



Spectrophotometric and Smartphone-based Dual Monitoring Method for the Determination of Al(III) Ions Using Fermented Black Carrot Juice (Şalgam/Shalgam) as a Green Chromogenic Agent

Batuhan Yardımcı^{1*} 

¹Zonguldak Bülent Ecevit University, Science and Technology Application and Research Center (ARTMER), Zonguldak, 67600, Turkey

Abstract: In this study, anthocyanin-rich fermented black carrot juice (şalgam/shalgam) was used as a chromogenic agent in order to develop eco-friendly, low-cost, simple, fast, and practical both visible spectrophotometric and smartphone-based methods for the determination of Al(III) ions in water samples. Formation of Al(III)-anthocyanin complex results in a color change from red to purple in direct proportion to the increasing Al(III) concentration. For the spectrophotometric analysis, the analytical response of the developed method between absorbance and logarithm of Al(III) concentration exhibits a satisfying wide linear concentration range from 37.0 to 1850.0 μM . The LOD and LOQ values are 6.67 μM and 22.0 μM , respectively. For smartphone-based analysis, the analytical response of the developed method between B values and Al(III) concentration obtained a linear concentration range from 18.5 to 111.0 μM . The LOD and LOQ values are 4.40 μM and 14.5 μM , respectively. LOD values are below the acceptable limit of Al(III) in water according to WHO (7.41 μM) for both methods. The interfering effect of common water ions was investigated, and the observed interferences from Sn^{2+} , Fe^{2+} , and Fe^{3+} were easily eliminated using enough concentration of Na_2EDTA without affecting the blank absorbance/B value of the Al(III)-anthocyanin complex for two monitoring methods. The selectivity of the developed method was investigated in the presence of possible species such as benzoic acid, lactic acid, amino acids, and salt. Developed spectrophotometric and smartphone-based methods applied to real water samples and validated against the reference ICP-OES method at 95% confidence level using Student's *t*- and *F*-tests.

Keywords: Chromogenic agent, aluminum(III) ions, fermented black carrot juice (Shalgam), anthocyanin, spectrophotometry, smartphone

Submitted: November 08, 2022. **Accepted:** January 11, 2023.

Cite this: Yardımcı B. Spectrophotometric and smartphone-based dual monitoring method for the determination of Al(III) ions using fermented black carrot juice (şalgam/shalgam) as a green chromogenic agent. JOTCSA. 2023;10(1):161-76.

DOI: <https://doi.org/10.18596/jotcsa.1201498>.

Corresponding author. E-mail: batuhan.yardimci@beun.edu.tr.

1. INTRODUCTION

Aluminum is not found free in nature. It ranks third among the most abundant metals in the earth's crust and constitutes approximately 8% of the crust's mass (1) and it is mainly found as silicate minerals (2). Due to the widespread presence of aluminum in the environment and industrial developments, its relationship with human health is becoming increasingly important. The amount of free Al(III) increases as the aluminum in the soil leaks into the environment and surface waters due

to acid rain (1). While aluminum is in ionic form; it can react with biological species. When it reacts, it causes harmful effects by suppressing or changing the function of the biological species (3). Aluminum ion is thought to be the reason for neurological disorders and it causes changes in enzymatic reactions by affecting neurofibrillary and neurotransmitters in the central nervous system. As a result; diseases such as Parkinson's disease, Alzheimer's disease, and dialysis encephalopathy occur (4). Aluminum is also thought to have the ability to replace iron and other metals in proteins

found in living things (5). Determination of Al(III) concentration is a critical issue for researchers due to its direct impact on the environment and human health. Different kinds of conventional analytical methods such as AAS (6), ICP-MS (7), ion-selective electrode-based potentiometry (ISE) (8) voltammetry (9), and fluorimetry (10) have been developed to determine the concentration of Al(III). Most of these methods are limited due to the requirements of time-consuming processes, expensive and complicated equipment, and a skilled person. Although fluorescent probes for the determination of Al(III) stand out among these methods due to their selectivity and sensitivity, the disadvantages of this method are that they have time-consuming complicated probe design and synthesis processes, are toxic, have low solubility in water, have low photostability and excess reagent consumption (11, 12).

Spectrophotometry is one of the versatile and good alternative analytical techniques to determine the concentration of many analytes, especially in water samples, and has many advantages such as low cost, easy applicability, fast analysis, reliability, high sensitivity at low concentrations, and wide analytical working range (13).

Recently, smartphone-based colorimetric and fluorometric methods have gained importance over traditional bulky devices due to their many properties such as quick analysis, low-cost and simple production, portability, and ease to use. The smartphone-based methods exhibit a highly comparable performance of analysis according to the traditional methods (14,15). Analysis of different kinds of samples by smartphone is based on the measurement of red (R), green (G), and blue (B) values with help of various software (15-17).

Anthocyanins are water-soluble flavonoid pigments that provide bright colors from light pink to dark blue when found in fruits and vegetables (18). In recent years, anthocyanins have gained great importance among researchers due to their antioxidant capacity, bioavailability properties, food coloring, and food stabilizer properties (19). In fact, according to studies no adverse events have been encountered in the intake of anthocyanins in high doses (20). Anthocyanins also have metal-chelating abilities such as dihydroxy B-ring substituted flavonoids (21). Fermented black carrot (şalgam/shalgam) juice, a dark red-colored and sour soft traditional Turkish beverage, is one of the sources of natural anthocyanin pigments originating from black carrot. It is very popular in the southern part of Türkiye. In addition, recently, fermented black carrot juice has taken its place in the markets of numerous European countries (22). Fermented black carrot juice is defined as a beverage formed as a result of lactic acid fermentation of black carrot, turnip, salt, extract of sourdough, and bulgur flour components (23). Turnip and bulgur are minor components of şalgam and turnip helps to develop the sensory character (24), while bulgur flour is used

as a source of microorganisms in the first fermentation stage. In addition, the reason for using salt is to control the fermentation flora (25). Fermented black carrot juice gets its red color from the main ingredient, black carrots. According to studies, cyanidin glycosides as an aglycone have been found in black carrots as the main anthocyanin compound (26,27), malvidin, and peonidin glycosides in black carrot roots (27,28). Furthermore, fermented black carrot juice has been reported to contain an average of 114.1 mg L^{-1} anthocyanin as cyanidin-3-glycoside (25). It is stated that commercially fermented black carrot contains the anthocyanins cyanidin-3-galactoside, cyanidin-3-glucoside, and cyanidin-3-arabinoside using LC/MS/MS and also its total phenolic content ($517.21 \text{ } \mu\text{g GAE/mL}$) and antioxidant capacity (in $\mu\text{mol Trolox equivalents/mL}$) were investigated with FRAP (2.26), DPPH (4.44) and ABTS (3.42) assays (29). Different types of metal determinations were studied from various anthocyanin-derived plants by utilizing the metal complexing properties of anthocyanins (30,31). In such studies, anthocyanins were extracted from natural plants, but the negative properties of isolated anthocyanins are time-consuming processes for the extraction stage, low extraction efficiency, instability, and degradation properties in the presence of light, heat, and oxygen (19,32). An important feature of anthocyanins from purple carrots is dominant that the cultivars are in the acyl form. Acylated anthocyanins are more stable compounds than other types of anthocyanins and degrade more slowly during storage (29). Fermented black carrot may also enhance the stability, absorption, and bioactivity of its anthocyanins (32), as well as metal complexes of anthocyanins, which are known to stabilize the structure more (19), and it can be concluded that by forming a complex with the metal, its repeatability will be better in the determination of the relevant metal.

In the light of this information, low-cost, non-toxic, anthocyanin-rich, sugar-free, and gluten-free, commercially fermented black carrot juice with good stability was preferred as a chromogenic agent. This chromogenic agent meets the at least three principles of green analytical chemistry (33) such as removing or replacing toxic reagents, using reagents obtained from a renewable source, and reducing the risk to the operator. In this way, the concentration of Al(III) in water samples can be determined quickly and simply via color changing from red to purple in direct proportion to the amount of Al(III) ions because of the formation of the Al(III)-anthocyanin complex, without any pre-treatment such as time-consuming extraction.

