

Enzyme-Assisted Extraction of Phenolics from *Capparis spinosa* Fruit: Modeling and Optimization of the Process by RSM and ANN

Rahman Qadir, Farooq Anwar,* Khalida Naseem, Mudassir Hussain Tahir,* and Hesham Alhumade

Cite This: *ACS Omega* 2022, 7, 33031–33038

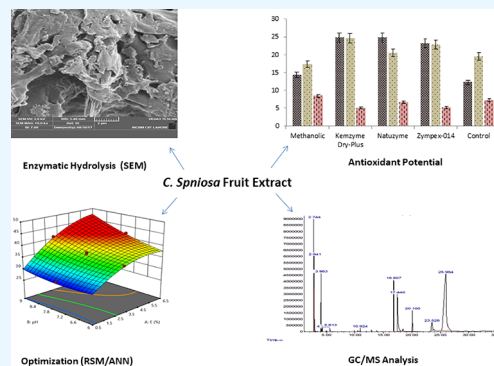
Read Online

ACCESS |

Metrics & More

Article Recommendations

ABSTRACT: The current study intends to appraise the effect of enzyme complexes on the recovery of phenolics from *Capparis spinosa* fruit extract using the response surface methodology (RSM) and artificial neural networking (ANN). Enzymatic treatment of *C. spinosa* fruit extract was optimized under a set of conditions (enzyme concentration, pH, temperature, and time) against each enzyme formulation such as Kemzyme Plus Dry, Natuzyme, and Zymplex-014. The extract yield observed for Kemzyme Plus Dry (42.00%) was noted to be higher than those for Zymplex-014 (39.80%) and Natuzyme (38.50%). Based on the higher results, the values of Kemzyme Plus Dry-based extract were further employed in different parameters of RSM. The F -value (16.03) and p -values (<0.05) implied that the selected model is significant. Similarly, the higher values for the coefficient of determination (R^2) at 0.9740 and adjusted R^2 (adj. R^2) at 0.9132 indicated that the model is significant in relation to given experimental parameters. ANN-predicted values were very close to the experimental values, which demonstrated the applicability of the ANN model. Antioxidant activities also exhibited profound results in terms of total phenolic content values (24.76 mg GAE/g), total flavonoid content values (24.56 mg CE/g), and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (IC_{50}) (5.12 mg/mL). Scanning electron microscopy revealed that after enzymatic hydrolysis, the cell walls were broken as compared with nonhydrolyzed materials. Five phenolics, namely, quercetin, *m*-coumaric acid, sinapic acid, kaempferol, and *p*-coumaric acid, were identified from *C. spinosa* extract by gas chromatography–mass spectrometry (GC/MS). The results of this study reveal that the proposed optimization techniques, using Kemzyme Plus Dry among others, had a positive effect on the recovery of phenolic bioactive compounds and thus increased the antioxidant potential of *C. spinosa* fruit extract.



1. INTRODUCTION

Plants are well-known reserves of nutrients and bioactive compounds with potential as drug and nutraceutical leads.¹ Plants have a long history of use in folk medicine in several civilizations across the world. About 80% of the population across the world, especially in South Asia and Africa, relies on medicine for their health care.² In recent years, there has been growing interest in screening medicinal plants and herbs as a potential source of bioactive compounds of therapeutic value.³ In fact, the physiological benefits of several herbs and medicinal plants are due to the presence of alkaloids, steroids, terpenoids, and especially polyphenols. It is now well accepted that some plant-derived bioactive compounds play a chemopreventive role against several disorders.⁴

Capparis spinosa, commonly known as caper bush and flinders rose, is a multipurpose plant. Out of 250 species from the family *Capparidaceae*, *C. spinosa* is a dicotyledonous perennial, 18–28 cm long climbing shrub that is widely dispersed in different arid areas of South-Western, East Africa, the Pacific Islands, Southeast Asia, Central Asia, Himalayas, Mediterranean, Madagascar, and Australia. *C. spinosa* is prevalent in dry/rocky areas and deserts of India and

Pakistan.^{5,6} A wide range of nutrients such as lipids, proteins, minerals, and tocopherol are documented and cited to be present in *C. spinosa*.

C. spinosa is locally an important and exotic plant due to its various nutritional characteristics. Globally, the incidence of metabolic disorders such as diabetes along with cardiovascular malfunctioning is increasing, and diabetes is mainly linked to the hypertensive condition. Both of the disorders discussed above can be simultaneously treated by eating dried fruits of *C. spinosa* on a daily basis.⁷ The leaves, fruits, and barks of *C. spinosa* have been shown to possess therapeutic properties to treat liver and spleen malfunctioning.⁵ The fruits of *C. spinosa* are frequently used in folk medicine. Dry powder of fruit also acts like a tonic, ridding gas and worms from the intestine/

Received: May 8, 2022

Accepted: July 15, 2022

Published: September 10, 2022



Table 1. Experimental Design, Response, and Analysis of Variance (ANOVA) for Optimization of Extract Yield from *C. spinosa* Fruit Using Different Independent Variables

run	pretreatment parameters				response (extract yield)			ANOVA		
	E (%)	pH	T (°C)	t (min)	KEM-DP	NAT	ZYM-014	source	F-value	p-value
1	3	7.5	50	30	31.50	31.00	26.40	model	16.03	0.0013
2	3	7.5	50	90	39.00	36.10	31.90	linear		
3	3	9	50	60	41.00	35.00	34.00	A ^a	69.73	0.0002
4	3	6	50	60	36.50	31.40	32.00	B ^b	10.28	0.0184
5	3	7.5	25	60	30.80	34.20	37.80	C ^c	15.72	0.0074
6	0.5	7.5	50	60	29.00	30.50	27.30	D ^d	21.65	0.0035
7	3	7.5	75	60	35.00	36.00	33.70	interaction		
8	6.5	7.5	50	60	42.00	35.20	39.00	AB	3.13	0.1275
9	5	6.5	70	75	35.00	38.50	32.90	AC	3.49	0.1111
10	1	8.5	30	75	33.80	35.40	38.40	AD	0.49	0.5086
11	5	8.5	30	45	31.80	38.00	39.60	BC	2.61	0.1576
12	1	6.5	70	45	30.50	36.70	35.20	BD	57.23	0.0003
13	1	6.5	30	45	30.20	34.60	33.60	CD	3.73	0.1018
14	1	8.5	70	75	34.60	31.40	39.80	quadratic		
15	5	8.5	70	45	32.50	25.40	32.10	A ²	17.91	0.0055
16	5	6.5	30	75	28.70	23.80	28.90	B ²	1.80	0.2284
17	3	7.5	50	60	37.80	34.80	29.80	C ²	54.44	0.0003
18	3	7.5	50	60	36.50	36.70	33.50	D ²	6.89	0.0393
19	3	7.5	50	60	37.40	35.20	28.60	LOF ^e	2.58	0.1905
20	3	7.5	50	60	37.20	32.90	31.40	R ²	0.9740	
21	3	7.5	50	60	35.50	35.60	30.50	CV (%)	3.23	

