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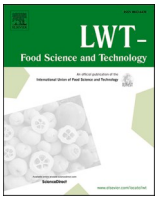

Optimisation of polyphenol extraction for the valorisation of spent gin botanicals

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ABSTRACT

An average of 0.21 kg spent gin botanicals (SGB) is generated for every 1 L of gin, making it the major by-product of gin distillation. The present circular economy dynamics and increased awareness on resource use presents an opportunity for valorisation of SGB. SGB was optimised for polyphenol extraction using a three-factor Box-Behnken Design in response surface methodology (RSM). This was used to both optimise and study how the extraction variables affected yields and values of polyphenol and antioxidant activity of SGB. The generated optimum conditions for polyphenol extraction from SGB were 50% (v/v) solvent concentration, 40 mL g^{-1} solvent: sample ratio, and 65 °C temperature. There is demand for polyphenols and natural antioxidants for use in both food and pharmaceuticals, and SGB is a natural bioresource material. The results from this study have shown a possible valorisation route for SGB and its potential for polyphenol extraction. This will create a sustainable pathway for the management of SGB and provide agro allied industries with a sustainable source of polyphenol. It will also provide a basis for process optimisation during gin distillation and possibility for reuse of botanicals materials.

1. Introduction

Botanicals are plants or plant parts (including leaf, root, stem, and flower) valued for their medicinal, therapeutic, or flavouring properties. They are utilised widely in food, pharmaceutical, cosmetics, and drink industries for their bioactive ingredients and antioxidant properties. In gin distillation, botanicals form the base raw material for flavour and aroma development. The different aroma notes in gin emanates from the respective aroma compounds corresponding to the individual botanicals utilised during the distillation process (Buck et al., 2020). In practice, gin distillers take advantage of diverse botanicals mix to develop different brands of gin characterised by distinct flavour to drive the market and consumer acceptance. Popular botanicals utilised in gin distillation ranges from juniper berries to coriander seeds, orris root, angelica root, citrus peels, liquorice root, cardamom seeds, cinnamon bark, fennel, aniseed, cumin, and a variety of other herbs and forages. Botanical utilisation in gin production ranges from 6 to 50 g per litre of grain neutral spirit (GNS) (Love Brewing, 2022; StillDragon, 2019). The by-product of gin distillation is spent gin botanicals (SGB). It contains the residue of botanicals after the gin distillation process with an average output of approximately 20 to 25 g generated for each litre of gin produced. The typical approach in many distilleries for managing SGB involves using it in compost. Current trends in sustainability initiatives as well as increased cognisance for optimal resource use and by-products management presents the need to explore more added value uses for SGB.

Polyphenols are plant metabolites with reported bioactivities. They have the capacity to boost oxidative and inflammatory stress, facilitate the absorption of macronutrients, and can exhibit properties in the gut microbiota like prebiotics (Bertelli et al., 2021). Polyphenols majorly owe their prominence to evidence that suggested that their ingestion can lower the likelihood of experiencing physiological disorders such as cancers, diabetes, cardiovascular health issues, and other chronic diseases. There is epidemiological and clinical evidence to support this claim (Debelo et al., 2020). This has increased the popularity of polyphenols and the study of its applications. The global sale for polyphenols is currently valued at 1.68 billion USD, and it is predicted to increase at a CAGR of 7.4% (Grand View Research, 2023). This was attributed to the following reasons: i. interest in the development of new products rich in polyphenol, ii. increased interest and awareness regarding the utilisation of non-synthetic anti-oxidative compounds in foods as well minimising the incorporation of artificial flavourings during food

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production, iii. demand for clean label and natural additives, iv. increased awareness among consumers regarding the importance of wellbeing, and v. growth in global functional food industry. This interest in natural antioxidants makes extraction of polyphenol from SGB a viable option. Bioresource materials such as SGB can be good sources of polyphenol because of their origin, hence, the need to study it for its polyphenolic content and antioxidant activity.

However, to study the potential of SGB as a polyphenol source as well as its antioxidant activity, the polyphenol, must first be extracted. This makes extraction a crucial step in the recovery and study of polyphenol. Optimal extraction of polyphenol depends on the extraction conditions which affect both yield and process efficiency. Hence, it is vital that these conditions are optimised for maximum recovery of polyphenol. This can be accomplished by utilisation of the statistical technique known as response surface methodology (RSM). RSM is used to understand how process variables affect a process and to also, optimise those variables for process enhancement and facilitation. It studies the interaction of parameters affecting the response variables and enables maximisation or minimisation of the process variables by simultaneously evaluating the different responses.

This study was aimed at valorising SGB as a sustainable source of polyphenol by optimisation of the extraction conditions. Natural and potent polyphenol sources are in demand for food, pharmaceutical, and cosmetic purposes. Study of the polyphenolic content of SGB and optimising the extraction conditions will provide the basis for its utilisation as a valuable polyphenol source in the various applications.

2. Materials and methods

2.1. SGB collection and chemicals procurement

The SGB sample was procured from an Irish gin distillery. Reagents (sodium carbonate, Folin-Ciocalteu's reagent, sodium nitrite, aluminium chloride, sodium hydroxide, sulphuric acid, sodium phosphate, ammonium molybdate, DPPH, methanol, sodium acetate, acetic acid, TPTZ, hydrochloric acid, and iron(III) chloride) and standards (gallic acid, quercetin, ascorbic acid, and trolox) that were utilised in the study were procured from Sigma-Aldrich (Merck – Wicklow, Ireland).

2.2. Sample preparation

Upon collection, the procured sample of SGB was dried immediately in a dehydrator (46 °C). Final moisture content of dried sample was ≤6% (d.b.). After dehydration, the dried sample was pulverised, transferred to a Ziploc bag, and then stored in the freezer at −20 °C until it is required for use.

2.3. Polyphenol extraction

Polyphenol extraction from the SGB was done by solvent extraction using ethanol. The dried SGB was suspended in different ethanol concentrations (20–80%, v/v), solvent: sample ratio (10–50 mL g^{-1}), and temperature (25–65 °C) in an incubator shaker for 14 h with continuous stirring at 200 rpm. Upon completion of the solvent extraction process, the sample containing the extract was then separated by centrifugation (4000 rpm for 15 min) and filtration, respectively (Iglesias-Carres et al., 2018).

2.4. Total phenolic content (TPC)

Folin-Ciocalteu assay was used for TPC measurement (Ganesan et al., 2008). Using gallic acid as a reference standard, 100 μL of the extract of SGB was combined with 2000 μL of 2% (w/v) Na_2CO_3 solution, and this allowed to stand for 2 min on the bench for the reaction to complete. This was followed by the addition of a 100 μL aliquot of 50% (v/v) Folin-Ciocalteu's reagent. The mixture was incubated for 30 min in the dark at ambient temperature and its absorbance reading was taken afterwards using UV-VIS spectrophotometer at 765 nm. TPC of SGB was then calculated according to Equation (1).

$$\frac{C \times V}{M} \qquad \text{Equation 1}$$

Equation (1). Calculation of TPC, TFC, TAC, DPPH, and FRAP per gram of SGB.

