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Antioxidant Profiling of Yemeni Varieties of *Opuntia ficus-indica* Pulp: Method Optimization, Validation, and Regional Evaluation

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Abstract

This study investigates the phytochemical properties and antioxidant potential of *Opuntia ficus-indica* growing in Yemen across diverse topographical regions, including Dhamar, Ibb, Sana'a, and Taiz. Despite its prevalence, Yemeni varieties remain scientifically under-characterized within the local context. This research focused on optimizing extraction protocols and quantifying the antioxidant content using spectrophotometric methods. The Extraction optimization identified deionized water as the superior solvent for bioactive recovery. The vortex extraction technique at 2 min was selected for its high efficiency and rapid processing, yielding 909.40±23.12 AAE/kg FW. Method validation demonstrated excellent linearity ($R^2 \geq 0.9967$), high precision (%RSD $< 3.665\%$), and recovery (%R 91.29–101.22%). Analysis of 25 real samples collected from various topographical regions in Yemen revealed that TAA ranged from 767.95 to 929.49 mg AAE/kg FW, TPC values ranged from 535.95 to 673.20 mg GAE/kg FW, while TFC varied between 28.75 and 87.50 mg QE/kg FW. These results establish a comprehensive phytochemical profile for Yemeni varieties of *Opuntia ficus-indica*, enhancing their economic and industrial value in the food and pharmaceutical sectors.

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Opuntia ficus-indica, Extraction Optimization, Antioxidants, Method Validation, Yemen.

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1. INTRODUCTION

The physiological balance of reactive oxygen species (ROS) is fundamental to cellular signalling and homeostasis, a state defined as oxidative eustress. However, excessive ROS production can disrupt redox homeostasis, leading to oxidative stress that damages vital biomolecules, including DNA, proteins, and lipids [1, 2]. Such biochemical disruptions are the primary drivers of chronic low-grade inflammation and mitochondrial dysfunction, which collectively underpin the pathogenesis of various non-communicable diseases, including cardiovascular disorders, type 2 diabetes, neurodegenerative conditions, and various malignancies [3].

In response to the limitations of synthetic antioxidants, biomedical research has focused on identifying natural plant-derived bioactive compounds. Dietary antioxidants, particularly polyphenols and carotenoids, have a profound ability to neutralize free radicals and modulate endogenous defense pathways, such as the Keap1/Nrf2/ARE signaling axis [4, 5]. Consequently, exploring the antioxidant profiles of underutilized flora is essential for developing novel functional foods and nutraceutical interventions. *Opuntia ficus-indica* (cactus). It is a tropical and subtropical plant that grows in arid and semi-arid regions [6].

Prickly pear (*Opuntia ficus-indica*) is found in many countries, including Mexico, the American hemisphere, Africa, and Mediterranean Basin countries [7]. Prickly pear belongs to the Cactaceae family, Opuntioideae subfamily, and Opuntia genus, which are characterized by their flat, structured leaves and stems [8]. The genus

Opuntia includes approximately 150–180 species [9].

Prickly pear fruit has the potential to provide food, beverages, and acceptable levels of nutrients, in addition to significant pharmacological potential and promising active ingredients in human nutrition and health. It is a good source of nutrients, such as minerals (Ca, Mg, K, and P), amino acids (alanine, arginine, and asparagine), and vitamins (vitamins C, E, K, and β-carotenes), and has antioxidant properties [10–12]. The medical and nutritional benefits of prickly pear fruits have been recognized for many centuries,as they contain many active biological nutrients and antioxidants [13, 14]. The medical and nutritional benefits of prickly pear fruits have been recognized for many centuries, as they contain many active biological nutrients and antioxidants [10–14].

In addition to its many nutritional benefits, cacti have surprising medicinal properties [14]. The fruit and cladodes of cactus are traditionally used to treat diabetes, hypertension, burns, edema, and indigestion in Oriental folk medicine [15]. In Turkey, the fruit of the cactus is used as a laxative and to relieve the pain of rheumatism and kidney stones [16, 17]. In addition, in Chinese folk medicine, cacti are used to accelerate the healing of wounds and ulcers and to treat gastrointestinal diseases and atherosclerosis [14, 18]. The fruit and pulp are shown in Figure 1.

Figure 1. A) Morphological Parts of the Opuntia fiscus-indica, B) fruit pulp

The *Opuntia ficus-indica*. was introduced to Yemen by the Turkish people during their occupation of Yemen in the late 16th century. For this reason, cactus (*Opuntia ficus-indica*) is known in Yemen as the “Turkish fig.” It was first planted in numerous villages at the border of mountains and highlands as a border to farmers’ fields and orchards. [19] However, it has now spread to several villages. Currently, Opuntia is being cultivated on an increasing amount of agricultural land [20]. *Opuntia ficus-indica*. It is the most famous prickly pear species that produces edible fruits [21].

Despite its prevalence and traditional use in Yemen, *Opuntia ficus-indica* remains scientifically undercharacterized in the local context. While the global literature extensively covers cultivars from the Americas and the Mediterranean, a significant data gap persists regarding the phytochemical potency and antioxidant capacity of the Yemeni genotypes. Furthermore, because the recovery efficiency of bioactive compounds depends heavily on extraction parameters, a standardized and validated methodology tailored to this specific region is currently lacking.