2. MATERIAL AND METHODS

2.1. Chemicals and Instrumentation

All reagents used in this study were of analytical grade except Lactic acid (80%). The fermented black carrot juice was bought from the local market. Aluminum nitrate nonahydrate ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$),

lead(II) nitrate ($\text{Pb}(\text{NO}_3)_2$), chromium(III) nitrate nonahydrate ($\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), cadmium acetate dihydrate ($\text{Cd}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), sodium benzoate ($\text{C}_6\text{H}_5\text{COONa}$), sodium acetate (CH_3COONa), L-glycine ($\text{NH}_2\text{CH}_2\text{COOH}$), L-lysine hydrochloride ($\text{H}_2\text{N}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{CO}_2\text{H} \cdot \text{HCl}$), and sodium bicarbonate (NaHCO_3) were purchased from Sigma Aldrich. Iron(III) nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), iron(II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), manganese (II) nitrate tetrahydrate ($\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), ammonium chloride (NH_4Cl), potassium nitrate (KNO_3), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), magnesium nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), copper(II) sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), Tin(II) chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$), zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), sodium nitrate (NaNO_3), sodium chloride (NaCl), sodium sulfate (Na_2SO_4), and sodium hydroxide (NaOH) were purchased from Merck. Lactic acid was obtained from Bioliv food industry and trade import export limited company. Potassium hydrogen phthalate ($\text{HOOC}_6\text{H}_4\text{COOK}$) was bought from CDH (Central drug house Ltd.). Sodium phosphate monobasic (NaH_2PO_4) was obtained from Riedel-de Haën. L-arginine ($\text{H}_2\text{NC}(=\text{NH})\text{NH}(\text{CH}_2)_3\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$) was purchased from sepenatural. L-cysteine hydrochloride monohydrate ($\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH} \cdot \text{HCl} \cdot \text{H}_2\text{O}$) was obtained from Akcan Kimya and L-glutamine ($\text{H}_2\text{NCOCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$) was bought from Hardline nutrition.

Precisa XB 220A Analytical Balance was used to weigh all the chemicals. Rayleigh VIS-723G visible (vis.) spectrophotometer and its glass cuvettes (optical thickness 5 mm) were used for all absorbance measurements. Only Shimadzu UV-1800 spectrophotometer was used for the characterization of anthocyanin. For RGB measurements, the Samsung Galaxy S10 Plus is used with its convenient black box that allows the phone to fit on it. Wisetherm-fuzzy control, wisd HB-48 dry bath was used to determine the optimum temperature for the proposed method. Both detection systems were validated against the ICP-OES (34) method using PerkinElmer Avio 200 ICP-OES.

2.2. Preparation of Solutions

Fermented black carrot juice bought from the market was stored at +4 °C and diluted with ultrapure water at a ratio of 1/2 (v/v) when necessary for the proposed method.

Working solutions of different initial concentrations of Al(III) were freshly prepared from the stock solutions of Al(III) at 3.7×10^{-2} M in ultrapure water. Potassium hydrogen phthalate (KHP)-NaOH buffer solutions were prepared from the appropriate amount of 0.1 M KHP and 0.1 M NaOH to adjust the pH 4.0, pH 5.0, and pH 6.0 values.

After preparing stock solutions of each common water ion (Cl^- , NO_3^- , HCO_3^- , SO_4^{2-} , PO_4^{3-} , CH_3COO^- , K^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Sn^{2+} , Fe^{2+} , Fe^{3+} , and Cr^{3+}) at the appropriate concentration, mixtures of each ion solution were prepared as certain fold of the Al(III) ions.

In order to observe the characteristic three absorption bands of anthocyanins, the solution was prepared by dissolving the precipitate in a mixture of MeOH and 2 M HCl (85:15 v/v) after the fermented black carrot juice was kept at 10.000 rpm for 10 min in an ultracentrifuge device.

For investigation of selectivity, stock solutions of 9.15 g L^{-1} of L-cysteine hydrochloride monohydrate, L-arginine, L-glycine, L-lysine hydrochloride, and L-glutamine were prepared. Additively, the stock solution of 40.25 g L^{-1} of lactic acid, 8.5%, w/v NaCl, and 0.1% (w/v) sodium benzoate were prepared.

2.3. Preparation of the Proposed Method for Al(III) Detection

For the spectrophotometric method, after adding 1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water, 1.0 mL of pH 6.0 KHP-NaOH buffer solution (0.1 M), and 0.5 mL of Al(III) solution at different initial concentrations (185.0-9250.0 μM) to the test tubes and waiting for 4.5 minutes at room temperature (RT), absorbance readings were recorded against the blank at a wavelength of 575 nm (Scheme 1). For the blank solution, 0.5 mL of ultrapure water is added instead of Al(III) solution.

Summarized procedure: for sample solutions, add 1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water + 1.0 mL of pH 6.0 KHP-NaOH buffer solution (0.1 M) + 0.5 mL of Al(III) solutions at different initial concentrations (185.0-9250.0 μM); wait 4.5 min. at RT.; record the absorbance at λ_{575} nm against the blank solution ($V_{\text{total}} = 2.5 \text{ mL}$).

For the smartphone-based method, the Samsung Galaxy S10 Plus was used with its smaller black colored box (16.5×8×3 cm) that comes out of the phone box when purchasing the phone and allows the phone to fit on. This box was evaluated by using it for RGB measurements (Figure 1A). A cardboard sample holder (8×3 cm) was designed to fit the square space of the black box (Figure 1B and Figure 1C). The blue (B) values were recorded via PhotoMetrix software obtained from the google play store without any payment for the android system. The region of interest was chosen 96×96 and flash mod was selected "on" to see and take the B values of samples in the box. Also, the 1.5 mL of glass vials were used instead of the cuvette (Figure 1C and Figure 1D).

1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water, 1.0 mL of pH 6.0 KHP-NaOH buffer solution (0.1 M), and 0.5 mL of Al(III) solution at different initial concentrations (92.5-

555.0 μM) were added the test tubes and waiting for 4.5 minutes at RT, the B value measurements of blank and sample solution were recorded using the black box with the smartphone (Scheme 1).

2.4. Investigation of Interference Effect of Common Ions and Selectivity

Dual monitoring methods were applied in the presence of common water ions and the recovery values of Al(III) were calculated. Interference effects were removed easily using Na_2EDTA for Sn^{2+} , Fe^{2+} , and L-ascorbic acid for Fe^{3+} (35). In addition, it was investigated whether possible species other than anthocyanin in fermented black carrot juice affect the proposed method.

First of all, the maximum concentration of Na_2EDTA that will not dissociate the Al(III)-anthocyanin complex in the proposed method was investigated. The reason for optimizing the amount of Na_2EDTA is to prevent the discoloration of the Al(III)-anthocyanin complex due to the excess EDTA in the medium. To this end, mixed Al(III) solutions at an initial concentration of 1850.0 μM and Na_2EDTA at different concentrations (1850.0, 3700.0, 5550.0, and 7400.0 μM) were prepared separately. The

appropriate Na_2EDTA concentration was determined by applying the proposed method to these mentioned mixture solutions and the solution containing only 1850.0 μM initial concentration of Al(III). Then, different mass ratios of Na_2EDTA were investigated along with Fe^{2+} and Sn^{2+} ions in 1:1 metal:Al(III) solutions to remove the interference due to Fe^{2+} and Sn^{2+} ions by masking (without exceeding the optimum amount of Na_2EDTA).

2.5. Application of the Proposed Method to Real Water Samples.

The proposed method was successfully applied to drinking water and tap water samples using vis. spectrophotometer and smartphone. Later, the recovery (%) and RSD (%) values of Al(III) were calculated.

2.6. Method Validation of the Developed Methods Against the ICP-OES Method for Al(III) Detection.

Both the spectrophotometric and smartphone-based proposed methods have been validated with the ICP-OES method (34). The confidence level was calculated using *t*- and *F*- tests via the proposed method and ICP-OES method.

