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Research article

Method optimization for the determinations of selected phytochemicals and antioxidant activities of wild Ethiopian *Syzygium guineense* fruit and seed under different drying conditions

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ABSTRACT

This study aimed to optimize the ultrasound-assisted extraction (UAE) parameters using response surface methodology (RSM) for the determination of ascorbic acid (AA), antioxidant activities/the half maximal effective concentration (EC50), total phenolic contents (TPC), and total flavonoid content (TFC) of Ethiopian wild *Syzygium guineense* fruit and seed processed under different drying conditions. The optimizations of the UAE methods for the determination of AA, antioxidant activities (EC50 values), TPC, and TFC were evaluated using response surface methodology (RSM). The extraction time of 15 min, the temperature of 35 °C, extraction solvent composition (methanol:water) of 75 to 25%, and solid-to-solvent ratios of 1:15 w/v were the optimum independent parameters. The experimental and the predicted data of TPC, EC50, and AA were in good agreement with the overall error below 0.01%. It also indicated a hypothesized distribution of predicted data fitted with experimental data. The average TPC, EC50, and AA content in *S. guineense* fruits and the seed varied from 581.25 ± 37.13 to 1917.40 ± 26.15 mg GAE/100 g, 4.02 ± 0.42 to 155.73 ± 5.11 mg/100 g, and 1.96 ± 0.02 to 0.94 ± 0.00 mg/mL, respectively. This study indicated that this underutilized wild fruit and its seeds can be an alternative source of AA and antioxidant compounds.

1. Introduction

Fruits and vegetables are the primary sources of antioxidant bioactive compounds such as phenolic compounds, ascorbic acid (AA),

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carotenoids, and essential oils [1]. *Syzygium guineense* is one of the important species from the genus *Syzygium*. It is an evergreen flowering forest plant widely distributed in the tropical regions of Australia, Asia, and Africa [2]. This evergreen tree, belonging to the family *Myrtaceae*, is also distributed throughout sub-Saharan Africa and grows to a height of 12–15 m. This species is naturally found in Senegal, stretching to Somalia in the east and extending southward to South Africa. *Syzygium guineense* Wall. leaf is being used as a traditional medicine against hypertension and diabetes mellitus [1]. In addition, this study showed that this underutilized wild fruit and its seeds could be an alternative source of AA and antioxidant compounds [1]. The plant is known by different local names in Ethiopia such as "Dokma" in Amharic, "Badeessaa" in Oromifa, and "Duwancho" in Sidama [3]. The plant is known by different English names such as water-berry, water-boom, or water-pear [2]. The fruits are edible in various parts of sub-Saharan African countries including Ethiopia, particularly in rural areas and during droughts.

According to a review made by Aung and co-workers [4] on plant description, phytochemical constituents, and bioactivities of the *Syzygium* genus, different parts of the plant are rich in phytochemical constituents. The review further showed that various extracts of the different parts exhibit various medicinal activities. For instance, examination of extracts from Cameroonian *S.* guineense fruits indicated strong activity against the proliferation and Wnt signaling in triple-negative breast and colon cancer cells [1]. Despite its importance, this plant has not been adequately investigated in Ethiopia.

For the detailed investigation of the contents of plant resources, we need to develop an efficient method of analysis, i.e., efficient extraction method, analysis techniques, and use valid data compilation statistical software. Method development, optimization, and validation are continuous processes to select appropriate extraction and analysis techniques for a particular target analyte in sample matrices. The state-of-the-art design of experiment (DoE) using response surface methodology (RSM) is one such advancement in assisting and controlling method development and optimization in sample preparation and analysis [5]. Because of the extraction efficiency and shorter extraction time for polar, non-polar, and thermolabile bioactive components than other techniques, UAE has been selected for such studies [6].

Several analytical methods have been reported for the determinations of phytochemical contents and effective concentrations (EC50) in different sample matrices, especially in fruits, vegetables, and their by-products. For instance, classical titration [7], electrochemical [8], enzymatic [9], spectrophotometric [10], capillary electrophoresis [11], and chromatographic [12] methods have been frequently used to determine phytochemicals including AA in various plant materials. However, there are limited reported studies on method development and optimization of extraction and determination of phytochemicals particularly AA using the HPLC-DAD technique in *Syzygium guineense* (willd.) fruit and its seed using RSM. On the other hand, the antioxidant activities of most plant extracts are evaluated by determining the EC50 values. EC50 values have been used to evaluate drug discoveries and their performance, the effects of pesticides and fungicides on pastes and fungi, stimulus toxicity dose-response relationships studies, and environmental toxicants [13]. Nowadays, the applications are extending to functional/nutraceutical foods to see the health effects of food or food products on humans [14,15]. For this purpose, a new statistical tool known as GraphPad prism software has been used for the estimation and evaluation of EC50 values of fruit extracts.

Therefore, this study aimed to optimize UAE and analytical conditions for the determination of AA content, TPC, TFC, and antioxidant activities of wild edible *S. guineense* fruit and its seed. In addition, the UHPLC-DAD method was developed and validated for the determination of AA.

2. Materials and methods

2.1. Raw material collection

About 5 kg of fresh *S. guineense* fruit samples were collected from North Gondar (Amhara region), Ethiopia in April 2020. The botanical specimens such as leaves, stems, and fruits were also collected and identified by their voucher number, 002/TBA/2020. The samples were collected in the morning sessions based on their accessibility or convenient sampling method. All the collected fruit samples were packed in a clean and air-tight polyethylene flexible plastic bag and transported using an ice box to Addis Ababa Science and Technology University (AASTU) laboratory. Ethiopia.

2.2. Sample pre-treatments

The collected fruits were washed with tap water followed by distilled water and the flesh edible parts of the fruits were detached from the seeds. Then *S. guineense* fruits and their seeds were finely chopped to smaller sizes and dried using three different drying methods, namely sun-drying (SD), drying at room temperature (room temperature/shade drying = RD), and vacuum freeze-drying (VFD) (Lablyo Plus, Britain). The drying methods were based on the reported study by Ghafoor and coworkers [16] with major modifications. The SD was performed in the temperature range from 25.8 to 38 °C which has not been studied in the reported method. The temperatures were recorded three times a day at 9:00 a.m., 12:30 a.m., and 3:30 p.m. The RD was performed in the temperature range from 19.9 to 23.7 °C. The temperatures were recorded at 9:00 a.m., 12:30 a.m., and 3:30 p.m. The properly chopped fruits were spread in thin layers on the plates/trays of VFD while the reported method has been sprayed sliced samples in the VFD dishes. The samples were frozen at −40 °C in a deep freezer for more than 6 h while in the reported method the slices have been frozen at −30 °C for 24 h at a pressure of −50 Pa. The initial temperatures of four probes were adjusted at −40 °C for 14 h, at −20 °C for 20 h, and at 0 °C for 14 h while the probe temperatures have not been reported in the reported study. The vacuum pressure and condenser temperatures were set at 1 Pa and −50 °C, respectively, while in the reported study the vacuum pressure and condenser temperature have been stated as 20 Pa and −58 °C, respectively. Then, the samples were assigned as SGFfd for freeze-dried *S. guineense* fruit, SGFrd for

room-dried *S. guineense* fruit, SGFsd for sun-dried *S. guineense* fruit, and SGS for *S. guineense* fruit seed. Both dried fruit and seed samples were ground by stainless steel grinding Mill (700 g Electric Grains, China) to a fine powder (250 μm sieve size) and stored in the refrigerator (4 °C) until extraction. The effects of drying methods on TPC, TFC, antioxidants, EC50, and AA constituents were investigated.

2.3. Optimization of extraction conditions for ultrasound-assisted extraction (UAE)

2.3.1. Optimization using response surface methodology (RSM)

Ultrasound-Assisted Extraction (UAE, Ultrasonic cell crusher and intelligent ultrasonic processor, SJIA-950W, China) was used for the extractions of AA, TPC, TFC, and DPPH antioxidant assay as EC50 (as responses) from the *S. guineense* fruit and its seed [17]. RSM optimization using UAE was done using Design Expert statistical software (model StatEase DesignExpert, v13). Face-centered central composite design (FC-CCD) was used to determine the number of experiments to investigate the effects of time (min, A: 5–25), temperature (°C, B: 20–45), methanol concentration relative to ultrapure water (%, C: 45–100), and solid-to-solvent ratio (ratio, D: 5–25) to obtain the optimum conditions for the determinations of TPC, TFC, antioxidant assay, and EC50 values in *S. guineense* fruit and its seed (Table 1). On the other hand, the effects of time (min, A: 5–25), temperature (°C, B: 10–30), and solid-to-solvent ratio (ratio, C: 5–25) on the determinations of AA were investigated. The extraction conditions for UAE were: pulse on was 4 s, pulse off was 2 s, probe type was @6 and power was 50% of 950 W. For the optimization processes, SGFrd and SGS were used after gross estimations of the responses. The experimental and coded levels of factors and second-order model/quadratic regression equation, which includes all interactions, are given in equation (1). All interactions were used to calculate the predicted response [7,18].

$$Y = \beta_o + \sum_{i=1}^{n} \beta_i A + \sum_{j=1}^{n} \beta_j B + \sum_{k=1}^{n} \beta_k C + \sum_{l=1}^{n} \beta_l D + \sum_{ii=1}^{n} \beta_{ii} A^2 + \sum_{jj=1}^{n} \beta_{jj} B^2 + \sum_{kk=1}^{n} \beta_{kk} C^2 + \sum_{ll=1}^{n} \beta l_l D^2 + \sum_{i\neq j=1}^{n} \beta_{ij} AB + \sum_{i\neq k=1}^{n} \beta_{ik} AC + \sum_{i\neq l=1}^{n} \beta_{il} AD + \sum_{j\neq k=1}^{n} \beta_{jk} BC + \sum_{j\neq l=1}^{n} \beta_{jl} BD + \sum_{k\neq l=1}^{n} \beta_{kl} CD + \varepsilon \quad (1)$$

where, Y is the response (TPC, EC50, or AA), A, B, C, and D are the independent variables, β_o is the intercept, β_i, β_j, β_k, and β_l *are linear terms,* β_{ii}, β_{jj}, β_{kk}, and β_{ll} are quadratic terms, β_{ij}, β_{ik}, βi_l, β_{jk}, βj_l, and β_{kl} are interaction coefficients of the factors, n is the number of variables (n = 4 for TPC and EC50, n = 3 for AA) and ε is the random error components that are normal and independently distributed with mean zero and constant variance.

