Journal of Applied Research on Medicinal and Aromatic Plants 29 (2022) 100371

Contents lists available at ScienceDirect

Journal of Applied Research on Medicinal and Aromatic Plants

journal homepage: www.elsevier.com/locate/jarmap

Bioprospection of underutilized wild *Cissus woodrowii* fruits for nutritional value and characterization of green-extracted antioxidant phenolic compounds

Rupali M. Kolap [a], Prachi S. Kakade [b], Ganesh D. Mankar [c], Rajkumar B. Barmukh [c], Rajesh N. Gacche [d], Saurabha B. Zimare [a,e]

[a] Naoroji Godrej Centre for Plant Research, Lawkim Motor Campus, Shindewadi, Post-Shirwal, Khandala, Satara 412801, India
[b] Department of Botany, Savitribai Phule Pune University, Pune 411007, India
[c] Modern College of Arts, Science and Commerce (Autonomous), Shivajinagar, Pune 411005, India
[d] Department of Biotechnology, Savitribai Phule Pune University, Pune 411007, India
[e] Sohuum Aroma Pvt. Ltd., Warje, Pune 411058, India

ARTICLE INFO

Keywords:
Box–Behnken design
Cissus woodrowii
Green extraction
Nutrition
Response surface methodology
Underutilized wild fruits

ABSTRACT

The nutritional value of underutilized wild *C. woodrowii* fruits was estimated, and a method for ultrasound-assisted extraction (UAE) of total phenolics content (TPC) and total flavonoids content (TFC) from fruits was established. Proximate analysis of fruits showed a calorific value of 168.86 kcal/100 g dry weight (DW). These fruits were found to be rich in macro- (sodium, potassium, calcium, magnesium, and phosphorus) and micro- (iron, manganese, zinc, and copper) mineral elements and vitamins (carotenoids and ascorbic acid). UAE was optimized with a Box-Behnken design (BBD) with three independent variables (ethanol concentration, temperature, and extraction time), each at three levels. The data was analyzed using response surface methodology (RSM). For UAE, the optimal combination for maximum recovery of phenolic compounds and antioxidant activity was 58.79% solvent concentration, 45 °C temperature, and 15 min ultrasonication time. The extract made under these conditions had higher TPC and TFC contents of 46.01 mg TAE/g DW and 32.65 mg QE/g DW, respectively. Furthermore, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays of the extract showed higher %RSA of 97.26% and 95.78%, respectively. Characterization of a potent extract using liquid chromatography–high-resolution mass spectrometry (LC–HRMS) discovered the presence of 20 different phenolic compounds.

1. Introduction

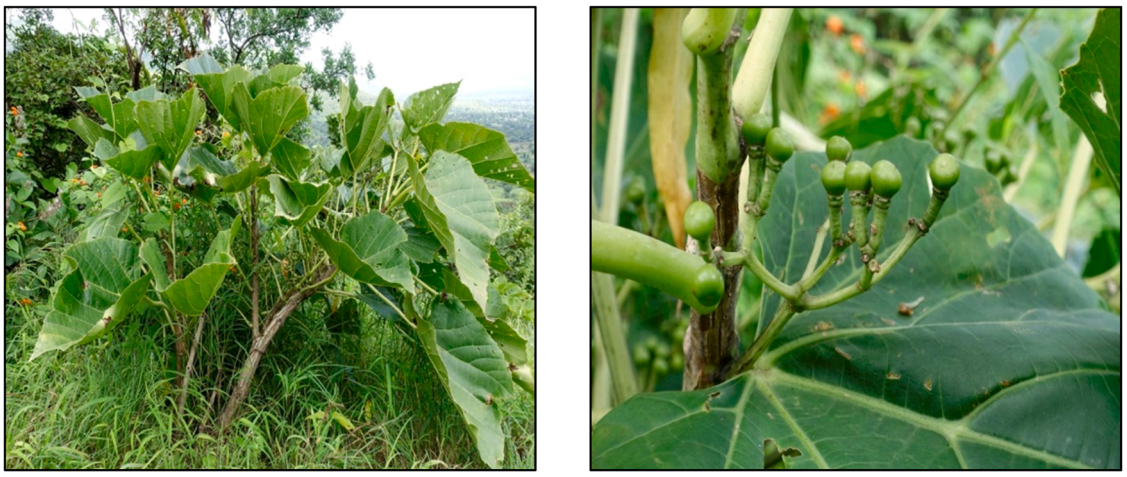
Fruits are a significant source of vitamins, minerals, dietary fiber, proteins, fats, and carbohydrates; hence considered a major source of energy and are important to reduce the risk of numerous non-communicable diseases (Li et al., 2016; Abeysuriya et al., 2020; Murthy and Bapat, 2020). Fruits contain different dietary phytochemicals, of which phenolics are important to reduce oxidative stress and related morbidity and mortality (Murthy and Bapat, 2020). India has a rich biodiversity. However, only eleven fruits are commercially cultivated and available for human consumption (Mitra et al., 2010). Thus, a significant pool of fruits is still underutilized and demands scientific exploration to ensure sustainability in food and medicines. Underutilized fruits have higher nutrients and bioactive phytochemicals, which can serve and nurture humankind (Murthy and Bapat, 2020).

The genus *Cissus* L., a member of the Vitaceae family, comprises 350 species, many of which are used in traditional medicines and by local healers (Tasadduq et al., 2017; Dhanasekaran 2020). Twenty-two species of *Cissus* are known from India, of which *Cissus woodrowii* (Stapf ex Cooke) Santapau (Woodrow's grape tree) is underutilized wild plant. This species is characterized by shrub-like habit (Fig. 1a and b) (Cooke, 1902). A recent study has explored antioxidant properties and phenolic compounds of *C. woodrowii* plant parts (Kolap et al., 2020). However, scientific studies describing the nutritional value, phenolic compounds,

Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BBD, Box-Behnken design; DPPH, 2,2-diphenyl-1-picrylhydrazyl; RSA, Radical scavenging activity; RSM, Response surface methodology; TFC, Total flavonoids content; TPC, Total phenolics content; UAE, Ultrasound-assisted extraction.

E-mail address: saurabhabot@gmail.com (S.B. Zimare).

https://doi.org/10.1016/j.jarmap.2022.100371
Received 4 September 2021; Received in revised form 26 December 2021; Accepted 20 January 2022
Available online 22 January 2022



a b

Fig. 1. Shrub-like habit (a) and fruiting twig (b) of *Cissus woodrowii*.

and antioxidants activity of *C. woodrowii* fruits are sparse.

The extraction of phenolic compounds from plants is critical as their yield is significantly influenced by extraction parameters (Okur et al., 2021; Zimare et al., 2021). For several decades, conventional (Soxhlet, maceration, infusion, and digestion) methods have been used to extract phenolic compounds. However, these methods need higher quantities of chemicals and energy and are prone to the oxidation, ionization, and hydrolysis of phenolic compounds, which eventually limits the recovery and bioactivity (Osorio-Tobón 2020; Okur et al., 2021; Zimare et al., 2021). To overcome such limitations, industrially and commercially important alternative methods called 'green extractions' are in advance (Okur et al., 2021; Zimare et al., 2021). The u**ltrasound-assisted extraction (**UAE) is a type of green extraction and is superior for the recovery of phenolic compounds compared with conventional methods (Osorio-Tobón 2020). Additionally, it is an eco-friendly approach since UAE requires comparatively lesser solvent, energy, time, and expenses for higher recovery of phenolic compounds (Altemimi et al., 2016; Aguilar-Hernández et al., 2020; Zimare et al., 2021). To understand the collective effects of inputs (extraction parameters) on the response (recovery of phenolics and antioxidant activity), UAE needs to be optimized and analyzed using mathematical and statistical tools (Altemimi et al., 2016; Aguilar-Hernández et al., 2020; Zimare et al., 2021).

