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A factorial design to optimise the extraction of phytochemicals from *Carpobrotus edulis*

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Background: *Carpobrotus edulis* is widely used in traditional medicine to treat several health problems in South Africa. Phytochemical screening of medicinal plants involves several assays and requires a reasonable amount of starting material. Several factors, including plant species and variety, growth conditions, plant age and harvesting time, and plant part, can influence the extraction yield and quality of medicinal plant extracts.

Aim: This research aimed to optimise extraction yields from *C. edulis* using an analytical 2^3 full factorial design.

Setting: The plant material for this study was collected from the dunes along the Bloubergstrand coast (latitude: –33°47′49.92″ S and longitude: 18°27′43.20″ E) in the Western Cape Province, South Africa.

Methods: The factorial design involved two extractants (aqueous and methanolic), two pH levels (7 and 9) and two extraction temperatures (25°C and 40°C) and two extraction periods of 72 and 168 h. Regression models and factorial analysis provided a robust framework for evaluating these variables, including interaction terms that allowed for a better understanding of how combined conditions affect yields differently from individual conditions.

Results: The most effective conditions were a pH of 9 and a temperature of 40°C, with extraction times of 72 h for the aqueous extracts and 168 h for the methanolic extracts.

Conclusion: A factorial design can systematically optimise the exact extraction parameters to produce the highest yield of bioactive compounds from plants.

Contribution: Obtaining the best yield in medicinal plant research can serve several purposes, including conservation of the plant and the need to drive innovation in herbal medicine research.

Keywords: *Carpobrotus edulis*; extraction yield; 2^3 factorial design; phytochemical analysis; regression models.

Introduction

Carpobrotus edulis L. Bolus (family Aizoaceae) is a succulent, drought-resistant, spreading plant with triangular and fleshy leaves. The plant is an element of the Materia medica of South Africa and is used traditionally for treating mouth and throat infections, diphtheria, dysentery, diarrhoea and tuberculosis (De Beer & Van Wyk 2011; Van Wyk 2008). The plant is also used to treat sores, scalds and skin diseases (De Beer & Van Wyk 2011). Omoruyi, Bradley and Afolayan (2012) reported that the plant is used to treat diabetes, hypertension, intestinal parasites and infrequent bowel movements in some regions of South Africa.

Phytochemical screening of medicinal plants involves several assays and requires a practical amount of starting material. Obtaining the best yield can serve several purposes, including economics, conservation of the plant, supply security, quality of the product and the need to drive innovation in herbal medicine research. Several factors, including plant species and variety, growth conditions, plant age and harvesting time, and plant part, can influence the extraction yield and quality of medicinal plant extracts.

The efficiency of any extraction technique is influenced by independent variables, including potential of hydrogen (pH), extraction time, temperature, solvent and solid-to-liquid ratio. These values cannot be generalised for all medicinal plants because of the diversity in their composition and bioactive compounds. Knowledge of the independent variables and their interactions in the

extraction process is required to understand the optimal method for the mining of phytochemicals. This information dictates the selection of variables and their values to obtain the maximum benefit from the technique, ensuring maximum efficiency (extraction yield). Traditionally, optimisation is achieved by examining the effect of a single variable at a time. Factorial designs for the extraction of plant compounds have been reported in our previous studies (Laloo et al. 2024; Terblanche et al. 2017) by several authors, including Kim et al. (2022), Suksaeree and Monton (2024), Cvitković et al. (2024) and Assaggaf et al. (2024).

This study aimed to investigate the effects of pH, extraction time and temperature on the yield of crude aqueous and methanolic extracts from *C. edulis* using two 2^3 full factorial designs. Furthermore, the extracts were screened to establish the presence of diverse phytochemicals obtained using the listed independent variables.

Research methods and design

Reagents used in this study

Many of the reagents were obtained from Sigma-Aldrich Corporation (St. Louis, MO, United States), including aluminum chloride, cholesterol, copper(II) acetate monohydrate, copper(II) sulphate, 3,5-dinitrobenzoic acid, Dragendorff reagent, gallic acid, iron(III) chloride, magnesium ribbon, methanol, 1-naphtol, ninhydrin, nitric acid, picric acid, pyridine, quercetin and sulphuric acid. Glacial acetic acid, chloroform, glucose, Lugol's iodine solution and sodium chloride were purchased from Rochelle, and blood agar plates (Selecta-Media Columbia Agar, ThermoScientific) were donated by the Biomedical Technology division of the Department of Health Sciences (VUT, South Africa). Acetic anhydride, aspartic acid, ethanol, gelatin, glycine, hydrochloric acid, lead acetate 3-hydrate, potassium acetate, potassium iodide, sodium carbonate anhydrous, sodium citrate dihydrate, sodium hydroxide, sucrose and tyrosine were obtained from Merck (Germany). Iodine and mercury(II) chloride were obtained from Thomas Baker Chemicals (Mumbai, India) and Radchem (Pty) Ltd (Gauteng, South Africa), respectively. All chemicals were analytical grade. We used Ultrapure water that was obtained from a Millipore Direct-Q water purifier system (Merck Millipore, Darmstadt, Germany).