Furthermore, validation experiments were carried out under optimized process conditions, and the percentage relative error (RE) between predicted and experimental values of responses was calculated using equation (2).

$$RE\ (\%) = \left[\frac{Predicted - Experimental}{Predicted}\right] \times 100 \quad (2)$$

in this FC-CCD, three axial points (−1, 0, +1) were used in the response surface model. Response surface graphs were obtained using the predicted values for the adjusted models at the 5% probability level (α = 0.05). Experiments using UAE were performed using the optimized parameters via FC-CCD to confirm the prediction of the mathematical model for all dependent variables (Table 1). As shown in Table 1, four independent variables with three-level of FC-CCD consisted of 30 experimental runs including 6 center points and 24

Table 1
Experimental variable ranges with experimental and coded levels using FC-CCD.

Independent variables	Units	Levels of coded variables for TPC and EC50				
		−α	Low	Center	High	+ α
		−2	−1	0	+1	+2
Time (A)	Min	7.5	5	17.5	30	42.5
Temperature (B)	°C	15	25	35	45	55
MeOH Conc. (C)	%	25	50	75	100	125
Solid-to-Solvent Ratio (D)	%	5	5	15	25	35
Independent variables	Units	Levels of coded variables for AA				
		−α	Low	Center	High	+ α
		−1.682	−1	0	+1	+1.682
Time (A)	Min	5	5	12.5	20	20
Temperature (B)	°C	10	10	20	30	30
Solid-to-Solvent Ratio (C)	%	5	5	15	25	25

FC-CCD = face-centered central composite design, the distance between the axial points to the center point was calculated using $\alpha = (2^n)^{1/4}$, where n is the number of variables, MeOH Conc. = Methanol concentration relative to ultrapure water. Point of prediction tool; α value was 0.05, the Tolerance proportion was 0.99 and the lower or upper-value interval was two-sided. The selection of optimum conditions was based on the high contents of AA, TPC, and antioxidant activities based on the desirability function of the Design Expert Software (Stat-Ease DesignExpert v13, Stat-Ease, Inc., USA). The optimized values of time and temperature were 10 min and 20 °C for AA (three factors), respectively.

non-center points were used for TPC and EC50 (Table 1). Additionally, three independent variables with three levels of FC-CCD consisting of 20 experimental runs including 6 center points and 14 non-center points were used for AA experimental conditions (Table 1).

Further, five levels of the two variables were coded to lie at ±1 for the factorial points, 0 for the center points, and ±α for axial points (Table 1). The distance between the axial points to the center point was ±2 and ± 1.682 for TPC/EC50 and AA, respectively (Table 1). The codes were calculated as a function of the range of interest of each factor. Experiments were performed as a single block and the order of runs within the block was randomized.

2.3.2. Ultrasound-assisted extraction

After optimization, about 1 g of SD, RD, and VFD powders of fruit and the seed samples were mixed separately with 15 mL of the mixture of methanol (MeOH) and ultrapure water (75:25 ratios). Then the mixtures were sonicated for 15 min at 35 °C and 50% of 950 W sonication power. On the other hand, 15 min of extraction time and 15 mL of 1% aqueous glacial acetic acid (AcOH) solid-to-solvent ratio of 1:15 at 20 °C were used for the determinations of AA. Then, the extract volume was made to 50 mL in a volumetric flask using methanol. The results of AA, TPC, TFC, DPPH-based antioxidant activities, and EC50 responses in triplicate measurements were fitted with a quadratic model. In particular cases, the DPPH and its EC50 activities were designed using 9–11 points of a multiple regression model (from the smallest concentration of 0.05 mg/mL to 80 mg/mL for samples and 0.025 mg/L to 500 mg/L of AA, respectively).

2.4. Soxhlet extraction

The solvent extractions of dried *S. guineense* fruit and its seed were carried out following the method reported by Belayneh and coworkers [19] with minor modifications. The dried fruit and seed samples were weighed (10 g each) and added to a Soxhlet extractor thimble (Bucher, v16, Germany) followed by methanol (MeOH, 150 mL, >99%, Sisco, India) in the ratio of 1:30 w/v. The methanol (MeOH) extraction was carried out at 50 °C for 4 h using a Soxhlet extractor. The sample extracts were cooled to room temperature (24 ± 2 °C) and filtered through Whatman filter paper (No 1, Sigma-Aldrich, Germany). Then the extract volume was made to 50 mL in a volumetric flask using methanol. Finally, the extracts of fruit and seed powders were used to evaluate the TPC, TFC, and DPPH/EC50 for comparison.

2.5. Determination of TPC, TFC, and free radical scavenging activities

2.5.1. Total phenolic content (TPC)

The TPC of the SD, RD, and VFD fruit and seed extracts were determined by UV-VIS spectrophotometer (Jasco – V-770, Japan) using the *Folin-Ciocalteu* reagent method [19] with minor modifications. Briefly, 400 μL of the extract was mixed with 400 μL of *Folin--Ciocalteu* reagent which was diluted with methanol (1:10 ratio, respectively). Then, after 5 min 400 μL of 0.2 mM Na_2CO_3 solution was thoroughly mixed with the prepared mixture. After 10 min 200 μL of 3% $NaNO_2$ solution was added. The mixture was left for 60 min and absorbance was measured at 765 nm using a UV–Vis Spectrophotometer (with 500 μL size quartz cuvette). Finally, the concentration of TPC in the fruit extract was calculated from the gallic acid (98%, Acros Organics, Belgium) standard calibration curve (ranging from 50 to 400 mg/L) with absorbance at 765 nm. Results were reported as gallic acid equivalent in milligrams per gram of dried extract (mg GAE/g d.w.).

2.5.2. Total flavonoid content (TFC)

The in-house method was used for the determination of TFC based on the procedure described by Belayneh et al. [19]. Briefly, an aliquot of 200 μL of SD, RD, and VFD fruit extracts solutions was thoroughly mixed with 200 μL of 2% $AlCl_3$ solution and allowed to stand for 30 min in the dark at room temperature. The absorbance of the clear-yellow colored solution was measured at 417 nm using a UV-VIS Spectrophotometer (with a 500 μL size quartz cuvette). Then, the concentrations of TFC in each extract were calculated from the standard calibration curve of quercetin (Reference standard, ANPEL Lab. Tech. Inc., Shangai, China) ranging from 50 to 500 mg/L. Results were reported as quercetin equivalent in milligrams per gram of dried extract (mg QE/g d.w.). The analysis was repeated three times.

2.5.3. Scavenging activity of DPPH radical

DPPH is a molecule containing a stable free radical that measures the free radical scavenging capacity of the *S. guineense* fruit and its seed under three drying conditions. The antioxidant scavenging activities of fruit extracts using Soxhlet and UAE methods were determined based on the scavenging effect on the stable DPPH free radical activity. The assay followed a method reported by Belayneh et al. [19]. Briefly, the DPPH (98%, Acros Organics, Belgium) stock solution was prepared by dissolving 1.9716 mg DPPH in methanol in a 50 mL brown volumetric flask, and the absorbance reading was adjusted to 1 ± 0.02. In 5 mL amber vials, 2850 μL DPPH solution was added to 150 μL each diluted fruit extract or vitamin C series standards. In the presence of an antioxidant, the purple color of DPPH free radical decay was formed which can donate an electron to DPPH. The AA standard curve was linear between 0.025 mg/L to 500 mg/L concentrations. After shaking properly, the mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank.

2.5.4. Effective concentration (EC50)

After determining the %RSA of the plant samples, the estimations of EC50 using DPPH free radical scavenging activities of the fruit

and its seed extracts were performed using the GraphPad Prism Statistical model [15]. The EC50 evaluations from different concentrations (9–11 different concentrations) were performed by non-linear regression that is Log[agonist - oxidant inhibitor conc.] in mg/mL versus normalized response – variable slope (% of inhibition – %RSA) for each antioxidant parameter. The model was effective [14,15,19] with real samples to estimate EC50 values within narrow ranges of maximum and minimum points.

2.6. Ascorbic acid extraction and determination by UHPLC-DAD

2.6.1. Extraction

Ascorbic acid (AA) is a water-soluble strong antioxidant compound mainly found in fruits and vegetables. The methods reported by Orsavová et al. [12] and Spínola et al. [20] were the initial bases for the current method development and optimization. In the reported studies, *meta*-phosphoric acid (mPA) [20], the mixtures of phosphoric and acetic acids, and their buffers [12,21] have been used as the main extraction solvents. In the current study, the method was developed after several repeated trials with different solvent systems. Such solvent systems were MeOH, a mixture of MeOH and H_2O, aqueous phosphate (MPA) buffer (pH = 2.5–4) alone, the mixture of MPA with MeOH, o-H_3PO_4 alone, and a mixture of MeOH, 0.5–2% AcOH (HPLC grade >99%, Sigma-Aldrich, Germany) alone and with MeOH. All trials were not successful due to different problems such as drift (negative) peaks, unresolved peaks, and lower yields of AA recorded in the fruit and seed. The extraction and analysis of AA in the fruit and seed were successful using 1% aq. AcOH. In brief, 30 mL of 1% aq. AcOH was added to around 2 g of fruit and peel powder. The mixtures were extracted with UAE for 10 min at the temperature of 20 ± 1 °C (the detailed optimization procedure has been mentioned in RSM optimization of UAE). The temperature was maintained with ice placed in the other beaker into which the sample containing beaker was placed. The extracted mixture was centrifuged at 3500×*g* for 15 min at room temperature. The supernatant solution was transferred into a volumetric flask and adjusted to 50 mL with 1% AcOH and kept in the dark place at room temperature (20–23 °C) and filtered with a 0.45 μm membrane syringe filter and taken for UHPLC-DAD analysis.