In light of this, the present study reports- i) assessment of the nutritional value of *C. woodrowii* fruits as per the guidelines of the Association of Official Agricultural Chemists (AOAC), ii) optimization of different UAE parameters for higher recovery of phenolic compounds and their subsequent antioxidant activity, and iii) characterization of phenolic compounds by liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS).

2. Materials and methods

2.1. Plant material

Fruiting twigs of *C. woodrowii* were collected from the Pasarni Ghat (17°56'14" N and 73°48'54") region of Satara district, India. Collected plant material was identified using the Flora of the Presidency of Bombay (Cooke, 1902). A herbarium specimen was deposited at Naoroji Godrej Centre for Plant Research (Voucher number NGCPR-003000).

2.2. Chemicals and equipment

Organic solvents (HPLC grade), tannic acid (GRM7541:100 G), quercetin (RM6191-100G), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (RM2798-1G), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (MB255-1G), and Folin–Ciocalteu's phenol reagent (RM10822-100ML) were procured from Himedia, India, and Sigma, USA. The ultrasonication unit and Soxhlet extractor (3840024) were purchased from Lifecare Equipment and Borosil India, respectively. The extracts were concentrated on a rotary evaporator (PBU-6D, Superfit, India) and were stored at 4°C till their further use.

2.3. Proximate composition and vitamins

Proximate analysis (moisture, ash, and crude fiber content) of *C. woodrowii* fruits was carried out by considering the guidelines of AOAC (AOAC, 2006). Fat, protein, and carbohydrate contents were determined by the methods of Kumari et al. (2017), Lowry et al. (1951), and Idris et al. (2019), respectively. The energy value was calculated by adding the values of carbohydrate, lipid, and protein (Idris et al., 2019) and expressed as kJ and kcal per 100 g fruits. The factor for carbohydrate and protein was 16.736 kJ, while for lipid, it was 37.656 kJ. Mineral contents were determined by using atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan). In addition to the proximate, two vitamins (carotenoids and ascorbic acid) were analyzed by the method of Kumari et al. (2017).

2.4. Pilot experiment

Total phenolics (TPC) and total flavonoids (TFC) were extracted using the UAE method. A pilot experiment was conducted to score the effects of ultrasound frequencies (30, 40, 50, and 60 kHz). At the same time, other factors such as ethanol concentration (75%), time (10 min), and temperature (40 °C) were kept constant. The efficiency of the UAE was compared with conventional methods of extractions [Hot (Soxhlet) and cold maceration]. For Soxhlet extraction, 50 g of sample was extracted with 500 ml of 75% aqueous ethanol for six h at 50 °C as described by Karami et al. (2015) with few modifications. For cold maceration, a method by Ojha et al. (2015) was used with slight modifications, where the parameters of extractions were similar to Soxhlet except temperature (20 °C). The antioxidant activity of these extracts was tested by using DPPH and ABTS assays.

2.5. Ultrasound-assisted extraction of phenolic compounds and antioxidant potential

2.5.1. Optimization of independent variables with Box Behnken design

Three independent variables *viz.*, ethanol concentration (X_1, %),

Table 1
Input variables and factors used to investigate the efficiency of ultrasound-assisted extraction.

Symbols	Independent Variables	Levels		
		-1	0	1
X_1	Ethanol concentration (%)	40	60	80
X_2	Ultrasonication time (min)	15	20	25
X_3	Temperature (°C)	45	55	65

Table 2
Effect of process variables on ultrasound-assisted extraction of total phenolics (TPC, mg TAE/g DW) and total flavonoids (TFC, mg QE/g DW) contents in Cissus woodrowii fruits, and their antioxidant activity (% Radical scavenging activity) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) assays.

Run	Independent Variables			Responses			
	Natural Values (Coded Values)						
	X_1	X_2	X_3	TPC	TFC	DPPH	ABTS
1	40 (-1)	15 (-1)	55 (0)	23.57	16.65	77.95	82.48
2	80 (1)	15 (-1)	55 (0)	27.89	19.65	79.23	78.29
3	40 (-1)	25 (1)	55 (0)	21.99	13.65	75.26	74.16
4	80 (1)	25 (1)	55 (0)	23.65	15.38	80.99	79.66
5	40 (-1)	20 (0)	45 (-1)	23.78	14.65	81.45	80.23
6	80 (1)	20 (0)	45 (-1)	25.09	17.77	83.29	76.59
7	40 (-1)	20 (0)	65 (1)	17.37	12.65	73.86	74.21
8	80 (1)	20 (0)	65 (1)	21.71	13.2	77.65	75.78
9	60 (0)	15 (-1)	45 (-1)	44.44	31.32	97.23	91.38
10	60 (0)	25 (1)	45 (-1)	43.23	29.78	94.29	90.45
11	60 (0)	15 (-1)	65 (1)	41.65	29.74	88.65	90.77
12	60 (0)	25 (1)	65 (1)	34.78	24.32	89.23	84.23
13	60 (0)	20 (0)	55 (0)	31.27	23.55	84.45	80.23
14	60 (0)	20 (0)	55 (0)	31.5	23.98	84.78	80.96
15	60 (0)	20 (0)	55 (0)	31.09	23.83	84.23	79.69

ultrasonication time (X_2, min), and temperature (X_3, °C) were used with three levels [low (-1), medium (0), and high (1)] (Table 1). A Box Behnken design (BBD) was used to optimize the UAE to recover higher yields of phenolic compounds (Table 2). Fifteen runs were employed, and all extractions and assays were performed in triplicate (Table 2).

2.5.2. Determination of phenolic compounds and antioxidant activity

Spectrophotometric analysis was carried out to determine the TPC, TFC, and antioxidants potential of *C. woodrowii* fruits as described previously for the plant parts of the same species (Kolap et al., 2020). The TPC and TFC were expressed as mg equivalent of tannic acid (mg TAE) and quercetin (mg QE) per gram dry weight (DW), respectively. The radical scavenging activity (%RSA) in ABTS (734 nm) and DPPH (518 nm) assays was measured spectrophotometrically and was calculated using the following formula:

$$\%\mathbf{RSA\ \ in\ \ ABTS\ \ or\ \ DPPH\ \ assay} = \frac{\mathbf{Ac - As}}{\mathbf{Ac}} \times \mathbf{100}$$

where 'Ac' is the absorbance of blank (ABTS/ DPPH solution), and 'As' is the absorbance of test solution. The %RSA of extracts was compared with that of ascorbic acid (control).

2.6. Characterization of extract

The fruit extract containing the highest recovery of phenolic compounds and antioxidant activity was further characterized by LC-HRMS (Kolap et al., 2020). In brief, Agilent Binary (LC 1260) Triple Quad LC-MS with an Agilent Zorbax Eclipse Plus (C18, 2.1 × 50 mm, 1.8 μm) column was used to screen the extract. Different ratios of the mobile phase comprising 95% water containing 0.1% formic acid (A) and 5% acetonitrile (B) were used with a flow rate of 0.3 ml/min. The electrospray ionization (ESI) was used in both positive and negative modes, and samples were scanned for 30 min. The Agilent 6540 Q-TOF MS system was used with a binary pump (G1312B), auto-sampler (G1312B), and mass spectrometer (G6540B).