Collection, authentication and preparation of the plant material

The plant material for this study was collected from the dunes along the Bloubergstrand coast (latitude: –33°47′49.92″ S and longitude: 18°27′43.20″ E) in the Western Cape Province, South Africa. The plant was authenticated by Professor Stefan Siebert, curator of the AP Goossens Herbarium (Northwest University, Potchefstroom), where two voucher specimens (numbers 148 300 and 148 301) are housed. The fresh leaves of the plant were prepared as described by Laloo et al. (2024). The dried pulverised plant material was stored at –20°C in an airtight container for further analysis.

Extraction and experimental design

The variables affecting the two solvents used to extract phytocompounds from *C. edulis* were screened using a factorial design comprising three independent variables, pH, extraction time and temperature, each at two levels. Box 1 shows the independent variables, their designated letters and the levels used in this study.

The solid-to-solvent ratio (1:20) was kept constant for all experimental runs. The experimental runs were randomised to minimise the effects of unexpected variability in the observed response. The extraction yield (%) was the dependent variable or response.

The independent variables (pH, extraction time and temperature) for each experimental run were chosen as dictated by the experimental design matrix (Table 1).

BOX 1: Independent variables and their levels used in the 2^3 factorial design.

Independent variables	Low level (–)	High level (+)
A: pH	5	9
B: Extraction time	72 h	168 h
C: Temperature	25°C	40°C

Source: Laloo, N., Terblanche, U., Ssemakalu, C. & Pillay, M., 2024, 'The effect of solvent, pH, extraction time and temperature on the extraction of phenolic compounds and antioxidant activity of *Carpobrotus edulis*', *Journal of Phytology* 16, 1–7. https://doi.org/10.25081/jp.2024.v16.8393

pH, potential of hydrogen.

TABLE 1: Design matrix, response and predicted values for the aqueous extraction conditions.

Run	Independent variables			Independent variables			Extraction yield (%) ± s.d.			
	A	B	C	A	B (h)	C (°C)	Aqueous extracts		Methanolic extracts	
							Actual	Predicted	Actual	Predicted
01	–	–	+	5	72	40	25.66 ± 1.22	25.57 ± 0.99	52.45 ± 3.06	52.93 ± 1.94
02	+	–	+	9	72	40	31.03 ± 0.58	31.11 ± 0.99	64.05 ± 0.85	63.13 ± 1.94
03	–	+	–	5	168	25	24.43 ± 0.85	24.34 ± 0.99	43.54 ± 0.80	42.62 ± 1.94
04	+	+	–	9	168	25	22.21 ± 1.08	22.29 ± 0.99	52.35 ± 1.26	52.83 ± 1.94
05	–	+	+	5	168	40	24.54 ± 1.57	24.45 ± 0.99	58.51 ± 3.55	59.09 ± 1.94
06	+	+	+	9	168	40	21.55 ± 1.12	21.64 ± 0.99	64.21 ± 2.12	64.06 ± 1.94
07	–	–	–	5	72	25	24.95 ± 0.77	24.86 ± 0.99	41.85 ± 1.18	41.70 ± 1.94
08	+	–	–	9	72	25	24.09 ± 0.42	24.18 ± 0.99	46.08 ± 0.82	46.67 ± 1.94

Source: Laloo, N., Terblanche, U., Ssemakalu, C. & Pillay, M., 2024, 'The effect of solvent, pH, extraction time and temperature on the extraction of phenolic compounds and antioxidant activity of *Carpobrotus edulis*', Journal of Phytology 16, 1–7. https://doi.org/10.25081/jp.2024.v16.8393

Note: Independent variables: A = pH; B = extraction time (h); C = temperature (°C). Low levels (–): pH 5, extraction time 72 h; temperature 25°C. High levels (+): pH 9, extraction time 168 h; temperature 40°C.

s.d., standard deviation.

A shaking incubator (Labotec, Model 355, Johannesburg, South Africa) was used for the extractions at 150 rpm. After filtering the extracts through Whatman No. 1 filter paper, the aqueous filtrates were lyophilised, and the methanolic extracts were concentrated using a rotary evaporator (HB 10 basic, IKA®-Werke GmbH & Co. KG, Staufen, Germany). The concentrated methanolic extracts were then dried further until the weight remained constant. The extracts were stored at −20°C in dark containers until further analysis. The extraction yield (%) was calculated using Equation 1:

$$\%\ Extraction\ yield = \frac{Amount\ (g)\ of\ dried\ crude\ extract\ obtained}{Amount\ (g)\ of\ finely\ ground\ plant\ material\ used} x100 \quad \text{[Eqn 1]}$$