2.6.2. Chromatographic conditions and analysis

AA content determination was performed using UltiMate 3000 Dionex UHPLC-DAD (Thermo Scientific, Germany) [12]. The reverse phase column (5 μm Fortis C18) with 250 × 4.6 mm was used and the mobile phase used was A – MeOH and B – 1% aq AcOH. The chromatographic linear gradient conditions were as follows: at 0 min equilibration starts and ends at 1 min with 100% of (B), up to 3 min–90% of (B), up to 6 min–60% (B), up to 10 min–40% of (B), up to 15 min–100% of (B), up to 20 min–80% of (B) and 25 min–90% of (B). The flow rate was 0.8 mL min^{-1}, injection volume was 10 μL, column temperature was 25 °C, and run time was 25 min. The measurement process and data analysis were controlled using Chromeleon v7.2 SR4. The analysis was recorded using multi-wavelength at 248, 254, 272, 278, and 450 nm, and the best results were recorded at 248, 272, and 278 nm. Finally, the optimum wavelength of 278 nm, the one having fewer interferences, was selected and the analysis was performed. The amounts of AA in the SD, RD, and VFD fruit extract samples were calculated using equation (3).

$$AA\left(\frac{mg}{100\,g}\right)=\frac{C\times V\times DF\times 100}{m\times 100} \tag{3}$$

where *C* is the concentration of *AA* obtained from the calibration curve ($C=(Y-b)/m$) in mg/L. *V* is the final volume of the sample in L, *DF* is the dilution factor and m is the mass (g) of the fruit and seed powders taken for extraction.

2.6.3. Method validation

A standard stock solution of AA was prepared by dissolving 100 mg in 100 mL of 1% aq. AcOH (HPLC grade). From this stock standard solution, 11 points of calibration standards (0.5, 1, 2.5, 5, 10, 20, 40, 60, 80, 100, 150 mg/L) were prepared in 1% aq. AcOH. The calibration curve standards were injected in triplicates and the standard curve was prepared by plotting the average of the concentrations of each standard versus peak area (mAU).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the repeated measurements of the blanks (n = 9) and calculated as ($LOD = 3S$ *and* $LOQ = 10S$, where 'S' is the standard deviation of the repeated measured blanks. The percent recovery experiment was done by spiking AA in the sample. In this experiment, 20 and 40 mg/L of AA standards were added to the 1 g of fruit powder samples. Then, the extractions and analyses of 7 replicates of recovery experiments were performed within the same and three different days. The inter- and intra-day recovery experiments (n = 7) were performed to see the precision (repeatability) and accuracy of the developed and optimized method within the same laboratory. All the recovery, sample extractions, and analysis were conducted within 24 h.

2.7. Statistical analysis

The results obtained in this study were expressed as mean ± standard deviation of at least three replicates. The analysis of variance and significant differences among the means of triplicate measurements were performed with one-way and two-way ANOVA using SPSS v24. Tukey Post Hoc multiple comparisons were used to compare each case. Additionally, ChemDraw (Ultra 12.0.2, CambridgeSoft), Design Expert (StatEase V13), and excel (2010) statistical tools were used based on the types of data obtained [22].

3. Results and discussion

3.1. Response surface methodology (RSM) for method development and optimization

3.1.1. Method optimization

Designed analytical experiments are a series of trials that can change the amounts of target responses against selected factors and their interaction effects. Thus, DoE in analytical methods can be used in method development and optimization with the help of statistical models such as RSM. In the present investigation, the relationships between the four independent variables and the responses (EC50 and TPC) using FC-CCD (Table 1) have been studied. Three factors were also identified for AA. The predicted and optimized independent variables for all responses were not significantly varied except for small changes with extraction time (predicted time was 17.5 min for EC50 and TPC, 12.5 min for AA, and optimized time was 15 min for EC50 and TPC and 10 min for AA) (Table 2).

Optimizations of extraction conditions were carried out by applying a quadratic model equation. The result of the optimization experiment showed that 1% aqueous AcOH was the best and the most selective extraction solvent giving high amounts of AA. The model indicated a statistically significant ($p < 0.001$) and goodness of fit for TPC, EC50, and AA contents in all three selected sample types. The predicted R^2 values (0.9979–1.00) of all of the regression quadratic models and their fit statistics for the determinations of TPC, EC50, and AA in *S. guineense* fruit and its seed were in reasonable agreement with the adjusted R^2 (the difference is less than 0.2). This indicated that the model was accurate and well-fitted with the data for the selected factors and responses [6,18]. The model F-values of the range from 98.477 to 568.684 (greater than 4 is desirable) imply the model is significant ($p < 0.01$). Moreover, the model *P*-values less than 0.01 indicated the model terms are significant at a 99% confidence interval. The coefficient of variance (C.V.) ranged from 0.11 to 1.69% also indicated the designed model was significant for the determination of all of the responses. In the cases of time, temperature, methanol concentration, solid-to-solvent ratio and all the interaction effects of these factors are significant for the optimization of the extraction processes. The experimental and the predicted data of TPC, EC50, and AA were in good agreement with the overall errors below 0.01%. It also indicated a hypothesized distribution of predicted data fitted with experimental data. This is to illustrate how the predicted data and the experimental data plots of independent factors could be useful in determining the optimum values of responses.

3.1.2. Response surface methodology (RSM) optimization for TPC and EC50

Experimental results were analyzed to determine the effect of extraction time (A), temperature (B), MeOH concentration (C), and solid-to-solvent ratio (D) on TPC and EC50. The experimental equations in terms of coded factors were fitted with the quadratic equations (4) and (5):

$$TPC = 1830.08 + 1.04A + 1.83B + 4.54C + 33.33D - 19.43AB + 14.76AC + 6.89AD + 5.57BC - 17.44BD + 6.80CD - 94.73A^2 - 24.69B^2 - 49.44C^2 - 47.48D^2 \quad (4)$$

$$EC_{50} = 0.94 + 0.06A + 0.06B + 0.06C - 0.41D + 0.13AB - 0.10AC - 0.10AD + 0.1BC + 0.21BD - 0.06CD + 0.21A^2 + 0.36B^2 + 0.09C^2 + 1.01D^2 \quad (5)$$

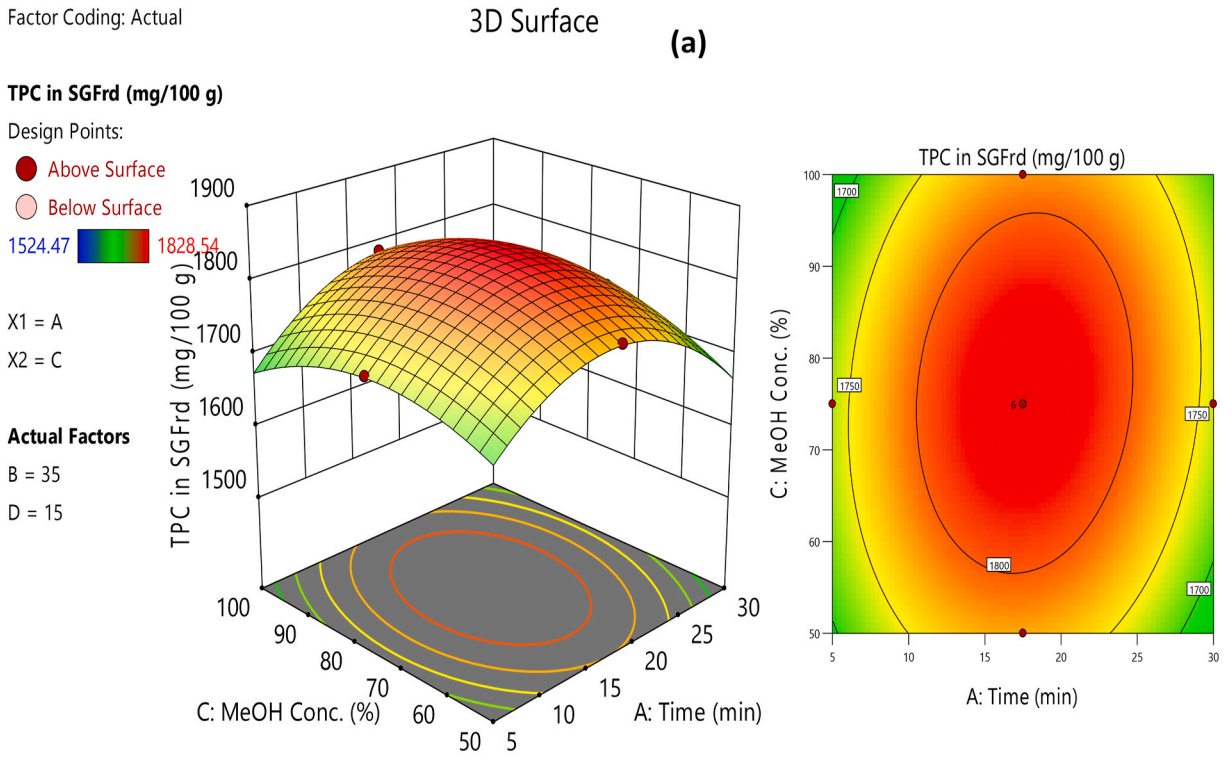
where; *A, B, C,* and *D* are the independent variables.