^aEnzyme conc. ^bpH. ^cTemp. ^dTime. ^eLack of fit.

stomach. Capers have also been reported to demonstrate powerful antioxidant behavior along with anticancer properties. These cited health/nutritive functions of *C. spinosa* can be linked to the presence of various bioactive compounds such as phenolics, alkaloids, organic acids, terpenoids, and flavonoids in its fruits/leaves.^{8,9}

Currently, due to the keen interest of researchers in natural compounds, the use of wild plants in the medicinal/pharmaceutical industries prompts the need to appraise the nutrients as well as the biological potential of underutilized parts of wild medicinal plants such as *C. spinosa*. Up to now, no detailed studies are available on optimized extraction/isolation of phenolic antioxidants from *C. spinosa* fruit grown in the Pothohar region. The present research project is mainly designed to screen out various enzyme formulations/combinations, i.e., Kemzyme Plus Dry (Kemin, Germany), Natuzyme (BioProton, Australia), and Zympex-014 (Impextraco, Belgium) against *C. spinosa* fruit extract for achieving optimum yield with potent antioxidant activities. Furthermore, the enzyme formulations found compatible with biological materials (substrates) would be further investigated using selected experimental parameters (time, temperature, pH, and enzyme concentration) following a rotatable central composite design (RCCD) using Design Expert software (10.0.1, Stat-Ease, Inc., Minneapolis). The prediction of the extract yield for Zympex-014 will also be calculated by developing another modular informatics soft computing technique, artificial neural network (ANN), using STATISTICA 10.

This proposed research work will provide information about the yield, chemical characterization, and biological activities of phenolic bioactive compounds in *C. spinosa* from the Pothohar region, leading to exploration of its potential utilization as a valuable ingredient for the local functional food/nutraceutical and pharmaceutical industries.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents. Commercial enzyme mixture “Kemzyme Plus Dry” (Kemin, Germany) composed of β -glucanase, xylanase, cellulase, α -amylase, and protease with a guaranteed minimum enzyme activity, i.e., 2350, 20,000, 4000, 400, and 450 units/g, respectively, was provided by Kemin, Islamabad. Another multienzyme complex “Natuzyme” (BioProton, Australia) composed of xylanase (10 000 unit/g), cellulase (5000 unit/g), β -glucanase (10 000 unit/g), pectinase (1400 unit/g), protease (6000 unit/g), phytase (500 unit/g), and α -amylase (1800 unit/g) was obtained from Meharban Poultry Services, Jhelum. “Zympex-014” (Impextraco, Belgium), composed of xylanase, β -glucanase, β -mannanase, α -galactosidase, amylase, and acid protease, respectively, was provided by BA Traders, Lahore. Chemicals used in the research were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2. Sample Preparation. Ripened fruits of *C. spinosa* plants were collected from Kallar Kahar, Punjab, Pakistan. After removing the debris and washing with distilled water, the collected samples were dried to constant mass at ambient temperature. After drying, the materials were ground into fine powder and then sieved (0.420 mm) and stored at $-20\text{ }^{\circ}\text{C}$.¹⁰

2.3. Enzymatic Pretreatment and Extraction. The preoptimization of enzyme mixture-based extracts under set conditions was executed by diluting the sample powder in phosphate buffer at a specific temperature. After that, it was subjected to solvent extraction using an orbital shaker and filtered under reduced pressure.¹¹ The solvent was separated from the extracts using a rotary evaporator.

2.4. Response Surface Methodology/Artificial Neural Networking. Enzyme concentration (EC), temperature (T), incubation time (t), and pH were examined at three points such as axial (eight runs), center (five runs), and factorial

(eight runs) with different levels ($-\alpha$, -1 , $+1$, $+\alpha$). Twenty-one treatments were employed to optimize the extract yield using a central composite design ($\alpha = 1.682$), as shown in Table 1. A second-order polynomial equation was employed to generate the relationship among dependent and independent variables using Design Expert Software.¹²

$$Y = b_0 + \sum bi Xi + \sum biiXi^2 + \sum bijXiXj$$

ANN design was modeled to create a link between dependent (yield) and independent factors (pH, enzyme concentration, temperature, and time) by neurons. In the current ANN architecture, we used a 4–7–1 distribution of neurons. The neural signals used seven hidden layers to achieve the optimized topology by means of four input layers, and the results of one output layer were noted. The degree of influence between neurons was developed by calculating weights, which were adjusted to generate the ANN model (with less test and training errors) using STATISTICA 10.¹³

2.5. Determination of Total Phenolic Content (TPC).

The Folin–Ciocalteu assay was used to measure total phenolic content (TPC) as previously reported by Qadir et al.¹¹ One milliliter of extract solution was combined with 0.5 mL of Folin–Ciocalteu reagent and 7.5 mL of deionized water for the TPC assay. To this, 1.5 mL of 20% (w/v) sodium carbonate (Na_2CO_3) was added; after keeping the solution at room temperature for 10 min, the solution was heated and then cooled. Spectrophotometric analysis was done at 755 nm. TPC was calculated using a gallic acid standard calibration curve (10–200 ppm).

2.6. Determination of Total Flavonoid Content (TFC).

Total flavonoid content (TFC) was estimated by the method described by Zahoor et al.¹⁴ Briefly, 1.0 mL of extract sample was mixed with distilled water (5 mL) and NaNO_2 (0.3 mL) in a flask for 5 min. After thorough mixing of AlCl_3 and NaOH with water, the absorbance was measured at 510 nm. The same procedure was utilized to prepare a series of catechin standard stocks in the range of 5–100 ppm. A standard calibration curve ($R^2 = 0.9911$) was prepared to measure the results and articulated as catechin equivalent (CE mg/g) on a dry weight basis.