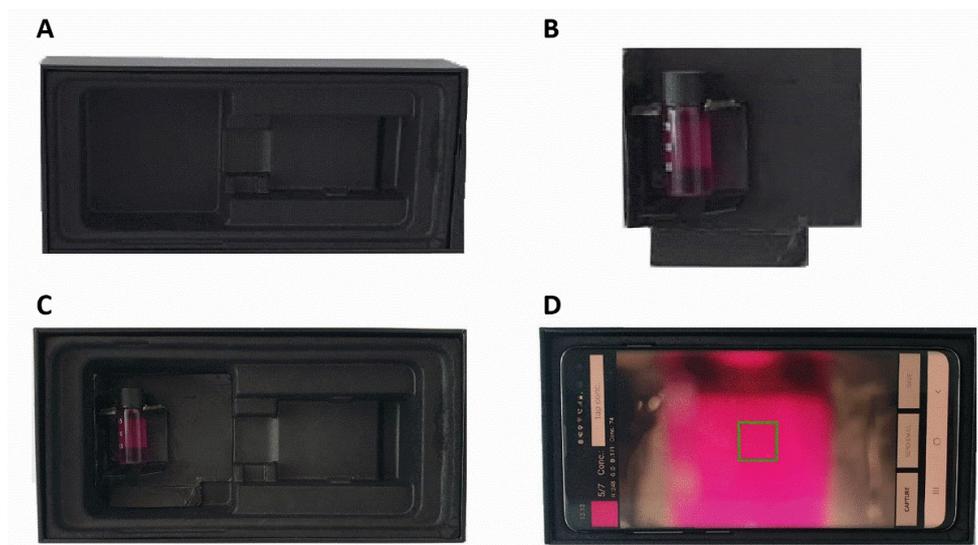
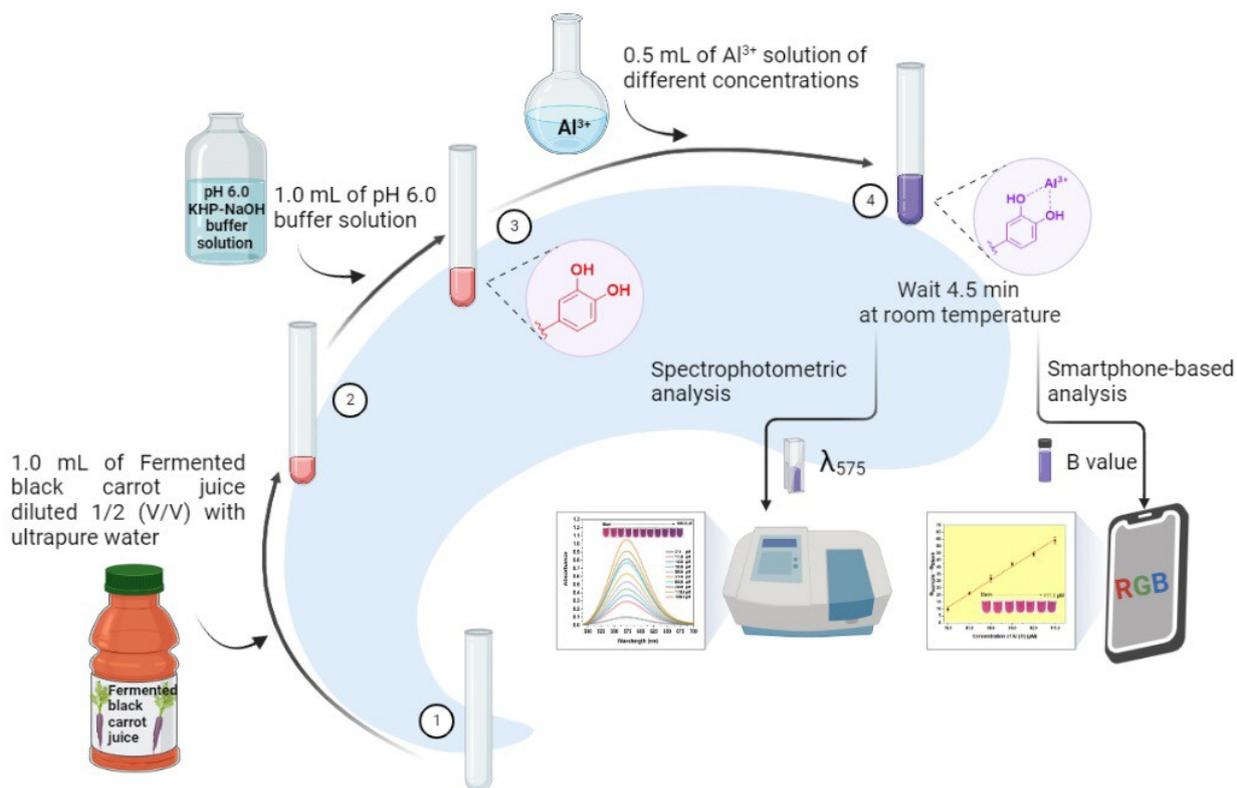


Figure 1: Representation of smartphone-based colorimetric detection of Al(III) ions (A) the original black box in which the phone was kept at the time of purchase and (B) sample holder made of cardboard; (C) sample holder placed in the black box; (D) reading the RGB values with the help of software by placing the phone in the black box designed for itself.



Scheme 1: Preparation of the proposed method for Al(III) detection using spectrophotometric and smartphone-based analysis.

2.7. Statistical Analysis

Excel software (Microsoft Office 2016) was used for performing the statistical analyses and calculating the means and the standard error of the mean. The mean \pm standard deviation (SD) was shown as a result. Validation of the proposed method for determining the Al(III) content against the ICP-OES method was made using the statistical tools of the same software.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Parameters

The figures obtained for the optimization of each parameter were formed as a result of three repetitive analyses ($N = 3$). Aluminum forms an insoluble complex known as the $\text{Al}(\text{OH})_3$ with increasing pH values and shows low solubility between pH 6.0-8.0 (36). It is also reported that cyanidin complexes formed with Al(III) in the pH range of 3.0-6.0 (32). Therefore, pH optimization studies were carried out between the pH range of 4.0-6.0 in order to select the optimal pH value. This experiment was applied for two different initial concentrations of Al(III) one at 555.0 μM and the other at 1850.0 μM for each pH value. For this purpose, after adding 1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water, 1.0 mL of KHP-NaOH (0.1M) buffer solution (pH 4.0 or pH 5.0 or pH 6.0), and 0.5 mL of 555.0 μM or 1850.0 μM initial concentrations of Al(III) to the test tubes, respectively ($V_{\text{total}} = 2.5 \text{ mL}$), solutions in the

test tubes were kept for 4.5 min at RT and the absorbances were recorded against the blank samples at 575 nm wavelength. As shown in Figure 2A, pH 6.0, where the absorbance differences are maximum for both 555.0 μM and 1850.0 μM initial concentration of Al(III), was chosen as the optimal pH value.

In order to select the optimal time, separate experiments were studied for two different initial concentrations of Al(III) one at 555.0 μM and the other at 1850.0 μM for each time interval. For this purpose, after adding 1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water, 1.0 mL of pH 6.0 KHP-NaOH (0.1M) buffer solution, 0.5 mL of 555.0 μM or 1850.0 μM initial concentrations of Al(III) to the test tubes, respectively ($V_{\text{total}} = 2.5 \text{ mL}$), solutions in the test tubes were kept separately for different time intervals starting from 0.5 min to 10 min at RT and the absorbances were recorded against the blank samples at 575 nm wavelength. According to Figure 2B, the optimal time for complex formation to reach equilibrium was determined as 4.5 minutes.

In order to select the optimal temperature, two different Al(III) solutions one at 555.0 μM and the other at 1850.0 μM initial concentrations were studied separately. After adding 1.0 mL of fermented black carrot juice diluted 1/2 (v/v) with ultrapure water, 1.0 mL of pH 6.0 KHP-NaOH (0.1M) buffer solution, 0.5 mL of 555.0 μM or 1850.0 μM

initial concentrations of Al(III) to the test tubes, respectively ($V_{\text{total}} = 2.5 \text{ mL}$), solutions in the test tubes were kept separately 4.5 min at different temperatures (25.0-100.0 °C) and the absorbances were recorded against the blank samples at 575 nm wavelength. As it is understood from Figure 2C, since there is no significant change in absorbance values at different temperatures, 25.0 °C (RT) was selected as the optimal temperature for the proposed method.

For the smartphone-based method, the R and G values were constant, and also it was observed that the calibration equation could be obtained by increasing the B values in direct proportion to the increasing Al(III) concentration between 18.5 and 111.0 μM so that, B value was selected to form calibration plot (Figure 2D).