In this case (Eqs. (4) and (5)), *A, B, C, D, AB, AC, AD, BC, BD, CD, A^2, B^2, C^2, D^2* are significant ($p < 0.001$) model terms for the investigations of TPC and EC50 in *S. guineense* dried fruit and its seed. The models indicated significant ($p < 0.001$) values with experimental data and the regression equation showed positive significant ($p < 0.001$) linear (A, B, C, and D, except D in EC50) effects. The interactive effects of time and MeOH conc. (AC), time and solid-to-solvent ratio (AD), and MeOH concentration and solid-to-solvent ratio (CD) showed a positive effect on the TPC model (Eqs. (3) and (4)). The negative interactions of linear AB and BD, and all of the quadratic terms showed the saddle contour plots or minimax and confined elliptical to the center of the contour plot or 3D

Table 2
Model validation with optimum time, temperature, MeOH conc., and solid-to-solvent ratio conditions on the TPC, EC50 and AA contents of SGF and SGS.

Predicted Vs Experimental	Time (min)	Temp. (°C)	MeOH Conc. (%)	SSR	75% MeOH UAE from SGFrd (mg/100 g)			75% MeOH UAE from SGS (mg/100 g)		
					TPC	EC50	AA	TPC	EC50	AA
Predicted	17.5/12.5	35/25	75	15	1830.08	0.94	160.53	1913.85	0.98	4.09
Experimental	15/10	35/25	75	15	1828.54 ± 6.74	0.94 ± 0.00	155.73 ± 5.11	1917.40 ± 26.15	0.97 ± 0.03	4.02 ± 0.42
RE (%)	14.29/20	0.00	0.00	0.00	0.08	0.00	2.99	0.19	1.02	1.71

RE – Relative error, Vs – versus, MeOH – methanol, Temp. – Temperature, SSR – Solid-to-Solvent Ratio.



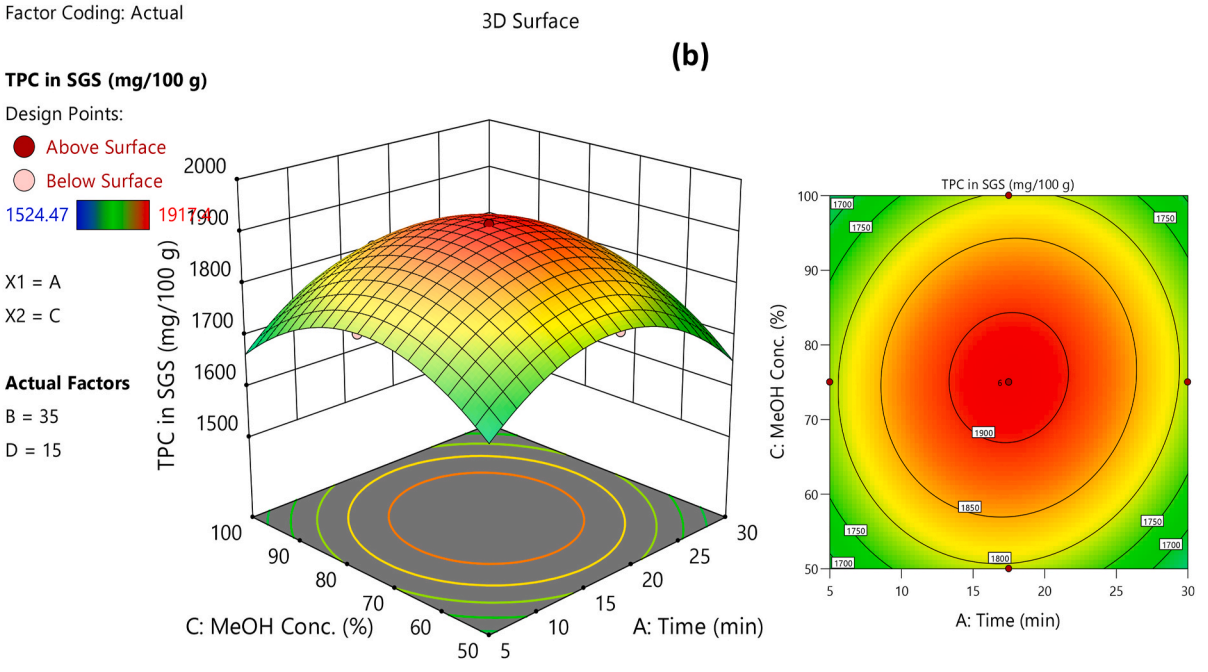
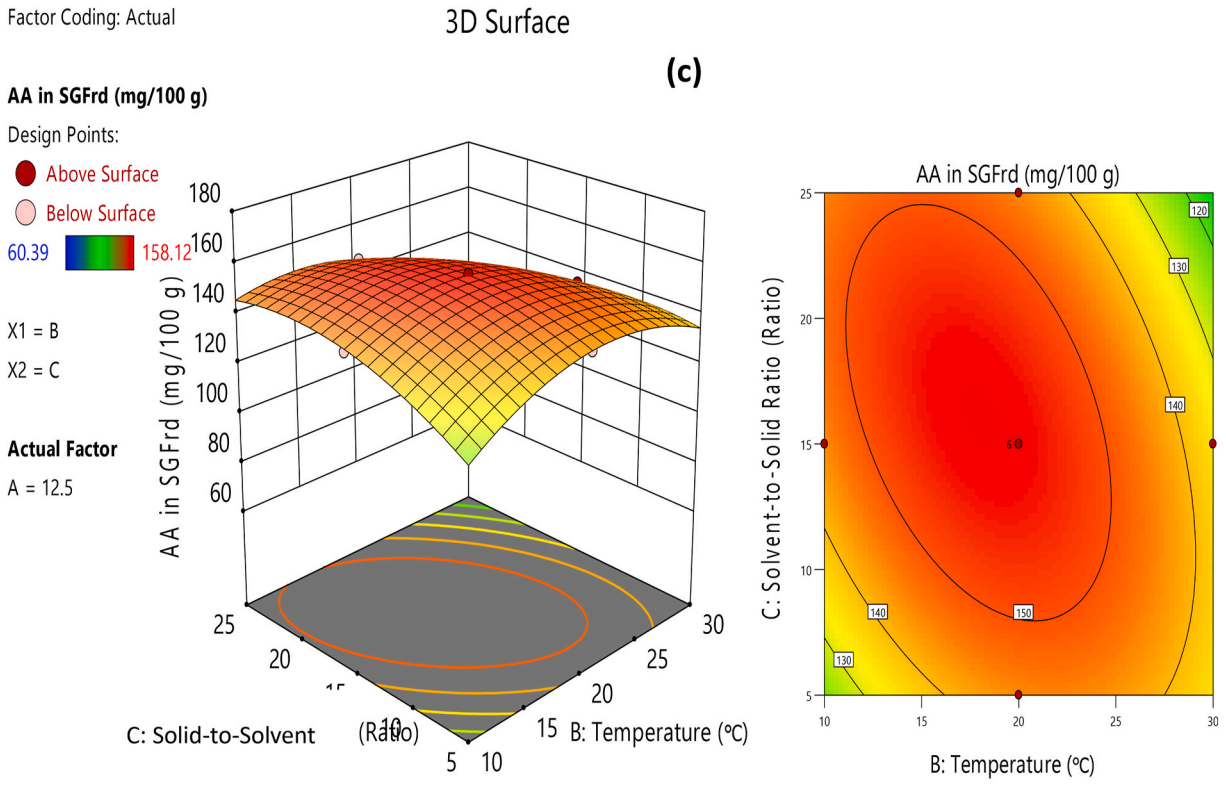


Fig. 1. Interaction effect of extraction variables (time, temperature, MeOH conc. and solid-to-solvent ratio) on TPC, EC50, and AA in SGFrd and SGS. These 3D surface graphs and contour plots show only two specific interaction effects as a function of time and MeOH Conc (A = TPC for SGFrd, B = TPC for SGS, C = EC50 for SGFrd, D = EC50 for SGS, E = AA for SGFrd, and F = AA for SGS), for the rest of the graphs it is available in supplementary files.