2.7. Antioxidant Activity. The following assay was performed to evaluate the antioxidant potential of *C. spinosa* extract.

2.7.1. DPPH Free Radical Scavenging Assay. A procedure reported by Asnaashari et al.¹⁵ was applied to evaluate free radical scavenging capability with minute changes. A 50 μL extract with varying concentrations (0.10–5.0 mg/mL) was mixed with a methanolic solution of DPPH (5 mL). The absorbance was recorded relative to a blank at 517 nm following 30 min of incubation in the dark.

$$\text{inhibition (\%)} = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the investigated component. IC_{50} , indicative of the 50% neutralizing capability of DPPH free radicals, was examined from the calibration curve of % DPPH against the concentration of extracted solutions.

2.8. Detection of Morphological Changes in Residues. The ultrastructural changes in the residues obtained after Zympex-014 treatment of plant extract were further analyzed using a TESCAN Vega 3 LMU scanning electron microscope (SEM). A liquid nitrogen-dried sample was placed

on a specimen holder by means of magnetic tape and precoated for 90 s with gold targets using a sputter coater. Morphological features of the cell wall of *C. spinosa* extract before and after enzymatic treatment were observed at the resolution of 2 and 5 μm .

2.9. Analysis of Individual Phenolic Compounds by GC/MS.

Before analyzing the phenolic compounds in *C. spinosa* fruit extract by GC/MS, the extract was first subjected to a derivatization process using trimethylchlorosilane (TMCS) to obtain the volatile form derivatives by GC/MS (Agilent Technologies 7890A). Helium was passed through the column at a flow rate of 0.5 mL/min, whereas the temperature of the Quadrupole MS detector was adjusted to 250 °C. After injecting the aliquot sample (0.2 μL) into the column, the ionization was set at 70 eV. The mass scanning range varied from 120 to 550 m/z . Turbo-Mass-OCPTVS-Demo SPL software was employed to measure the peak areas and data processing. 3-Hydroxy benzoic acid (5 ng/ μL) was used as the internal standard.

3. RESULTS AND DISCUSSION

3.1. Optimization of Extract Yield from *C. spinosa* Fruit Using RSM.

Optimization of extract yield from *C. spinosa* fruit was employed by synergizing the effects of selected parameters such as E (%), T , t , and pH (Table 1). A layout of 21 treatments was designed to execute the effect of enzyme cocktails (Kemzyme Plus Dry, Natuzyme, and Zympex-014) on the extract yield. The response (extract yield) observed for Kemzyme Plus Dry (42.00%) was noted to be higher than those for Zympex-014 (39.00%) and Natuzyme (35.00%) at the conditions (6.5% enzyme concentration, 7.5 pH, 50 °C temperature, and 60 min reaction time) given in Table 1.

The cumulative view of results of output variable/response against the preoptimized set of conditions for input variable using RSM revealed that Kemzyme Plus Dry exhibited the maximum yield for selected variables as compared to Zympex-014 and Natuzyme, which might be attributed to its active composition (β -glucanase, xylanase, cellulase, α -amylase, and protease). Based on the higher efficiency to extract the free/bound components from *C. spinosa* fruit, Kemzyme Plus Dry was modeled through a second-order polynomial equation. These findings are in line with the investigations of Mushtaq et al.,^{12,16} who also suggested that Kemzyme can be a promising enzyme cocktail for the effective release of bioactive compounds from sweet lime, watermelon, and pomegranate peel extracts.

The statistical results of the current design were also calculated in terms of F -value (16.03), p -value (<0.0500), and lack of fit. The F -value at 2.58 relative to the pure error also confirmed the significance of the model applied (Table 1). Likewise, the large values of the coefficient of determination (R^2) at 0.9740 and adjusted R^2 (adj. R^2) at 0.9132 also authenticated the robustness of the model. The reliability of the results was also verified by the coefficient of variation at 3.23 and found comparable to the results reported previously by some researchers during the optimization of mulberry leaf extract, *Gordonia axillaris* fruit extract, carob pulp extract, and sunflower seed extract.^{17,18} According to their RSM model results, high F -values along with low p -values and coefficient of variation in the range of 1–5 supported the applicability of RSM models used for improving extract yields from subject plants.

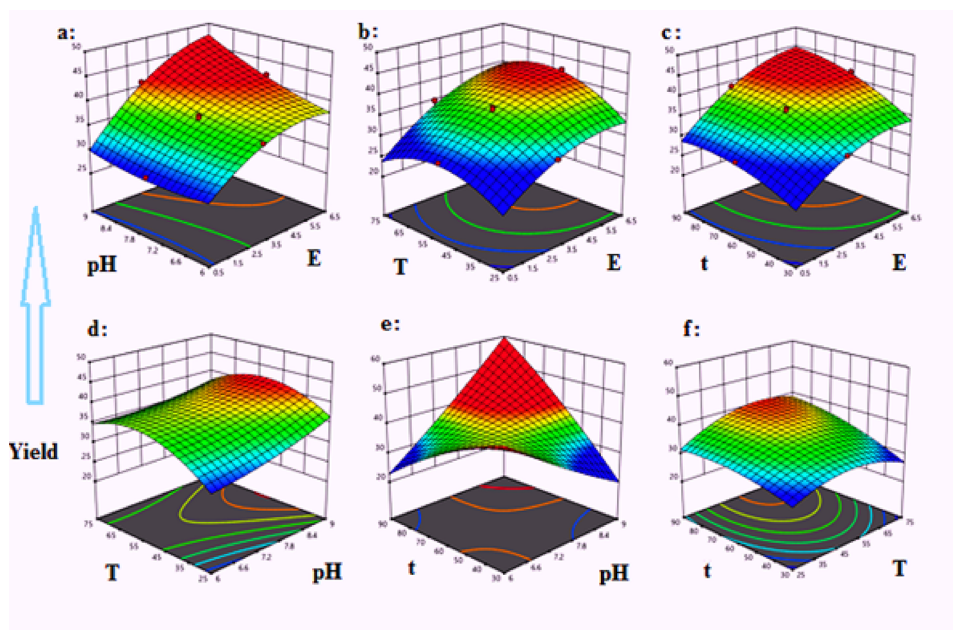


Figure 1. Surface plots showing the effect of selected variables on the extract yield of *C. spinosa* fruit.