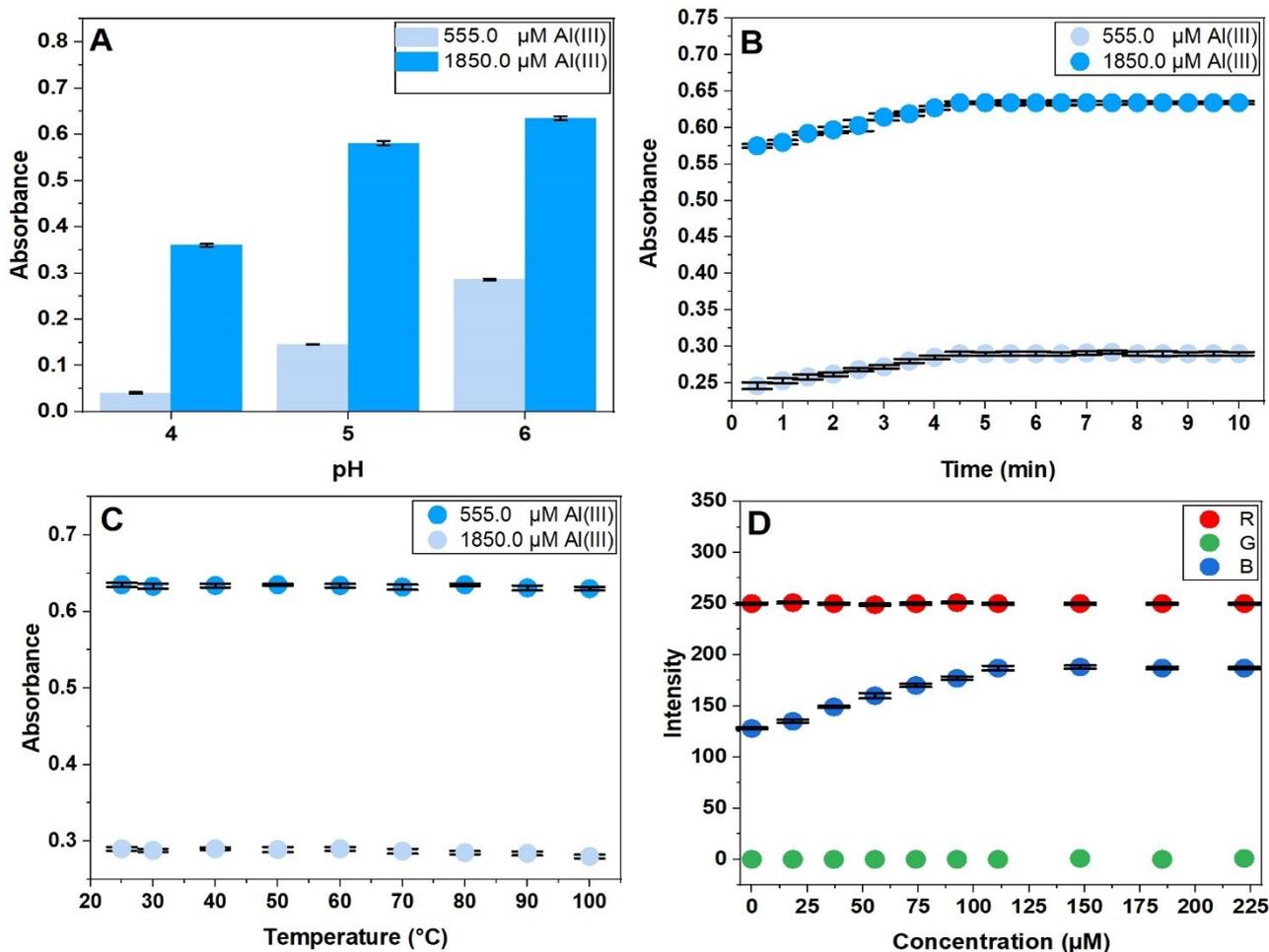


Figure 2: Optimization of the proposed method parameters: (A) optimal pH; (B) optimal time; (C) optimal temperature for spectrophotometric method and (D) selecting optimal RGB value for constructing calibration equation for smartphone-based method.

3.2. Characterization and Working Principle of the Proposed Method

Anthocyanins are known as colorful flavonoids including a flavylium cation (37) and they have a metal binding ability due to their two vicinal or three hydroxyl (-OH) substitutes located at the B ring (catechol or pyrogallol moiety). Polyvalent metal ions cause the loss of hydrogen ions bound to these rings and convert the flavylium cation to a quinoid base as shown in Figure 3A (38). Anthocyanins consist of a completely delocalized π -conjugated system, which gives a maximum absorption band in the wavelength range of 500-550 nm. Metal binding to anthocyanins causes a decrease in the energy

required for light-induced electron transfer in this chromophore system and also both maximum absorption shift towards the larger wavelength (bathochromic shift) and the increasing intensity of maximum absorbance (hyperchromic effect) are observed (21). According to some researchers, a bathochromic shift occurs due to the strong charge transfer from the ligand to the metallic center (LMCT), and according to others, it depends on the decrease in the HOMO-LUMO gap in the flavonoid molecule rather than a LMCT (39).

Furthermore, this phenomenon causes the color changing of the solution and enables the

determination of relevant metal via UV-vis. spectroscopy (21). When the proposed method was applied and absorbances values were recorded against the water, a 20 nm bathochromic shift was (from 536 to 556 nm) observed between blank solution and 1295.0 μM initial concentration of Al(III) due to the formation of Al(III)-anthocyanin complex (Figure 3B). The bathochromic shift proved the presence of the ortho-dihydroxyl group of anthocyanin. The fermented black carrot juice was centrifuged at 10.000 rpm for 10 minutes and the

supernatant phase was decanted, then the precipitant was dissolved in 2 mL of methanol, and its flavonoid properties were controlled by applying the Shinoda's test (40). After a few pieces of magnesium ribbon and 1 mL of concentrated HCl were added to the methanolic solution, pink color was observed a few minutes later. Additionally, the observation of maximum absorption at 280, 330, and 535 nm wavelengths in the MeOH/HCl solution, confirms the phenolic and flavonoid groups in the anthocyanin compound.

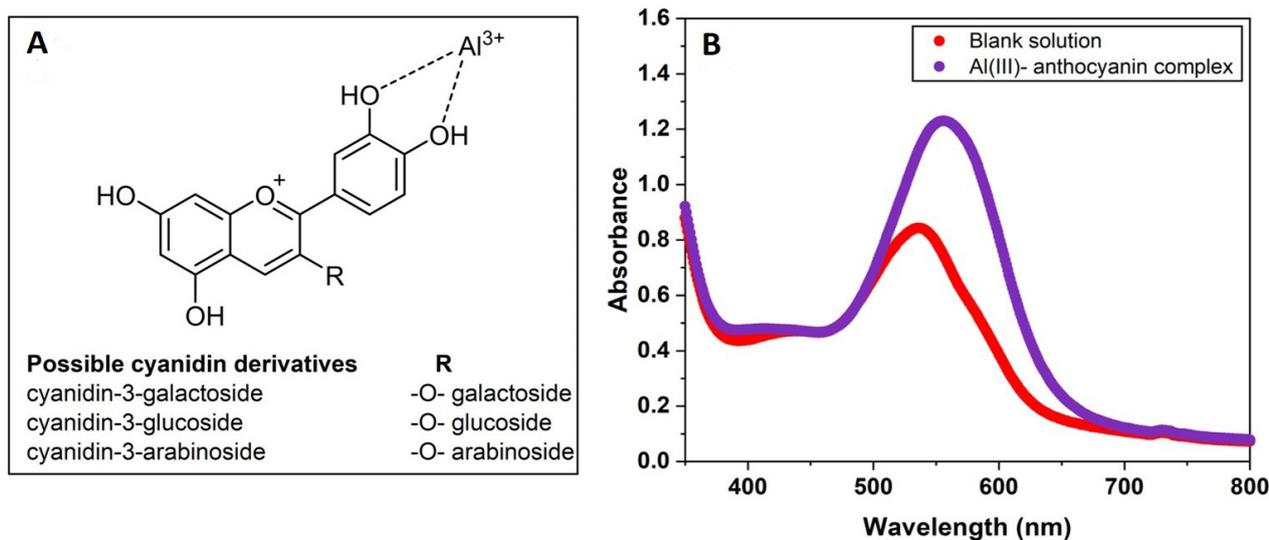


Figure 3: Working principle of the proposed method: (A) Al(III) complexation sites of possible cyanidin derivatives; (B) demonstration of the vis. spectra taken against the water as a result of applying the proposed method to Al(III) at an initial concentration of 1295.0 μM and blank solution.

3.3. Analytical Performance of the Proposed Method for the Determination of Al(III) Using Both Vis. Spectrophotometer and Smartphone

When the proposed method was applied to Al(III) solutions at different initial concentrations, a color change from red to purple was observed in direct proportion to the increasing logarithm of the concentration of Al(III) (Figure 4A and Figure 4B). There are many studies in which the absorbance values vary linearly with the logarithm of the analyte concentration (41, 42). All the absorbance measurements were recorded against the blank solution at 575 nm wavelength. The linear calibration equation was obtained with the absorbance versus the logarithm of the concentration of the Al(III) at the wavelength of 575 nm.

Linear calibration equation for Al(III):

$A_{575\text{ nm}} = 0.585 \log C_{\text{Al(III)}} - 0.87$ ($N = 10$, $r = 0.9963$)
where $C_{\text{Al(III)}}$ is the final concentration of Al(III) (in micromole L^{-1} (μM)).

The linear final concentration range is from 37.0 to 1850.0 μM . Furthermore, the limit of detection (LOD) and the limit of quantification (LOQ) values are 6.67 μM and 22.0 μM , respectively. The limit of detection was calculated in micromole per liter units according to the literature (43, 44). ($\text{LOD} = 3\sigma_{\text{blank}}/\text{antilog } m$,

$\text{LOQ} = 10\sigma_{\text{blank}}/\text{antilog } m$ where σ_{blank} denotes the standard deviation of a blank and m shows the slope of the calibration line). The coefficients of variation (CVs) of intra- and inter-assay calculations for Al(III) were 0.96 and 1.07%, respectively ($N = 5$).