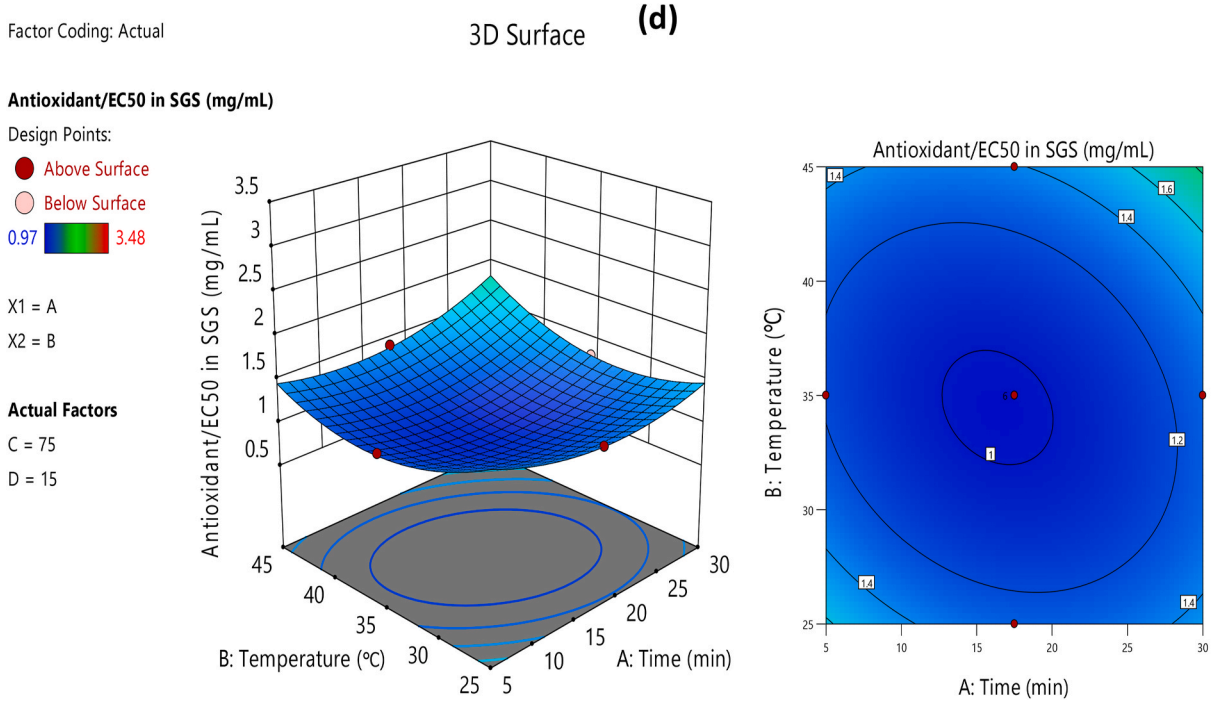
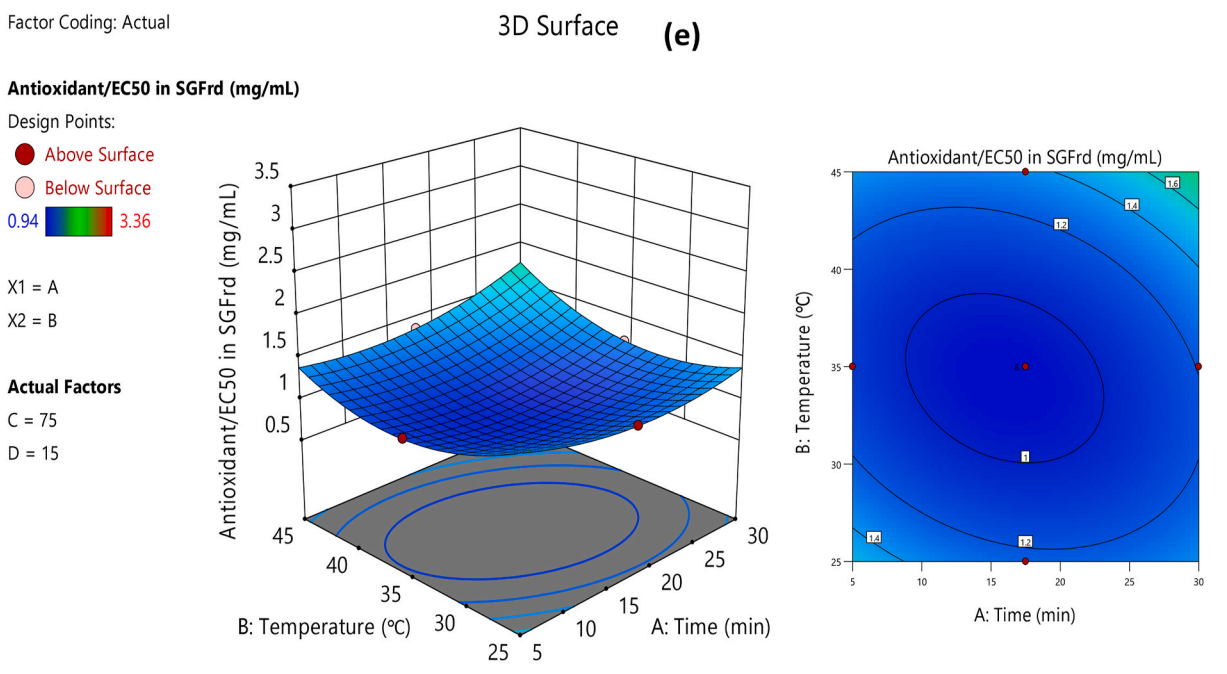


Fig. 1. (*continued*).



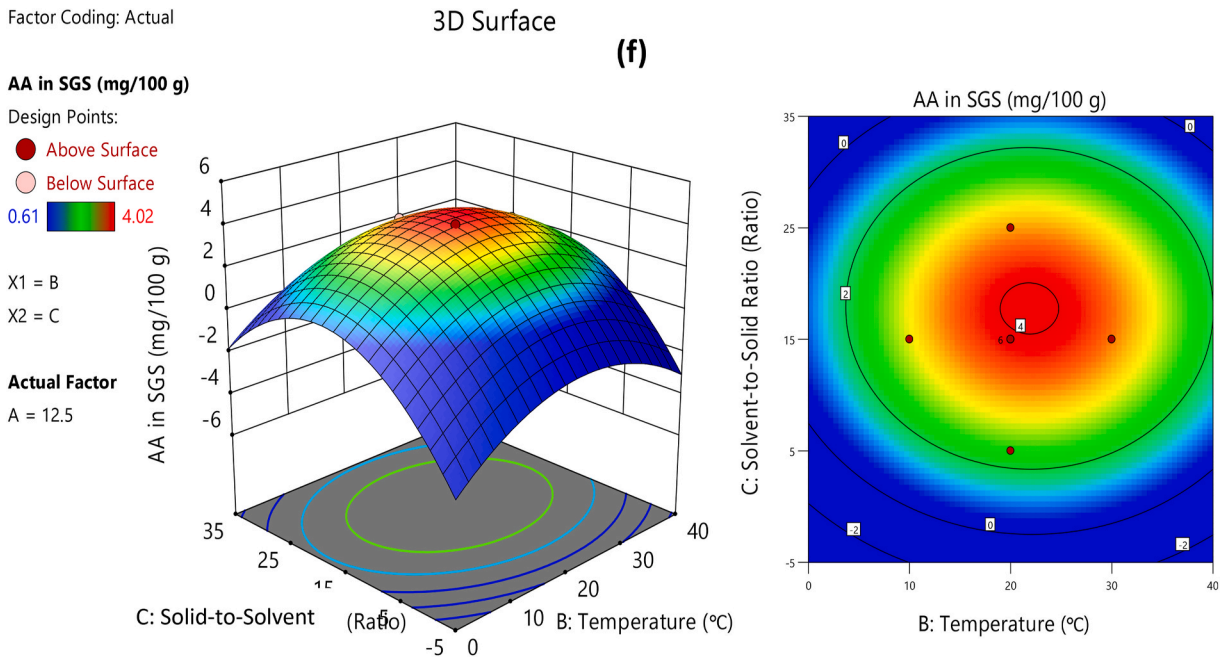


Fig. 1. (*continued*).

graph surfaces [23].

The 3D surface graphs and contour plots of TPC and EC50 in SGFrd (Fig. 1a and 1c) and SGS (Fig. 1b and 1d) showed that there was a significant ($p < 0.001$) positive effect on extraction time, temperature, solvent mixture, and solid-to-solvent ratio in all of the responses. The contour and 3D surface graphs of TPC and EC50 change by time vs. MeOH conc. and temperature vs. solid-to-solvent ratio has a highly significant effect and shifts to the maximum, and minimum amounts (Fig. 1a, 1b, and 1c, 1d), respectively. With increasing extraction time and MeOH conc. from 5 to 17.5 min and 45–75%, the TPC amount for *S. guineense* dried fruit and seed increased from 1524.47 to 1828.54 mg/100 g and 1524.47–1917.40 mg/100 g then decreased after optimum values. The TPC values of *S. guineense*

fruit and its seed were increased to the maximum (15 min) inhibition concentrations with increased values of temperature and solid-to-solvent ratio from 25 to 35 °C and from 1:5 to 1:15 (w/v).

The three factors, namely time, temperature, and solid-to-solvent ratio also showed a significant effect on the experimental results of AA. The 2nd order polynomial equation in terms of coded factors was fitted with equation (6):

$$AA = 155.43 + 13.71A - 5.09B + 0.40C + 12.75AB + 12.81AC - 10.82BC - 10.77A^2 + 15.56B^2 - 10.57C^2 \quad (6)$$

where; *A, B, C,* and *D* are the independent variables.

In this case (Eqs. (4) and (5)), *A, B, C, AB, AC, A^2, B^2, C^2* are significant model terms for the investigations of AA in *S. guineense's* dried fruit and seed. Fig. 1e and 1f shows the effect of extraction time and temperature, temperature, and solid-to-solvent ratio on the AA values of the fruit and the seed. The model indicated significant ($p < 0.001$) values with experimental data and the regression equation showed a positive significant ($p < 0.001$) linear (A, B, and C) effect. The interactive effects of time and temperature, temperature, and solid-to-solvent ratio showed a positive effect on the AA model (Eq. (5)). All of the quadratic terms showed significant ($p < 0.001$) positive effects on AA in both SGFrd and SGS (Eq. (6)).

The 3D surface graphs and contour plots (Fig. 1e and 1f) showed that there was a strong significant ($p < 0.001$) positive effect on AA extraction time, temperature, and solid-to-solvent ratio. The contour and 3D surface graphs of AA in both SGFrd and SGS dried powders were more significantly affected by temperature vs. solid-to-solvent ratio to the maximum (Fig. 1e and 1f). With increasing extraction temperature vs. solid-to-solvent ratio from 10 to 20 and 1:5 to 1:15, the AA amount for *S. guineense* dried fruit and its seed increased from 60.39 to 158.12 and 0.61–4.02 mg/100 g, respectively and then decreased after 10 min.