Table 3
Proximate composition, analysis of minerals and vitamins in *Cissus woodrowii* fruits.

Proximate composition	Fruits
Moisture (%)	13.71 ± 1.78
Ash (%)	23.88 ± 1.01
Fiber (g/100 g DW)	21.15 ± 2.13
Fat (g/100 g DW)	2.17 ± 1.11
Protein (g/100 g DW)	5.78 ± 0.21
Carbohydrate (g/100 g DW)	15.23 ± 1.65
Energy	
(kJ/100 g DW)	706.54
(kcal/100 g DW)	168.86
Mineral contents	
Macro minerals (mg/100 g DW)	
Na	37.23 ± 2.54
K	71.9 ± 2.47
Ca	78.29 ± 3.01
Mg	118.77 ± 1.80
P	30.45 ± 2.31
Micro minerals (μg/100 g DW)	
Fe	12.45 ± 1.24
Mn	6.41 ± 0.89
Zn	3.11 ± 0.13
Cu	1.22 ± 0.34
Vitamins	
Carotenoids (μg/100 g DW)	15.29 ± 0.14
Ascorbic acid (mg/100 g DW)	63.78 ± 0.78

2.7. Statistical analysis

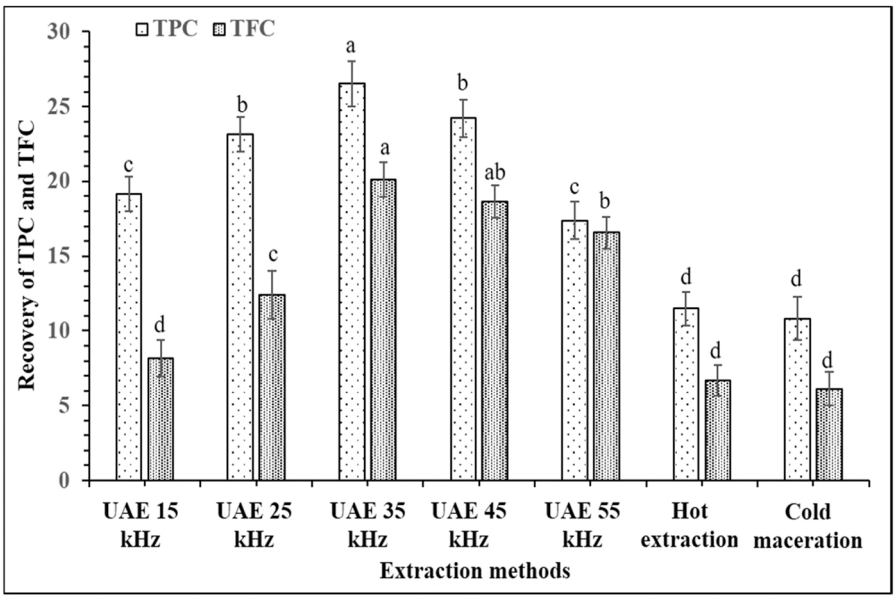
All experiments were replicated thrice, and the results were reported as the mean of three observations. Minitab 17 was used for constructing a design matrix, graph, and data analysis. The t-test and f-test (ANOVA) were performed at $P = 0.05$ (significant) and $P = 0.01$ (highly significant). The predicted optimized condition of the mathematical model was verified using the percentage of relative change (PRC) between experimental and predicted values.

3. Results and discussion

3.1. Proximate composition and nutritional values of C. woodrowii fruits

The moisture, ash, and crude fiber content of *C. woodrowii* fruits were 13.71%, 23.88%, and 21.15 g/100 g DW, respectively (Table 3). Analysis of proximate composition is vital for the food industry. The moisture content must be below 15% to avoid bacterial and fungal contamination (Hammond et al., 2015; Thangaraj, 2016). Though the ash and crude fiber contents do not play an important role in the nutritional values, their analysis is important to describe the intestinal tract's mineral content and peristaltic action (Aurand et al., 1987). Consumption of a high fiber diet helps to reduce constipation and related complications (Busuttil-Griffin et al., 2015). Analysis of fat, protein, and carbohydrates is important to score the oxidizable energy of food (Idris et al., 2019). In *C. woodrowii* fruits, fat, protein, and carbohydrates contents were 2.71 g/100 g DW, 5.78 g/100 g DW, and 15.23 g/100 g DW, respectively (Table 3). Based on this analysis, the oxidizable energy of the *C. woodrowii* fruits was 706.54 kJ/100 g DW or 168.86 kcal/100 g DW.

Presence of sodium (37.23 mg/100 g DW), potassium (71.9 mg/100 g DW), calcium (78.29 mg/100 g DW), magnesium (118.77 mg/100 g DW), and phosphorus (30.45 mg/100 g DW) was recorded in *C. woodrowii* fruits (Table 3). Clinical trials have shown that the intake of these macro-minerals helps to reduce the chances of non-communicable diseases (Bruins et al., 2019; Schiefermeier-Mach et al., 2020;



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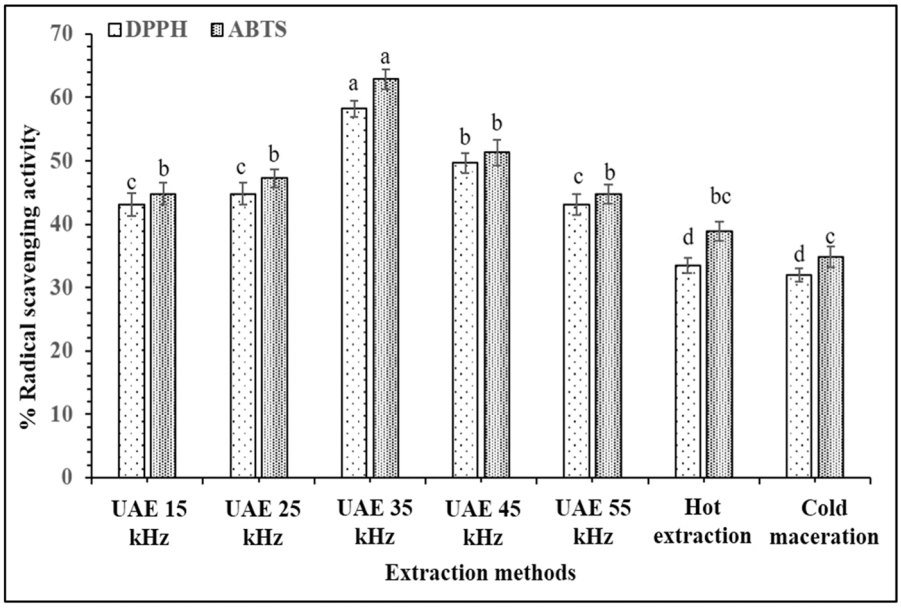

b

Fig. 2. Effects of extraction methods on (a) recovery of total phenolics (TPC, mg TAE/g DW) and total flavonoids (TFC, mg QE/g DW) contents from *Cissus woodrowii* fruits and their (b) antioxidant activity (% Radical scavenging activity) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

Angeles-Agdeppa et al., 2020). In the present investigation, the micronutrients of *C. woodrowii* fruits were also quantified. Higher contents of iron (12.45 μg/100 g DW) followed by manganese (6.41 μg/100 g DW), zinc (3.11 μg/100 g DW), and copper (1.22 μg/100 g DW) (Table 3) were observed. Iron, manganese, and zinc are the important components of proteins, enzymes, haemoglobin, amino acids, and lipids. These mineral elements also play an important role in carbohydrate metabolism and gene regulation (Trumbo et al., 2001). The biochemical role of copper is related to copper metalloenzymes which help to reduce molecular oxygen (Trumbo et al., 2001). The fruits also contain carotenoids (15.29 μg/100 g DW) and ascorbic acid (41.23 mg /100 g DW) (Table 3), which are important to reduce oxidative stress, inflammatory, diabetes, and different types of cancer (Tan et al., 2018; Bohn, 2019). Thus, the fruits of *C. woodrowii* have good nutritional value and may be used to provide food security and sustainability in the natives.