For the smartphone-based measurements, color changing from red to purple enabled to form calibration plot between B values and different concentrations of Al(III) in the concentration range of 18.5-111.0 μM by applying the proposed method (Figure 4C).

In smartphone-based colorimetric methods, there are studies in which calibration plots are created by subtracting the relevant values (R, G, or B values) of the sample and blank solution (45, 46). The calibration equation was obtained by subtracting the B values of the blank solution (B_{blank}) from the B values of the samples (B_{sample}).

Linear calibration equation for Al(III) using a smartphone:

$y = 0.529 C_{\text{Al(III)}} + 1.35$ ($N = 6$, $r = 0.9972$)
where $C_{\text{Al(III)}}$ is the final concentration of Al(III) (in micromole L^{-1}).

The limit of detection (LOD) and the limit of quantification (LOQ) values are 4.40 μM and 14.50

μM , respectively. The limit of detection was found in micromole per liter units. ($\text{LOD} = 3\sigma_{\text{blank}}/m$, $\text{LOQ} = 10\sigma_{\text{blank}}/m$ where σ_{blank} denotes the standard deviation of a blank and m shows the slope of the calibration line). The coefficients of variation (CVs) of intra- and inter-assay calculations for Al(III) were 1.69 and 2.11%, respectively ($N = 5$). Both spectrophotometric and smartphone-based developed method measurements have good precision according to the CVs results and also the

LOD values are below $7.41 \mu\text{M}$, which is the acceptable limit for Al(III) ions in water by the WHO (47).

When the analytical performance of the proposed sensor method and recent studies were compared (Table 1), the proposed sensor method stands out with its not preparation time, short analysis time, and quite good wide linear response range.

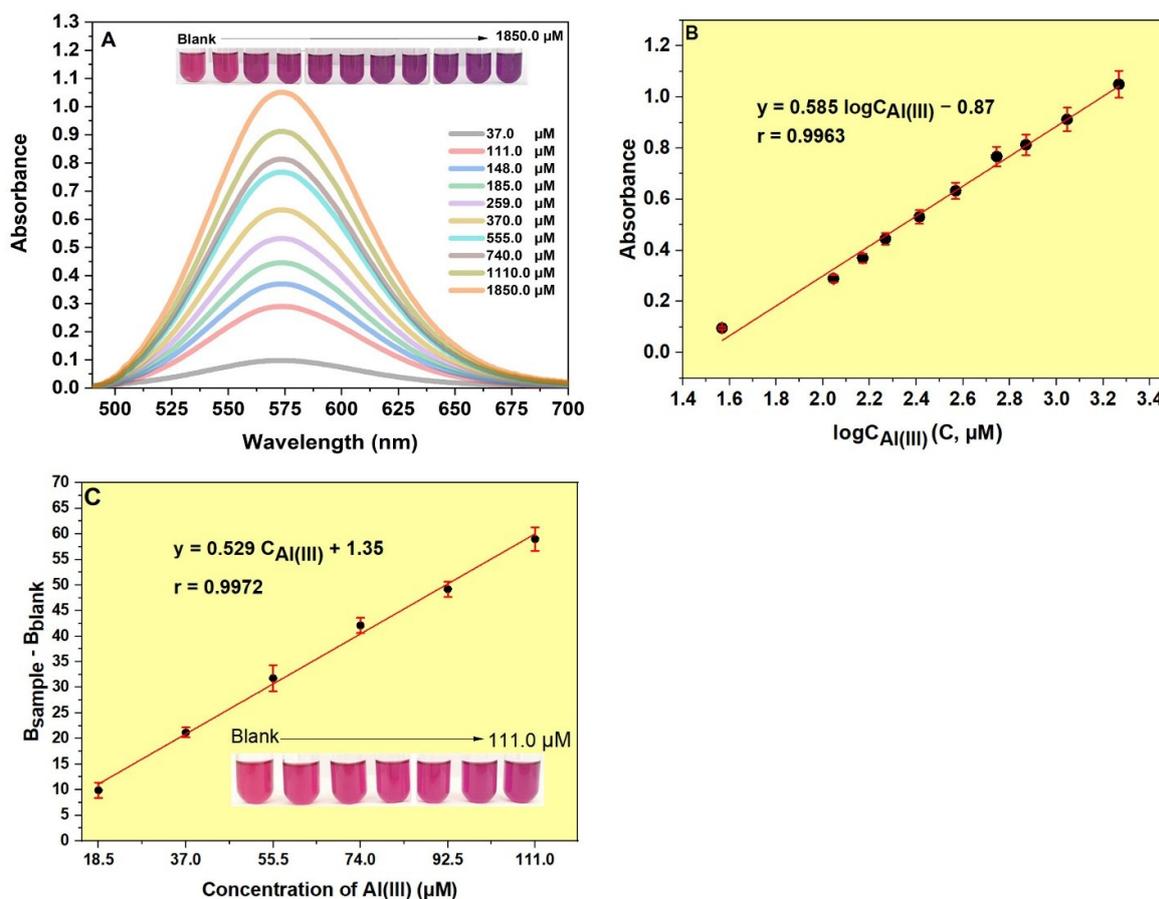


Figure 4: (A) The vis. spectrum of fermented black carrot juice containing different concentrations of Al(III) in an aqueous medium and their photograph (inset); (B) calibration plot of different concentrations of Al(III) solutions mixed with fermented black carrot juice for vis. spectrophotometer; (C) formed calibration plot via Blue (B) values using a smartphone and also the photograph of (inset) different concentrations of Al(III) solutions mixed with fermented black carrot juice.

Table 1: Comparison of the analytical performance of some optical and paper-based methods for the determination of Al(III) ions.

No	Detection method	Probe	Probe Preparation Time	Analysis Time	Linear response range	LOD	Real water application	Ref.
1	Paper-based fluorometric	Rhodamine grafted CDs	84 h (~3.5 days)	10 min	1×10^{-4} - 1×10^{-2} M	3.89×10^{-5} M	x	48
2	Fluorometric	PAA-AuNPs	135 min	10 min	50-150 μ M	2 μ M 8 μ M (with bare eye)	x	49
3	Fluorometric	CDs	140 min	10 min	0.15-38.46 μ M	113.8 nM	✓	50
	Smartphone based-method	CDs	140 min	15 min	15.39-153.85 μ M	5.55 μ M	✓	50
4	Colorimetric	Pectin-rich apple extract-based AuNPs	50 min	1 min	0-100 μ M	20 μ M	✓	51
5	Colorimetric	Cyanidin extracted from red cabbage as a chelating agent	73 h (~3 days)	a few min	n.a	50 μ M	✓	31
6	Colorimetric	Anthocyanin immobilization in CMC/starch films	~ 67 h (~2.8 days)	60 min ($C \leq 3$ mg L^{-1}), 20 min ($C \geq 5$ mg L^{-1})	n.a	1.9×10^{-4} M	x	52
7	Proposed Colorimetric method	Fermented black carrot juice	Direct (0 min.)	4.5 min	37.0-1850.0 μ M	6.67 μ M	✓	This work
	Proposed smartphone-based method	Fermented black carrot juice	Direct (0 min.)	4.5 min	18.5-111.0 μ M	4.40 μ M	✓	This work

n.a: not available, C: concentration, CDs: carbon nanodots, PAA-AuNPs: polyacrylate functionalized gold nanoparticles, CMC: carboxymethylcellulose, Conc.: concentration.

3.4. Investigation of Interference Effect of Common Ions and Selectivity

The interference effect of different common water ions on the proposed method was studied (Table 2), each containing a different mass ratio of Cl^- , NO_3^- , HCO_3^- , SO_4^{2-} , PO_4^{3-} , CH_3COO^- , K^+ , NH_4^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Cu^{2+} , Ni^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} , Sn^{2+} , Fe^{2+} , Fe^{3+} , and Cr^{3+} with the initial concentration of $1850.0 \mu\text{M}$ of Al(III) (e.g., 1-, 200- fold of Al(III)) for spectrophotometric method and $370.0 \mu\text{M}$ of Al(III) for the smartphone-based method. Additively, the Al(III) recovery (%) values were calculated in the range of 87.29-112.93 and 88.00-111.29% by the spectrophotometric and smartphone-based methods, respectively, as shown in Figure 5. First, the proposed method was applied to mixed solutions including the initial concentration of $1850.0 \mu\text{M}$ of Al(III) mixed with different concentrations of Na_2EDTA solutions to find the maximum concentration of Na_2EDTA that would not interact with Al(III) in the Al(III) -anthocyanin complex. A tolerable maximum initial concentration of Na_2EDTA was determined as $5550.0 \mu\text{M}$ in the mixed solution with the initial concentration of Al(III) at $1850.0 \mu\text{M}$ when the proposed method was applied, and no difference in absorption was observed when compared with the Al(III) -anthocyanin complex solution. Later the interference effects of Fe^{2+} and

Sn^{2+} were easily removed with Na_2EDTA ($\text{Fe}^{2+}:\text{EDTA}$ ratio 1:3 (w/w) and $\text{Sn}^{2+}:\text{EDTA}$ ratio 1:3 (w/w)) as a masking agent without affecting Al(III) -anthocyanin complex before applying the proposed method.