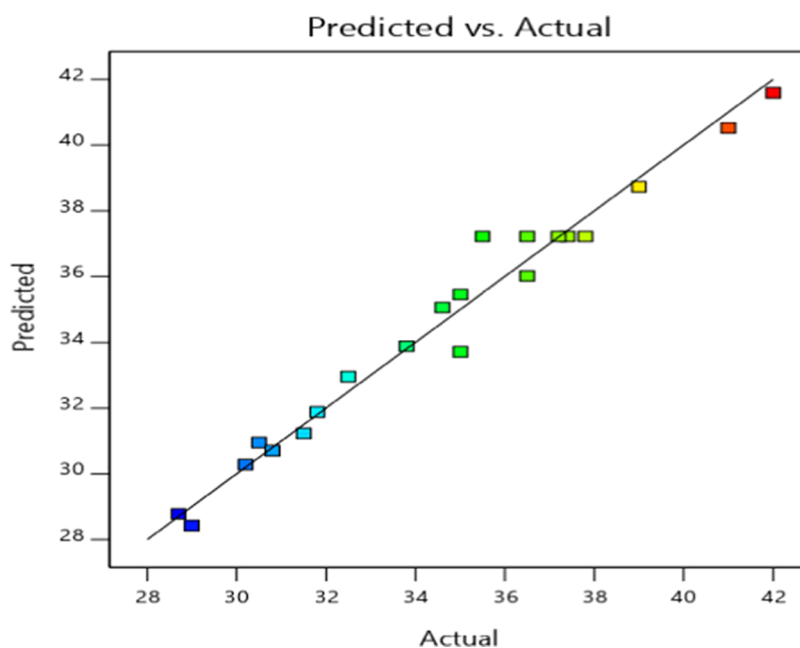


Figure 2. Graph showing the relation between predicted and actual values.

Surface plots of *C. spinosa* fruit extract depicted that three factors *E* (%), pH, and time equally affected the extract yield. However, the increase in temperature could not positively affect the yield. The trends shown by variables in the case of *C. spinosa* fruit extract are nearly similar, as discussed for *Morus alba* leaves by Samavati and Yarmand.¹⁸ Here, in this study, enzyme concentration showed promising effects on the increased yield as compared with other factors, as easily seen in Figure 1a,b,e. However, higher values of temperature and time were reported to impart a negative effect on the response applied in this model.

The model applied for recovering optimum extract yield from *C. spinosa* fruit using Kemzyme Plus Dry was further confirmed by validation experiments that exhibited a response

value of extract yield as $41.74 \pm 1.46\%$, which clearly promises robustness and suitability of the quadratic model. In agreement with our present study, Mushtaq et al.¹⁶ also devised the best conditions (8% of the cocktail enzyme at 6.7 pH and 41 °C for 85 min) during the pretreatment of pomegranate peel and found that an optimal yield ($65.89 \pm 2.64\%$) was obtained under the given experimental conditions.

Three enzyme cocktails, namely, Kemzyme Plus Dry, Natuzyme, and Zympex-014, were employed in this study to enhance phenolic extract yield from selected medicinal plant extracts. It can be concluded that a higher extract yield was obtained through Kemzyme Plus Dry, followed by Zympex-014 and Natuzyme. The higher yield with Kemzyme Plus Dry may be due to its active ingredient components such as β -

Table 2. Parameters of ANN (Weights of Hidden and Output Layers) Utilized for Model Generation

neurons	hidden layer (weights)				input bias	output layer (weights)	
	X1 (conc.)	X2 (pH)	X3 (temp.)	X4 (time)		neuron weights	
1	2.17454	-0.24249	0.46886	-0.37071	0.04142	1	2.09591
2	-0.24051	-1.32823	0.20767	0.97329	0.91830	2	-1.79084
3	-0.40771	0.43486	0.36499	-0.21225	-0.74985	3	1.93794
4	2.36715	5.95571	-0.06257	-0.36590	0.23608	4	-0.39453
5	-0.58844	-0.30177	-0.35277	0.34028	-0.63848	5	0.18892
6	-0.78504	0.07150	0.23122	1.08546	1.83706	6	-1.08386
7	1.56441	0.49900	-0.11323	0.21417	0.37329	7	-1.03156

glucanase, xylanase, cellulase, α -amylase, and protease, which are thoroughly affected in hydrolyzing the bound phenolics and thus their effective release into the solvent media. Figure 2 also shows that the values of yield predicted out of the model are in line with the actual values observed under the specified experimental parameters by depicting a straight line of normality plot that strengthens the ANOVA result validation. Moreover, the high value of the coefficient of determination also strengthens the model validity.¹⁶

3.2. ANN Data for the Prediction of Extract Yield from *C. spinosa* Fruit. The ANN model generated for the prediction of extract yield from *C. spinosa* fruit was employed to compare the authenticity and robustness of the RSM used. In architecture, the neurons selected in input, hidden, and output layers were 4, 7, and 1, respectively. Training error and test error were found to be very low, i.e., 0.002721 and 0.007951, respectively. Test performance at 0.997668 also authenticated the suitability of the model generated. Weights of hidden and output layers as well as the results obtained from ANN are given in Tables 2 and 3. ANN-predicted values were very close to the experimental values, which demonstrated the applicability of the ANN model. It can be claimed that neural network modeling can be employed for the successful prediction of phenolic content extraction by employing

Table 3. Experimental and Predicted Yield for Phenolic Extract from *C. spinosa* Fruit Using ANN

run	X1	X2	X3	X4	experimental response	ANN predicted	% variation for ANN
1	3	7.5	50	30	31.50	30.98	0.52
2	3	7.5	50	90	39.00	39.66	-0.66
3	3	9	50	60	41.00	40.25	0.75
4	3	6	50	60	36.50	36.09	0.41
5	3	7.5	25	60	30.80	30.10	0.70
6	0.5	7.5	50	60	29.00	31.27	-2.27
7	3	7.5	75	60	35.00	34.34	0.66
8	6.5	7.5	50	60	42.00	42.03	-0.03
9	5	6.5	70	75	35.00	35.53	-0.53
10	1	8.5	30	75	33.00	33.91	-0.91
11	5	8.5	30	45	31.00	31.67	-0.67
12	1	6.5	70	45	30.50	29.17	1.33
13	1	6.5	30	45	30.20	29.05	1.15
14	1	8.5	70	75	34.60	33.07	1.53
15	5	8.5	70	45	32.50	33.34	-0.84
16	5	6.5	30	75	28.70	29.90	-1.2
17	3	7.5	50	60	37.80	36.01	1.79
18	3	7.5	50	60	36.50	36.65	-0.15
19	3	7.5	50	60	37.40	36.65	0.75
20	3	7.5	50	60	37.20	36.65	0.55
21	3	7.5	50	60	35.50	36.65	-1.15

experimental data. Similar investigations were carried out by Mojtaba et al.¹⁹ on the optimized oil extraction (56.52%) under suitable conditions from *Pistacia khinjuk* by the application of enzyme using ANN. The input variables selected were temperature, enzyme concentration, pH, and ultrasonic time.