3.2. *Pilot experiment for recovery of phenolic compounds and antioxidant activity*

The efficiencies of conventional extraction Soxhlet and cold maceration and green extraction (ultrasonication) were compared for the recovery of TPC, TFC, and their subsequent antioxidant activities (Fig. 2a and b). The recovery of TPC and TFC ranged between 17.37 – 26.52 mg TAE/ g DW and 8.17 – 20.11 mg QE/ g DW, whereas the %RSA in the

Table 4
Analysis of variance, regression coefficients, quadratic polynomial models, and regression coefficients for the recovery of total phenolics content (TPC) and total flavonoids content (TFC) from *Cissus woodrowii* fruits and subsequent antioxidant activity (% Radical scavenging activity) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

Term	TPC		TFC		DPPH		ABTS	
	Coef	*p*-Value	Coef	*p*-Value	Coef	*p*-Value	Coef	*p*-Value
β0 (Intercept)	31.29	0	23.79	0	80.29	0	84.49	0
Linear								
β1 (Solvent)	1.45	0.0003	1.05	< 0.0001	-0.095	0.707	1.58	< 0.0001
β2 (Ultrasonic)	-1.74	0.0001	-1.78	< 0.0001	-1.8	< 0.0001	-0.4113	0.0245
β3 (Temp.)	-2.63	< 0.0001	-1.7	< 0.0001	-1.71	< 0.0001	-3.36	< 0.0001
Quadratic								
β11	-13.02	< 0.0001	-10.84	< 0.0001	-7.08	< 0.0001	-9.71	< 0.0001
β22	6.01	< 0.0001	3.38	< 0.0001	5.43	< 0.0001	3.58	< 0.0001
β33	3.73	< 0.0001	1.62	< 0.0001	3.48	0.0002	4.28	< 0.0001
Cross Product								
β12	-0.665	0.031	-0.3175	0.0425	2.42	0.0008	1.11	0.0017
β13	0.7575	0.0195	-0.6425	0.0028	1.3	0.0119	0.4875	0.0445
β23	-1.41	0.0015	-0.97	0.0004	-1.4	0.0088	0.88	0.0048
P-Value of Model		< 0.0001		< 0.0001		< 0.0001		< 0.0001
Lack of fit		0.1241		0.4711		0.4847		0.3236
S	0.4471	NA	0.2347	NA	0.6744	NA	0.3655	NA
R Sq %	0.999	NA	0.9995	NA	0.995	NA	0.9989	NA
R Sq (Adj) %	0.9972	NA	0.9987	NA	0.986	NA	0.997	NA

NA - Not available, * Significant at $p \leq 0.05$, and **Significant at $p \leq 0.01$.

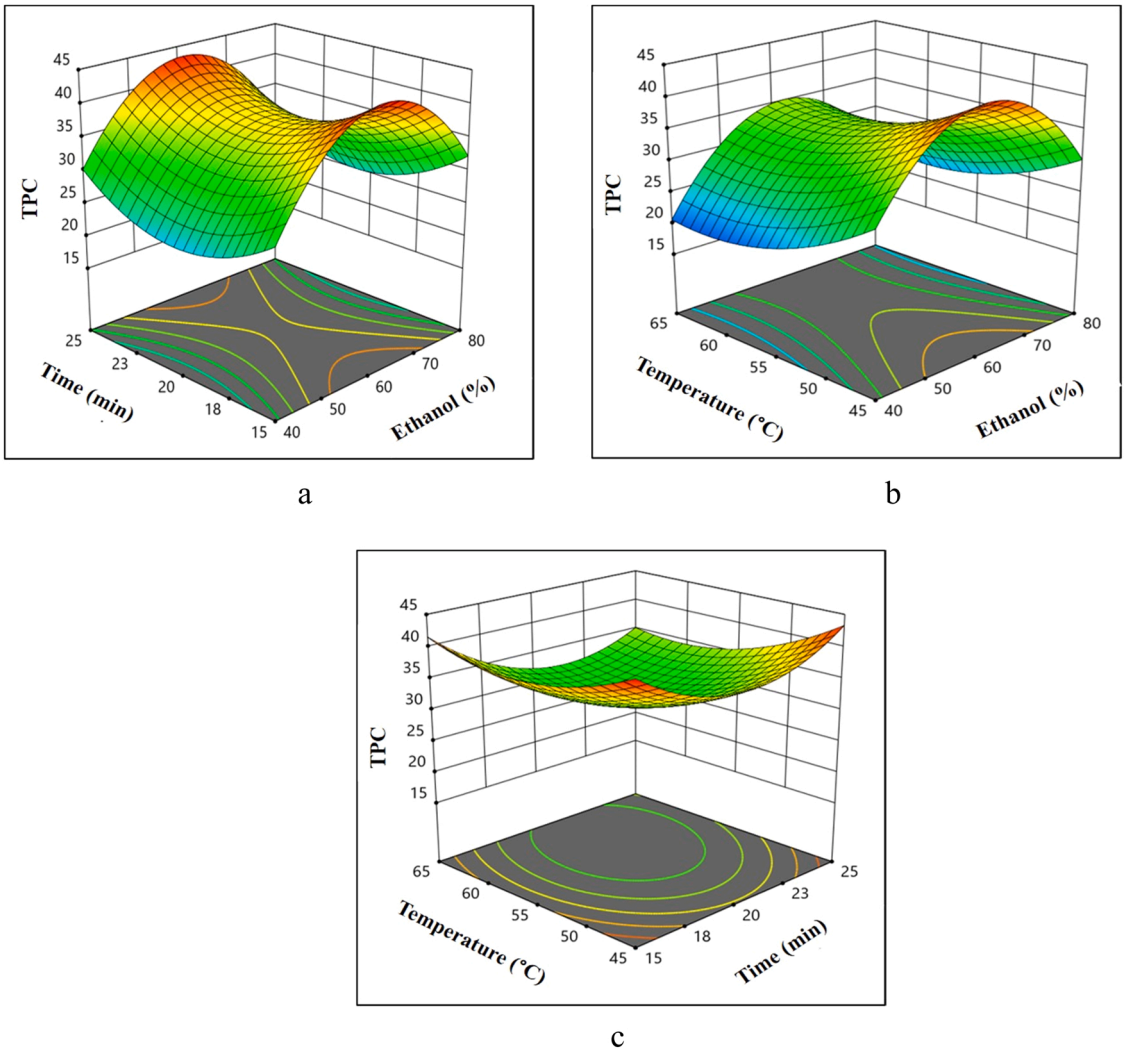

Fig. 3. Response surface plot showing the effects of (a) ethanol concentration (X_1, %), and extraction time (X_2, min), (b) X_1 and temperature (X_3, °C), and (c) X_2 and X_3 on the recovery of total phenolics content (TPC, mg TAE/g DW) from *Cissus woodrowii* fruits.