- C is concentration obtained from calibration curve
- V is volume of solvent
- M is sample mass

2.5. Total flavonoid content (TFC)

Aluminium assay was used for TFC measurement (Liu et al., 2009). Using quercetin as reference standard, a 250 μL aliquot of the extract of SGB was combined with 75 μL 5% (w/v) sodium nitrite and this was allowed to stand for 6 min on the bench for the reaction to complete. Afterwards, 150 μL of 10% (w/v) $AlCl_3$ solution was added to the reaction mixture, and this was allowed to stand for 5 min on the bench. This was followed by the addition of 500 μL 1 mol dm^{-3} NaOH and thoroughly mixing them together. The absorbance reading was then taken at 510 nm using UV-VIS spectrophotometer and TFC was calculated according to Equation (1).

2.6. Total antioxidant capacity (TAC)

TAC was measured using the procedure outlined by Brewer et al. (2014). Using L-(+)-ascorbic acid as reference standard, in one test tube, a 300 μL aliquot of the extract of SGB was combined with 3000 μL reagent comprising of equal proportions of sulphuric acid (0.6 mol dm^{-3}), sodium phosphate (0.028 mol dm^{-3}), and ammonium molybdate (0.004 mol dm^{-3}). This was allowed to incubate at 95 °C for 90 min. The absorbance reading of the reaction mixture at the end of the incubation and after it has been allowed to cool down was taken at 695 nm using UV-VIS spectrophotometer. TAC of SGB was calculated according to Equation (1).

2.7. 1,1-Diphenyl-2-picrylhdrazyl (DPPH) radical scavenging activity (DPPH)

DPPH measurement was carried out using the method of Brewer et al. (2014). DPPH reagent (0.004%, w/v) was prepared using methanol on the day of analysis (Liyana-Pathirana & Shahidi, 2006). The prepared DPPH reagent (1900 μL) was transferred to an Eppendorf tube followed by the addition of SGB extract (100 μL). This was then allowed to incubate for half an hour at ambient temperature while avoiding light interference. After incubation, absorbance reading was taken using UV-VIS spectrophotometer at 517 nm wavelength. Reference standard for the assay was L-(+)-ascorbic acid. DPPH was calculated according to Equation (1).

2.8. Ferric-reducing antioxidant power (FRAP)

FRAP of the SGB sample was conducted using the method of Brewer et al.(Brewer et al., 2014). The FRAP reagent comprised of a mixture of 0.3 mol dm^{-3} acetate buffer (pH 3.6), 0.01 mol dm^{-3} 2,4,6-tripyridyl--S-triazine in 0.04 mol dm^{-3} hydrochloric acid, and 0.02 mol dm^{-3} iron (III) chloride hexahydrate in a ratio of 10:1:1 (Benzie & Strain, 1999). Using Trolox as reference standard, 1800 μL of the FRAP reagent, 300 μL of the extract, and 180 μL of distilled water were mixed. This was allowed to incubate at 37 °C for 4 min. After incubation, the reaction mixture was allowed to cool down to room temperature. Afterwards, absorbance reading was taken using UV-VIS spectrophotometer at 593 nm wavelength. FRAP was calculated according to Equation (1).

2.9. Experimental design

The optimisation of polyphenol extraction from SGB involved the utilisation of response surface methodology (RSM) to obtain optimal values for extraction conditions of solvent concentration, solvent: sample ratio, and temperature. A three-factor Box-Behnken design was used to study how these factors (independent variables) affected the yield and values of polyphenol as well as their interactions. The levels of the variables (Table 1) were influenced by findings from a preliminary study and information from existing literature. The response variables were TPC, TFC, TAC, DPPH and FRAP. Experimental data were fitted using the second-order polynomial function (Equation (2)).

$$Y = \beta_\Theta + \sum_{i=0}^{n} \beta_1 X_1 + \beta_1 X_2 + \beta_1 X_3 + \sum_{i=0}^{n} \beta_2 X_i^2 + \beta_2 X_2^2 + \beta_2 X_3^2 + \sum_{i=0}^{n} \beta_3 X_1 X_2 + \beta_3 X_1 X_3 + \beta_3 X_2 X_3$$

Equation 2

Equation (2). Second-order polynomial function.

- Y represents the responses.
- β_Θ represents the model intercept
- β_1, β_2, and β_3 are coefficients of the linear, quadratic, and interaction effects, respectively.
- X_1, X_2, and X_3 are the independent variables of solvent concentration, solvent: sample ratio, and temperature, respectively.

2.10. Statistical analysis

Experiments were carried out in triplicates. Experimental data were reported as mean of triplicate analysis. Box-Behnken Design and data analysis using RSM were conducted using Design-Expert version 13.

3. Results and discussion

3.1. Model fitting

The optimisation of extraction conditions for polyphenol extraction from SGB was done by fitting the second-order polynomial function (Table 2). Regression equation showing the impact of the independent variables as well as their interactions on the response variables was generated. All the generated models were significant for the corresponding responses ($p < 0.05$) and fitted well with the experimental data. The coefficient of determination (R^2) for the responses as well as their corresponding F-values and p-values (lack of fit) were all used to validate the models. The fitted models were used to generate response surface plots to show how the extraction variables affected polyphenol yield and antioxidant property of SGB.

3.2. Effect of extraction variables on total phenolic content

TPC is used for the estimation of polyphenol with important aglycones that have reducing properties which are known to account for majority of the antioxidant property of plants and plants products (Sulaiman & Balachandran, 2012). Folin-Ciocalteu assay which is utilised for the assessment of TPC relies on electron transfer under alkaline conditions between phenolic compounds and phosphomolybdic/phosphotungstic acid complexes. This gives rise to blue complexes which induce λ_{max} at 765 nm (Molole et al., 2022). The calibration curve equation is shown in Equation (3). Result from the study showed that TPC of SGB varied from 4.87 to 14.42 mg GAE/g of SGB (Table 3).

$$y = 0.0012x - 0.0106; R^2 = 0.9955$$

Equation 3

Equation (3). Curve equation for gallic acid standard (measure range is 75–500 mg/L).