RSM and ANN have been cumulatively used by various researchers such as Zahedi and Azarpour²⁰ and Fullana et al.²¹ in different studies to predict the best operating conditions, thus resulting in the maximum extraction yield of bioactive compounds from plant materials, i.e., *Citrus sinensis* and *P. khinjuk*, respectively. Another study was carried out recently by Pilkington et al.,²² who observed the efficacy of selected input variables (temperature, duration, and solvent) on the notable yield of artemisinin from *Artemisia annua* leaves. Hence, it can be revealed that both techniques such as ANN and RSM can be efficiently employed to predict the optimized extract yield from plant materials.

3.3. Morphological Features of *C. spinosa* Fruit Residue Before and After Enzymatic Treatment (Kemzyme Plus Dry). Before the enzymatic treatment, the cell wall remained intact with reduced release of the bound phenolics (Figure 3a); however, as the treatment with enzyme mixture such as Kemzyme Plus Dry was executed, the cell walls of *C. spinosa* fruit got ruptured due to enzymatic hydrolysis by releasing the bound phenolics into the solvent as can be seen with the appearance of wrinkles on the cell wall surface of *C. spinosa* fruit in Figure 3b. In this case, the conventional solvent extraction used alone is unable to release the bound phenolics from glycosidic linkages. Mushtaq et al.¹² analyzed sweet lime peels before and after the enzymatic treatment and observed that the plant cell wall was broken down effectively after treatment. In another study, during optimization of enzyme-assisted extraction of silybin, Liu et al.²³ observed similar changes in the seeds of *Silybum marianum* by SEM and TEM. They concluded that after enzymolysis, the cell walls of seeds were broken effectively.

3.4. Antioxidant Potential of Enzyme-Assisted *C. spinosa* Fruit Extract. Total phenolic content (TPC) values quantified in solvent and control extracts of *C. spinosa* were 14.34 and 11.56 mg GAE/g, respectively. Among enzymes used for extraction, Kemzyme Plus Dry (24.76) recovered the highest phenolic content, followed by Zympex-014-based extract (23.75) and Natuzyme extracts (22.67 mg GAE/g) (Figure 4). Estimation of total phenolic content in *C. spinosa* fruits is rarely reported in the literature. However, our results for *C. spinosa* were relatively lower as compared to TPC values (445 mg GAE/g) recorded in fruit extracts of *C. spinosa* by Gull et al.⁵ In *C. spinosa* fruit, flavonoid contents were 17.34 and 19.54 (mg CE/g) for methanolic and control extracts, respectively. Enzyme-assisted extracts exhibited greater TFC

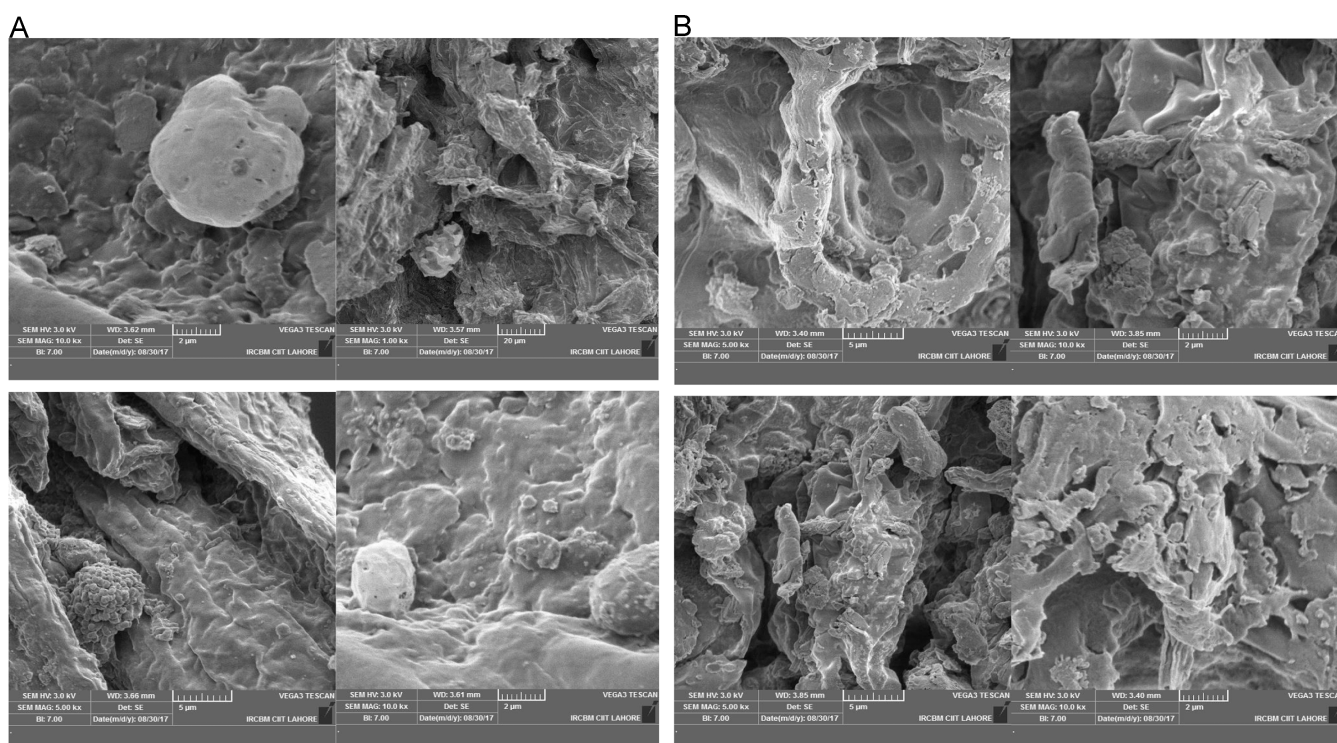


Figure 3. (a) Morphological features of *C. spinosa* fruit residue before enzymatic treatment. (b) Morphological features of *C. spinosa* fruit residue after enzymatic treatment.