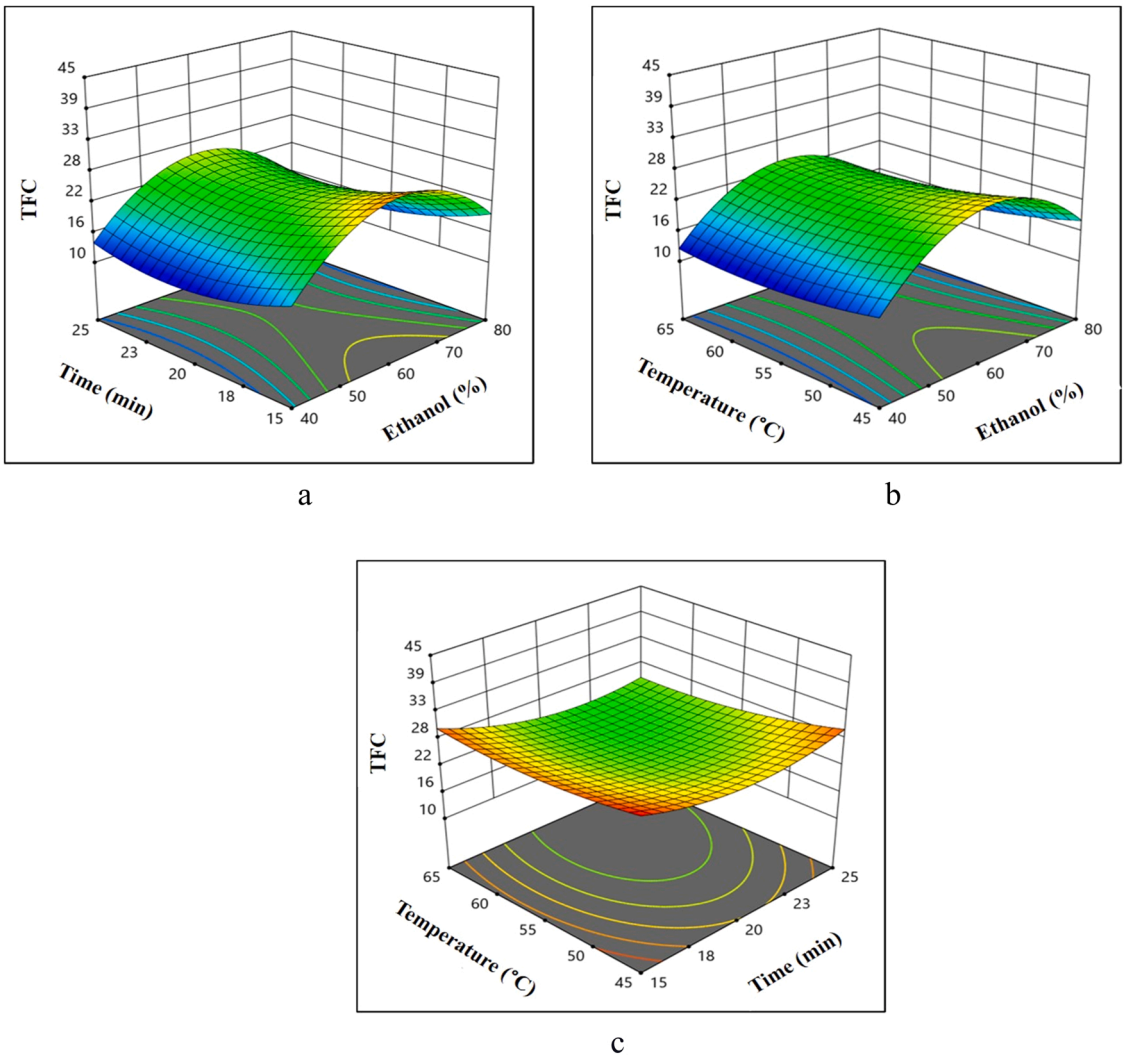

Fig. 4. Response surface plot showing the effects of (a) ethanol concentration (X_1, %) and extraction time (X_2, min), (b) X_1 and temperature (X_3, °C), and (c) X_2 and X_3 on the recovery of total flavonoids content (TFC, mg QE/ g DW) from *Cissus woodrowii* fruits.

DPPH and ABTS ranged between 43.09 – 58.24% and 44.73 – 62.89%, respectively (Fig. 2a and b). The extracts prepared by ultrasonication were superior in TPC and TFC contents and antioxidant activities. In the UAE, 35 kHz ultrasound frequency was optimal for the highest recovery of TPC and TFC. The extract thus prepared contained 2.31 and 2.44 fold higher TPC than observed in the extracts from the Soxhlet and cold maceration, respectively (Fig. 2a). In contrast, the recovery of TFC was 3.01 and 3.27 times greater (Fig. 2a). Consequently, the %RSA in the extract from UAE was 1.73 and 1.73 folds higher in the DPPH assay and 1.61 and 1.80 folds higher in the ABTS assay than Soxhlet and cold maceration, respectively (Fig. 2b). These results concur with Karami et al. (2015), Altemimi et al. (2016), Dobrinčić et al. (2020), and Saifullah et al. (2020), who have put forth that green extraction improves recovery of phenolics and antioxidant activity than conventional methods. The formation of cavitation bubbles in the UAE causes physical and mechanical damage to the cells, increasing the discharge of phenolic compounds in the extracting solvent (Aguilar-Hernández et al., 2020). Since lower ultrasonication frequencies are insufficient to cause such damage (Mahalleh et al., 2019), the yield of phenolic compounds (TPC and TFC) and antioxidant activity was lower for the frequencies below 35 kHz (Fig. 2a and b). On the contrary, a reduced response was noted at higher frequencies of 45 and 55 kHz (Fig. 2a and b), possibly because of collapsing cavitation bubbles (Altemimi et al., 2016;).

3.3. Experimental design, optimization of ultrasonic-assisted extraction and model fitting

BBD was employed to optimize the independent variables to achieve higher phenolic compounds and antioxidant activity (Tables 1 and 2). The choice of independent variables was based on their significant role in the recovery of bioactive compounds and antioxidants in different plant species (Agnieszka et al., 2020; Ahmed et al., 2020). The design matrix of 15 runs and the observed responses are presented in Table 2. In addition, Response surface methodology (RSM) was used to understand the interactions among the independent variables. A second-order polynomial model was developed by multiple regression analyses between phenolic compounds (TPC and TFC) and antioxidant assays (DPPH and ABTS), which showed the significance and good fit of the model based on analysis of variance (ANOVA) (Table 4). All models thus obtained were highly significant ($p < 0.01$), and the lack of fit statistics was insignificant ($p > 0.05$), suggesting reliability and adequacy of all the models.