Regression and statistical analysis

The experiments were performed in triplicate to estimate experimental error, reduce noise and minimise bias in the observed response values (Vaux, Fidler & Cumming 2012). The Design Expert version 11 (Stat-Ease Inc., MN, United States) statistical software was used to analyse the response variables, as described in Laloo et al. (2023) The following equation expressed the regression model in this study (Equation 2):

$$y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{123} ABC + \varepsilon \quad \text{[Eqn 2]}$$

In Equation 2, the predicted response is represented by y, and β_n represents the regression coefficient correlated with variable n. The regression coefficients were obtained from the analysis of the experimental data. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e. Analysis of variance (ANOVA) was used to determine the statistical significance ($p < 0.05$) of the model, and the accuracy was determined using the coefficients of determination (R^2). The Fisher F-test was used to determine whether the constructed models were adequate to describe the observed response values. The relationship between the independent variables (main effects), their intervariable effects (interaction effects) and the response variable was demonstrated through statistical plots.

Qualitative phytochemical screening

Qualitative phytochemical screening was performed on all reconstituted (50 mg/mL) aqueous (Terblanche et al. 2017) and methanolic crude extracts using standard methods (Godghate, Sawant & Sutar 2012; Nayak et al. 2011; Obouayeba et al. 2015; Samejo et al. 2013). The following phytochemicals were analysed: carbohydrates, proteins, amino acids and secondary metabolites, including alkaloids, anthocyanins, diterpenes, flavonoids, glycosides, phenols, phytosterols, saponins, tannins and terpenoids.

Ethical considerations

Ethical clearance to conduct this study was obtained from the Vaal University of Technology Faculty Research Ethics Committee (No. FACSREC-16102020-A00015).

Results and discussion

Extraction optimisation

This study determined how varying the pH, extraction time and temperature can affect the extraction yield of phytochemicals from the leaves of *C. edulis* using maceration. A factorial design shows the influence of the independent variables and their interactions. Table 1 shows the design matrix with the values of the independent variables, the experimental response values (extraction yield as a percent) and predicted values (based on the regression analysis) for each of the 16 experimental runs (8 aqueous extracts and 8 methanolic extracts).

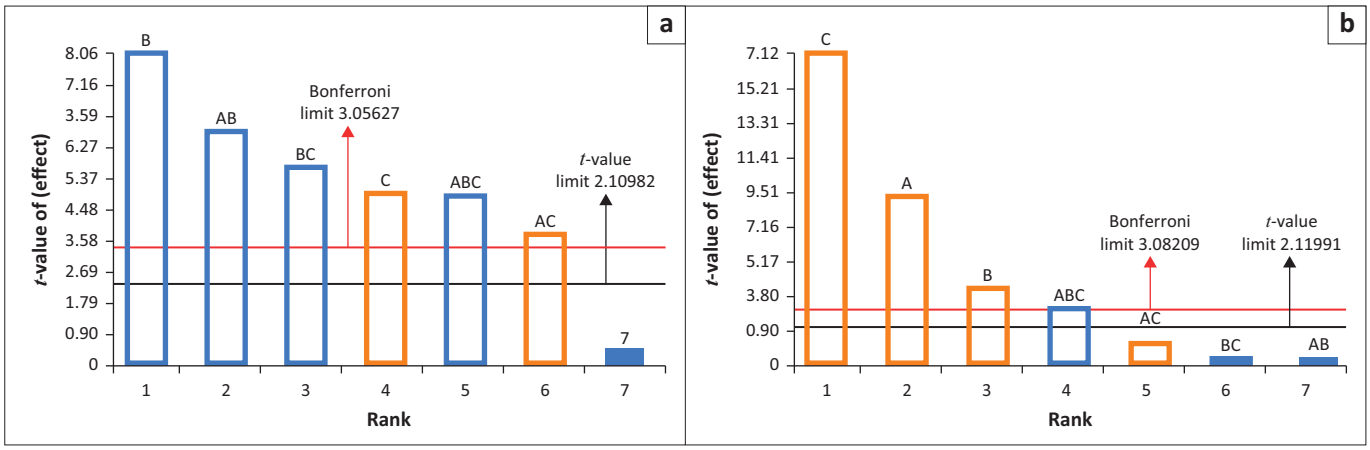
Conspicuously higher extraction yields (41.70% – 64.21%) were obtained for the methanolic extracts than the aqueous extracts (21.55% – 31.11%). The amphiphilic nature of methanol can form intermolecular forces between polar and nonpolar functional groups, resulting in the solubilisation and extraction of a larger range of phytochemicals (Tiwari et al. 2011). Lapornik, Prošek and Wondra (2005) stated that methanol is more efficient in the degradation of cell walls, which have nonpolar characteristics, releasing more phytochemicals from the cells. Methanol was also found to be a better extraction medium by Magar et al. (2023) and Nortjie et al. (2024). The observation from this study underscores the importance of extracting solvents in medicinal plant research.