To address these limitations, this study aimed to optimize the extraction solvents and techniques required to establish a comprehensive chemical profile of Yemeni *Opuntia ficus-indica*. Concurrently, we aimed to validate spectrophotometric methods in accordance with the Association of Official Analytical Collaboration (AOAC) guidelines to evaluate the plant’s TAA using the ferric (III) 2,2’-bipyridine reducing capacity (FBRC) assay alongside its TPC and TFC. Ultimately, this study aims to provide an analytical foundation necessary to elevate the economic and industrial value of this cactus as a potent source of natural antioxidants within the Yemeni food and pharmaceutical sectors.

2. MATERIALS AND METHODS

2.1. Instrumentation

Quantitative analyses of TAA, TPC, and TFC were performed using a Specord 200 double-beam UV-Vis spectrophotometer (Analytik Jena, Germany). A calibrated digital analytical balance, Mettler-Ae160, made in Switzerland, was used for weighing purposes, pH-Meter Biotech Engineering HM Digital pH-80, made in Germany, was used for measuring and adjusting the pH of the solutions, Centrifuge MSE Mistral 2000, made in the UK, Variable mechanical shaker 3006, GFL, made in Germany, Memmert water bath, Rost Fret, made in Germany, A manual/continuous vortex instrument (Velp Scientific, 60 Hz, 45 W, made in Europe), and Bransonic Ultrasonic Cleaner, Model B-5200 E4, Model No. Wuv-D22h Power Supply, 450 W,220 V, 50-60 Hz, made in China.

2.2. Chemicals and Reagents

All chemicals used were of analytical grade. Standards included ascorbic acid (99.7%, w/w; BDH), gallic acid (99.8%; ACS), quercetin (99.9%; Pspark Scientific), sodium carbonate (99.5%), aluminum chloride (99.7%), 2,2-bipyridine (99.5%; Pspark Scientific), and ferric chloride hexahydrate (99%; Merck). The solvents used were methanol (99%v/v), acetone (99.5%v/v), ethanol (96%v/v), and acetonitrile (99.97%v/v), which were obtained from Scharlau. Deionized water (DIW) was prepared using a Direct-Q3 purification system (Millipore, Bedford, MA, USA).

2.3. Preparation of Standard Solutions

Primary stock standards for ascorbic acid, gallic acid, and quercetin were prepared at a concentration of 1000

ppm. Intermediate and working solutions were prepared daily through serial dilution with appropriate solvents to establish calibration curves and spike the Opuntia ficus indica samples.

2.4. Sample Collection and Pretreatment

Opuntia ficus-indica samples were collected during the summer of 2024 from four governorates of Yemen: Ibb, Taiz, Dhamar, and Sana'a. The samples were transported to the laboratory, and the fruits were washed with tap water followed by DIW. The peels were removed, seeds were separated from the pulp using a fine sieve, and the pulp was homogenized using an electric blender. The resulting homogenized pulp was stored at –4 °C until further analysis.

2.5. Optimization of the Extraction Parameters

The efficiency of bioactive compound recovery from a pulp matrix is highly dependent on the solvent polarity, mechanical methods applied, and extraction time. Three-stage optimization was conducted to maximize the yield of total antioxidants from *Opuntia ficus-indica*.

2.5.1. Selection of Extraction Solvent

Twenty-four solvent systems were screened to identify the most effective medium for bioactive compound recovery. These include pure solvents such as methanol, ethanol, acetone, acetonitrile, and deionized water, as well as various aqueous organic mixtures.

For the initial screening, 0.5 g of homogenized *Opuntia ficus-indica* pulp was accurately weighed and mixed with 10 mL of the respective solvent. The mixture was then shaken for 2 min. at 25±2 °C. The extracts were then centrifuged at 3500 rpm for 10 min. The resulting supernatant was separated and used for the TAA assay [22–25].

2.5.2. Evaluation of Extraction Techniques

Five distinct extraction techniques were comparatively evaluated to determine the most efficient approach for routine analysis, focusing on balancing the yield with the processing time.

Homogenized *Opuntia ficus-indica* pulp samples (0.5 g) were extracted with 10 mL of the chosen solvent (except for 5 g in 100 mL with the maceration technique) for several time intervals using different extraction techniques at room temperature.

Following each extraction procedure, all the mixtures were centrifuged at 3500 rpm for 10 min. To ensure the stability of the bioactive compounds, spectrophotometric analysis was performed within one hour of extract preparation.

2.5.3. Effect of Ionic Strength

Based on the preliminary findings of the technique evaluation, the most effective extraction parameters were selected for further study. The influence of ionic strength on the extraction efficiency was investigated by evaluating the salting-out effect.

Varying weights of sodium chloride, ranging from 0.1 to 1.0 g, were added to 10 mL of the optimized extraction solvent containing 0.5 g of *Opuntia ficus-indica* pulp. The mixtures were agitated and centrifuged for 10 min. at 3500 rpm. The supernatants were then analyzed for antioxidant content.

2.6. Quantitative Determination of TAA, TPC, and TFC

The antioxidant activity of *Opuntia ficus-indica* was analyzed spectrophotometrically in triplicate using the following methods:

2.6.1. Determination of Total Antioxidant

The total antioxidant activity was evaluated using the ferric (III) 2,2'-bipyridine reducing capacity (FBRC) [22–25]. A 500 μL aliquot of the extract (0.5 g/10 mL) was mixed in a 10 mL volumetric flask with 2 mL acetate buffer (pH 4), 1.5 mL $FeCl_3$ (0.01 M), and 1.5 mL 2,2-bipyridine (1000 ppm). The solution was diluted up to the mark with DIW, incubated at 50 °C for 30 min, and the absorbance was measured at 520 nm after cooling against the reagent blank. The results are expressed as mg ascorbic acid equivalents per kilogram of fresh weight (mg AAE/kg FW).