In order to remove the interference effect of Fe^{3+} on the proposed method L-ascorbic acid (35) was used with the 1-fold of Fe^{3+} ($\text{Fe}^{3+}:\text{L-ascorbic acid}$ ratio 1:1 (w/w)).

Fermented black carrot juice contains a maximum amount of 8.05 g L^{-1} lactic acid, 1.97% NaCl (w/v), 1.83 g L^{-1} total amino acids (53), and 0.02% sodium benzoate (54) (w/v) known as the preservative, apart from black carrot, which is the main ingredient. Additionally, it is also stated that it does not contain gluten and sugar. It was tested whether colored complexes were formed with Al(III) at $9250.0 \mu\text{M}$ initial concentration when the proposed method was applied to other species at the specified concentration in the content without using fermented black carrot juice for selectivity. L-cysteine hydrochloride monohydrate, L-arginine, L-glycine, L-lysine hydrochloride, and L-glutamine were used separately as amino acids. As can be seen in Figure 6, no coloration was observed when the proposed method was applied to other ingredients.

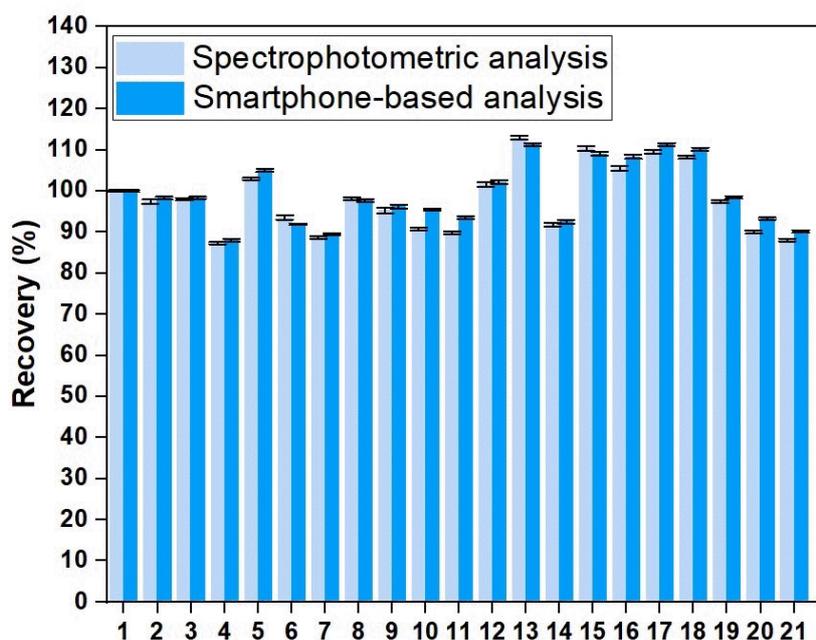


Figure 5: The response of Al(III) ions (1) and possible interference species $\{\text{Cl}^-$ (2), NO_3^- (3), HCO_3^- (4), CH_3COO^- (5), SO_4^{2-} (6), PO_4^{3-} (7), K^+ (8), NH_4^+ (9), Ca^{2+} (10), Mg^{2+} (11), Mn^{2+} (12), Cu^{2+} (13), Ni^{2+} (14), Zn^{2+} (15), Cd^{2+} (16), Pb^{2+} (17), $[\text{Sn}^{2+} + \text{EDTA}]$ (18) $[(\text{Fe}^{2+} + \text{EDTA})]$ (19), $[(\text{Fe}^{3+} + \text{L-ascorbic acid})]$ (20), and Cr^{3+} (21)} in the presence of Al(III) at the initial concentration of $1850.0 \mu\text{M}$ for spectrophotometric method and $370.0 \mu\text{M}$ for smartphone-based method.

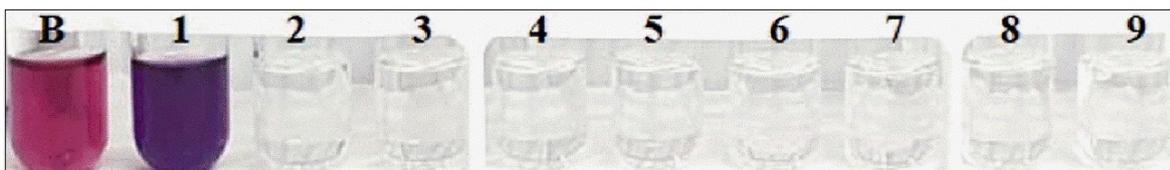


Figure 6: Color states of solutions in the test tubes with the proposed method in the presence of blank solution (B), (1) Al(III) (9250.0 μM), and mixture solutions without using fermented black carrot juice (2) lactic acid (8.05 g L^{-1}), (3) NaCl (1.97%, w/v), (4) sodium benzoate (0.02%, w/v), (5) L-cysteine hydrochloride monohydrate (1.83 g L^{-1}), (6) L-arginine (1.83 g L^{-1}), (7) L-glycine (1.83 g L^{-1}), (8) L-lysine hydrochloride (1.83 g L^{-1}) and (9) L-glutamine (1.83 g L^{-1}).

3.5. Application of the Proposed Method to Real Water Samples

Tap water and drinking water were filtered (CHROMAFIL, PET-20/25) before applying the sensor method. Drinking water and tap water were spiked separately with different concentrations of Al(III) solutions by standard addition method and then the proposed method was applied to real samples using both spectrophotometric and smartphone-based analyses. For the spectrophotometric analysis, Al(III) recoveries (%) and RSD % values for drinking water were found between 97.14-100.99 and 0.77-2.11%, respectively, while these values for tap water were found between 94.16-99.91 and 0.92-2.23% (Table 3). For the smartphone-based analysis, Al(III) recoveries (%) and RSD % values for drinking water were found between 97.43-100.27 and 0.80-2.35%, respectively, while these values for tap water were found between 97.43-94.23 and 1.24-2.65% (Table 3). According to these results, the proposed methods could be used for the determination of Al(III) in real water samples.

3.6. Method Validation of the Proposed Sensor Against the ICP-OES Method for Al(III) Detection

In order to obtain the calibration equation, the concentration range of 18.5-740.0 μM standard

Al(III) solutions were analyzed according to US EPA method 200.7 with ICP-OES (34).

Three repetitive analyses were performed for each standard concentration to generate the calibration equation. The calibration equation between intensity (CPS) and concentration was:

$$\text{Intensity} = 6480 C_{\text{Al(III)}} + 56674 \quad (r = 0.9999) \text{ for Al(III).}$$

($C_{\text{Al(III)}}$: final concentration of Al(III) in micromole L^{-1} (μM)).

Statistical comparison between the results of the proposed and ICP-OES methods applied to both 370.0 μM and 74.0 μM of Al(III) spiked-drinking water as a real sample was made on $N = 5$ repetitive analyses, substantially showing no important difference in precision and accuracy between the results. The population means and variances were compared using t - and F - tests, respectively. The confidence levels used in the validation of findings were 95% (nominal 0.05 significance level) for both of t - and F - tests. Statistical parameters of the proposed methods and the reference ICP-OES method were shown in Table 4.

Table 2: Al(III) recovery (%) results when the proposed method is applied to ionic species commonly found in the water of different mass ratios with Al(III).

Interferent	Mass ratio (Al ³⁺ :ions (w/w))	Error (%)	
		For spectrophotometer	For smartphone
Cl ⁻	1200	-2.57	-1.70
NO ₃ ⁻	1200	-2.00	-1.60
HCO ₃ ⁻	5	-12.71	-12.00
CH ₃ COO ⁻	300	+2.97	+5.02
SO ₄ ²⁻	1200	-6.44	-8.10
PO ₄ ³⁻	7	-11.31	-10.55
K ⁺	1200	-1.95	-2.31
NH ₄ ⁺	600	-4.75	-3.82
Ca ²⁺	300	-9.29	-4.51
Mg ²⁺	200	-10.14	-6.49
Mn ²⁺	300	+1.53	+2.13
Cu ²⁺	10	+12.93	+11.29
Ni ²⁺	500	-8.20	-7.53
Zn ²⁺	60	+10.39	+9.01
Cd ²⁺	250	+5.52	+ 8.32
Pb ²⁺	50	+9.60	+ 11.27
Sn ²⁺	1	before EDTA masking: +42.90 after EDTA masking: +8.23	before EDTA masking: +31.30 after EDTA masking: + 10.15
Fe ²⁺	1	before EDTA masking: +14.32 after EDTA masking: -2.61	before EDTA masking: +26.40 after EDTA masking: -1.54
Fe ³⁺	1	before using L -ascorbic acid masking: +26.85 after L-ascorbic acid masking: +10.0	before using L-ascorbic acid masking: +38.2 after L-ascorbic acid masking: +6.72
Cr ³⁺	10	-11.89	-9.82

Table 3: Determination of Al(III) in drinking water and tap water samples by applying the proposed method.