The effects of the independent variables on the extraction yield

Statistical plots (i.e. Pareto plots, main effect and interaction plots) for the water and methanol extraction conditions were generated to: (1) identify the statistically significant variables; and (2) describe the effect of each of the independent variables and their interactions on the percent extraction yield.

The weight and statistical significance of the independent variables and the intervariable interactions are displayed on Pareto charts (Figure 1), main effect plots (Figure 2) and interaction plots (Figure 3).

According to Ozbay and Yargic (2015), Pareto charts show the absolute values of the standardised effects in decreasing order and establish two limit lines, namely, the Bonferroni limit line (t-value of effect = 3.08209) and the t limit line (t-value of effect = 2.11991), which defines the minimum statistically significant effect magnitude for the 95% confidence level. Regression coefficients with t-value effects above the Bonferroni line are labelled as significant, while those with t-value effects below the t-limit line are considered statistically nonsignificant (Shah & Pathak 2010). If the t-value of the effect of a regression coefficient lies between the Bonferroni and t-limit lines, the coefficient is likely to be significant. Main effect and interaction plots refer to simple line graphs obtained from connecting the mean values of each treatment. An effect with a zero-slope horizontal line (main effect plots) and an interaction displaying parallel lines



Note: The colour of the bar denotes a positive (orange) or negative (blue) effect on the response value. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e.

FIGURE 1: Pareto chart for the (a) aqueous extraction conditions and (b) methanolic extraction conditions.

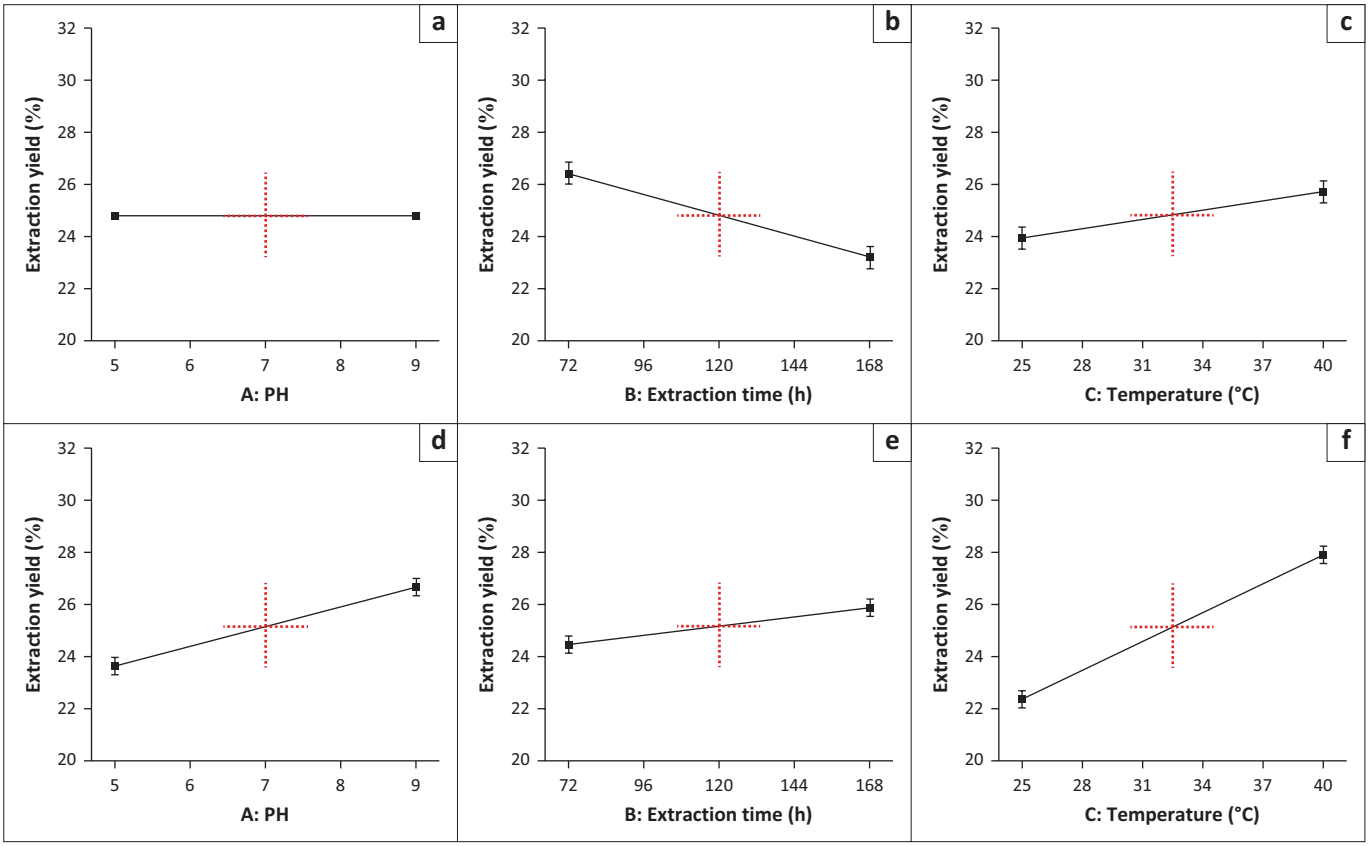


FIGURE 2: Main effect plots illustrating the influence of each independent variable on the extraction yield (%) for the aqueous (a–c) and methanolic (d–f) extraction conditions.