The coefficient of determination (R^2) value of the model was higher than 99%, indicating a higher correlation between the predicted values and the experimental data (Liu et al., 2018). Additionally, the adjusted R^2 values were also very high (>98%), justifying the model's higher significance (Han et al., 2016; Yan et al., 2016). According to this statistical analysis, TPC, TFC, DPPH, and ABTS follow the reduced

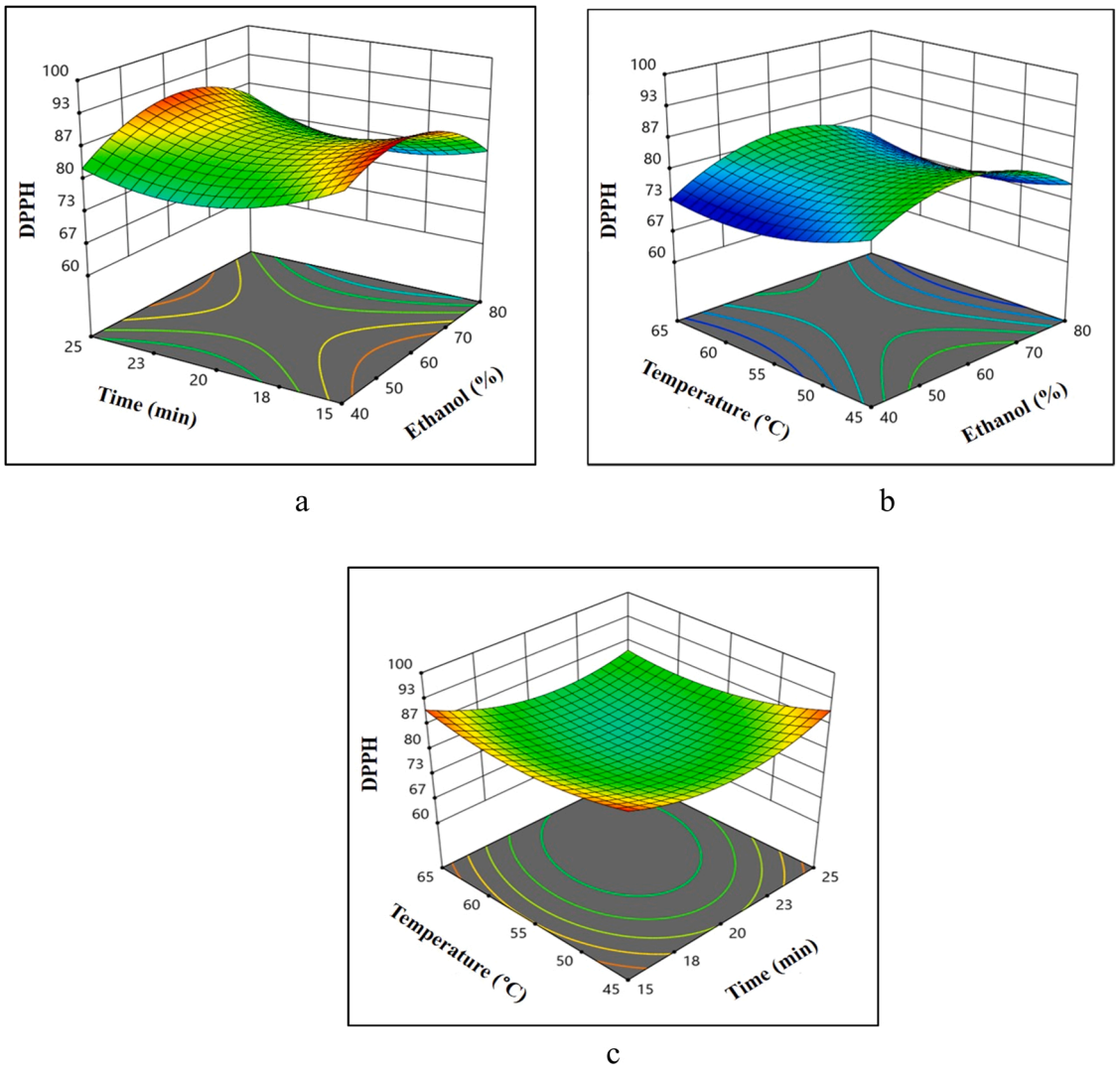

Fig. 5. Response surface plot showing the effects of (a) ethanol concentration (X_1, %) and extraction time (X_2, min), (b) X_1 and temperature (X_3, °C), and (c) X_2 and X_3 on the radical scavenging activity (%) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of *Cissus woodrowii* fruits.

second-order polynomial equation:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j$$

wherein y is the response (outputs); β_0 is the constant (or model intercept); β_i is the linear coefficient of the coded variables; β_{ii} represents the coefficient of quadratic parameters; β_{ij} the cross-product coefficient; and x_i and x_j are the coded independent variables (Zimare et al., 2021).

3.4. Response surface analysis

3.4.1. Analysis of phenolic compounds

The 3-dimensional surface plot was drawn with one variable kept at a medium level and the other two variables with their experimental range. The significance of each coefficient for the models is shown in Table 4, which confirms the fitting of the model. Response surface plots that describe the effects of independent variables on the extractability of phenolic compounds (TPC and TFC) are illustrated in Figs. 3 and 4. TPC was in the range of 17.37–44.44 mg TAE/g DW (Fig. 3a–c). It indicates that linear and quadratic and interactive effect of solvent concentration, ultrasonication time, and the temperature was significant ($p < 0.01$) (Table 4). Fig. 3a shows a plot having the surface like a saddle with two maxima and two minima. TPC is decreasing as X_1 is increasing or decreasing from 60 for any X_2 value. Maximum TPC is around 43 mg TAE/g DW observed at $X_1 = 60$, $X_2 = 15$ and $X_1 = 60$, $X_2 = 25$. Minimum TPC is around 25 mg TAE/g DW which is observed at $X_1 = 40$, $X_1 = 80$ and for $X_2 = 20$. Fig. 3b shows maximum TPC around 43 mg TAE/g DW near $X_1 = 60$, $X_2 = 45$ and two minimum TPC values, 20 mg TAE/g DW near $X_1 = 40$, $X_3 = 61$ and 22 mg TAE/g DW at $X_1 = 80$, $X_3 = 61$. TPC is decreasing with increasing or decreasing X_1 from 61. Fig. 3c has an inverted umbrella pattern where only one minimum value and multiple maximum values are seen. The minimum TPC is 30 mg TAE/g DW, observed at $X_2 = 20$, $X_3 = 60$. Two maximum TPC values, 43 mg TAE/g DW at the two corners $X_2 = 15$, $X_3 = 45$ and $X_2 = 15$, $X_3 = 65$ and 41 mg TAE/g DW near $X_2 = 25$, $X_3 = 45$ can be seen.

In this study, the amount of TFC ranged between 13.20 – 31.32 QE/g DW for different extraction parameters (Fig. 4). The response surface plots (Fig. 4a–c) demonstrate the effects of independent variables on the extractability of TFC, which had significant linear and quadratic effects ($p < 0.01$). In this analysis, the interactive effect of solvent concentration ($p < 0.05$), ultrasonication time, and temperature were the most important influential factors ($p < 0.01$). Fig. 4a shows the maximum TFC observed for all values of X_2 where $X_1 = 60$. Maximum TFC is 28 mg QE/g DW observed near $X_1 = 60$, $X_2 = 15$. Minimum TFC is 11 mg QE/g DW at $X_1 = 40$, $X_2 = 21$. Fig. 4b represents maximum values of TFC ranging