Solvent concentration is an important factor in polyphenol extraction as solvent polarity affects extraction efficiency and yield (Prasad et al., 2011). The optimum solvent concentration (50%) recorded in this study for TPC yield correlates with findings from previous studies that have also indicated that the use of water/alcohol mixture is more effective in the extraction and isolation of plant polyphenol, rather than the utilisation of an absolute alcohol system (Gandolpho et al., 2021; Gunathilake et al., 2019). This could be attributed to interactions and mechanism of action between organic solvents and bioactive compounds polarities. Water is a universal solvent and acts as the swelling agent for plants while alcohol on the other hand, separates the linkages or bonds holding phenolic compounds within a sample matrix and as a result, aids their solubility and subsequent recovery (Ghitescu et al., 2015). Therefore, the combination of water/alcohol mixture creates a synergistic effect for higher extraction yields (Wang & Weller, 2006). Additionally, alcohols in solvent extraction reduce the dielectric constant of the solvent mixture. This helps to minimise the rate of interaction of the solute and solvent thereby increasing mass transfer efficiency by molecular diffusion (Cacace & Mazza, 2003). However, at very high

Table 1
Levels of variables for the experimental design.

Independent variables	Levels		
	−1	0	+1
X_1; Solvent concentration (%)	20	50	80
X_2; Solvent to sample ratio (mL g^{-1})	10	30	50
X_3; Temperature (°C)	25	45	65

Table 2
Regression coefficients of the predicted quadratic models for the responses.

Regression coefficients, β	TPC mg GAE/g	TFC mg QE/g	TAC mg AAE/g	DPPH mg AAE/g	FRAP mg TE/g
Intercept, X_0	11.10	23.93	9.70	6.37	6.21
Linear					
X_1	1.14	4.56	1.53	0.47	1.05
X_2	2.19	3.23	2.52	1.16	3.70
X_3	1.20	3.19	0.13	0.52	−0.13
Quadratic					
X_1	−2.64	−3.10	−1.41	−2.08	0.32
X_2	−0.53	−0.99	−0.67	0.65	−0.27
X_3	−0.22	−1.10	−0.31	0.54	0.01
Interaction					
X_1. X_2	−0.03	2.99	0.90	0.10	0.47
X_1. X_3	−0.16	−0.09	−0.06	−0.11	−0.33
X_2. X_3	0.37	0.91	0.32	0.04	0.10
F-value (model)	96.05	63.96	152.40	187.37	150.86
p-value (model)	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
p-value (lack of fit)	0.0621	0.0702	0.1394	0.1230	0.1932
R^2	0.9942	0.9914	0.9964	0.9970	0.9963
Adjusted R^2	0.9839	0.9759	0.9898	0.9917	0.9897
Sum of squares	87.02	410.41	82.00	34.61	120.66
Residual sum of squares	0.5033	3.56	0.2989	0.1026	0.4443
Mean square	9.67	45.60	9.11	3.85	13.41
Residual mean square	0.1007	0.7130	0.0598	0.0205	0.0889
Residual error mean square	0.0105	0.0844	0.0142	0.0043	0.0296

X_1 = Solvent concentration (%).
X_2 = Solvent to sample ratio (mL g^{-1}).
X_3 = Temperature (°C).
TPC = Total phenolic content.
TFC = Total flavonoids content.
TAC = Total antioxidant capacity.
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.
FRAP = Ferric reducing antioxidant power.

Table 3
Box-Behnken design for extraction of polyphenol from SGB.

Run	Factors			Responses				
	Solvent concentration %	Solvent: sample ratio mL g^{-1}	Temperature °C	TPC mg GAE/g	TFC mg QE/g	TAC mg AAE/g	DPPH mg AAE/g	FRAP mg TE/g
1	20	10	45	4.87	15.90	4.70	3.48	2.15
2	80	10	45	7.04	17.88	5.77	4.01	3.39
3	20	50	45	8.88	15.81	7.65	5.67	8.18
4	80	50	45	10.95	29.74	12.34	6.60	11.30
5	20	30	25	5.76	11.28	6.09	3.63	5.33
6	80	30	25	8.52	21.76	9.44	4.99	8.02
7	20	30	65	8.28	17.88	6.63	4.89	5.71
8	80	30	65	10.39	27.99	9.74	5.80	7.09
9	50	10	25	7.02	16.06	6.33	5.96	2.25
10	50	50	25	11.08	21.28	11.01	8.12	9.89
11	50	10	65	8.89	20.59	5.79	6.92	1.81
12	50	50	65	14.42	29.43	11.76	9.23	9.84
13	50	30	45	11.13	24.23	9.60	6.30	6.28
14	50	30	45	11.19	23.65	9.83	6.43	6.01
15	50	30	45	10.99	23.91	9.66	6.38	6.33

TPC = Total phenolic content.
TFC = Total flavonoids content.
TAC = Total antioxidant capacity.
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.
FRAP = Ferric reducing antioxidant power.

alcohol concentrations, denaturation of cell wall proteins (including those coupled with phenolic compounds) can occur, which will affect the process (Chen et al., 2013). Using lower volumes of solvent during extraction processes is one step towards achieving green extraction for a more sustainable process. Result from this study has shown that solvent for polyphenol extraction from SGB as measured by TPC can be reduced by half for optimal yield. Similar values have also been observed in other studies with respect to solvent concentration and TPC. Carciochi et al. (2018) and Gandolpho et al. (2021) recorded optimal solvent concentrations in the range of 60–68% for TPC of brewers' spent grain and brewing wastes (trub) samples. While Spigno et al. (2007) and Silva et al. (2016) observed higher TPC in grape seeds and lychee peels respectively, at 50% solvent concentration.

For solvent: sample ratio, TPC of SGB increased with increase in solvent: sample ratio. This may be because greater concentration gradient between solid and liquid components creates stronger driving force for the movement and diffusion of compounds from the sample matrix into the solvent (Carciochi et al., 2018). As a result, adequate volume of solvent is required such that it should be able to provide sufficient hydration and swelling of the solid inter-phase. Higher values for TPC of the SGB sample were observed at solvent: sample ratio ≥30 mL g^{-1}. This corresponded to the values observed in the study by Carciochi et al. (2018) which reported increased extraction yield with increased solvent: sample ratio.

With respect to temperature, highest value of TPC for the SGB sample was observed at 65 °C. Like the other variables, temperature also plays significant role in the extraction of polyphenol. Elevated temperatures can be beneficial in relaxing of the plant tissues, swelling of the sample matrix by enhancing solvent uptake, weakening of bonds holding the phenolic compounds within the plant material, enhancing the permeability of the cells, reducing surface tension, and increasing the rates of diffusion in the sample matrix (Arruda et al., 2017). Excessively elevated temperatures, on the other hand, may result in the thermal degradation of certain phenolic compounds and encourage undesired reactions between these compounds and the sample matrix (Yim et al., 2013). Gunathilake et al. (2019) and Prasad et al. (2011) reported optimal temperature of 60 °C and 58 °C for TPC in leaves of *Centella asiatica* and *Mangifera pajang* Kosterm. peels, respectively.