(interaction plots) were interpreted as not significant effects, whereas increasing (positive effect – the response is higher at the high level) or decreasing (negative effect – the response is lower at the high level) effect lines were considered significant.

Figure 1 for the aqueous extraction conditions revealed that the extraction time (B) had the most significant effect in a negative mode (Figure 2) on the extraction yield (%), whereas it showed a positive significant effect (Figure 2) on the percent extraction yield (%) for the methanolic extraction conditions. The temperature (°C) had the most significant effect in a positive mode (Figure 2) on the extraction yield (%) for the methanolic extraction conditions (Figure 1) and exhibited a significantly positive effect (Figure 2) for the aqueous extraction conditions. The interactive effects, AB (pH*extraction time) and BC (extraction time*temperature), demonstrated both negative significant effects (Figure 3) for the aqueous extraction conditions but displayed no significant effects (Figure 3) on the extraction yield (%) for the methanolic extraction.

The contribution of pH (A) was positively significant (Figure 2) for the methanolic extraction conditions but was not significant (Figure 2) for the aqueous extraction conditions. The interaction between pH and temperature

(AC) exhibited a weak, positively significant effect (Figure 3) on the extraction yield for the aqueous extraction conditions but had no significant role (Figure 3) during the methanolic extraction conditions. The three-way interaction ABC (pH*extraction time*temperature) was above the Bonferroni limit and displayed a significantly negative effect on the extraction yield (%) for both the aqueous and methanolic extraction conditions.

Factorial regression model development

After fitting the obtained response values for each extraction condition tested to the regression model equation (Equation 2) and performing ANOVA to determine the accuracy of the suggested models, the *p*-values for the three independent variables and the intervariable interactions were obtained and are presented in Table 2 (aqueous), Table 3 (aqueous), Table 4 (methanolic) and Table 5 (methanolic), respectively. The variables and interactions with *p*-values greater than 0.05 were considered not significant and excluded from the model development. These mathematical models, based on the regression equation (Equation 2), were built and are presented in Equation 3 (aqueous conditions) and Equation 4 (methanolic conditions):

$$\hat{y} = \beta_0 - \beta_2 B + \beta_3 C - \beta_{12} AB + \beta_{13} AC - \beta_{23} BC - \beta_{123} ABC$$
$$Yield(\%) = 24.81 - 1.62 * ET + 0.8871 * T - 1.21 * pH * ET + 0.6838 * pH * T - 1.02 * ET * T - 0.8738 * pH * ET * T \quad \text{[Eqn 3]}$$

$$\hat{y} = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C - \beta_{123} ABC$$
$$Yield(\%) = 52.88 + 3.79 * pH + 1.77 * ET + 6.92 * T - 1.31 * pH * ET * T \quad \text{[Eqn 4]}$$

where $\hat{y}$ represents the predicted extraction yield, *ET* the extraction time and *T* the temperature.

The regression coefficients (β_n) were obtained from the ANOVA and describe the potential influence of each of the independent variables and their interactions on the response value (Montgomery 2013). The higher the value of the regression coefficient, the more significant the corresponding effect (Alcheikh Hamdon, Darwish & Hilal 2015). The positive sign of the regression coefficients for the aqueous extraction condition (β_0, β_3 and β_{13}) and the methanolic

TABLE 2: Analysis of variance report for the full model for the aqueous extraction conditions.

Source	Sum of squares	Degree of freedom	Mean squares	*F*	*p*
Model	172.5100	7	24.6400	24.0600	< 0.0001
A: pH	0.1820	1	0.1820	0.1777	0.6790
B: Extraction time	63.3400	1	63.3400	61.8300	< 0.0001
C: Temperature	18.8900	1	18.8900	18.4300	0.0006
AB	35.4100	1	35.4100	34.5600	< 0.0001
AC	11.2200	1	11.2200	10.9500	0.0044
BC	25.1500	1	25.1500	24.5500	0.0001
ABC	18.3200	1	18.3200	17.8800	0.0006
Pure error	16.3900	16	1.0200	-	-
Cor total	**188.90**	**23**	-	-	-

Note: s.d. = 1.01; R^2 = 0.9132; R^2_{adj} = 0.8753. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e.

