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Anatomical characteristics, thermal stability of betalains, and ABTS antioxidant activity of nopal cactus (*Opuntia ficus-indica*) from Phu Yen, Vietnam

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Abstract
The Nopal cactus (*Opuntia ficus-indica* (L.) Mill.) is a succulent plant renowned for its exceptional biological adaptability in semi-arid environments. It represents a valuable botanical resource rich in bioactive compounds with potential applications in pharmaceuticals, cosmetics, and functional foods [1]. Despite its vigorous growth in the harsh climate of Phu Yen Province, Vietnam, systematic studies detailing its anatomical traits, phytochemical profile, and post-harvest thermal processing dynamics remain scarce. This study investigates the anatomical characteristics of the cladode, the effect of drying temperatures on moisture desorption kinetics, experimental repeatability (%RSD), and the stability of the nitrogenous betalain pigments. Furthermore, the *in vitro* $ABTS^{\bullet+}$ radical scavenging capacity of the extracts was evaluated.
Histological examination via double-staining light microscopy revealed a highly developed water-storage parenchyma composed of large, thin-walled cells, a stomata-bearing epidermis, vascular bundles containing spiral xylem vessels, and abundant calcium oxalate druses (sphaeraphides) [3]. Thermal drying kinetics demonstrated that moisture desorption was temperature-dependent. However, betalains proved thermolabile, undergoing accelerated thermal degradation at higher processing windows. The fresh extract (M2) demonstrated an outstanding $ABTS^{\bullet+}$ radical scavenging capacity with an IC_{50} value of 48.39 µg/mL, which closely approached the antioxidant capacity of pure ascorbic acid (IC_{50} = 43.43 µg/mL). Convective oven-drying at a moderate temperature of 40°C achieved high experimental repeatability (%RSD < 1%) while protecting the thermolabile betalains against severe degradation. These findings highlight the tremendous potential of the Phu Yen Nopal cactus as a high-quality source of natural antioxidants for functional and dermo-cosmetic applications.

Keywords: *Opuntia ficus-indica*, anatomy, betalains, thermal stability, ABTS assay, Phu Yen

Introduction

Opuntia ficus-indica (L.) Mill., colloquially designated as the Nopal cactus or prickly pear, belongs to the class Magnoliopsida, order Caryophyllales, and family Cactaceae. Native to the arid and semi-arid regions of Mexico, this species has naturalized extensively across tropical and subtropical zones worldwide [1]. Characterized by flattened, succulent stems termed cladodes and leaves modified into protective spines, the plant minimizes transpirational water loss while maximizing photosynthetic efficiency through Crassulacean Acid Metabolism (CAM). In Vietnam, the plant has successfully adapted to survive the harsh sandy terrains and microclimatic stresses typical of coastal areas, such as Phu Yen Province [3].
Beyond fundamental macronutrients, *Opuntia ficus-indica* synthesizes a dense array of high-potency secondary metabolites. The defining chemotaxonomic feature of the *Opuntia* genus is the accumulation of betalains. These water-soluble, nitrogenous pigments are divided into two main sub-classes: betacyanins (infusing red-violet hues) and betaxanthins (yielding yellow-orange reflections) [4]. The radical-neutralizing mechanism of betalains is attributed to their highly conjugated resonance structure, which readily donates electrons or hydrogen atoms ($H^{\bullet}$) to electron-deficient free radical species, thereby terminating free-radical chain reactions before cellular injury occurs [2, 4].
However, the structural integrity of betalains is heavily influenced by physicochemical variables, including temperature, pH, light exposure, and oxygen availability. In industrial processing, thermal energy represents the most critical destabilizing factor. Elevated temperatures drive the hydrolytic cleavage of the betalain aldimine bond, yielding betalamic acid, alongside concurrent decarboxylation pathways [4]. Establishing a precise thermal processing window is therefore essential to balance moisture removal rates against the structural preservation of native betalains.
Despite its vigorous growth in the harsh climate of Phu Yen Province, Vietnam, systematic studies detailing its anatomical traits, phytochemical profile, and post-harvest thermal processing dynamics remain scarce. Therefore, this study investigates the anatomical characteristics of the cladode, the effect of drying temperatures on moisture desorption kinetics, experimental repeatability (%RSD), and the stability of the nitrogenous betalain pigments. Furthermore, the *in vitro* $ABTS^{\bullet+}$ radical scavenging capacity of the extracts was evaluated to provide a comprehensive scientific foundation for utilizing this valuable botanical resource.

Materials and Methods

Plant Materials and Reagents

Fresh, young cladodes of *Opuntia ficus-indica* were harvested from Van Hoa, Phu Yen Province, Vietnam (Figure 1). The analytical-grade chemicals utilized in this study included ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), $K_2S_2O_8$ (Potassium persulfate), Ethanol, NaOH, and Vitamin C (ascorbic acid), all sourced from standard commercial distributors.