From the response surface analysis, the studied extraction variables were all significant ($p < 0.05$) and had positive effects on the TPC of SGB (Table 2). Solvent concentration and solvent: sample ratio both had significant ($p < 0.05$) linear and quadratic effects. This means that the polyphenol yield of the SGB sample as measured by TPC was favoured by their increase. However, their quadratic effect was negative, an indication that higher values for both factors beyond the optimal, can have negative effect on TPC of SGB. Solvent: sample ratio was the most significant variable for the response of TPC with a p value of <0.0001. The ANOVA results showed that F value (96.05) of the model corresponding to a p value of <0.0001 was highly significant. Therefore, the final model (Equation (4)) can be used to predict responses for TPC as presented in Table 4. The R^2 value of 0.9942 for the model is an indication of strong positive correlation between input variables and TPC, with the model accounting for 99.42% of the responses (Fig. 2a). Response surface plot resulting from the model is graphically illustrated in Fig. 1a.

$$TPC = 11.10 + 1.14X_1 + 2.19X_2 + 1.20X_3 - 2.64X_1^2 - 0.53X_2^2 - 0.22X_3^2 - 0.03X_1X_2 - 0.16X_1X_3 + 0.37X_2X_3$$

Equation 4

Equation (4) (see Fig. 2). Model fitting for TPC of the SGB sample.

Where X_1, X_2, and X_3 are the independent variables of solvent concentration, solvent: sample ratio, and temperature, respectively. The values and arithmetic signs of the linear terms (X_1, X_2, X_3), quadratic terms (X_1^2, X_2^2, X_3^2), and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model indicated the magnitude and type of effect each of the studied extraction variables had on the generated model, and how this influenced the values for TPC of SGB.

From the study, the SGB showed good capacity for TPC yield, an indication of its potential as a source of polyphenol and for use in ingredient formulation to boost polyphenol profile. Polyphenols are known to offer health benefits due to their antioxidant activity. With respect to human physiology, they play a role in health and body defence mechanisms with their ingestion being linked with alleviation of various physiological disorders. As a result, a lot of diets are taking advantage of this and are incorporating foods rich in polyphenol in their formulation. The intake of such diets has been correlated with decreased incidence of chronic ailments like diabetes, cancer, and cardiovascular diseases through the management of oxidation (Lin et al., 2016). In addition to food sources, some by-products materials are now being exploited for their polyphenol profile. Some of these include spent grain, lentil straw, grape pomace, olive cake/leaves, tomato/grape/apple pomace, etc. These have been utilised in animal feed, nutrition, and cosmetics with reported bioactivities (Bertelli et al., 2021). Therefore, if

Table 4
Observed experimental and predicted data for responses.

Run	Factors			Responses									
	Solvent conc. %	Solvent: sample ratio mL g^{-1}	Temp. °C	TPC mg GAE/g		TFC mg QE/g		TAC mg AAE/g		DPPH mg AAE/g		FRAP mg TE/g	
				Obsd.	Pred.	Obsd.	Pred.	Obsd.	Pred.	Obsd.	Pred.	Obsd.	Pred.
1	20	10	45	4.87	4.58	15.9	15.03	4.70	4.47	3.48	3.42	2.15	1.97
2	80	10	45	7.04	6.91	17.88	18.18	5.77	5.72	4.01	4.15	3.39	3.14
3	20	50	45	8.88	9.01	15.81	15.51	7.65	7.70	5.67	5.53	8.18	8.43
4	80	50	45	10.95	11.24	29.74	30.61	12.34	12.57	6.60	6.66	11.30	11.48
5	20	30	25	5.76	5.74	11.28	11.88	6.09	6.26	3.63	3.73	5.33	5.29
6	80	30	25	8.52	8.34	21.76	21.19	9.44	9.43	4.99	4.89	8.02	8.05
7	20	30	65	8.28	8.46	17.88	18.45	6.63	6.64	4.89	4.99	5.71	5.68
8	80	30	65	10.39	10.41	27.99	27.39	9.74	9.57	5.80	5.70	7.09	7.13
9	50	10	25	7.02	7.33	16.06	16.33	6.33	6.39	5.96	5.92	2.25	2.47
10	50	50	25	11.08	10.97	21.28	20.98	11.01	10.79	8.12	8.16	9.89	9.68
11	50	10	65	8.89	9.00	20.59	20.90	5.79	6.01	6.92	6.88	1.81	2.02
12	50	50	65	14.42	14.11	29.43	29.16	11.76	11.70	9.23	9.27	9.84	9.62
13	50	30	45	11.13	11.10	24.23	23.93	9.60	9.70	6.30	6.37	6.28	6.21
14	50	30	45	11.19	11.10	23.65	23.93	9.83	9.70	6.43	6.37	6.01	6.21
15	50	30	45	10.99	11.10	23.91	23.93	9.66	9.70	6.38	6.37	6.33	6.21

TPC = Total phenolic content.
TFC = Total flavonoids content.
TAC = Total antioxidant capacity.
DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity.
FRAP = Ferric reducing antioxidant power.

properly harnessed as a bioresource material, SGB can become an important source of polyphenol which can be utilised in ingredients formulation and new product development.

3.3. Effect of extraction variables on total flavonoid content

TFC measures flavonoid polyphenol. Flavonoid polyphenol are the predominant polyphenolic compounds found in human diets and are primarily categorised into two groups: anthocyanins and anthoxanthins, which encompass flavones, flavans, flavonols, flavanols, isoflavones, and their glycosides (Durazzo et al., 2019). The calibration curve equation is shown in Equation (5).

$$y = 0.0009x + 0.0001; R^2 = 0.9986 \quad \text{Equation 5}$$

Equation (5). Curve equation for quercetin standard (measure range is 75–200 mg/L).

TFC of SGB ranged from 11.28 to 29.74 mg QE/g of SGB and increased consistently with increase in solvent concentration. This may be due to the non-polar nature of flavonoids which make them to be more extractable in solvents with low polarity Yusof et al. (2020). In addition, quercetin which was used as the calibration standard for TFC in this study reportedly has numerous hydroxyl groups which predispose it to have a greater affinity for extraction at higher alcohol concentrations (Maulana et al., 2019). Yusof et al. (2020) reported optimum yield of TFC in propolis at 80% solvent concentration while Sun et al.(Sun et al., 2015) reported an optimum TFC yield at 75% solvent concentration also for propolis. Optimum TFC yields have also been reported at 60% and 64% solvent concentration in herbs and grape by-products, respectively (Y. Liu et al., 2015).

For solvent: sample ratio, highest value for TFC of the SGB sample was observed at 50 mL g^{-1}. Increase in solvent: sample ratio enhances surface contact area between sample and solvent. This helps to accelerate mass transfer and extraction efficiency by movement of compounds of interest into the extraction matrix. Furthermore, for materials that have been dried (like the SGB in this study), more solvent is required to hydrate the sample matrix. This allows for more solubilisation and subsequent extraction of the polyphenol. Thus, a higher solvent: sample ratio will mean greater extraction efficiency.

