2023). https://doi.org/10.3390/molecules28052283
27. N. Saptarini, D. Suryasaputra, H. Nurmalia, Application of butterfly pea extract (Clitoria Ternatea Linn) as an indicator of acid-base titration. J. Chem. Pharm. Res. **7**, 275–280 (2015)
28. R. Guiné, F. Gonçalves, C. Lerat, T. Idrissi, E. Rodrigo, P. Correia, J.C. Gonçalves, Extraction of phenolic compounds with antioxidant activity from beetroot (*Beta vulgaris* L.). Curr. Nutr. Food Sci. **14**, 350–357 (2018). https://doi.org/10.2174/1573401313666170609102336
29. E.H. Loukili, M. Merzouki, M. Taibi, A. Elbouzidi, B. Hammouti, K. Kumar Yadav, M. Khalid, M. Addi, M. Ramdani, P. Kumar, J. Ryeol Choi, Phytochemical, biological, and nutritional properties of the prickly pear, *Opuntia Dillenii*: a review. Saudi Pharm. J. **32**(10), 102167 (2024). https://doi.org/10.1016/j.jsps.2024.102167

30. W.L. Cheng, C. Markus, C.Y. Lim, R.Z. Tan, S.K. Sethi, T.P. Loh, Calibration practices in clinical mass spectrometry: review and recommendations. Ann. Lab. Med. **43**(1), 5–18 (2023). https://doi.org/10.3343/alm.2023.43.1.5
31. S. Chavan, D. Desai, Analytical Method Validation: A Brief Review. World Journal of Advanced Research and Reviews **16**, 389–402 (2022). https://doi.org/10.30574/wjarr.2022.16.2.1165

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.