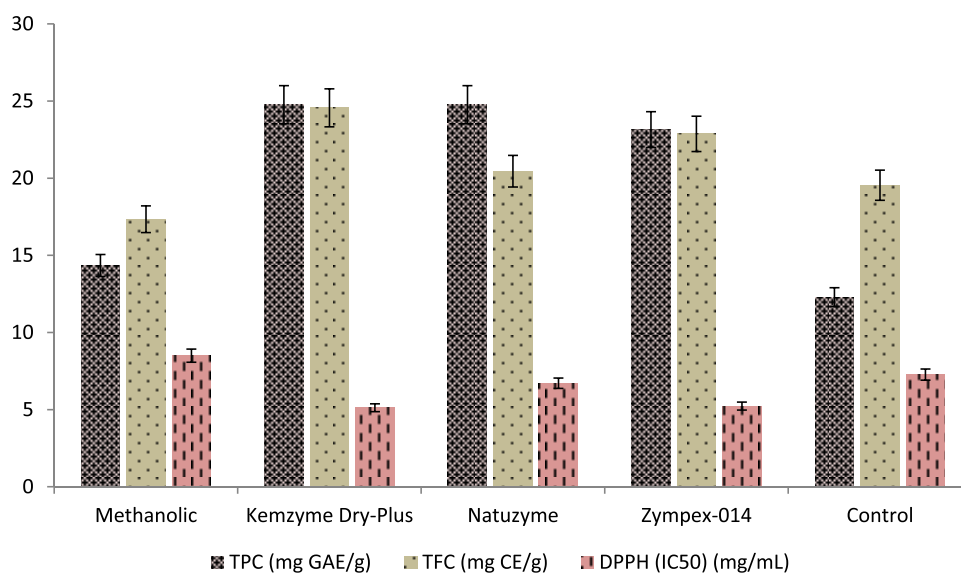


Figure 4. TPC, TFC, and DPPH radical scavenging methanolic, control, and enzyme-assisted *C. spinosa* fruit extracts.

values from 20.45 mg CE/g (Natuzyme), 24.56 mg CE/g (Kemzyme Plus Dry), and 22.87 mg CE/g (Zympex-014). The results of TFC reported by Gull et al.⁵ for *C. spinosa* fruit extract (189.4 mg CE/g) were quite higher than those reported in the present work, which may be linked to the varied nature of the sample and extraction procedure employed. Variations in the amounts of TP and TF between different parts, i.e., leaves/fruits of medicinal plants, may be attributed to the varied genetic makeup of the parts and the level of maturity at the harvesting stage.

The fruit extract of *C. spinosa* exhibited an appreciable level of DPPH free radical scavenging activity, with IC₅₀ (the extract

concentration that scavenges 50% of the DPPH free radicals) values ranging from 5 to 9 mg/mL. When comparing the efficiency of enzyme formulations used in extractions, Kemzyme Plus Dry-based *C. spinosa* extract (5.12 mg/mL) was found to be the most efficient radical scavenger of DPPH, followed by Zympex-014-assisted (5.23 mg/mL) and Natuzyme-assisted extracts (6.71 mg/mL). DPPH values of methanolic (8.5 mg/mL) and control extracts (7.27 mg/mL) were higher (lower activity) than those of enzyme-assisted extracts. Our results regarding the DPPH scavenging activity of *C. spinosa* are in line with the findings of Baghiani et al.,²⁴ who reported high values for *Capparis* species. Hence, the use of

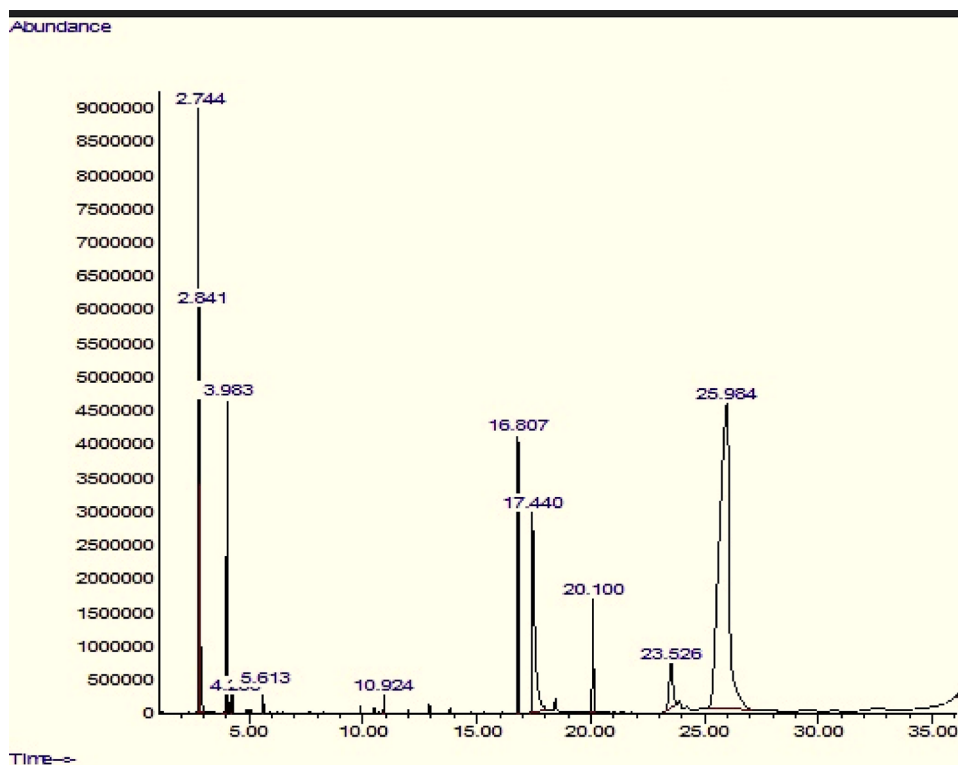


Figure 5. Typical GC/MS chromatogram showing separation of phenolic compounds in *C. spinosa* fruit extract.

enzymes for the optimized yield of phenolics produced extracts with increased levels of antioxidants as compared to conventional solvent extracts (CSEs) can be explored as a viable biotechnological process to maximize the nutraceutical benefits of plant products.