Optimum extraction temperature for TFC yield of the SGB sample was observed at 45 °C. Usually, better liberation of bioactive compounds from plants have been observed at elevated temperatures. A reasonably high temperature will reduce solvent viscosity and increase molecular movement of solutes. However, it is important to note that relatively elevated temperatures do not favour flavonoid extraction as it tends to destroy the flavonoids and interfere with their efficacy. Reportedly, optimum extraction temperature for flavonoids have been observed to vary from 50 to 70 °C (X. min Liu et al., 2022; Xu et al., 2019) with a maximum of 75 °C as reported by Rodríguez De Luna et al. (2020).

In the response surface analysis for TFC of SGB, the proposed model had an F value of 63.96 and was highly significant with a corresponding p value of 0.0001. Analysis of variance (Table 2) of the model showed that all the variables had significant ($p < 0.05$) positive linear effects on TFC yield from SGB. In addition to a significant ($p < 0.05$) positive linear effect of the variables, the interaction of solvent concentration and solvent: sample ratio was also significant ($p < 0.05$). For the quadratic effect of the variables, they all had negative effects which meant that excessive increase of either of the variables can diminish the yield of TFC in the sample. The model (Equation (6)) corresponding R^2 value of 0.9914 and very low p value indicated that it can be used to adequately predict responses for TFC of SGB (Table 4 and Fig. 2b). Response surface plot of the responses is illustrated in Fig. 1b.

$$TFC = 23.93 + 4.56X_1 + 3.23X_2 + 3.19X_3 - 3.10X_1^2 - 0.99X_2^2 - 1.10X_3^2 + 2.99X_1X_2 - 0.09X_1X_3 + 0.91X_2X_3 \quad \text{Equation 6}$$

Equation (6). Model fitting for TFC of the SGB sample.

Where X_1, X_2, and X_3 are the independent variables – solvent concentration, solvent: sample ratio, and temperature, respectively. The values and arithmetic signs of the linear terms (X_1, X_2, X_3), quadratic terms (X_1^2, X_2^2, X_3^2), and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model indicated the magnitude and type of effect each of the studied extraction variables had on the generated model, and how this influenced the values for TFC of SGB.

The SGB sample had shown good capacity for TFC. As part of phenolic compounds, flavonoids also have defensive properties against physiological conditions like diabetes, cancer, and cardiovascular diseases, with reports of positive correlation of their intake with reduced mortality for cardiovascular disease (Babu & Liu, 2009). Thus, flavonoids are becoming a focus for current nutritional and therapeutic

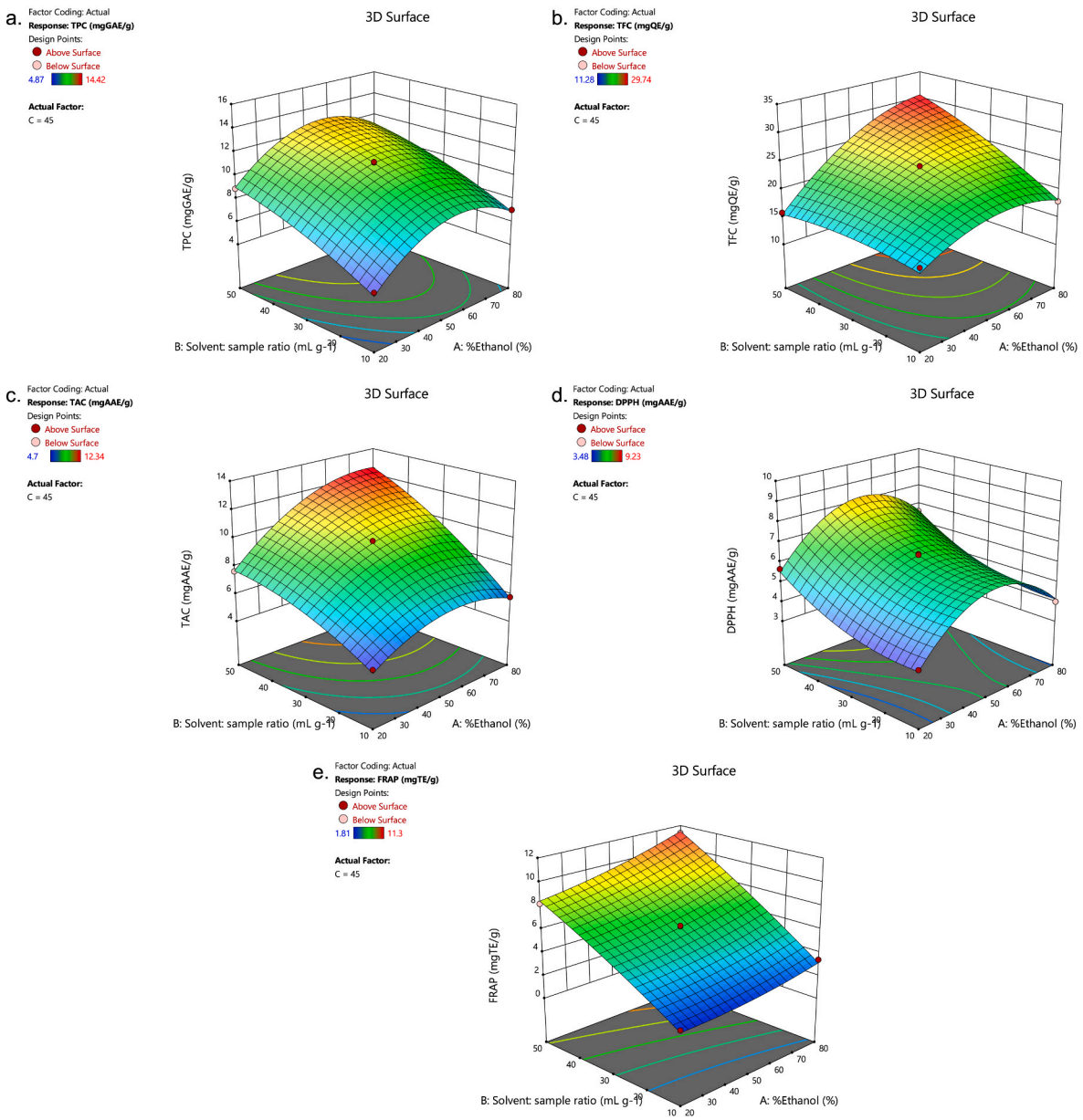

Fig. 1. Response surface plots showing the effect of the extraction variables on: a. total phenolic content; b. total flavonoid content; c. total antioxidant capacity; d. DPPH radical scavenging activity; e. ferric reducing antioxidant power. Temperature was kept at centre point value.

interest. Also, the high TFC of SGB is an indication that an opportunity exists for process optimisation for increased flavour infusion from the botanicals during gin distillation. Flavonoids are known to exert certain organoleptic properties such as colour, aroma, and taste to foods and food products (Ruiz-Cruz et al., 2017). These are often exploited during the process of gin distillation. One of the goals of gin distillation is flavour infusion into ethyl alcohol utilising botanicals. Factors that can affect flavour infusion from botanicals include but not limited to solvent concentration, botanical ratio, and bulk charge (Hodel et al., 2020). Hence, the findings of this study can also be used to enhance the gin distillation process for optimal resource use.