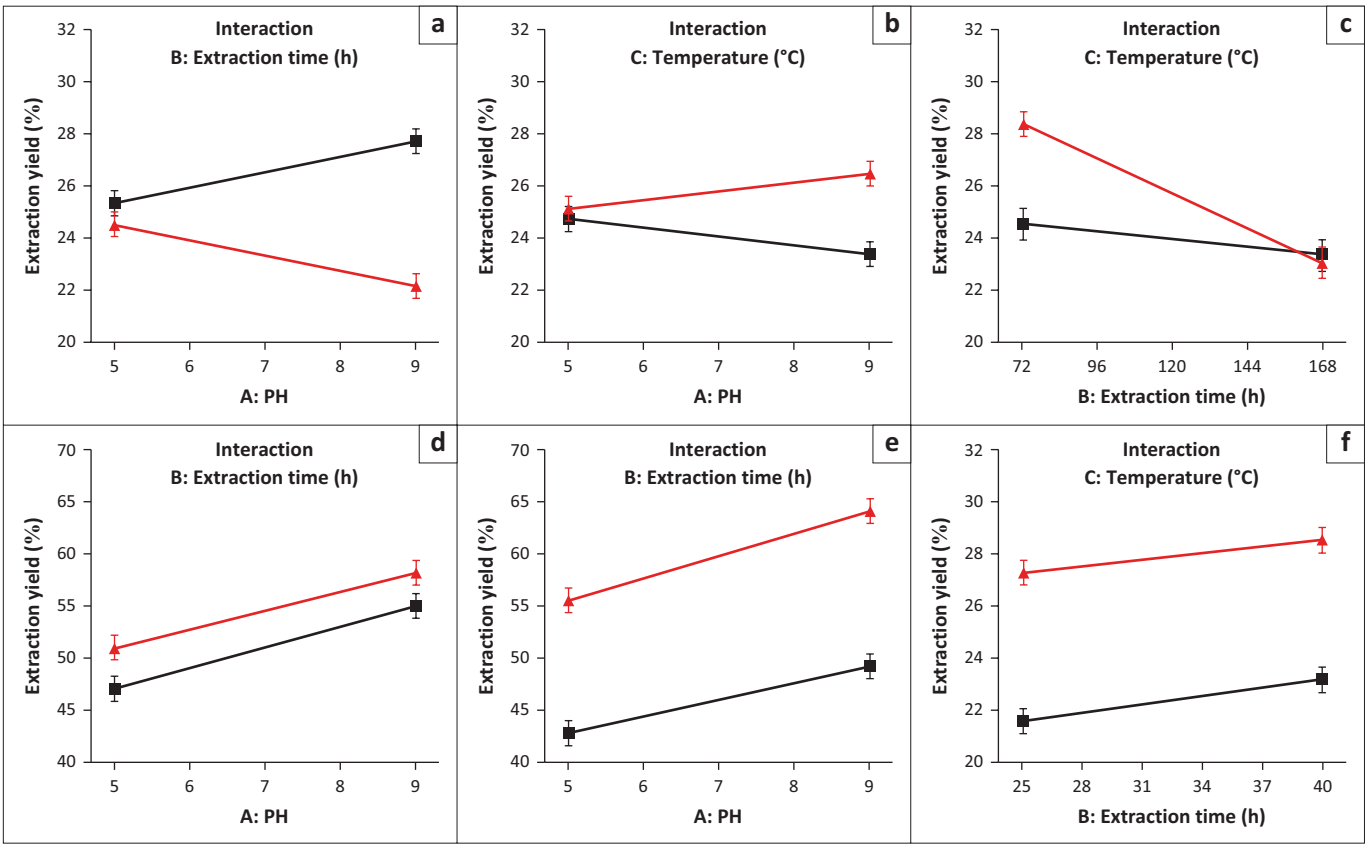


FIGURE 3: Interaction plots for the aqueous (a–c) and methanolic (d–f) extraction conditions. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC.

TABLE 3: Analysis of variance report for the reduced model for the aqueous extraction conditions.

Source	Sum of squares	Degree of freedom	Mean squares	*F*	*p*
Model	172.3300	6	28.7200	29.4600	< 0.0001
B: Extraction time	63.3400	1	63.3400	64.9700	< 0.0001
C: Temperature	18.8900	1	18.8900	19.3700	0.0004
AB	35.4100	1	35.4100	36.3200	< 0.0001
AC	11.2200	1	11.2200	11.5100	0.0035
BC	25.1500	1	25.1500	25.8000	< 0.0001
ABC	18.3200	1	18.3200	18.7900	0.0004
Residual	16.5700	17	0.9749	-	-
Lack of fit	0.1820	1	0.1820	0.1777	0.6790
Pure error	16.3900	16	1.0200	-	-
Cor total	**188.9000**	**23**	-	-	-

Note: s.d. = 0.9874; R^2 = 0.9123; R^2_{adj} = 0.8813. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e.

TABLE 4: Analysis of variance report for the full model for the methanolic extraction conditions.