2.6.2. Total Polyphenol Content

Total polyphenol content was determined using the Folin-Ciocalteu method [25, 26]. *Opuntia ficus-indica* extract (500 μL; 0.5 g/10 mL) was mixed with 3.6 mL DIW and 0.4 mL Folin-Ciocalteu reagent. After 5 min, 4 mL of 7% Na_2CO_3 was added, and the volume was adjusted to 10 mL with deionized water (DIW). Following incubation at 50 °C for 20 min, the absorbance was measured at 750 nm against a reagent blank. The results are expressed as mg gallic acid equivalents per kilogram of fresh weight (mg GAE/kg FW).

2.6.3. Total Flavonoid Content

The total flavonoid content was quantified using the aluminum chloride ($AlCl_3$) method [25, 27]. A 3 mL aliquot of the extract (0.5 g/10 mL) was mixed with 2 mL of 2% $AlCl_3$ and diluted to 10 mL with ethanol/water (1:1 v/v). The cells were then incubated at room temperature for 10 min. The absorbance was recorded at 415 nm against a reagent blank. The results are expressed as mg quercetin-equivalent per kilogram of fresh weight (mg QE/kg FW).

2.7. Method Validation

The reliability of the spectrophotometric methods for TAA, TPC, and TFC assays was verified through method validation using *Opuntia ficus-indica* samples spiked with ascorbic acid, gallic acid, or quercetin standard solutions in triplicate. Validation parameters were established in accordance with the AOAC [28], including linearity (determined for R^2 of calibration curve), sensitivity (limit of detection (LOD) and limit of quantification (LOQ), precision expressed as relative standard deviation %RSD, and accuracy was assessed through percentage recovery (%R). Calibration was performed between the absorbance and the concentration of the spiked sample [22–27].

2.8. Analysis of Real Samples

The optimized analytical method was applied to determine the TAA, TPC, and TFC of 25 uniform-sized and fully ripened *Opuntia ficus-indica* samples collected between May and September 2024. Samples were sourced from different geographical regions of Yemen.

2.9. Statistical Analysis

Analytical assays for TAA, TPC, and TFC were performed in triplicate (n = 3) for each sample, and the data are expressed as the mean ± standard deviation (SD). To assess the variation in phytochemical accumulation across the four geographical regions (Dhamar, Ibb, Sana'a, and Taiz), we used a One-Way Analysis of Variance (ANOVA). We then applied Tukey's Honestly Significant Difference (HSD) post-hoc test to pinpoint specific differences between the regional group means, with statistical significance defined as $p < 0.05$. Finally, we conducted a two-tailed Pearson correlation test on the 25 individual samples to determine the strength and direction of the linear relationships between TAA, TPC, and TFC. We evaluated these correlation coefficients (r) for significance at both $p < 0.05$ and $p < 0.01$ levels.

3. RESULTS AND DISCUSSION

3.1. Solvent Selection Results

The efficacy of various solvent systems for the recovery of TAA using mechanical shaking is illustrated in Figure 2. The experimental data demonstrated that deionized water was the most effective solvent, yielding the highest TAA recovery of 892.31±18.49 mg AAE/kg FW, followed by ethanol (789.74±31.19 mg AAE/kg FW). A ternary mixture of water/acetone/acetonitrile (1:1:1 v/v/v) provided moderate recovery values of 788.03 ± 12.90 mg AAE/kg FW, and water/ethanol/ methanol (1:1:1 v/v/v) was 786.32±12.90 mg AAE/kg FW. Conversely, pure acetonitrile exhibited the lowest solubilizing capacity, recovering only 317.09±5.922 mg AAE/kg FW.

These results indicate that the antioxidant constituents in *Opuntia ficus-indica* pulp are highly polar, making an aqueous solvent the optimal choice for achieving maximum yield. Consequently, deionized water was selected as the extraction medium for all the subsequent experiments. This choice aligns with the chemical profiles of primary bioactive compounds, such as betalains and hydrophilic phenolics, which require a polar environment for efficient extraction. However, aqueous extraction characteristically co-extracts high-molecular-weight hydrocolloids and mucilage. While this phenomenon can increase extract viscosity, potential mechanical and optical limitations were minimized in this study by employing high-speed centrifugation followed by fine-porosity membrane filtration (0.45 μm) to obtain a clear supernatant. Crucially, the spectrophotometric reagents used exhibit satisfactory chemical selectivity toward the targeted phenolic functional groups, minimizing potential interference from the carbohydrate backbone of the co-extracted components. This analytical approach is supported by the excellent recovery rates and exceptionally low standard deviation (±0.012 mg/kg) achieved in the deionized water extracts, suggesting that the presence of hydrocolloids did not significantly compromise quantification reproducibility. Consequently, the use of DIW ensures the complete solubilization and retention of these highly polar antioxidant fractions, providing an eco-friendly and effective medium for hydrophilic compound recovery without the use of organic solvents.

3.2. Evaluation of Extraction Techniques

Once the solvent was established, five extraction techniques were compared to determine the balance between maximum recovery and operational efficiency (Table 1).