3.4. Effect of extraction variables on total antioxidant capacity

Phosphomolybdenum assay utilised for the measurement of TAC is a quantitative assay that is characterised by the reduction of molybdenum (VI) to molybdenum(V) and the resulting production of a green phosphate/molybdenum(V) complex at acidic pH (Sadeer et al., 2020). The calibration curve equation is shown in Equation (7).

$$y = 0.0045x - 0.0174; R^2 = 0.9969 \quad \text{Equation 7}$$

Equation (7). Curve equation for ascorbic acid standard for TAC (measure range is 75–250 mg/L).

TAC of SGB ranged from 4.70 to 12.34 mg AAE/g of SGB. The highest value for TAC of SGB was observed at 50% solvent concentration, 50 mL g^{-1} solvent: sample ratio, and 45 °C temperature. The results of this study showed that elevated sample: solvent ratio and temperature aided TAC of the SGB sample. And just like TPC, an intermediate balance of water and solvent favoured TAC as compared to a relatively high solvent concentration. Elevated extraction conditions for the studied variables of solvent concentration, solvent: sample ratio and temperature has been found to favour TAC. Bamba et al.(Bamba et al., 2018) observed that 50% solvent concentration and 40 mL g^{-1} solvent: sample ratio favoured TAC of blueberry pomace.

The proposed model for TAC of SGB is shown in Equation (8). The model terms showed that the effect of solvent concentration and solvent:

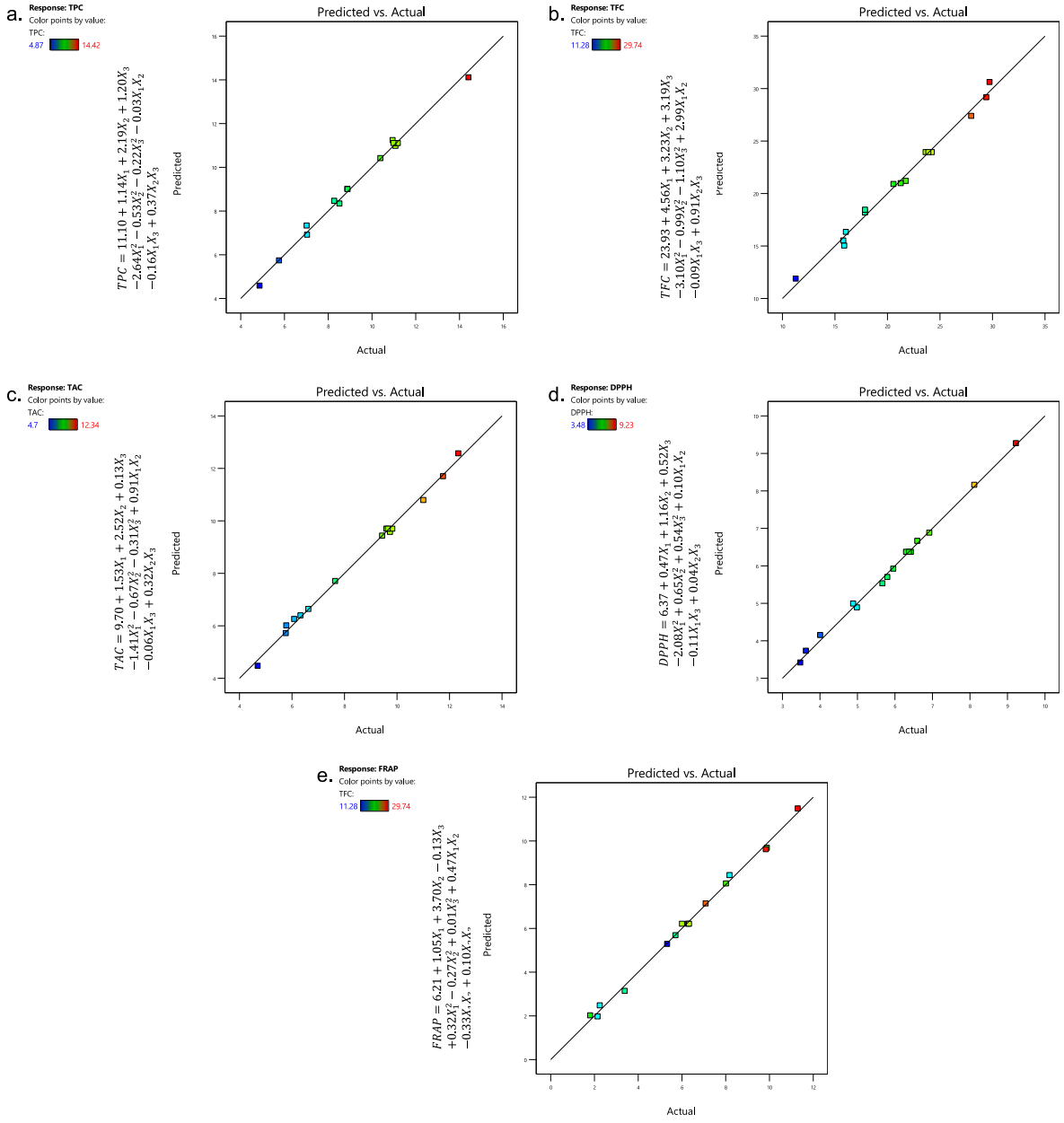

Fig. 2. Predicted vs. actual responses for: a. total phenolic content; b. total flavonoid content; c. total antioxidant capacity; d. DPPH radical scavenging activity; e. ferric reducing antioxidant power.

sample ratio as well their interaction was linear, and their increase affected the TAC of SGB significantly ($p < 0.05$). Their quadratic terms were also significant ($p < 0.05$); however, they were both negative, an indication that very high concentrations of both factors will affect the TAC of SGB negatively. The effect of temperature for the model was not significant ($p > 0.05$) which corresponded to the findings of Bamba et al. (2018). The model had F value of 152.40, p value of <0.0001, and R^2 value of 0.9964 (Equation (8) and Table 4). The surface plot of the model with temperature as the centre point is shown in Fig. 1c.

$$TAC = 9.70 + 1.53X_1 + 2.52X_2 + 0.13X_3 - 1.41X_1^2 - 0.67X_2^2 - 0.31X_3^2 + 0.91X_1X_2 - 0.06X_1X_3 + 0.32X_2X_3 \quad \text{Equation 8}$$

Equation (8). Model fitting for TAC of the SGB sample.