Samples	Spectrophotometric Analysis				Smartphone-based Analysis			
	Spiked (µM)	Found (µM)	Recovery %	RSD % (N=3)	Spiked (µM)	Found (µM)	Recovery %	RSD % (N= 3)
Drinking water	37.0	36.9	99.73	1.25	18.5	18.2	98.38	1.54
	111.0	112.1	100.99	2.11	37.0	37.1	100.27	0.96
	185.0	184.7	99.84	0.98	55.5	54.6	98.38	0.80
	370.0	359.4	97.14	0.77	74.0	72.1	97.43	2.35
Tap water	37.0	36.5	98.65	2.23	18.5	18.0	97.30	1.63
	111.0	110.9	99.91	0.92	37.0	35.2	95.13	2.65
	185.0	183.7	99.30	1.12	55.5	52.3	94.23	1.24
	370.0	348.4	94.16	0.99	74.0	72.1	97.43	1.72

Table 4: Statistical comparison of the proposed method with ICP-OES reference method for the determination of Al(III).

Method	Mean Conc. (µM)	SD (σ)	S ^{a,b}	t ^{a,b}	T _{table} ^b	F ^b	F _{table} ^b
Proposed method (For spectroscopy)	372.96	0.108	-	-	-	-	-
ICP-OES reference method (For spectroscopy)	368.15	0.046	0.051	1.80	2.306	5.60	6.39
Proposed method (For smartphone)	75.92	0.051	-	-	-	-	-
ICP-OES reference method (For smartphone)	75.55	0.050	0.056	0.47	2.306	0.78	6.39

^a $S^2 = ((n_1 - 1)s_1^2 + (n_2 - 1)s_2^2) / (n_1 + n_2 - 2)$ and $t = (\bar{a}_1 - \bar{a}_2) / (S(1/n_1 + 1/n_2)^{1/2})$, where S is the pooled standard deviation, s_1 and s_2 are the standard deviations of the two populations with sample sizes of n_1 and n_2 , and sample means of \bar{a}_1 and \bar{a}_2 respectively (t has $(n_1 + n_2 - 2)$ degrees of freedom); here, $n_1 = n_2 = 5$.

^bStatistical comparison made on paired data produced with proposed and reference methods; the results given only on the row of the reference method.

4. CONCLUSION

In this study, in accordance with the principles of green analytical chemistry, anthocyanin-rich fermented black carrot juice was used as a green and chromogenic agent instead of polluting chemicals in order to determine the concentration of Al(III) ions in water samples. The application of this developed method to real water samples is eco-friendly, simple, fast, and practical. In addition, fermented black carrot juice is a healthy beverage rich in antioxidants and anthocyanins, and it is a chromogenic agent that can be used safely in the laboratory and is quite cheap (approx. 0.44 €). As can be seen from Table 1, the preparation of the chromogenic agent by other methods is very time-consuming and complex steps, and furthermore, the

proposed spectrophotometric method allows a very wide linear Al(III) concentration range (37.0 to 1850.0 µM) without any preliminary preparation. Thanks to the closed box system for analysis, the method developed based on a smartphone ensures usability in any field due to its portability and it can be easily applied to samples. Smartphones are more reachable and cheaper than other portable analytical devices. The high recoveries obtained by the application of both detection methods demonstrate their accuracy and applicability to real samples (tap water and drinking water) for the determination of Al(III). Additively, the recorded relative standard deviation values were very low (<5.0%) for spectrophotometric and smartphone-based methods. LOD values of spectrophotometric and smartphone-based methods are 6.67 µM and

4.40 μM , respectively, and both proposed methods obtain the required WHO permissible detection limit for Al(III) in drinking water. This method may open the way to novel low-cost Al(III) quantification methods without any extraction processes utilizing anthocyanin-rich fermented black carrot juice obtained from local markets.

5. CONFLICT OF INTEREST

The author declares no competing financial interest.

6. REFERENCES

- Goswami S, Paul S, Manna A. Selective “naked eye” detection of Al (III) and PPI in aqueous media on a rhodamine-isatin hybrid moiety. *RSC Adv.* 2013;3(27):10639-10643. [<DOI>](#).
- Imadi SR, Waseem S, Kazi AG, Azooz MM, Ahmad P. Aluminum toxicity in plants: an overview. *Plant Metal Interaction.* 2016;1-20. [<DOI>](#).
- Maity D, Govindaraju T. Naphthaldehyde-Urea/Thiourea conjugates as turn-on fluorescent probes for Al³⁺ based on restricted C=N isomerization. *European Journal of Inorganic Chemistry.* 2011;(36):5479-5485. [<DOI>](#).
- Al-Kindy SM, Al-Hinai A, Al-Rasbi NK, Suliman FEO, Al-Lawati HJ. Spectrofluorimetric determination of aluminium in water samples using N-((2-hydroxynaphthalen-1-yl) methylene) acetylhydrazide. *Journal of Taibah University for Science.* 2015;9(4):601-609. [<DOI>](#).
- Michael JK. Powder-Metallurgy Aluminum Alloys. *Aluminum Alloys-Contemporary Research and Applications: Contemporary Research and Applications.* 2012;1:323.
- Frankowski M, Ziola-Frankowska A, Sienkiewicz J. New method for speciation analysis of aluminium fluoride complexes by HPLC-FAAS hyphenated technique. *Talanta.* 2010;80(5): 2120-2126. [<DOI>](#).
- Melnyk LJ, Morgan JN, Fernando R, Pellizzari ED, Akinbo O. Determination of metals in composite diet samples by inductively coupled plasma-mass spectrometry. *Journal of AOAC International.* 2003;86(2):439-448. [<DOI>](#).
- Mahdavi M, Nezamzadeh-Ejhieh A. An aluminum selective electrode via modification of PVC membrane by modified clinoptilolite nanoparticles with hexadecyltrimethyl ammonium bromide (HDTMA-Br) surfactant containing Arsenazo III. *Journal of colloid and interface science.* 2017; 494:317-324. [<DOI>](#).
- Zuziak J, Reczyński W, Baś B, Jakubowska M. Voltammetric determination of aluminum (III) as Al-Alizarin S complex in tea leaves and infusions. *Analytical biochemistry.* 2018;558:69-79. [<DOI>](#).
- Guha S, Lohar S, Sahana A, Banerjee A, Safin DA, Babashkina MG et al. Coumarin-based “turn-on” fluorescent sensor for the determination of Al³⁺: single crystal X-ray structure and cell staining properties. *Dalton Transactions.* 2013;42(28):10198-10207. [<DOI>](#).
- Wang B, Liu X, Duan W, Dai S, Lu H. Visual and ratiometric fluorescent determination of Al³⁺ by a red-emission carbon dot-quercetin system. *Microchemical Journal.* 2020;156: 104807. [<DOI>](#).
- Huang P, Li J, Liu X, Wu F. Colorimetric determination of aluminum (III) based on the aggregation of Schiff base-functionalized gold nanoparticles. *Microchimica Acta.* 2016;183(2): 863-869. [<DOI>](#).
- Zezi-Arruda MA, Poppi RJ. Spectrophotometry / Inorganic Compounds. *Encyclopedia of Analytical Science.* 2005;351-358. [<DOI>](#).
- Alawsi T, Mattia GP, Al-Bawi Z, Beraldi R. Smartphone-based colorimetric sensor application for measuring biochemical material concentration. *Sensing and Bio-Sensing Research.* 2021;32:100404. [<DOI>](#).
- Liu T, Zhang S, Liu W, Zhao S, Lu Z, Wang Y, Wang G, Zou P, Wang X, Zhao Q, Rao H. Smartphone based platform for ratiometric fluorometric and colorimetric determination H₂O₂ and glucose. *Sensors and Actuators B: Chemical.* 2020;305:127524. [<DOI>](#).
- Serhan M, Jackemeyer D, Long M, Sprowls M, Perez ID et al. Total iron measurement in human serum with a novel smartphone-based assay. *IEEE Journal of Translational Engineering in Health and Medicine.* 2020;8:1-9. [<DOI>](#).
- Saranchina NV, Slizhov YG, Vodova YM, Murzakasymova NS, Ilyina AM et al. Smartphone-based colorimetric determination of fluoride anions using polymethacrylate optode. *Talanta.* 2021;226:122103. [<DOI>](#).
- Fang J. Classification of fruits based on anthocyanin types and relevance to their health effects. *Nutrition.* 2015;31(11-12):1301-1306. [<DOI>](#).
- Silva S, Costa EM, Calhau C, Morais RM, Pintado ME. Anthocyanin extraction from plant tissues: A review. *Critical reviews in food science and nutrition.* 2017;57(14):3072-30833. [<DOI>](#).
- Clifford MN. Anthocyanins-nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture.* 2000;80(7):1063-1072. [<DOI>](#).
- Fedenko VS, Shemet SA, Landi M. UV-vis spectroscopy and colorimetric models for detecting anthocyanin-metal complexes in plants: An overview

of in vitro and in vivo techniques. *Journal of plant physiology*. 2017;212:13-28. [<DOI>](#).