- **Mechanical and Manual Shaking Methods:** The automated shaker and manual shaking methods yielded statistically similar results, producing 892.31±18.49 mg AAE/kg FW and 881.20±38.49 mg AAE/kg FW, respectively, within a 2 min extraction period.
- **Maceration and Sonication Methods** The two techniques, maceration (at 25±2°C for 120 min) and sonicated (at 25±2 °C for 30 min). yielded recovery values of 866.67±15.38 mg AAE/kg FW and 870.94±20.72 mg AAE/kg FW, respectively. However, these methods require significantly longer processing times (60 times longer than the vortex method).
- **Vortex Method** Achieved The highest overall TAA recovery of 909.402±23.15 mg AAE/kgFW was achieved in just 2 min. Extending the vortexing time to 60 min did not yield a significant increase in concentration, indicating that a 2 min vortexing period is sufficient, offering the benefits of high throughput,

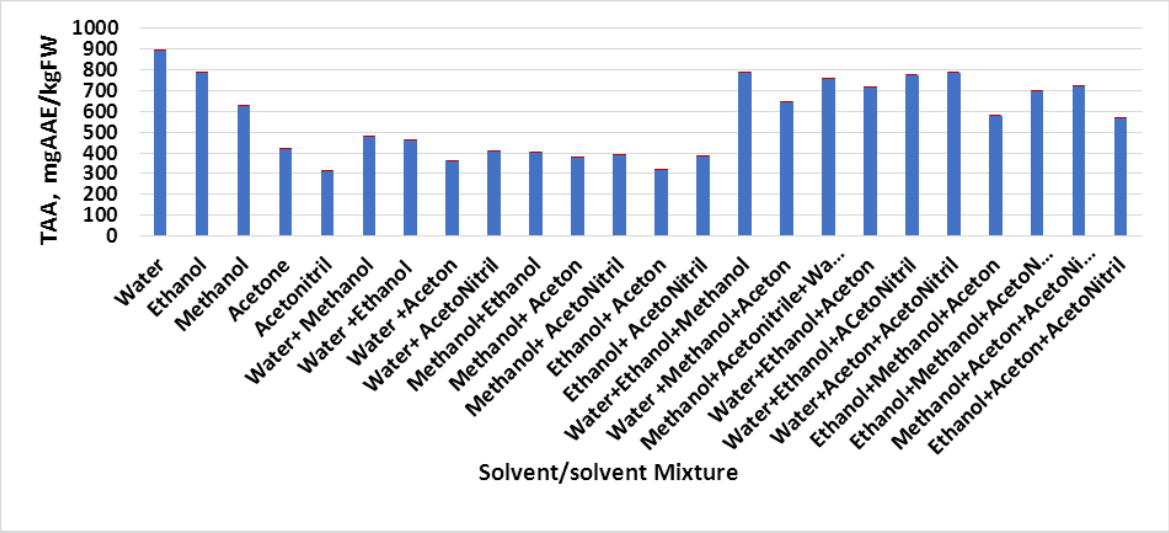

Figure 2. Capacity of different extraction solvents for TAA from *Opuntia ficus-indica* pulp samples, binary and tertiary mixtures of solvents were in equal ratios.

reduced energy consumption, and high reproducibility for the analysis. Therefore, the vortex method was selected as the optimal technique.

Table 1. Optimized extraction techniques (n=3)

No.	Extraction Techniques	Extraction Time (min)	Average TAA mg AAE/Kg FW,
1	Hand shaking	2	881.20±38.49
2	Shaker	2	892.31±18.49
3	Vortex	2	909.40±23.12
4	Sonication	30	870.94±20.72
5	Maceration	120	866.67±15.38

3.3. Influence of Ionic Strength

The addition of sodium chloride was investigated to evaluate the "salting-out" effect across all tested techniques. The results consistently showed that varying the ionic strength had no significant impact on the extraction efficiency of the antioxidants.

3.4. Method Validation Results

The proposed spectrophotometric methods for TAA, TPC, and TFC were validated based on linearity, precision, accuracy, and sensitivity (LOD/LOQ), according to the AOAC guidelines [28].

3.4.1. Validation Method Results of TAA Determination

To assess the method performance, *Opuntia ficus-indica* samples were spiked with ascorbic acid at concentrations ranging from 0.313–3.125 ppm, as shown in Table 2. The calibration curve (Figure 3) exhibited excellent linearity, with a correlation coefficient (R^2) of 0.9996. The precision was calculated using Equation (1). The obtained values confirmed high repeatability, with %RSD values ranging from 0.56% to 1.89%.

$$\%RSD = \left(\frac{SD}{X}\right) \times 100 \quad (1)$$

where SD represents the standard deviation, and X corresponds to the mean absorbance of the replicate measurements.

The accuracy of the method was calculated using Equation (2). The recovery rates ranging from 92.26–100.07% demonstrated high method accuracy.

$$\%R = \left(\frac{C_{\text{spiked}} - C_{\text{unspiked}}}{C_{\text{added}}}\right) \times 100 \quad (2)$$

Where C spiked is the total concentration determined in the spiked sample, C unspiked is the concentration in the original sample, and C added is the nominal concentration of the pure ascorbic acid standard added to the sample.

The LOD and LOQ were determined to be 0.061 ppm and 0.203 ppm, respectively, as shown in Table 2. Calculated according to Equations (3 and 4).

$$LOD = \frac{3 \times SD}{S} \quad (3)$$

$$LOQ = \frac{10 \times SD}{S} \quad (4)$$

S is the slope of the corresponding calibration curve.