3.5. Identification of Phenolics in *C. spinosa* Fruit Extract by GC/MS. In the case of *C. spinosa*, Kemzyme Plus Dry-based extract was characterized by GC/MS. The total ion chromatograms of the extract samples obtained under the optimized enzymatic conditions are shown in Figure 5. Chemical derivatization of phenolic compounds is usually required to facilitate the separation by GC. Through derivatization, compounds were converted into trimethylsilyl derivatives with better thermal stability and enriched volatility. 3-Hydroxy benzoic acid was used as an internal standard, whereas pyridine was added as an aprotic organic solvent. Five phenolic compounds, namely, quercetin, *m*-coumaric acid, sinapic acid, kaempferol, and *p*-coumaric acid, were identified from *C. spinosa* extract (Table 4). Sinapic and benzoic acids were identified as the major phenolic bioactive compounds in

Table 4. Identification of Phenolic Compounds in *C. spinosa* Fruit by GC/MS

sr. no.	phenolic components	retention time (min)	concentration ($\mu\text{g/g}$)
1	quercetin	5.61	0.14
2	<i>m</i> -coumaric acid	10.92	0.07
3	sinapic acid	16.80	0.87
4	kaempferol	20.10	0.12
5	<i>p</i> -coumaric acid	23.52	0.45
6	3-hydroxy benzoic acid (IS) ^a	25.98	5.20

^aInternal standard.

C. spinosa fruit by Rezzan et al.²⁵ Similarly, Gull et al.⁵ observed various phenolic compounds such as *p*-coumaric acid, gallic acid, *p*-hydroxy benzoic acid, caffeic acid, and sinapic acid ranging from 0.25 to 95.23 mg/100 g in different parts of *C. spinosa* using RP-HPLC. Various studies reported that the phenolic compounds observed in this study are capable of exhibiting carcinogenic, antibacterial, and anti-inflammatory effects in addition to antioxidant behavior.^{26,27}

4. CONCLUSIONS

The present research work optimized the extraction of phenolic bioactive compounds from *C. spinosa* fruit extract using predictive modeling techniques such as RSM and ANN, followed by the characterization of these bioactive compounds by GC/MS. Among the commercial enzyme cocktails, Kemzyme Plus Dry was found to be the most efficient in carrying out the hydrolysis of bound phenolic moieties, resulting in a higher yield of antioxidant phenolics. Enzyme concentration strongly increased the extract yield out of the selected parameters (time, pH, enzyme concentration, and temperature). ANOVA results also authenticated the suitability of the model used. Rupturing of the cell wall of *C. spinosa* fruit by Kemzyme Plus Dry, comprising β -glucanase, xylanase, cellulase, α -amylase, and protease was also visualized with SEM. Overall, the results of the present research work support that the *C. spinosa* fruit extract is a viable source of phenolic antioxidants for the development of functional foods and nutraceuticals with the perspective of value-addition.

AUTHOR INFORMATION

Corresponding Authors

Farooq Anwar – Institute of Chemistry, University of Sargodha, Sargodha 41000, Pakistan; Email: fjanwar@yahoo.com

Mudassir Hussain Tahir – Department of Chemistry, Division of Science and Technology, University of Education, Lahore 54000, Pakistan; orcid.org/0000-0002-9413-3757; Email: mhtahir@mail.ustc.edu.cn

Authors

Rahman Qadir – Institute of Chemistry, University of Sargodha, Sargodha 41000, Pakistan

Khalida Naseem – Department of Chemistry, Faculty of Science and Technology, University of Central Punjab, Lahore 54000, Pakistan

Hesham Alhumade – K.A.CARE Energy Research and Innovation Center and Department of Chemical and Materials Engineering, Faculty of Engineering, King Abdulaziz University, Jeddah 21589, Saudi Arabia; orcid.org/0000-0002-9382-242X

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsomega.2c02850>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the support provided by King Abdullah City for Atomic and Renewable Energy (K.A.CARE) under K.A.CARE-King Abdulaziz University Collaboration Program.