Where X_1, X_2, and X_3 are the independent variables – solvent concentration, solvent: sample ratio, and temperature, respectively. The values and arithmetic signs of the linear terms (X_1, X_2, X_3), quadratic terms (X_1^2, X_2^2, X_3^2), and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model indicated the magnitude and type of effect each of the studied extraction variables had on the generated model, and how this influenced the values for TAC of SGB.

3.5. Effect of extraction variables on 1,1-diphenyl-2-picrylhdrazyl (DPPH) radical scavenging activity

DPPH is a synthetic free radical product that is widely utilised in biosciences for antioxidant activity measurement (Rahman et al., 2021). The capacity of biological regents to scavenge DPPH radical can be expressed as its magnitude of antioxidant potential. The calibration curve equation is shown in Equation (9).

$$y = -0.0031x + 0.7675; R^2 = 0.9943 \quad \text{Equation 9}$$

Equation (9). Curve equation for ascorbic acid standard for DPPH

(measure range is 75–225 mg/L).

DPPH of the SGB ranged from 3.48 to 9.23 mg AAE/g of SGB. The optimum value for DPPH radical scavenging activity of the SGB sample was observed at extraction conditions of 50% solvent concentration, 50 mL g^{-1} solvent: sample ratio, and 65 °C temperature. The obtained result was consistent with reports that elevated conditions of extraction variables will favour extraction efficiency of samples and consequently, a higher antioxidant activity (Hikmawanti et al., 2021; Sun et al., 2015).

The response surface analysis for DPPH radical scavenging activity showed that all the variables had significant ($p < 0.05$) linear and quadratic effects on the response. The linear effects were positive for all variables, while quadratic effect for solvent concentration was negative. This meant that the combined effect of solvent: sample ratio and temperature were greater than that of solvent concentration. This relationship is shown graphically in Fig. 1d. Interaction effects for the generated model were not significant ($p > 0.05$). The model F value of 187.37 was very highly significant with a p value of <0.0001 and R^2 value of 0.9970 (Table 4 and Equation (10)).

$$DPPH = 6.37 + 0.47X_1 + 1.16X_2 + 0.52X_3 - 2.08X_1^2 + 0.65X_2^2 + 0.54X_3^2 + 0.10X_1X_2 - 0.11X_1X_3 + 0.04X_2X_3$$

Equation 10

Equation (10). Model fitting for DPPH of the SGB sample.

Where X_1, X_2, and X_3 are the independent variables – solvent concentration, solvent: sample ratio, and temperature, respectively. The values and arithmetic signs of the linear terms (X_1, X_2, X_3), quadratic terms (X_1^2, X_2^2, X_3^2), and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model indicated the magnitude and type of effect each of the studied extraction variables had on the generated model, and how this influenced the values for DPPH of SGB.

DPPH radical scavenging activity of the SGB sample is an indication of its free radicals scavenging. Natural antioxidants present in foods have gained wide interest in the scientific community as well as in the food industry due to indications from epidemiological studies that their regular ingestion can reduce the risks associated with certain chronic diseases, and they can replace synthetic antioxidants in food products (Faustino et al., 2019). The high retention of antioxidant property of the SGB sample shows its potential as a natural source for the development of oxidants/free radicals' scavengers. Extracts of SGB can be used to prevent oxidative reactions in food and biological systems for different purposes such as preservation and storage. SGB and its extract could therefore serve as a valuable resource for human health improvement and at the same time could be utilised as an antioxidant in food preparations. Their incorporation in diet and new products development could be beneficial in the fight against free radicals.

3.6. Effect of extraction variables on ferric-reducing antioxidant power

FRAP assay is an electron transfer assay based on the reduction of Fe^{3+} to Fe^{2+} by antioxidants in the presence of tripyridyltriazine (TPTZ) (Qasim et al., 2016). In FRAP assay, the reduction property of the analyte is used as an index to measure its antioxidant property (khatoon et al., 2013). The calibration curve equation is shown in Equation (11).

$$y = 0.0093x - 0.0373; R^2 = 0.9910$$

Equation 11

Equation (11). Curve equation for Trolox standard (measure range is 75–175 mg/L).

The FRAP of the SGB sample ranged from 1.81 to 11.30 mgTE/g of SGB. The highest value for FRAP of SGB was observed at 80% solvent concentration, 50 mL g^{-1} solvent: sample ratio, and 45 °C temperature. The result showed that high solvent concentration and high solvent: sample ratio favoured FRAP values of the SGB. This could be because of relatively higher values observed for TPC and TFC at elevated extraction conditions which both contribute to antioxidant activity. In the evaluation of antioxidant of propolis by Sun et al.(Sun et al., 2015), FRAP value was highest at 75% solvent concentration. However, unlike the other parameters, FRAP of the SGB did not depreciate at 80% solvent concentration. Belwal et al. (2020) reported that at 50 mL g^{-1} solvent: sample ratio, FRAP of Berberis roots was increased beyond which a decrease was recorded.

Response surface analysis for FRAP of SGB showed that the significant ($p < 0.05$) model terms were solvent concentration and solvent: sample ratio both of which had a positive linear effect. Their interaction was also positive and significant ($p < 0.05$). This indicated that elevated conditions for these variables favoured FRAP antioxidant activity of the SGB. The effect of temperature was not significant ($p > 0.05$) for FRAP. Vergara-Salinas et al. (2012) reported that FRAP of deodorised thyme was not affected by increase in temperature. The generated model had F value of 150.86, a p value of <0.0001, and R^2 value of 0.9963 (Table 4 and Equation (12)). The surface plot for FRAP is shown in Fig. 1e.

$$FRAP = 6.21 + 1.05X_1 + 3.70X_2 - 0.13X_3 + 0.32X_1^2 - 0.27X_2^2 + 0.01X_3^2 + 0.47X_1X_2 - 0.33X_1X_3 + 0.10X_2X_3$$

Equation 12

Equation (12). Model fitting for FRAP of the SGB sample.

Where X_1, X_2, and X_3 are the independent variables – solvent concentration, solvent: sample ratio, and temperature, respectively. The values and arithmetic signs of the linear terms (X_1, X_2, X_3), quadratic terms (X_1^2, X_2^2, X_3^2), and interaction terms (X_1X_2, X_1X_3, X_2X_3) of the model indicated the magnitude and type of effect each of the studied extraction variables had on the generated model, and how this influenced the values for FRAP of SGB.

4. Conclusion

The study evaluated the valorisation of the bioresource of SGB by studying and optimising its potential for polyphenol content and antioxidant property using RSM. Box-Behnken Design was able to describe how these factors and their interactions affected yields and values of TPC, TFC, TAC, DPPH and FRAP. Optimised conditions for the extraction variables were gotten using the desirability function in Design-Expert version 13, and these were: 50% solvent concentration, 40 mL g^{-1} solvent: sample ratio, and 65 °C temperature (Table 5). Solvent concentration had the most effect on polyphenol yield from SGB followed by solvent: sample ratio and temperature, respectively (Fig. 3). Pearson correlation coefficient (Table 6) showed the relationship between polyphenolic content and antioxidant activity.