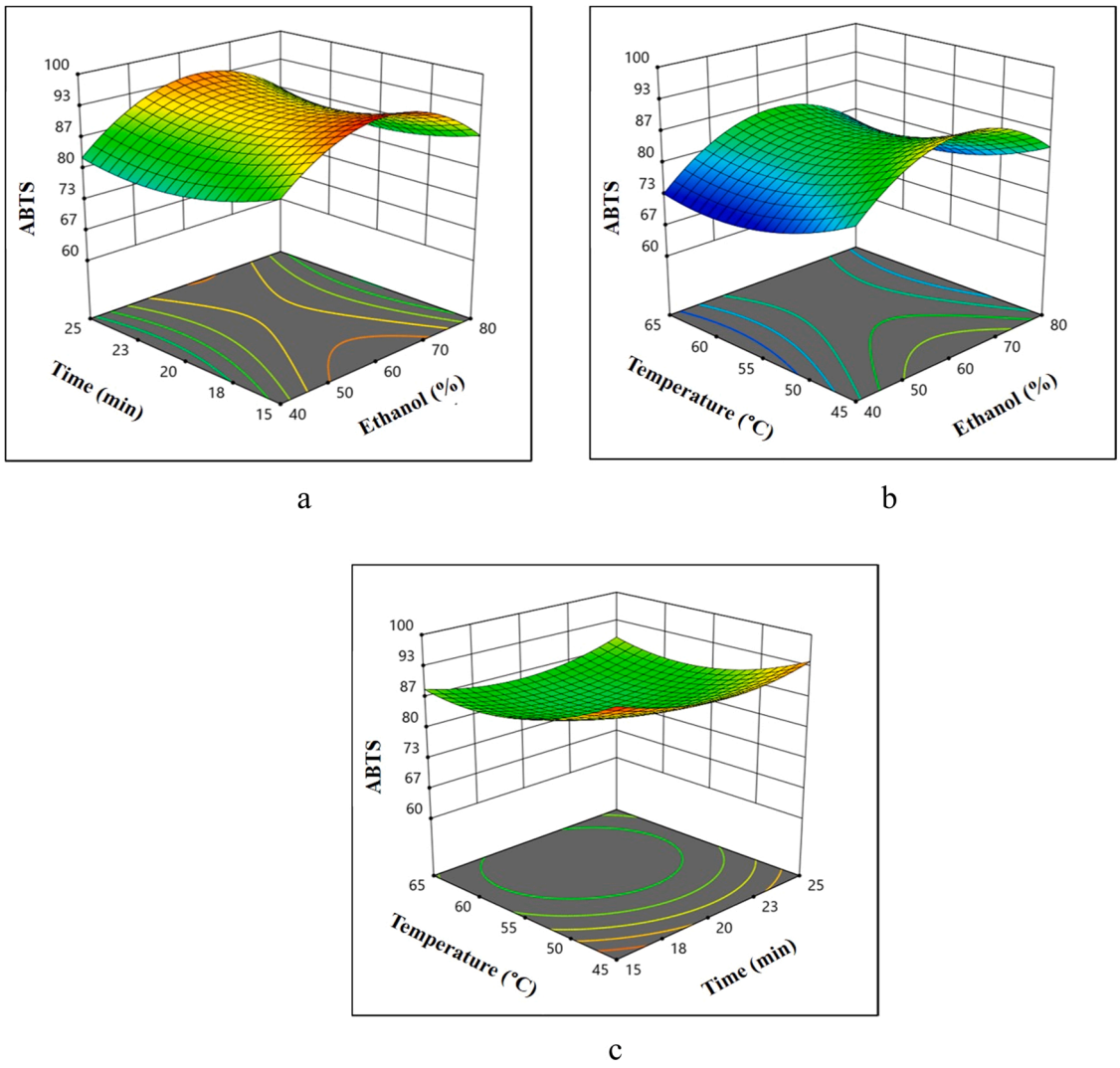

Fig. 6. Response surface plot showing the effects of (a) ethanol concentration (X_1, %) and extraction time (X_2, min), (b) X_1 and temperature (X_3, °C), and (c) X_2 and X_3 on the radical scavenging activity (%) in 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay of *Cissus woodrowii* fruits.

from 23 to 27 mg QE/g DW for $X_1 = 60$ as X3 is decreasing from a higher level of $X_3 = 65$ to a lower level of $X_3 = 45$. The values are decreasing for the increase or decrease of X_1 from 60. Maximum TFC of 27 mg QE/g DW is located near $X_1 = 60$, $X_3 = 15$ and minimum TFC is near $X_1 = 40$, X2 = 56. Fig. 4c indicates that TFC is in a narrow range of 22 to 30 mg QE/g DW. The surface looks like a shallow bowl. Max TFC is around 30 mg QE/g DW observed at three corners ($X_3 = 45$, $X_2 = 15$), ($X_3 = 45$, $X_2 = 25$), and ($X_3 = 65$, $X_2 = 15$), whereas the minimum value is observed at the center ($X_3 = 55$, $X_2 = 20$).

To date, several methods have been reported for the extraction of phenolics, of which UAE is a better one (Osorio-Tobón et al., 2020). Different ratios of a binary solvent system (ethanol and water) were used to extract phenolic compounds from fruits of *C. woodrowii*. Ethanol is food grade organic solvent and commonly used to recover TPC and TFC (Bamba et al., 2018; Martínez-Patiño et al., 2019). Furthermore, the use of water improves the polarity and swelling of the plant material, which assists in the higher extraction of TPC and TFC (Bamba et al., 2018; Martínez-Patiño et al., 2019). The reduction in the yield (TPC and TFC) at higher temperatures and longer extraction time was also observed, which showed resemblance with the previous studies in different plants (Bamba et al., 2018; Zimare et al., 2021). The longer extraction time and higher temperature can cause the oxidative pyrolysis of phenolic compounds (; Zimare et al., 2021).

3.4.2. Analysis of antioxidant assay

In addition to the analysis of phenolic compounds, a relationship between extraction parameters (other than ultrasonic frequency) and antioxidant activity (%RSA) was studied (Figs. 5 and 6). The DPPH and ABTS assays showed %RSA in the range of 73.86 – 97.23% and 74.21 – 91.38%, respectively (Table 2). For the DPPH assay, a linear effect of ultrasonication time and the temperature was significant at $p < 0.01$, but for solvent concentration, it was insignificant. In contrast, the quadratic and interaction effect of solvent concentration, ultrasonication time, and temperature was significant at $p < 0.01$ (Table 4). Fig. 5a represents two maximum and one minimum %RSA in the DPPH assay. This maximum DPPH %RSA is 91% which is near $X_1 = 55$, $X_2 = 15$, and $X_1 = 60$, $X_2 = 25$. Minimum DPPH %RSA is 79% which is near $X_1 = 40$, $X_2 = 20$. Fig. 5b also shows two maximum and one minimum DPPH %RSA. One maximum DPPH %RSA is 85% which is near $X_1 = 57$, $X_3 = 45$, and another is 81% which is near $X_1 = 60$, $X_3 = 65$. Minimum DPPH %RSA is 72% which is near $X_1 = 40$, $X_3 = 60$. Fig. 5c shows three maximum and one minimum DPPH %RSA. All three maximum DPPH %RSA were observed near ($X_2 = 15$, $X_3 = 45$), ($X_2 = 25$, $X_3 = 45$) and ($X_2 = 15$, $X_3 = 65$). In contrast, the minimum DPPH %RSA is 79% which is near $X_2 = 20$ and $X_3 = 60$.

The response surface plot shown in Fig. 6 describes the effects of independent variables on the %RSA in ABTS assay where a liner,

Table 5
The predicted and experimental values at the optimal settings for ultrasound-assisted extraction of total phenolics content (TPC) and total flavonoids content (TFC) from Cissus woodrowii fruits and their antioxidant activity (% Radical scavenging activity) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

Response	Fit	SE Fit	95% CI	95% PI	Experimental value
TPC	43.961	0.387	(42.966, 44.956)	(42.441, 45.481)	46.01
TFC	31.279	0.203	(30.756, 31.801)	(30.481, 32.077)	32.65
DPPH	91.353	0.584	(89.851, 92.854)	(89.059, 93.646)	95.78
ABTS	96.999	0.317	(96.186, 97.813)	(95.756, 98.242)	97.26

quadratic and interactive effects of solvent concentration, ultrasonication time and temperature were significant at ($p < 0.01$). It shows two maximum and one minimum ABTS %RSA. One maximum ABTS % RSA is 96% which is near $X_1 = 60$, $X_2 = 15$, and another is 94% which is near $X_1 = 60$, $X_2 = 25$ (Fig. 6a). In contrast, the minimum DPPH %RSA is 79% which is near $X_2 = 20$, $X_3 = 60$, whereas another minimum DPPH % RSA is 81% which is near $X_1 = 40$, $X_2 = 20$. Fig. 6b shows maximum ABTS %RSA is 92% which is near $X_1 = 60$, $X_3 = 45$. In contrast, the minimum ABTS %RSA is 72% which is near $X_1 = 40$, $X_3 = 60$. Fig. 6c shows maximum ABTS %RSA (96%) near $X_2 = 15$, $X_3 = 45$, whereas the minimum ABTS %RSA is 83%, which is near $X_2 = 20$, $X_3 = 60$.