22. Tanguler H, Erten H. Chemical and microbiological characteristics of shalgam (salgam); A traditional Turkish lactic acid fermented beverage. *Journal of Food Quality*. 2012;35(4):298-306. [<DOI>](#).

23. Tanguler H, Selli, S, Sen K, Cabaroglu T, Erten, H. Aroma composition of shalgam: a traditional Turkish lactic acid fermented beverage. *Journal of Food Science and Technology*. 2017;54(7):2011-2019. [<DOI>](#).

24. Canbaş A, Fenercioglu H. Salgam suyu üzerinde bir araştırma. *Gıda*. 1984;9(5):279-286 (in Turkish).

25. Erten H, Tanguler H, Canbaş A, A traditional Turkish lactic acid fermented beverage: Shalgam (Salgam). *Food Reviews International*. 2008;24(3):352-359. [<DOI>](#).

26. Kammerer D, Carle R, Schieber A. Quantification of anthocyanins in black carrot extracts (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) and evaluation of their color properties. *European Food Research and Technology*. 2004;219(5):479-486. [<DOI>](#).

27. Narayan MS, Venkataraman LV. Characterisation of anthocyanins derived from carrot (*Daucus carota*) cell culture. *Food Chemistry*. 2000;70:361-363. [<DOI>](#).

28. Canbas A. Siyah havucun renk maddesi üzerine bir araştırma. *Doğa*. 1985;9(3):394-398 (in Turkish).

29. Ekinci FY, Baser GM, Özcan E, Üstündağ ÖG, Korachi M. Characterization of chemical, biological, and antiproliferative properties of fermented black carrot juice, shalgam. *European Food Research and Technology*. 2016;242(8):1355-1368. [<DOI>](#).

30. Porrawatkul P, Pimsen R, Kuyyogsuy A, Nuengmatcha P. Simple and selective naked-eye and visual detection of Cu²⁺ and Al³⁺ ions using Hibiscus Rosa-Sinensis Linn flower Extract. *Oriental Journal of Chemistry*. 2018;34(1):188. [<DOI>](#).

31. Khaodee W, Aeungmaitrepirom W, Tuntulani T. Effectively simultaneous naked-eye detection of Cu (II), Pb (II), Al (III) and Fe (III) using cyanidin extracted from red cabbage as chelating agent. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2014;126:98-104. [<DOI>](#).

32. Park S, Kang S, Jeong DY, Jeong SY, Park JJ et al. Cyanidin and malvidin in aqueous extracts of black carrots fermented with *Aspergillus oryzae* prevent the impairment of energy, lipid and glucose metabolism in estrogen-deficient rats by AMPK activation. *Genes & nutrition*. 2015;10(2):1-14. [<DOI>](#).

33. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. *TrAC Trends in Analytical Chemistry*. 2013;50:78-84. [<DOI>](#).

34. U. USEPA, Method 200.7: determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Rev. 4.4, 1994; EPA600/R-94-111.

35. Zolgharnein J, Shahrjerdi A, Azimi G, Ghasemi J. Spectrophotometric determination of trace amounts of fluoride using an Al-xylenol orange complex as a colored reagent. *Analytical Sciences*. 2009;25(10):1249-1253. [<DOI>](#).

36. Krupińska I. Aluminium drinking water treatment residuals and their toxic impact on human health. *Molecules*. 2020;25(3):641. [<DOI>](#).

37. Grotewold, E. The genetics and biochemistry of floral pigments. *Annual review of plant biology*. 2006;57(1):761-780. [<DOI>](#).

38. Schreiber HD, Swink AM, Godsey TD. The chemical mechanism for Al³⁺ complexing with delphinidin: A model for the bluing of hydrangea sepals. *Journal of inorganic biochemistry*. 2010;104(7):732-739. [<DOI>](#).

39. Ren J, Meng S, Lekka CE, Kaxiras E. Complexation of flavonoids with iron: structure and optical signatures. *The Journal of Physical Chemistry B*. 2008;112(6):1845-1850. [<DOI>](#).

40. Ravishankara MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. (Anantmul). *Phytomedicine*. 2002;9(2):153-160. [<DOI>](#).

41. Jiang Y, Miao Y, Ding Z, Lu Y. In situ formed silicon-based nanoparticles enabled highly efficient dual-mode biosensing of chlorpyrifos. *Food Chemistry*. 2023;403:134243. [<DOI>](#).

42. Shang C, Li Y, Zhang Q, Tang S, Tang X, Ren H, Hu P, Lu S, Li P, Zhou, Y. Alkaline phosphatase-triggered dual-signal immunoassay for colorimetric and electrochemical detection of zearalenone in cornmeal. *Sensors and Actuators B: Chemical*. 2022;358:131525. [<DOI>](#).

43. Durmazel S, Üzer A, Apak, R. Naked-Eye Detection of 3-Nitro-1, 2, 4-triazole-5-one at Sub-Femtomolar Levels with Melamine and Unlabeled Au Nanoparticles. *ACS Applied Nano Materials*. 2022;5(4): 5244-5257. [<DOI>](#).

44. Ular N, Uzer A, Durmazel S, Ercag E, Apak R. Diaminocyclohexane-functionalized/thioglycolic acid-modified gold nanoparticle-based colorimetric sensing of trinitrotoluene and tetryl. *ACS sensors*. 2018;3(11): 2335-2342. [<DOI>](#).

45. Shahvar A, Shamsaei D, Saraji M. A portable smartphone-based colorimetric sensor for rapid determination of water content in ethanol. *Measurement*. 2020;150:107068. [<DOI>](#).
46. Bandi R, Alle M, Park CW, Han SY, Kwon GJ, Kim NH, Kim JC, Lee SH. Cellulose nanofibrils/carbon dots composite nanopapers for the smartphone-based colorimetric detection of hydrogen peroxide and glucose. *Sensors and Actuators B: Chemical*. 2021;330:129330. [<DOI>](#).
47. Han T, Feng X, Tong B, Shi J, Chen L et al. A novel "turn-on" fluorescent chemosensor for the selective detection of Al³⁺ based on aggregation-induced emission. *Chemical communications*. 2012;48(3):416-418. [<DOI>](#).
48. Kim Y, Jang G, Lee TS. New fluorescent metal-ion detection using a paper-based sensor strip containing tethered rhodamine carbon nanodots. *ACS applied materials & interfaces*. 2015;7(28):15649-15657. [<DOI>](#).
49. Kumar A, Bhatt M, Vyas G, Bhatt S, Paul P. Sunlight induced preparation of functionalized gold nanoparticles as recyclable colorimetric dual sensor for aluminum and fluoride in water. *ACS applied materials & interfaces*. 2017;9(20):17359-17368. [<DOI>](#).
50. Wei W, Huang J, Gao W, Lu X, Shi X. Carbon dots fluorescence-based colorimetric sensor for sensitive detection of aluminum ions with a smartphone. *Chemosensors*. 2021;9(2):25. [<DOI>](#).
51. Park H, Kim W, Kim M, Lee G, Lee W et al. Eco-friendly and enhanced colorimetric detection of aluminum ions using pectin-rich apple extract-based gold nanoparticles. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2021;245:118880. [<DOI>](#).
52. da Silva HM, Mageste AB, e Silva SJB, Ferreira GMD, Ferreira GMD. Anthocyanin immobilization in carboxymethylcellulose/starch films: A sustainable sensor for the detection of Al(III) ions in aqueous matrices. *Carbohydrate Polymers*. 2020;230:115679. [<DOI>](#).
53. Canbas A, Deryaoglu A. Şalgam suyunun üretim tekniği ve bileşimi üzerinde bir araştırma. *Doga-Turkish Journal of Agricultural and Forestry* 1993;17:119-129.
54. Coskun F. A traditional Turkish fermented non-alcoholic beverage, "Shalgam". *Beverages* 2017; 3: 49. [<DOI>](#).