In general, the contour plots that indicated the center of the plots showed symmetrical surfaces with maximum responses of TPC and AA in all fruit samples at the center of the peak. A similar shape could be found for minimum responses of EC50 values for all of the dried fruit and seed powder extracts since the contours would have smaller values of the responses as it is closer to the contour center (Fig. 1a–f). Such results indicate where optimal interaction effects of time, temperature, MeOH conc. and solid-to-solvent ratio towards a maximum (TPC and AA) and minimum (EC50) amount in the investigation. Very few plots such as effects of time vs solid-to-solvent ratio (TPC in SGS), temperature vs solid-to-solvent ratio (TPC in SGS), time vs MeOH conc. (EC50 in SGFrd) and time vs solid-to-solvent ratio (AA in SGFrd showed saddle contour plots or minimax which indicated that the responses could decrease from the center of the region. The maximum values predicted by the surfaces were confined elliptical to the center of the contour plot which indicated the reasonable interactions between the independent variables. Therefore, in conclusion, the optimized significant combinations of time, temperature, solvent mixture (MeOH conc.), and solid-to-solvent ratios were obtained for the maximum recoveries of AA, TPC, and EC50 (eqs. (4)–(6)).

The same conclusions can be made for 3D surface graphs; however, such a plot allows us to better visualize the increasing and decreasing levels of factors (Fig. 1a–f). These experimental factors on the recorded parameters (using a different model) have been reported on other foodstuffs and by-products such as bioactive compounds in wood apple fruit [24], and antioxidants in ternary mixtures of green, yellow, and red teas [25]. Additionally, the improvements in phenolic contents and antioxidant activities of *Berberis asiatica* fruits have been fitted with the quadratic model of CCD [23]. All of the factors and their interactive effects on the model significantly affected all of the response variables (Fig. 1a–f). In general, the FC-CCD was good (because R^2 is in reasonable agreement with the adjusted R^2) with symmetrical 3D surfaces graphs and contour plots to investigate the amounts of TPC, TFC, EC50 values, and AA in SGFfd, SGFrd, SGFsd, and SGS extracts. Additionally, the lack of fit is significant ($p < 0.001$) for the quadratic models.

3.1.3. Model validation

The criteria chosen to process model validation of independent and response variables were based on desirable predicted and

Table 3
Experimental values and comparison of total phenolic, flavonoid, antioxidant activities, EC50 values, and AA of SGFfd, SGFrd, SGFsd, and SGS fruit powders after optimization using RSM.

Fruits and Drying Methods	MeOH Soxhlet extract (mg/100 g)		75% MeOH UAE (mg/100 g)		1% aq. AcOH AA UAE (mg/100 g)
	TPC	TFC	TPC	TFC	
SGFfd	540.49 ± 24.28[a]	117.08 ± 10.08[a]	581.25 ± 37.13[a]	308.23 ± 15.94[a]	40.48 ± 2.50[c]
SGFrd	1130.11 ± 32.33[c]	498.143 ± 4.93[c]	1828.54 ± 6.74[c]	1324.20 ± 13.31[c]	155.73 ± 5.11[d]
SGFsd	937.98 ± 8.92[b]	404.65 ± 15.34[b]	937.98 ± 8.92[b]	1072.02 ± 41.41[b]	15.39 ± 2.03[b]
SGS	1285.49 ± 31.70[d]	556.22 ± 21.49[d]	1917.40 ± 26.15[d]	1428.36 ± 55.97[d]	4.02 ± 0.42[a]
	DPPH (mM/100 g)	EC50 for Soxhlet (mg/mL)	DPPH UAE (mM/100 g)	EC50 for UAE (mg/mL)	
SGFfd	341.43 ± 18.69[a]	2.55 ± 0.03[b]	464.93 ± 6.87[a]	1.96 ± 0.02[b]	
SGFrd	333.12 ± 6.23[a]	1.20 ± 0.02[a]	459.63 ± 4.51[a]	0.94 ± 0.00[a]	
SGFsd	346.41 ± 5.55[a]	2.25 ± 0.01[b]	467.74 ± 2.18[a]	1.87 ± 0.01[b]	
SGS	440.57 ± 3.68[b]	1.21 ± 0.02[a]	466.90 ± 1.95[a]	0.97 ± 0.03[a]	
AA Std	–	0.23 ± 0.04	–	0.23 ± 0.04	

SGF = *Syzygium guineense* fruit, SGS = *Syzygium guineense* fruit seed, fd = freeze dried, rd = room dried and sd = sun dried. The concentrations of DPPH Assays were based on the percent (%) radical scavenging activity (%RSA) results at maximum values (%RSA >90%); Letters with different superscripts and with no letter assignation in the same row are significantly different at $p \leq 0.05$. The Post Hoc Descriptive multiple comparisons of DPPH assay, and EC50 values were based on the %RSA of multiple point concentration graphs of regression graphs (Fig. 2).

response values as presented in Table 2. By running the optimum solution with the highest desirability, the criteria for numerical solution were analyzed to evaluate the validation of the model. Validation experiments were carried out based on the optimized solution and average values of responses compared with predicted ones. Based on the results, the percentage of RE varied from 0.00 to 2.99% (Table 2). All of the predicted and experimental values are less than 5% of RE. Thus, the results verify that the experimental data were in good agreement with the predicted data. Moreover, optimization of independent variable conditions of TPC and EC50 suggests that the maximum desirability function (1.00) that meets all the goals presented in Table 2 can be achieved through treatment of the SGFrd and SGS at 15 min, 35 °C, 75% of MeOH, and 1:15 of solid-to-solvent ratio. The non-significant ($p > 0.05$) value of lack of fit ($F = 0.99$) showed the model fitted well to the spatial influence of the independent variable on the responses [6,18].

3.2. In vitro-antioxidant analysis

3.2.1. Antioxidant activities

As shown in Table 3, the *S. guineense* fruit and seeds have high antioxidant potential. The TPC and TFC in the three sample powders were also determined. In addition, the three drying techniques, namely, SD, RD, and VFD were evaluated against these antioxidant activities. TPCs in *S. guineense* fruit varied between 540.49 ± 24.28 (SGFfd = *S. guineense* fruit freeze dried) and 1285.49 ± 31.70 (SGS *S. guineense* seeds) mg GAE/100 g using Soxhlet extraction. On the other hand, in the case of UAE of *S. guineense* fruit and the seed varied between 581.25 ± 37.13 (SGFfd) and 1917.40 ± 26.15 (SGS) mg GAE/100 g. There were significant ($p < 0.05$) differences between soxhlet extraction and UAE in terms of all of the studied parameters. The amount of TPC in SGFrd was the highest and the SGFfd TPC was the lowest among all of the other dried fruits (Table 3).

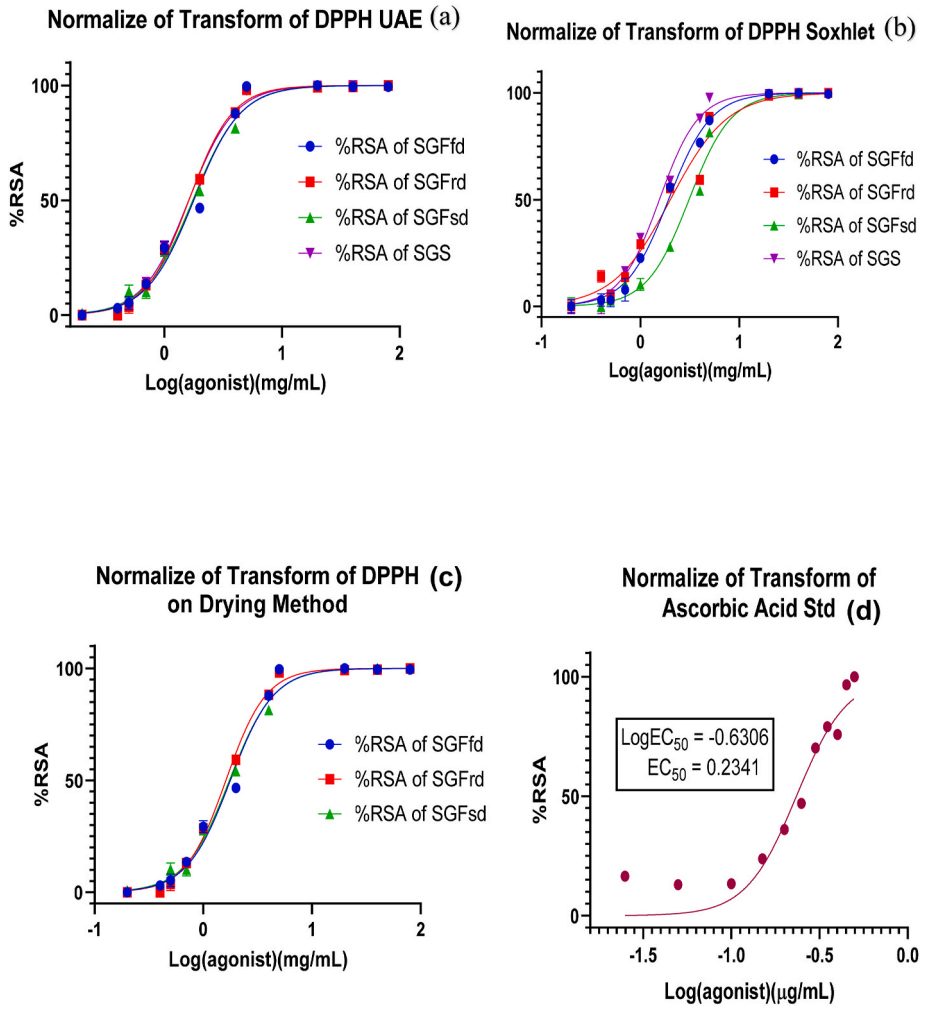


Fig. 2. Comparison of antioxidant activities of different concentrations of MeOH Soxhlet and UAE extracts of fruit species and ascorbic acid standard by spectrophotometric detection of the DPPH with increased concentration at 517 nm, respectively. Comparison of antioxidant activities of different concentrations of RSM optimized UAE extract of fruit drying methods by spectrophotometric detection of the DPPH with increased concentration at 517 nm (Std = Standard).