Source	Sum of squares	Degree of freedom	Mean squares	*F*	*p*
Model	1621.0000	7	231.5700	58.9600	< 0.0001
A: pH	345.1900	1	345.1900	87.8900	< 0.0001
B: Extraction time	75.3300	1	75.3300	19.1800	0.0005
C: Temperature	1150.6600	1	1150.6600	292.9800	< 0.0001
AB	0.6534	1	0.6534	0.1664	0.6888
AC	6.8500	1	6.8500	1.7400	0.2053
BC	1.1300	1	1.1300	0.2869	0.5996
ABC	41.1900	1	41.1900	10.4900	0.0051
Pure error	62.8400	16	3.9300	-	-
Cor total	**1683.8400**	**23**	-	-	-

Note: s.d. = 1.98; R^2 = 0.9627; R^2_{adj} = 0.9464. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e.

TABLE 5: Analysis of variance report for the reduced model for the methanolic extraction conditions.

Source	Sum of squares	Degree of freedom	Mean squares	*F*	*p*
Model	1612.37	4	403.09	107.1700	< 0.0001
A: pH	345.19	1	345.19	91.7700	< 0.0001
B: Extraction time	75.33	1	75.33	20.0300	0.0003
C: Temperature	1150.66	1	1150.66	305.9100	< 0.0001
ABC	41.19	1	41.19	10.9500	0.0037
Residual	71.47	19	3.76	-	-
Lack of fit	8.63	3	2.88	0.7323	0.5478
Pure error	62.84	16	3.93	-	-
Cor total	**1683.84**	**23**	-	-	-

Note: s.d. = 1.94; R^2 = 0.9576; R^2_{adj} = 0.9486. The main variables are represented by A, B and C, the two-way interactions are by AB, AC and BC, the three-way interaction by ABC and the experimental error by e.

extraction condition (β_1, β_2 and β_3) is indicative of a synergistic effect, whereas the negative sign of the regression coefficients β_2, β_{12}, β_{23} and β_{123} (aqueous) and β_{123} (methanolic) denotes an antagonistic effect.

Significance and validation of the regression models

The Fisher F-test was used to evaluate the statistical significance of the constructed regression models. The *F*-values of 24.06 for the full model (Table 2) and 29.46 for the reduced model (Table 3), both with *p*-values of < 0.0001, suggested that the models were highly significant in describing the response values for the aqueous extraction. The improved *F*-value obtained for the reduced model was because of the elimination of the nonsignificant model terms.

The coefficient of determination (R^2) and adjusted coefficient of determination (R^2_{adj}) were used to validate the accuracy of the model. The coefficient of determination (R^2) determines the proportion of variability in the response value that can be explained by the independent variables, indicating the proximity of the predicted values to the fitted regression line (Ogee et al. 2013). The R^2 for the full (R^2 = 0.9132) and reduced (R^2 = 0.9123) models (aqueous extraction conditions) indicated that the predicted values from the models are a good fit.

Note that the accuracy of the model should not solely be based on R^2, as R^2 always increases with an increase in the number of model variables, even if these variables are not significant (Kukreja et al. 2011). The presence of multiple variables and intervariable effects warrants the evaluation of the adjusted R^2 values, which compensates for the introduction of additional variables on the normal R^2 calculation by only increasing the value, should the additional variables significantly contribute to the model (Grant & Kenton 2019). The value of R^2_{adj} for the full (R^2_{adj} = 0.8753) and reduced (R^2_{adj} = 0.8813) models, even though lower than R^2, still affirms an acceptable model fit.

Similar to the validation criteria used for the aqueous extraction conditions, *F*-tests, R^2 and R^2_{adj} were used to ascertain the significance and validity of the regression models for the methanolic extraction conditions. The *F*-values of 58.96 for the full model (Table 4) and 107.17 for the reduced model (Table 5), both with *p*-values of < 0.0001, affirm the statistical significance in describing the response values for the methanolic extraction conditions. The R^2 for the full (R^2 = 0.9627) and reduced (R^2 = 0.9576) models (methanolic extraction conditions) points to a better model prediction than that of the aqueous extraction conditions. This is underscored by the improved R^2_{adj} calculations (R^2_{adj} = 0.9464) and reduced (R^2_{adj} = 0.9486) for the methanolic extraction models.

Numerical optimisation of the extraction conditions

The regression models for maximum extraction yield were optimised by using the maximum values of the aqueous and methanolic extraction conditions. The optimal yield for the aqueous model was obtained at a pH of 9, an extraction time of 72 h and a temperature of 40°C. The methanolic model produced the best yield at a pH of 9, extraction time of 168 h and temperature of 40°C.