REFERENCES

- (1) Gull, T.; Sultana, B.; Anwar, F.; Nouman, W.; Mehmood, T.; Sher, M. Characterization of Phenolics in Different Parts of Selected Capparis Species Harvested in Low and High Rainfall Season. *J. Food Meas. Charact.* **2018**, *12*, 1539–1547.
- (2) Cos, P.; Vlietinck, A. J.; Vanden Berghe, D.; Maes, L. Anti-Infective Potential of Natural Products: How to Develop a Stronger in Vitro “Proof-of-Concept”. *J. Ethnopharmacol.* **2006**, *106*, 290–302.
- (3) Liaudanskas, M.; Noreikiene, I.; Zymonė, K.; Juodytė, R.; Žvikas, V.; Janulis, V. Composition and Antioxidant Activity of Phenolic Compounds in Fruit of the Genus *rosa* L. *Antioxidants* **2021**, *10*, 545.
- (4) Sahib, N. G.; Anwar, F.; Gilani, A. H.; Hamid, A. A.; Saari, N.; Alkharfy, K. M. Coriander (*Coriandrum Sativum* L.): A Potential Source of High-Value Components for Functional Foods and Nutraceuticals- a Review. *Phytother. Res.* **2013**, *27*, 1439–1456.
- (5) Gull, T.; Anwar, F.; Sultana, B.; Alcaide, M. A. C.; Nouman, W. Capparis Species: A Potential Source of Bioactives and High-Value Components: A Review. *Ind. Crops Prod.* **2015**, *67*, 81–96.
- (6) Zhao, G.; Fang, C.; Hu, J.; Zhang, D. Platinum-Based Electrocatalysts for Direct Alcohol Fuel Cells: Enhanced Performances toward Alcohol Oxidation Reactions. *ChemPlusChem* **2021**, *86*, 574–586.
- (7) Sakcali, M. S.; Bahadir, H.; Ozturk, M. Eco-Physiology of Capparis Spinosa L.: A Plant Suitable for Combating Desertification. *Pakistan J. Bot.* **2008**, *40*, 1481–1486.
- (8) Pascual, B.; San Bautista, A.; Ferreros, N.; López-Galarza, S.; Maroto, J. V. Analysis of Germination of Caper Seeds as Influenced by the Position of Fruit on the Mother Plant, Fruit Maturation Stage and Fruit Weight. *J. Hortic. Sci. Biotechnol.* **2003**, *78*, 73–78.
- (9) Allaith, A. A. Assessment of the Antioxidant Properties of the Caper Fruit (*Capparis Spinosa* L.) from Bahrain. *J. Assoc. Arab Univ. Basic Appl. Sci.* **2016**, *19*, 1–7.
- (10) Qadir, R.; Anwar, F.; Gilani, M. A.; Zahoor, S.; Rehman, M. M.; Mustaqeem, M. RSM/ANN Based Optimized Recovery of Phenolics from Mulberry Leaves by Enzyme-Assisted Extraction. *Czech J. Food Sci.* **2019**, *37*, 99–105.
- (11) Qadir, R.; Anwar, F.; Batool, F.; Mushtaq, M.; Jabbar, A. Enzyme-Assisted Extraction of *Momordica Balsamina* L. Fruit Phenolics: Process Optimized by Response Surface Methodology. *J. Food Meas. Charact.* **2019**, *13*, 697–706.
- (12) Mushtaq, M.; Sultana, B.; Bhatti, H. N.; Asgher, M. Optimization of Enzyme-Assisted Revalorization of Sweet Lime (*Citrus Limetta* Risso) Peel into Phenolic Antioxidants. *BioResources* **2014**, *9*, 6153–6165.
- (13) Mittermayr, S.; Olajos, M.; Chovan, T.; Bonn, G. K.; Guttman, A. Mobility Modeling of Peptides in Capillary Electrophoresis. *TrAC, Trends Anal. Chem.* **2008**, *27*, 407–417.
- (14) Zahoor, S.; Anwar, F.; Mehmood, T.; Sultana, B.; Qadir, R. Variation in Antioxidant Attributes and Individual Phenolics of Citrus Fruit Peels in Relation to Different Species and Extraction Solvents. *J. Chil. Chem. Soc.* **2016**, *61*, 2884–2889.
- (15) Asnaashari, S.; Delazar, A.; Alipour, S.; Nahar, L.; Williams, A.; Pasdaran, A.; Mojarab, M.; Azad, F.; Sarker, S.D. Chemical composition, free-radical-scavenging and insecticidal activities of the aerial parts of *Stachys byzantina*. *Arch. Biol. Sci.* **2010**, *62*, 653–662.
- (16) Mushtaq, M.; Sultana, B.; Anwar, F.; Adnan, A.; Rizvi, S. S. H. Enzyme-Assisted Supercritical Fluid Extraction of Phenolic Antioxidants from Pomegranate Peel. *J. Supercrit. Fluids* **2015**, *104*, 122–131.
- (17) Wang, W.; Wang, X.; Ye, H.; Hu, B.; Zhou, L.; Jabbar, S.; Zeng, X.; Shen, W. Optimization of Extraction, Characterization and Antioxidant Activity of Polysaccharides from *Brassica Rapa* L. *Int. J. Biol. Macromol.* **2016**, *82*, 979–988.
- (18) Samavati, V.; Yarmand, M. S. Statistical modeling of process parameters for the recovery of polysaccharide from *Morus alba* leaf. *Carbohydrate Polymers* **2013**, *98*, 793–806.
- (19) Mojtaba, A.; Fardin, K. Optimization of Enzymatic Extraction of Oil from Pistacia Khinjuk Seeds by Using Central Composite Design. *Food Sci. Technol.* **2013**, *1*, 37–43.
- (20) Zahedi, G.; Azarpour, A. Optimization of Supercritical Carbon Dioxide Extraction of Passiflora Seed Oil. *J. Supercrit. Fluids* **2011**, *58*, 40–48.
- (21) Fullana, M.; Trabelsi, F.; Recasens, F. Use of neural net computing for statistical and kinetic modelling and simulation of supercritical fluid extractors. *Chem. Eng. Sci.* **2000**, *55*, 79–95.
- (22) Pilkington, J. L.; Preston, C.; Gomes, R. L. Comparison of response surface methodology (RSM) and artificial neural networks (ANN) towards efficient extraction of artemisinin from *Artemisia annua*. *Ind. Crops Prod.* **2014**, *58*, 15–24.
- (23) Liu, H.; Du, X.; Yuan, Q.; Zhu, L. Optimisation of Enzyme Assisted Extraction of Silybin from the Seeds of *Silybum Marianum* by Box-Behnken Experimental Design. *Phytochem. Anal.* **2009**, *20*, 475–483.
- (24) Baghiani, A.; Ameni, D.; Boumerfeg, S.; Adjadj, M.; Djarmouni, M.; Charef, N.; Khennouf, S.; Arrar, L. Studies of Antioxidants and Xanthine Oxidase Inhibitory Potentials of Root and Aerial Parts of Medicinal Plant *Capparis Spinosa* L. *Am. J. Med. Med. Sci.* **2012**, *2*, 25–32.
- (25) Rezzan, A.; Ozan, E. E.; Huseyin, S.; Oktay, Y.; Nimet, B. Phenolic Components, Antioxidant Activity, and Mineral Analysis of *Capparis Spinosa* L. *Afr. J. Biotechnol.* **2013**, *12*, 6643–6649.
- (26) Babotă, M.; Frumuzachi, O.; Găvan, A.; Iacoviță, C.; Pinela, J.; Barros, L.; Ferreira, I. C. F. R.; Zhang, L.; Lucini, L.; Rocchetti, G.; et al. Optimized ultrasound-assisted extraction of phenolic compounds from *Thymus comosus* Heuff. ex Griseb. et Schenk (wild thyme) and their bioactive potential. *Ultrason. Sonochem.* **2022**, *84*, No. 105954.
- (27) Liaudanskas, M.; Noreikiene, I.; Zymonė, K.; Juodytė, R.; Žvikas, V.; Janulis, V. Composition and Antioxidant Activity of Phenolic Compounds in Fruit of the Genus *rosa* L. *Antioxidants* **2021**, *10*, 545.