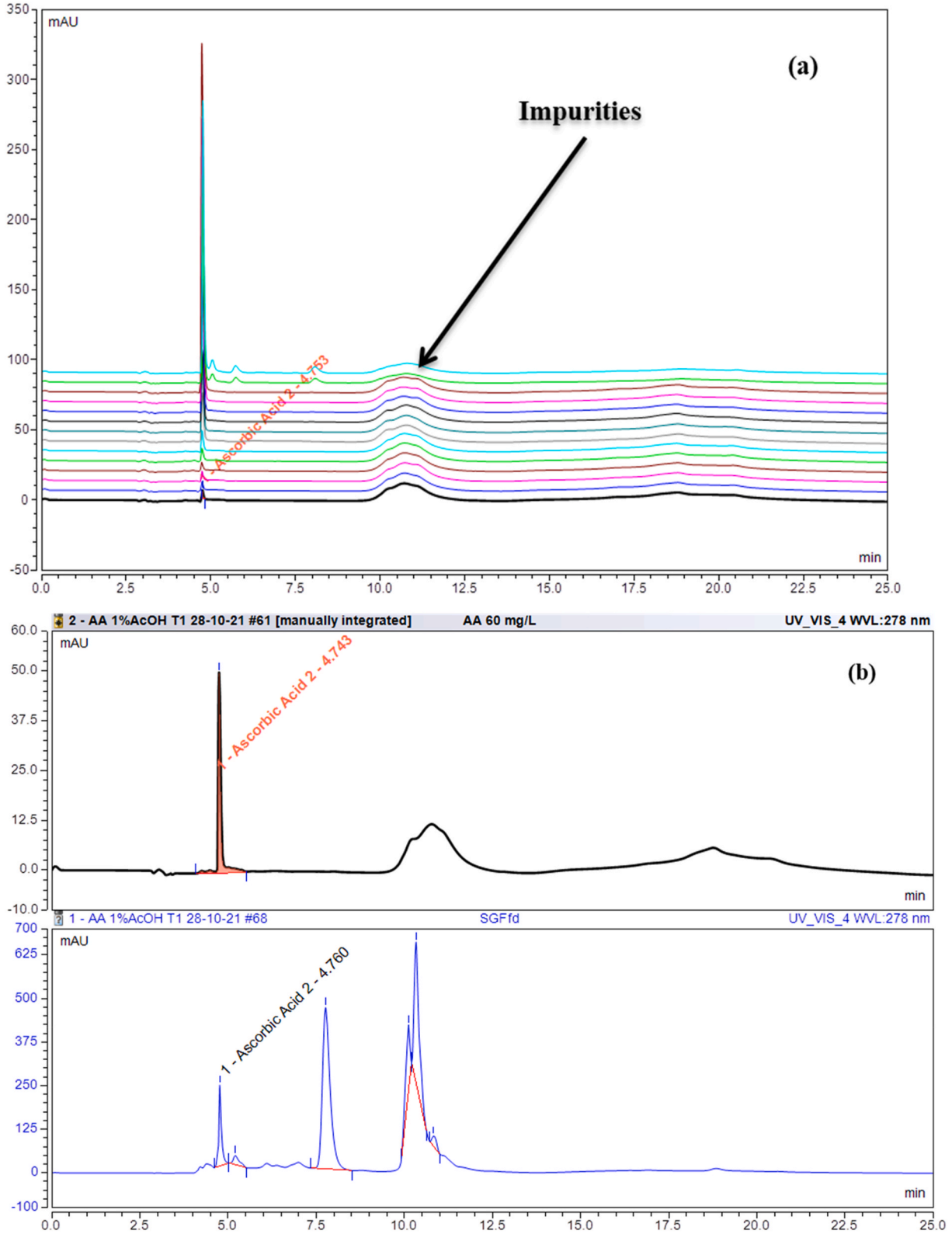


Fig. 3. The optimization process of UHPLC–DAD conditions for ascorbic acid analysis: chromatograms of L-AA standards (A), the stacked chromatogram of standard AA with sample extract (B), and overlaid chromatogram of samples with standards at optimized λ-max of 278 nm.

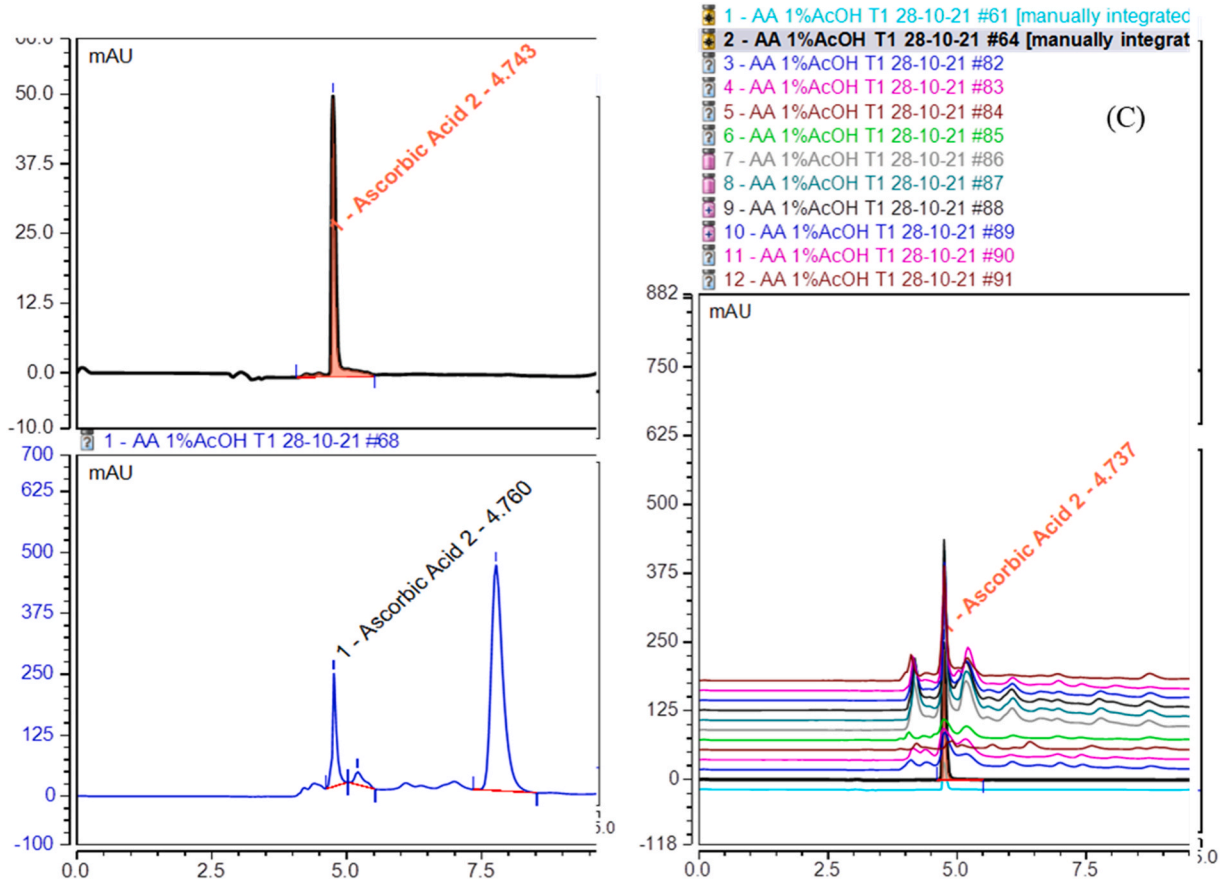


Fig. 3. (*continued*).

The TPC in the optimized methanol-water mixture (75:25 V/V) extracts of *S. guineense* fruit and its seed along with their drying effects were found in the decreasing orders of SGS > SGFrd > SGFsd > SGFfd for both Soxhlet and UAE methods. Similar results were obtained for TFC in both extraction methods and drying methods. This indicated that the contents of AA and the antioxidant potentials of fruits were well preserved in RD than in other drying processes [26]. In contrast, according to a reported study by Saifullah et al. (2019) [27], freeze-drying has preserved more amounts of bioactive components of the plant parts than room drying. On the other hand, it preserves more pigments than other bioactive components [28]. Recently, the effects of several drying methods such as VFD, RD (shade), SD, hot air, microwave, ultrasound, vacuum, and infrared drying techniques have been evaluated and reported on the other food matrices [27] in which freeze-drying has more preservative effects than others. The drying preservative effects depend mainly on the nature of the plants and their bioactive constituents [26–28].

The statistically significant tests ($\alpha = 0.05$) were performed among fruit samples and types of extraction methods used (Table 3). As a result, there were statistically significant differences ($p \leq 0.05$) between each fruit drying condition and seed powder as well as between the two extraction methods. Additionally, there were significant differences between and among each of the dried fruit powders at $p \leq 0.05$ which indicates that drying conditions affect the compositions of nutrients and bioactive compounds in the fruits.

The antioxidant activities of extracted samples were evaluated against commonly accepted in vitro assay of the DPPH method (Table 3). There were high DPPH activity differences between the RD and SD as well as between RD and VFD extracts. The radical scavenging activities of SGFrd and SGS extracts were recorded in higher amounts than the other counterparts. From the current study, it is possible to see that this plant is a good source of antioxidants.