3.4.2. Validation Methods Results of TPC Determination

The linearity and accuracy of TPC were evaluated by spiking samples with gallic acid (0.625–3.125 ppm). The method showed a strong linear response (Figure 4) with an R^2 value of 0.9967. The repeatability was excellent,

Table 2. Method validation results for TAA, in spiked *Opuntia ficus-indica* samples, n=3

Spiked Conc. of Ascorbic Acid (ppm)	Ave. Ast	Abs. unspiked sample	As.	Abs. Difference	SD	%RSD	%R	LOD (ppm)	LOQ (ppm)
0.313	0.056	0.345	0.397	0.052	0.008	1.89	92.26	0.061	0.203
0.625	0.110	0.345	0.448	0.104	0.003	0.72	95.14		
1.250	0.204	0.345	0.545	0.197	0.003	0.56	96.89		
1.875	0.299	0.345	0.649	0.292	0.012	1.89	97.88		
2.500	0.388	0.345	0.725	0.379	0.004	0.58	97.68		
3.125	0.470	0.345	0.821	0.470	0.008	0.97	100.07		

Conc.= Concentration, Ast.=Absorbance of standard, As.=Average absorbance of spiked sample, Abs. Difference = Absorbance of Spike - Absorbance of Unspike,

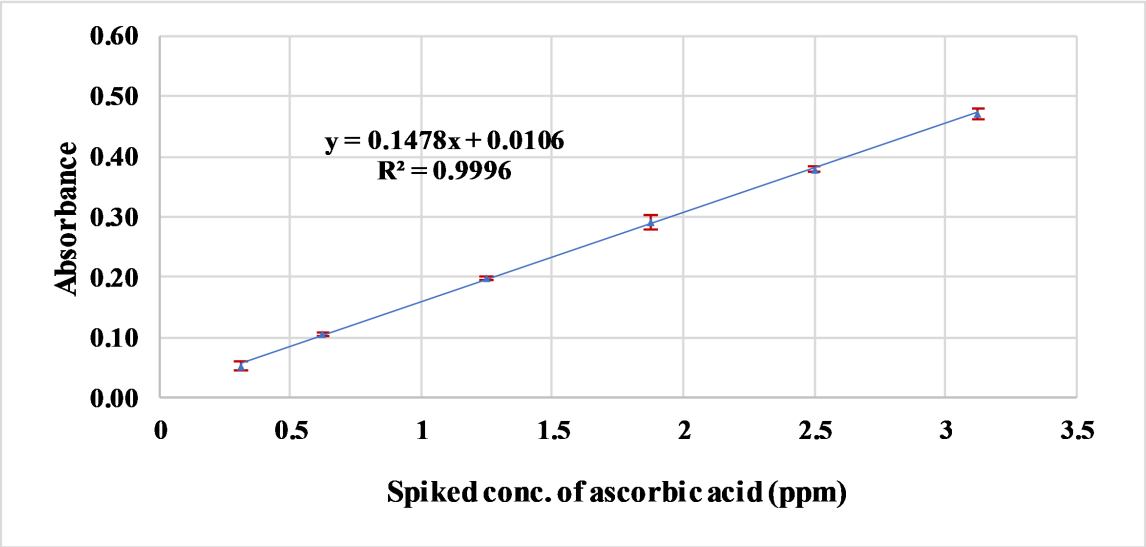

Figure 3. Calibration curve of TAA of spiked *Opuntia ficus-indica* sample.

with %RSD values ranging from 0.57% to 1.27%. The accuracy was high, with recovery rates between 94.25% and 100.78%. The analysis Sensitivity yielded an LOD of 0.072 ppm and an LOQ of 0.241 ppm, as shown in Table 3.

3.4.3. Validation Methods of TFC Determination

For the total flavonoid content (TFC), the samples were spiked with quercetin (1.25–12.50 ppm). The calibration curve (Figure 5) demonstrated a superior linearity (R^2 = 0.9992). The precision was high, with %RSD values ranging from 0.360% to 3.665%. The recovery of quercetin was between 91.29% and 101.22%, confirming the accuracy of the method. The LOD and LOQ were determined to be 0.133 ppm and 0.443 ppm, respectively, as shown in Table 4.

3.5. Real Samples Analysis Results

The optimized method was applied to quantify the bioactive constituents of 25 *Opuntia ficus-indica* samples collected from various topographical regions in Yemen. The comprehensive results for the TAA, TPC, and TFC are summarized in Table 5.

3.5.1. Phytochemical Composition Determination Results

Phytochemical profiling of Yemeni *Opuntia ficus-indica* pulp revealed substantial TPC and TAA, underscoring the profound influence of regional geography and environmental stressors. Comparing these findings with international studies highlights notable variations driven by agro-climatic conditions.

The TPC results obtained for Yemeni varieties (ranging from 535.95 to 673.20 mg GAE/kg FW) were in strong concordance with those reported by Nadia et al. [29] for North Algerian cultivars (472.30 to 666.00 mg GAE/kg FW). However, the Yemeni samples demonstrated superior antioxidant capacity compared to the values reported by Benattia and Arrar [30] in Algeria (∼420 mg AAE/kg FW), likely due to the intense solar radiation and high-altitude microclimates characteristic of the Yemeni highland region.