With respect to bioresource use and valorisation route, results from this study presents SGB as a high value bioresource material. This is in line with a Mintel study that had reported on the effectiveness, health-boosting properties, and natural attributes of botanicals. Natural and potent antioxidants are in demand for food and pharmaceutical products, and SGB has shown potentials that it can be exploited for such

Table 5
Optimal conditions for polyphenol extraction and antioxidant activity of SGB.

Extraction variables	
Solvent concentration	50%
Solvent: sample ratio	40 mL g^{-1}
Temperature	65 °C
Predicted responses	
TPC	13.14 mg GAE/g
TFC	27.75 mg QE/g
TAC	10.69 mg AAE/g
DPPH	8.10 mg AAE/g
FRAP	7.76 mg TE/g

TPC = Total phenolic content.
TFC = Total flavonoids content.
TAC = Total antioxidant capacity.
DPPH = 2,2-diphenyl-1-picrylhydrazyl antioxidant activity.
FRAP = Ferric reducing antioxidant power.

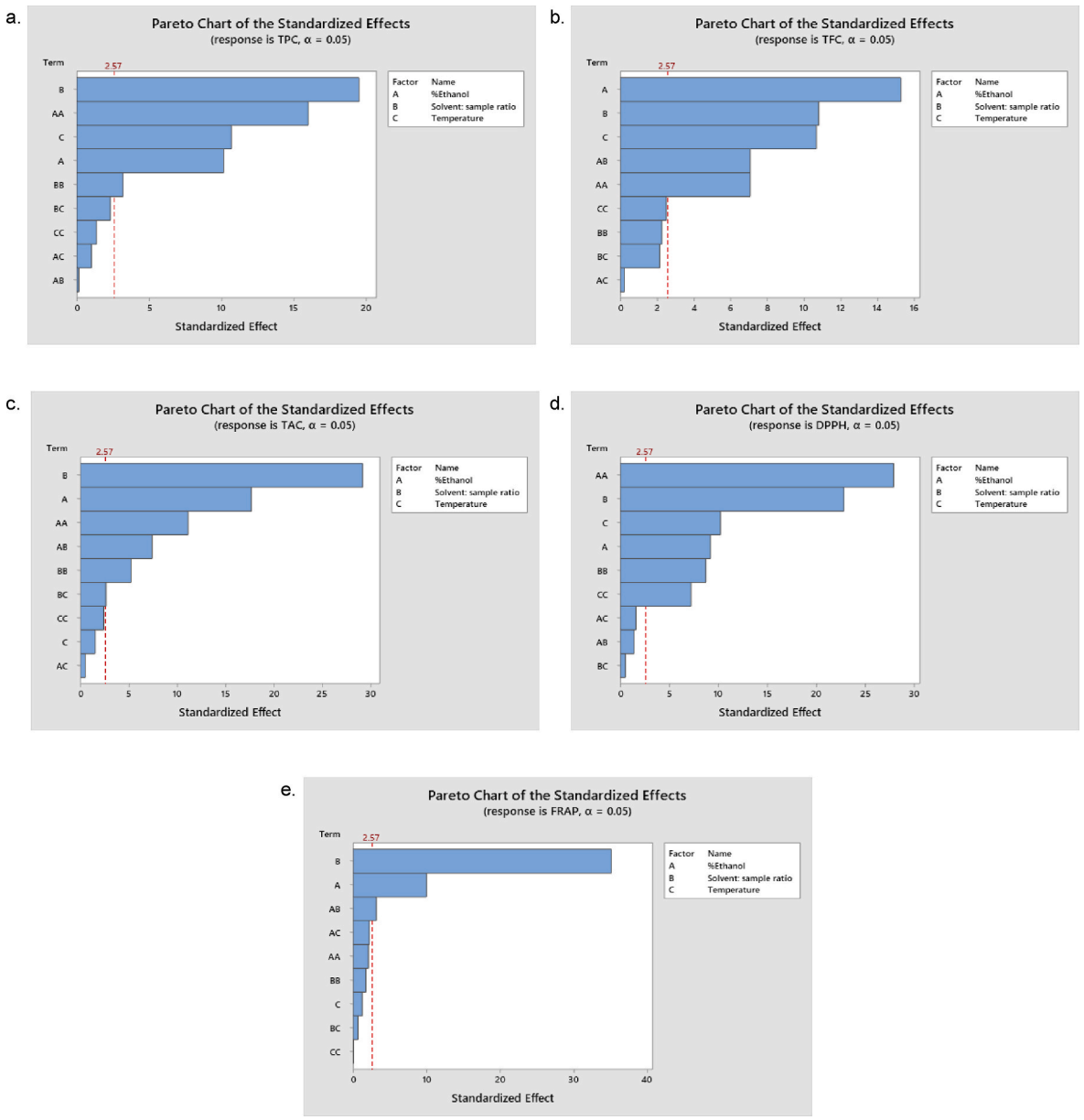

Fig. 3. Pareto chart showing magnitude of factors for: a. total phenolic content; b. total flavonoid content; c. total antioxidant capacity; d. DPPH radical scavenging activity; e. ferric reducing antioxidant power.

Table 6
Pearson's correlation coefficient calculated for the response variables.

	TPC	TFC	TAC	DPPH	FRAP
TPC	1	0.836**	0.877**	0.887**	0.693*
TFC		1	0.830**	0.674*	0.596*
TAC			1	0.727*	0.888**
DPPH				1	0.535*
FRAP					1

Level of significance *p < 0.05; **p < 0.01.
TPC = total phenolic content.
TFC = total flavonoids content.
TAC = total antioxidant capacity.
DPPH = 2,2-diphenyl-1-picrylhydrazyl antioxidant activity.
FRAP = ferric reducing antioxidant power.

purposes. In other words, opportunities abound for SGB and its extracts. These include but not limited to: i. utilisation in the delivery of functional foods and ingredients in new products development, ii. utilisation in the "preventative health" space for dietary supplements, iii. utilisation for the delivery of "clean labels" and natural alternatives to artificial/manufactured additives/ingredients, and iv. utilisation of the extracts as natural antioxidants in foods. In addition, optimisation of the polyphenol extraction process can provide a basis for optimal use of botanicals, water, and energy resources during gin distillation for optimal flavour extraction and development. The interest in polyphenol and polyphenol ingredients can see SGB become an important source of polyphenol to meet the growing demands of the market. However, the study has not identified the individual polyphenols contained in SGB. To fully harness this valorisation potential of SGB, the individual polyphenols in SGB will be analysed and identified for further exploration of their benefits.

CRediT authorship contribution statement

Ekene Christopher Umego: Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Catherine Barry-Ryan:**

Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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