Qualitative phytochemical analysis

Table 6 shows the results of the qualitative phytochemical screening of the extracts, showing the impact of the various extraction conditions. The extraction variables did not affect the presence of carbohydrates (Molisch and Benedict's test), phenols, tannins, flavonoids, cardiac glycosides, diterpenes, phytosteroids and triterpenoids because they were present in

TABLE 6: Qualitative phytochemical screening of aqueous and methanolic crude extracts of *Carpobrotus edulis*.

Compound	Phytochemical test	Aqueous extracts (Run)								Methanolic extracts (Run)								Positive indication
		1	3	5	7	9	11	13	15	2	4	6	8	10	12	14	16	
Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Violet ring at the junction
	Benedict's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Reddish brown ppt‡
	Iodine test	–	–	–	–	–	+	–	+‡	–	–	–	–	–	–	–	–	Black-blue
Proteins	Biuret test	–	+	–	–	+a	–	+a	–	+	+	+	+	+	+	+	+	Violet
	Sulphur test	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	Black
	Xanthoproteic test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Yellow to orange ppt
Amino acids	Ninhydrin test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Blue-violet
Alkaloids	Dragendorff test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Orange-brown ppt
	Mayer's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Cream to yellow ppt
	Wagner's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Reddish-brown ppt
Flavonoids	Alkaline reagent test	+a	+a	+a	+a	+a	+a	+a	+a	+	+	+	+	+	+	+	+	Yellow to orange
	Shinoda test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Pink, red or orange-red
	Anthocyanin test	–	+a	–	–	–	–	+	+	–	–	–	–	–	–	–	–	Blue-violet after the addition of ammonia
	Leucoanthocyanin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Red in the upper layer
Anthraquinone glycosides	Modified Borntrager's test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Pink, red or violet in the ammonia layer
Cardiac glycosides	Keller Kiliani test	+a	+	+a	+a	+a	+a	+a	+a	+	+	+a	+	+a	+	+	+	Bluish and brown ring at the junction
	Kedde's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Purple that fades to a brownish-yellow
	Baljet test	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	Orange or red
Saponin glycosides	Foam test	–	–	–	+a	–	–	–	–	–	–	–	–	–	+a	–	+a	Persistent foam
	Haemolysis test	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Blood haemolysis
Phenols and tannins	Gelatin test	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	White ppt
	Braymer's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Bluish-black or brownish-green
	Phlobatannins	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Red ppt
Diterpenes	Copper acetate test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Emerald green
Phytosteroids and triterpenoids	Salkowski's test	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	Red, reddish-brown
	Liebermann Burchard's test	+	+	–	–	+a	+a	+a	+	+	+	+	+	+a	+	+	+	Red in lower layer and greenish in upper layer

ppt, precipitate; ‡, aTrace amounts.

all extracts under all experimental conditions. Many of the therapeutic properties of *C. edulis* are attributed to the presence of these metabolites. Phytochemical tests for the xanthoproteic reaction, ninhydrin alkaloids, anthraquinone glycosides, leucoanthocyanins, haemolysin test for saponins and phlobatannins were negative for both extracts. On the contrary, anthocyanins, starch, phytosteroids, triterpenoids, the Biuret test for proteins and saponins were present in some of the experimental runs. The sulphur test showed the presence of proteins only in the methanolic extracts. The presence or absence of these phytochemicals provides evidence that a factorial design is essential in obtaining a substantial quantity of a desired active compound from plants. The results also show that certain solvents are required to isolate specific compounds, as shown in the case of sulphur in this study. The negative results for starch, aromatic amino acids, free amino acids, alkaloids, anthocyanins, leucoanthocyanins, anthraquinone glycosides, saponins and phlobatannins may be because of the choice of extraction solvent or the absence of these compounds in detectable quantities in the extracts.

Conclusion

Two 2^3 full factorial designs were used to determine the contribution of pH, extraction time and temperature to the extraction yield (%) of phytochemicals from the leaves of *C. edulis*. Statistical analysis of data for the aqueous extraction of *C. edulis* showed that the percentage extraction yield was significantly affected by the extraction time and temperature and their intervariable interactions. Although the effect of pH was not statistically significant, the pH*extraction time and pH*temperature interactions strongly affected the extraction yield. The methanolic extraction of *C. edulis* strongly depended on all three investigated extraction parameters (pH, extraction time and temperature) as well as the three-way interaction between them. The presence or absence of some of the phytochemicals observed in this study suggests the power of a factorial design to isolate specific compounds from *C. edulis*.

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Competing interests

The authors reported that they received funding from the Vaal University of Technology, which may be affected by the research reported in the enclosed publication. The authors have disclosed those interests fully and have implemented an approved plan for managing any potential conflicts arising from their involvement. The terms of these funding arrangements have been reviewed and approved by the affiliated university in accordance with its policy on objectivity in research.

Authors' contributions

U.T. and C.C.S. designed the research, analysed the data and wrote the manuscript. N.L. contributed to the research. M.P. supervised the work.

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Data availability

The data that support the findings of this study are available from the corresponding author, M.P., upon reasonable request.

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