Our results are in excellent agreement with those from South America, where Coria Cayupán et al. [31] documented TPC values between 540.00 and 1120.00 mg

Table 3. Method Validation Results for TPC in Spiked *Opuntia ficus-indica* Samples, n=3

Spiked Conc. of Gallic Acid (ppm)	Ave. Ast	Abs. unspiked sample	As.	Abs. Difference	SD	%RSD	%R	LOD (ppm)	LOQ (ppm)
0.625	0.075	0.247	0.318	0.071	0.003	1.21	94.25	0.072	0.241
1.25	0.153	0.247	0.397	0.150	0.004	1.27	98.25		
1.875	0.225	0.247	0.468	0.221	0.004	0.98	98.08		
2.500	0.296	0.247	0.539	0.292	0.003	0.57	98.87		
3.125	0.386	0.247	0.636	0.389	0.005	0.96	100.78		

Conc.= Concentration, Ast.=Absorbance of standard, As.=Average absorbance of spiked sample, Abs. Difference = Absorbance of Spike - Absorbance of Unspike,

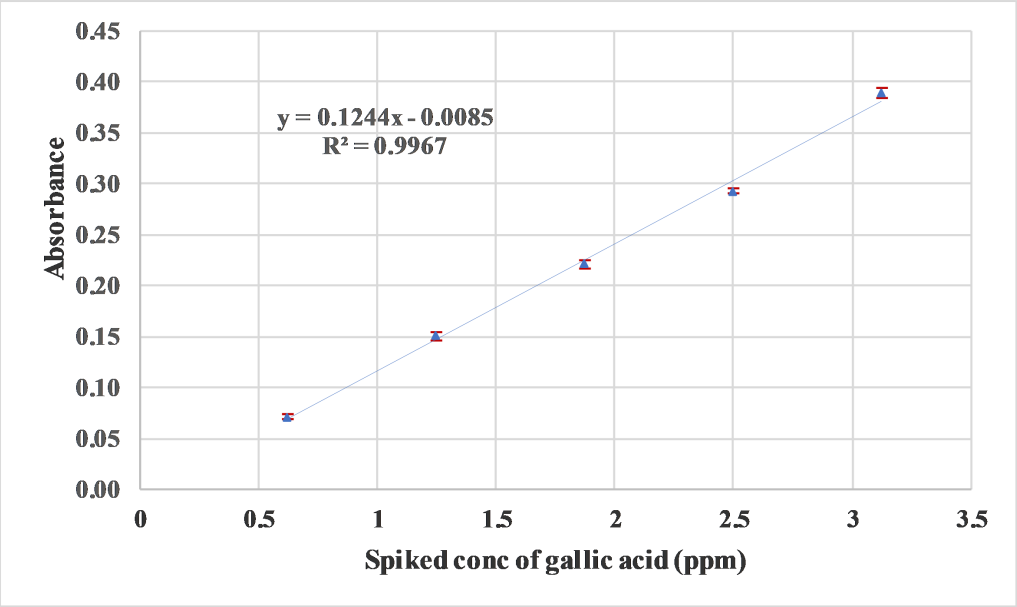

Figure 4. Calibration Curve of TPC of *Opuntia ficus-indica* Sample.

Table 4. Method Validation Result for TFC in Spiked *Opuntia ficus-indica* Samples, n=3

Spiked Conc. for Quercetin (ppm)	Ave. Ast	Abs. unspiked sample	As.	Abs. Difference	SD	%RSD	%R	LOD (ppm)	LOQ (ppm)
1.250	0.073	0.002	0.07	0.066	0.003	3.67	91.29	0.133	0.443
2.500	0.160	0.002	0.16	0.155	0.003	1.50	96.88		
3.750	0.252	0.002	0.25	0.247	0.002	0.80	98.01		
5.000	0.331	0.002	0.33	0.325	0.003	0.77	98.39		
6.250	0.428	0.002	0.43	0.428	0.009	1.99	100.00		
9.375	0.619	0.002	0.62	0.620	0.003	0.48	100.05		
12.500	0.821	0.002	0.833	0.831	0.003	0.36	101.22		

Conc.= Concentration, Ast.=Absorbance of standard, As.=Average absorbance of spiked sample, Abs. Difference = Absorbance of Spike - Absorbance of Unspike,

GAE/kg FW for fresh prickly pears from Santiago del Estero, Argentina. This biochemical alignment suggests that plants cultivated in semi-arid, high-stress environments tend to accumulate comparable concentrations of protective secondary metabolites.

The phenolic concentrations in Yemeni cactus pulp substantially exceeded those reported by Maataoui et al. [32] for Moroccan Chaouia varieties (300.00 mg GAE/kg FW). This variance highlights that the rugged topography, specific soil composition, and diurnal temperature fluctuations in Yemen stimulate the phenylpropanoid biosynthetic pathway more intensely than in milder coastal or plain regions.

Furthermore, the Yemeni cultivars exhibited markedly higher phenolic content than that recorded by Medina et al. [33] for fruits from Tenerife Island, Spain (172.00