3.2.2. Effective concentrations of fruit and seed extracts

The EC50 can be defined as the effective concentration of agonist that provokes a response halfway between the normalized 20% baseline and a maximum response of up to 100% [15]. The EC50 value is the concentration of the logarithm of the agonist that provokes a response halfway between the base-line (x-axis) and maximum response (y-axis) (Fig. 2). The logarithm of the agonist (log [agonist]) aimed to determine the EC50 of the agonist – the concentration that provokes a response equals 50% of the activities using multiple concentrations. The logarithm of the agonist means that the process of normalization (baseline noise – "all curves begin at 0 and plateau at 100%") and transform (best fit) of the data uphill (larger y and x values) by subtracting the base-line noise (minimum and maximum responses become plateaus (Fig. 2) – nearly constant responses).

Calculations of EC50 values of dried fruit powders for MeOH Soxhlet and RSM optimized MeOH-water mixture (75:25) extracts against DPPH assay were performed using the GraphPad Prism software model and presented in Table 3. The Soxhlet MeOH and UAE MeOH-water mixture extracts of *S. guineense* fruit and its seed had the highest inhibitory activity against DPPH radical as compared with EC50 estimation values of AA as a standard (Table 3). The tests were performed using 9–11 points of extract concentrations by decreasing the concentrations of each dried fruit and seed extract (80, 40, 20, 8, 4, 1, 0.8, 0.7, 0.5, 0.4, 0.2) mg/g or mg/mL of extracts of each fruit). The scavenging potentials of 100% MeOH Soxhlet extract fruit against EC50 values ranged from 1.55 ± 0.03 (SGFfd) to 1.10 ± 0.02 mg/mL (SGFrd) mg/mL. The scavenging potentials of RSM-optimized UAE ranged from 1.96 ± 0.02 (SGFfd) to 0.94 ± 0.00 (SGFrd) mg/mL.

The DPPH scavenging activities of SGFrd showed a significantly higher value than its seed (0.97 ± 0.03 mg/mL, Table 3) for both extraction methods. The EC50 value of SGFrd was significantly (at $p \leq 0.05$) lower than the SGFsd and SGFfd. The calculations of EC50 values were based on the method which was proposed for the first time by Chen et al. [14], Sridhar and Linton [15], and then recently by Belayneh et al. [19]. These estimations of EC50 values in *S. guineense* fruit and its seed were effective and can be applied to real samples (Fig. 2).

3.3. Ascorbic acid determination by UHPLC-DAD

Ascorbic acid is a water-soluble strong antioxidant component of fruits. In most cases, AA has been determined under acidic conditions (pH < 6 – strong acidic conditions mostly at pH values of 2.5–4.5) to reduce the degradation of AA during sample preparation, storage, and analysis [12]. As a result, extractions using *meta*-phosphoric acid and HPLC analysis using buffer solutions prepared from di- and mono-basic phosphate salts [20] have been frequently used to prevent the oxidation of AA. However, there are limited reported studies on extraction and the HPLC-DAD method of analysis for AA using aqueous acetic acid in any of the sample matrices. There are also limited reported studies on method development, optimization, and validation using RSM in underutilized wild edible fruits.

The extraction and analysis methods of L-AA (vitamin C) in dried *S. guineense* fruit and its seed powder extracts (SGFfd, SGFrd, SGFsd, and SGS) were developed and optimized. The UAE based on FC-CCD followed by UHPLC analyses were used for the optimization and method development of AA in the *S. guineense* fruit and its seed. In this study, a new method of extraction and analysis of AA was developed and optimized for different dried fruit powders using UAE and UHPLC-DAD methods. The dried fruit samples were extracted with 1% of aqueous AcOH and the recovery of AA (89.74–105.02%) in fruit was comparatively high (Table 3).

The method dynamic range was from 0.5 to 150 mg/L comprising 11 calibration points as depicted in Fig. D2 (Supplementary files) with a correlation coefficient, $r^2 = 0.9994$. The LOD and LOQ were 0.029 mg/100 g (29.01 μg/mL), and 0.0967 mg/100 g (96.71 μg/mL), respectively. The average recovery was 95.54 ± 4.33%. The ranges of recoveries in two days (n = 7 for each day) were 90.67–98.72% and 89.74–105.02%, respectively. The intra- and inter-day precisions of the method were 3.32% and 5.49% on different measurement days. As can be observed from the results, the method has shown good repeatability, reproducibility (within the same laboratory and analyst), sensitivity, and accuracy.

As shown in Fig. 3, selective and specific detection systems were developed using UHPLC-DAD. The maximum wavelengths were 230, 248, 254, 272, and 278 nm after scanning from 200 to 600 nm. The maximum absorbances of AA were obtained at the wavelengths of 248 (Fig. 3c and 3e, 272, and 278 nm (Fig. 3a, 3b, and 3d). The chosen optimized wavelength was 278 nm for subsequent analysis (Table 3). In this study, simple, selective, sensitive, accurate, and precise extraction methods were developed for the analysis of AA using UHPLC-DAD methods. Fig. 3 shows the optimization process of UHPLC–DAD conditions for AA analysis in SGFfd, SGFrd, SGFsd, and SGS. Fig. 3a, 3b, 3c, 3d, and 3e show the stacked chromatogram of L-AA standards, stacked chromatogram of standards AA with the sample extract, SGS sample chromatogram, overlaid and stacked chromatogram of AA standards with the sample extract, and 3D-field chromatogram of the standard (to show the purity level of the peak) at λ-max of 278 nm, respectively.

The lowest amount of AA obtained was 4.02 ± 0.42 mg/100 g in SGS and the highest amount was 155.73 ± 5.11 mg/100 g using the UAE method. The content of AA in SGFrd was the highest among all of the other dried fruit and seed samples. The comparison among SD, RD, and VFD treatments of fruits and the peel (Table 3) indicated that the RD method preserves more AA (about 4 times) than SGFfd. This indicated that the contents of AA and the antioxidant potentials of fruits were well preserved in RD than in other drying processes [26]. That is due to the RD method can cause less damage to the nutrient and bio-nutrient compositions of fruits and vegetables [26]. Therefore, SGFrd was significantly different ($p \leq 0.05$) from SGFfd, SGFsd, and SGS. There were significant differences ($p \leq 0.05$) between SGFrd and SGFfd and SGFrd and SGFsd. Even though there were higher amounts of TPC and TFC in seeds than in fruits, the antioxidant activity of the fruit was higher than its seed. This is due to the fact that the AA content was about 40 times higher in the fruit than in its seed. The amount of AA obtained in *S. guineense* fruit was higher than Broccoli (113 mg/100 g), Brussels sprouts (87–109 mg/100 g), green pepper (128 mg/100 g), and lower than Blackcurrant (200–210 mg/100 g) and Guava (230–300 mg/100 g) as stated by Davey et al. [29]. The content of AA in SGFrd was higher than AA in grapefruit (40 mg/100 g), lemon and orange (50 mg/100 g), and many other fruits [29]. The studied *S. guineense* fruit and its seed were also higher than the reported average values of 47.9 ± 4.7 mg/100 g in peeled lemons, 59.8 ± 4.7 mg/100 g in peels of lemons, 47.7 ± 4.9 mg/100 g in peeled orange, 59.6 ± 5.2 mg/100 g in peels of orange, 35.1 ± 3.5 mg/100 g in peeled grapes, and 43.8 ± 4.1 mg/100 g in peels of grapes [30].

The current study showed that *S. guineense* contains a comparable amount of AA, antioxidant potential (minimum EC50 values), TPC, and TFC values with the other fruits known for their strong antioxidant activities. For instance, SGF and its seed are comparable with *Vaccinium corymbosum* (1079–1921 mg/100 g), *Photinia melanocarpa* (1018 mg/100 g), *Passiflora tarminiana* (1161 mg/100 g), *Myrciaria dubia* (2086 mg/100 g), and lower than *Ximenia Americana* (2664 mg/100 g), *Phyllanthus emblica* (3185 mg/100 g), *Sandoricum macropodum* (3240 mg/100 g), *Ziziphus mauritiana* (3404 mg/100 g) and others [30]. This remarkably higher AA, strong

antioxidant potential (minimum EC50 values), TPC, and TFC found in SGF and its seed are helpful for the prevention and treatment of various human chronic and degenerative diseases [20,30,31]. The variations also depend on the method used to estimate the content of AA in fruits and vegetables.

4. Conclusion

This study indicated that the underutilized wild fruits and seeds of *S. guineense* found in Ethiopia can be very good alternative sources of AA, strong antioxidant compounds, TPC, and TFC. The DoE using RSM for TPC, TFC, antioxidant potential, and recovery of AA in the dried *S. guineense* fruits and its seeds showed that the extractions of the bioactive compounds are affected by extraction time, temperature, and MeOH concentration relative to ultrapure water and solid-to-solvent ratio. RSM of the FC-CCD quadratic model was successfully employed to optimize the interactive effect of these independent variables. This study also showed that the mode of drying methods can affect the contents of TPC, TFC, AA, and the antioxidant potentials of *S. guineense* fruit and its seed. Out of the three drying methods (SD, RD, and VFD), room drying (RD) methods significantly preserved the TPC, TFC, antioxidant potential, and AA compared to SGFfd, SGFsd, and SGS. The 1% of aqueous AcOH extracts of dried fruit samples showed acceptable recovery of AA in the fruit and its seed as a new method of extraction of AA in dried fruit and seed samples.

The current study showed that S. *guineense* fruit and its seeds can be used as a source of valuable bioactive compounds and can be utilized for the development of value-added products such as nutraceuticals, alcoholic beverages, marmalade, jams, candied peels, syrup, and colorants. In addition to this, the quantification of individual bioactive compounds (phenolic acids, flavonoids, anthocyanins, and other components) should be done further to see the full potential of the fruit and the seed.

Authors contribution

Tilahun Belayneh Asfaw: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Henock Woldemichael Woldemariam, Fekade Beshah Tessema, Zelalem Gizachew Admassie: Analyzed and interpreted the data; Wrote the paper.

Mesfin Getachew Tadesse: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Tarekegn Berhanu Esho: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16227.

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