The present study suggests that the independent variables govern the yield of phenolic compounds from *C. woodrowii* fruits and their antioxidant capacities. A strong correlation between the concentration of phenolic compounds and the antioxidant property has been reported in a recent study (Kainama et al., 2020). The ABTS and DPPH are calorimetric assays and are mainly used to score the antioxidant potential of the test sample (Sujarwo and Keim, 2019; More and Makola, 2020). Due to antioxidants, purple ethanolic DPPH solution changes to yellow color in these assays while the blue-green color of ABTS solution is turned colorless (Bibi Sadeer et al., 2020). Plant phenolic compounds are characterized by hydroxyl groups, which mainly contribute to the antioxidant activity (Sujarwo and Keim, 2019). The antioxidant activity of *C. woodrowii* fruit extracts are influenced by different ratios of binary solvents, which concurs with the previous studies carried in other plants (Waszkowiak and Gliszczyńska-Świgło 2016; Saifullah et al., 2020). In addition to the solvents system, temperature and time also regulated the antioxidant property of *C. woodrowii* fruit extract. These factors are crucial for extracting phenolic compounds qualitatively and quantitatively (Rashad et al., 2021; Zimare et al., 2021). A reduction in the antioxidant activity at higher values of these variables might be because of degradation and oxidations of phenolic compounds (González-Centeno et al., 2015; Altemimi et al., 2016).

3.5. Verification of the model-predicted optimal condition

The model-predicted for the optimum condition was determined by following Derringer and Suich (1980), wherein 59.80% ethanol, 45 °C temperature, and 15 min ultrasonic time is desirable to accomplish maximum phenolic compounds and antioxidant activity. Considering the optimum conditions, the predicted response values ($p = 0.05$) for TPC and TFC were 43.96 mg TAE/g DW and 31.28 mg QE/g DW, respectively. At the same time, such values for DPPH and ABTS assay were 91.35% and 97%, respectively (Table 5). The experiments (extraction and assays) were performed in triplicates to find the experimental values. The observed values in these studies were 46.01 mg TAE/g DW and 32.65 mg QE/g DW for TPC and TFC (Table 5). However, it was 95.78% and 97.26% in DPPH and ABTS assays, respectively (Table 5). Based on the comparison of predicted and observed values, one can conclude that the optimized UAE model is reasoned and repeatable to get a higher yield.

Table 6
Putative identification of phenolic compounds using liquid chromatography–high-resolution mass spectrometry of *Cissus woodrowii* fruit extract.

Name	m/z	RT (min)	Formula	Mass (g/mol)	Mass error*
Catechin	289.07	5.47	$C_{15}H_{14}O_6$	290.07	1.07
Rutin	609.14	5.53	$C_{27}H_{30}O_{16}$	610.15	-0.12
Gallic acid	169.01	5.76	$C_7H_6O_5$	170.02	1.32
Diosmetin	327.05	6.33	$C_{16}H_{12}O_6$	300.06	-0.74
Phloridzin	435.12	6.53	$C_{21}H_{24}O_{10}$	436.13	0.49
Ellagic acid	300.99	6.57	$C_{14}H_6O_8$	302.00	3.59
Rhoifolin	605.15	6.66	$C_{27}H_{30}O_{14}$	578.16	1.84
Cosmosiin	431.09	6.68	$C_{21}H_{20}O_{10}$	432.10	1.45
Epicatechin	441.08	6.76	$C_{22}H_{18}O_{10}$	442.09	1.03
Centaurein	539.09	6.94	$C_{24}H_{26}O_{13}$	522.13	-2.38
Norstictic acid pentaacetate	599.10	7.1	$C_{28}H_{24}O_{15}$	600.11	0.24
Quercitrin	507.11	7.15	$C_{21}H_{20}O_{11}$	448.10	0.74
Naringenin-7-O-glucoside	433.11	7.4	$C_{21}H_{22}O_{10}$	434.12	0.26
Lomatin	227.07	7.85	$C_{14}H_{14}O_4$	246.08	-2.13
Cosmosiin hexaacetate	665.15	8.01	$C_{33}H_{32}O_{16}$	684.16	1
Dihydrorobinetin	285.04	8.5	$C_{15}H_{12}O_7$	304.05	-1.16
Hesperetin	283.06	11.27	$C_{16}H_{14}O_6$	302.07	-2.74
Embelin	293.17	11.67	$C_{17}H_{26}O_4$	294.18	0.32
Harderoporphyrin	607.25	18.36	$C_{35}H_{36}N_4O_6$	608.26	-2.44
Rhoifolin	605.15	6.66	$C_{27}H_{30}O_{14}$	578.16	1.84

3.6. Characterization of extract

The fruit extract that showed the highest yield and antioxidant activities (35 kHz, 60% ethanol, 45 °C temperature, and 15 min) was characterized using LC-HRMS. In this analysis, 20 phenolic compounds such as catechin, rutin, gallic acid, diosmetin, phloridzin, ellagic acid, rhoifolin, cosmosiin, epicatechin, centaurein, norstictic acid pentaacetate, quercitrin, naringenin-7-O-glucoside, lomatin, cosmosiin hexaacetate, dihydrorobinetin, hesperetin, embelin, harderoporphyrin, and rhoifolin were putatively identified (Table 6). These compounds are associated with various bioactivities, such as antioxidants, and play an important role in treating non-communicable diseases (Kolap et al., 2020). These phenolic compounds were also reported from the leaf, stem, and root of *C. woodrowii* (Kolap et al., 2020) and other genera of Vitaceae such as *Vitis vinifera* (Somkuwar et al., 2018).

The present paper is the first comprehensive study describing the variety of nutrients, phenolic compounds, and higher antioxidant potential of *C. woodrowii* fruits. This study has revealed that the fruit of *C. woodrowii* is a rich source of fiber, fat, protein, carbohydrate, energy, mineral (macro and micro), and vitamins which can serve as a non-conventional food source to reduce malnutrition. In addition, it demonstrates the potential of green extraction of phenolic compounds from *C. woodrowii* fruits and their antioxidant activity. Ultrasonic assisted extraction has revealed that the phenolic and flavonoid contents are influenced by the ratio of binary solvent system, extraction temperature, and duration of extraction. These factors also govern the antioxidant potential of *C. woodrowii* fruits. Furthermore, the characterization of the potent extract revealed the presence of 20 phenolic compounds, many of which are enlisted as dietary phenolic compounds. The presence of a wide array of phenolic compounds suggests that the *C. woodrowii* fruits can be used to treat non-communicable diseases in addition to their nutritional value.

Acknowledgment

The authors are thankful to Mr. Vijay Mohan Crishna (Director, NGCPR) and Dr. Mayur D. Nandikar (Head and Scientist) for providing

research funds and necessary facilities under the in-house funding scheme.

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