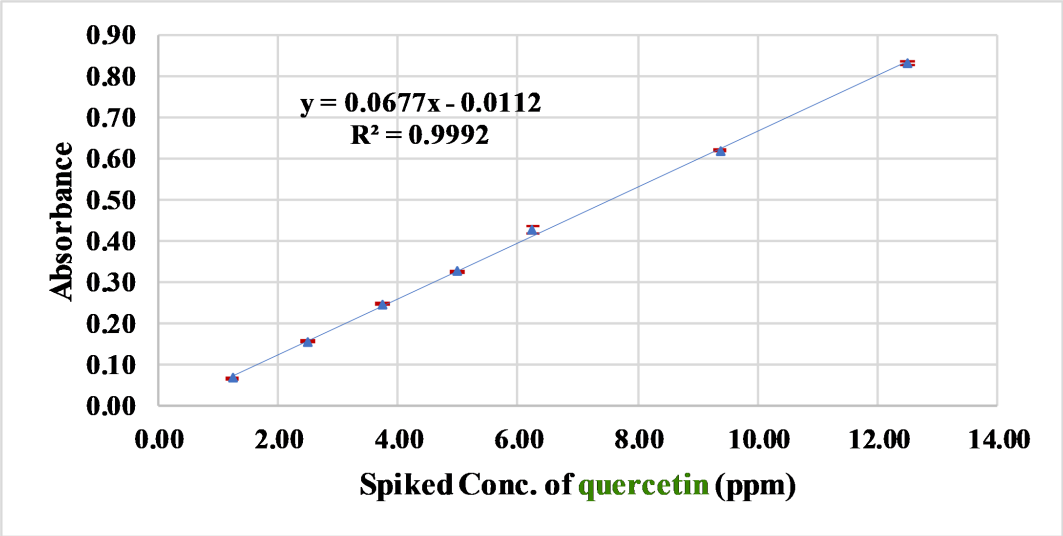

Figure 5. Calibration Curve of TFC of *Opuntia ficus-indica* Sample.

mg GAE/kg FW). Drawing parallels with foundational literature from Mexico (the center of origin), it is well established that water deficit and high-altitude exposure synergistically enhance the accumulation of bioactive compounds, rationalizing the elevated antioxidant profiles observed in high-altitude Yemeni regions such as Dhamar and Sana'a.

The total flavonoid content of the analyzed samples ranged from 28.75±3.54 to 87.50±14.14 mg QE/kg FW, with the Dhamar Governorate yielding the highest flavonoid levels, reaching a mean value of 68.50 mg QE/kg FW. These values are comparable to the total flavonoid concentrations reported by El Mannoubi [34] for Tunisian yellow-orange *Opuntia ficus-indica* pulp, which ranged from 25.05 to 243.75 mg QE/kg FW, depending on the extraction solvent. Notably, our samples exhibited higher flavonoid levels than their 80% methanol (25.05 mg QE/kg FW) and 80% ethanol (49.95 mg QE/kg FW) extracts, but lower than the yields obtained via 80% acetone extraction (243.75 mg QE/kg FW).

3.5.2. Statistical Analysis and Correlation of Phytochemical Assays

3.5.3. Geographical Variance of Phytochemical Content

The geographical origin of the Yemeni *Opuntia ficus-indica* played significantly influenced the accumulation of bioactive compounds in the plants. ANOVA results confirmed statistically significant differences ($p < 0.05$) in TAA, TPC, and TFC among the four governorates (Table 6). Tukey's post-hoc comparisons revealed that samples from the Dhamar region had the highest concentrations, particularly peaking in TPC (642.35±14.13 mg/kg) and TAA (920.00±18.49 mg/kg). In contrast, samples harvested from the Ibb governorate demonstrated significantly lower values in all three assays. This clear variation in secondary metabolite accumulation highlights the strong influence of regional environmental factors, such as altitude, soil composition, and climatic stress, on the plant's biosynthetic pathways.

Correlation between assays To better understand the biochemical drivers behind the observed antioxidant capacity, Pearson correlation analysis was conducted on the individual samples (n = 25), with the correlation matrix detailed in Table 7. We found a strong and highly significant positive correlation ($r = 0.842$, $p < 0.01$) between TAA and TPC. This robust linear relationship strongly suggests that phenolic compounds are the primary bioactive secondary metabolites responsible for the free radical scavenging capacity of the extracts, and the high density of electron-donating hydroxyl groups on these phenolic structures likely governs this antioxidant mechanism.

The relationship between TAA and TFC was also positive, although more moderate ($r = 0.415$, $p < 0.05$). While flavonoids make up a significant portion of the antioxidant pool, this weaker correlation reveals an interesting dynamic. For instance, Sana'a and Taiz exhibited nearly identical TFC averages but completely divergent TAA capacities. This indicates that the total antioxidant activity does not rely solely on the flavonoid fraction but rather on a synergistic effect across the broader phenolic profile.

Finally, the weak positive correlation between TPC and TFC ($r = 0.334$) aligns with the standard phytochemical hierarchy, as flavonoids are a subclass of total polyphenols. Interestingly, the varying TFC to TPC ratios across the governorates suggest that distinct regional stressors may selectively enhance the phenylpropanoid biosynthetic pathway, driving increased flavonoid biosynthesis in specific environments, such as Dhamar.

Table 5. TAA, TPC, and TFC content in *Opuntia ficus-indica* real samples

No.	Governorate	Code	Average, mg/Kg FW, n=3		
			TAA	TPC	TFC
1	Dhamar	Dh 1	916.67±18.13	630.07±14.79	58.75±10.61
2		Dh 1	907.69±29.01	645.75±14.75	77.50±14.14
3		Dh 3	929.49±10.87	657.52±11.0	52.50±14.14
4		Dh 4	920.51±14.50	643.14±14.74	81.25±10.61
5		Dh 5	925.64±14.50	635.29±14.71	72.50±7.07
Average			920.00±18.49	642.35±14.13	68.500±11.62
1	Ibb	Ib 1	885.90±18.13	632.68±14.79	35.00±7.07
2		Ib 2	788.46±32.63	535.95±22.15	45.00±7.07
3		Ib 3	893.59±10.87	626.14±25.81	56.25±10.61
4		Ib 4	767.95±32.63	567.32±22.11	67.50±14.14
5		Ib 5	888.46±18.13	623.53±18.44	30.00±7.07
Average			844.87±24.11	597.12±21.05	46.75±9.62
1	Sana'a	Sa 1	905.13±14.51	673.20±18.49	37.50±7.07
2		Sa 2	900.03±14.52	609.15±14.74	28.75±3.54
3		Sa 3	891.03±18.13	619.61±7.392	56.25±10.61
4		Sa 4	917.93±14.54	618.30±18.41	65.00±14.14
5		Sa 5	900.03±14.55	627.45±29.57	37.50±7.07
6		Sa 6	923.03±14.56	627.45±14.72	63.75±10.61
7		Sa 7	911.53±10.87	632.68±14.77	45.00±7.07
8		Sa 8	887.13±14.58	628.76±11.04	71.25±10.61
9		Sa 9	905.13±21.79	623.53±18.40	50.00±7.07
10		Sa 10	892.33±14.510	631.37±11.05	87.50±14.14
Average			903.33±15.47	629.15±16.90	54.25±9.75
1	Taiz	Ta 1	869.23±7.25	600.00±11.09	42.50±7.07
2		Ta 2	855.13±10.8	610.45±11.09	37.50±14.1
3		Ta 3	888.46±18.1	601.30±14.78	52.50±7.07
4		Ta 4	893.59±10.8	626.14±18.48	67.50±14.1
5		Ta 5	900.00±14.5	616.99±14.78	70.00±14.1
Average			881.28±12.87	610.98±14.32	54.00±11.83

Table 6. Geographical variation in the antioxidant activity and phytochemical profiles of the analyzed samples.

Governorate	TAA (mg/Kg FW)	TPC (mg/Kg FW)	TFC (mg/Kg FW)
Dhamar	920.00 ± 18.49^{a}	642.35 ± 14.13^{a}	68.50 ± 11.62^{a}
Sana'a	903.33 ± 15.47^{ab}	629.15 ± 16.90^{ab}	54.25 ± 9.75^{b}
Taiz	881.28 ± 12.87^{bc}	610.98 ± 14.32^{bc}	54.00 ± 11.83^{b}
Ibb	844.87 ± 24.11^{c}	597.12 ± 21.05^{c}	46.75 ± 9.62^{c}
Where a is the highest statistically significant value, b is significantly lower than a, c is the lowest statistically significant value, ab represents a transitional statistical group that shares similarities with both a and b, and bc represents a transitional statistical group that shares similarities with both b and c. Values are expressed as mean ± standard deviation (n=3 technical replicates for each sample). Different superscript letters within the same column indicate significant differences ($p < 0.05$) according to Tukey's post hoc test.			

Table 7. Pearson correlation matrix for TAA, TPC, and TFC assay

Assay	TAA	TPC	TFC
TAA	1		
TPC	0.842^{**}	1	
TFC	0.415^{*}	0.334	1
* Correlation is significant at the 0.05 level (2-tailed). *Correlation is significant at the 0.01 level (2-tailed).			

4. PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF *OPUNTIA FICUS-INDICA*

4.1. STATISTICAL CORRELATIONS AND REGIONAL VARIATIONS ACROSS YEMENI GOVERNORATES

The experimental data revealed a robust, positive linear correlation between TAA and TPC across all evaluated samples, demonstrating that hydrophilic phenolics and flavonoids extracted from *Opuntia ficus-indica* fruit pulp drive the primary antioxidant mechanism via ferric ion (Fe^{3+} to Fe^{2+}) reduction and free radical scavenging. Comparative regional profiling exhibited distinct geographical variations dictated by environmental and microclimatic disparities among Yemeni governorates. Samples collected from the Dhamar Governorate consis-

tently displayed the highest levels of TAA, TPC, and TFC. This phytochemical abundance is linked to the region's high-altitude topography, lower average temperatures, and intense solar ultraviolet (UV) radiation [35]. ambient conditions that induce environmental stress and accelerate the biosynthesis of protective secondary metabolites within the fruit pulp matrix. Samples from the Sana'a Governorate followed closely behind Dhamar, followed by Taiz, reflecting the similarities in their semi-arid highland conditions, which modulate the phytochemical profiles of local samples. Conversely, the samples from Ibb recorded the lowest average phytochemical parameters. This could be attributed to the region's high annual rainfall, high humidity, and temperate climate, which minimize environmental stress, thereby reducing the physiological demand for synthesizing substantial amounts of antioxidant compounds within the fruit pulp matrix [36].

5. CONCLUSION

This study successfully optimized the extraction and quantification methods for bioactive compounds in the Yemeni genotype of *Opuntia ficus-indica*. Water was identified as the superior extraction solvent for total antioxidant content compared to various organic solvent mixtures. Notably, the 2 min vortex method yielded the highest overall total antioxidant recovery, demonstrating its enhanced extraction efficiency and practicality. The validated spectrophotometric methods were accurate and reliable for measuring TAA, TPC, and TFC in fruit pulp. By addressing the research gap regarding Yemeni varieties, this study provides a foundation for the systematic categorization and broader adoption of *Opuntia ficus-indica* in food and pharmaceutical industries.

DECLARATIONS

Ethical Approval

This article does not contain any studies involving animals or human participants performed by the authors. We declare that this manuscript is original and is not currently being considered for publication elsewhere. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Conflict of interest

The authors declare no conflicts of interest.

Competing interests

The authors have no financial or proprietary interest in any material discussed in this article.

Authors' contributions

All authors contributed to the study's conception and design. All authors performed simulations, data collection and analysis, and commented on the present version of the manuscript. All authors have read and approved the final manuscript.

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