Fig 1: Morphological appearance of *Opuntia ficus-indica* cultivated in Phu Yen province

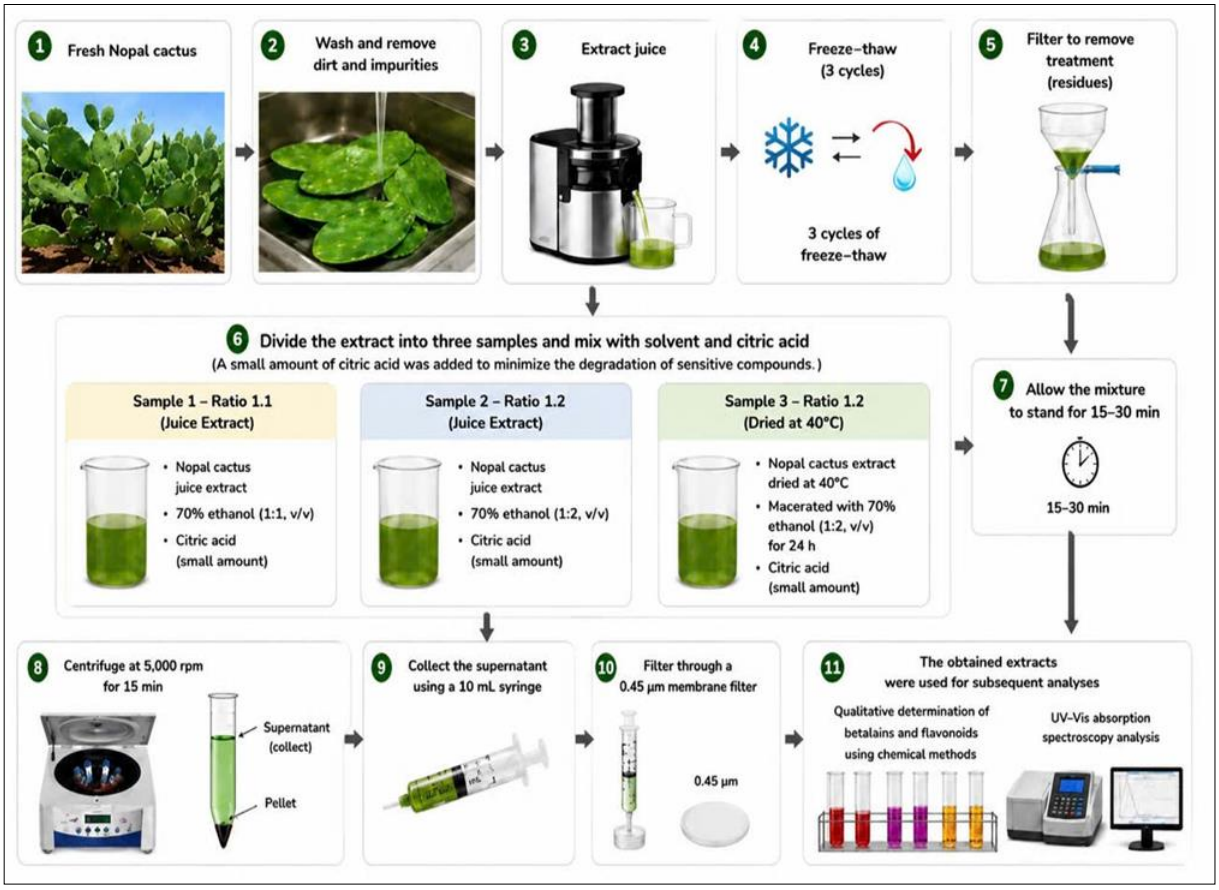

Fig 2: Schematic workflow of the experimental procedure, including post-harvest drying, extraction, phytochemical screening, and antioxidant evaluation of *Opuntia ficus-indica* cladodes

Histological and Anatomical Characterization

Fresh cladode specimens were fixed, cut into thin transverse cross-sections (10-20 µm), and cleared using a sodium hypochlorite (NaOCl) solution to remove endogenous chlorophyll. The sections were then subjected to double-staining using iodine green and carmine alum, mounted on glass slides, and examined under a high-resolution optical microscope according to botanical microtechnique protocols adapted from Nguyen (2019) [3].

Drying Kinetics and Repeatability Analysis

To evaluate moisture desorption behavior, uniform cladode samples were dehydrated in a forced-convection laboratory oven at four controlled temperatures: 30°C, 40°C, 50°C, and 60°C. Sample weights were logged at predetermined intervals to plot drying curves (performed in triplicate, n=3). The precision and repeatability of the thermal drying procedure were monitored by calculating the Relative Standard Deviation (%RSD):

$$RSD(\%) = (\frac{SD}{\bar{x}}) \times 100\%$$

where SD represents the sample standard deviation and x denotes the sample mean value. Lower %RSD metrics indicate higher reproducibility and stability within the experimental drying assembly.

Phytochemical Screening and ABTS Radical Scavenging Assay

Phytochemical Screening

The presence of betalains and flavonoids was evaluated using characteristic colorimetric reactions with sodium hydroxide (NaOH) and confirmed by sweeping the absorbance profiles via UV-Vis spectrophotometry.

$ABTS^{\bullet+}$ Radical Scavenging Assay

The $ABTS^{\bullet+}$ radical cation was generated by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was incubated in the dark for 12-16 hours at room temperature. Before the assay, the $ABTS^{\bullet+}$ solution was diluted with ethanol to achieve an optical density (OD) of approximately 0.700 at 734 nm. Aliquots of the extracts at varying concentrations were mixed with the working $ABTS^{\bullet+}$ solution. After exactly 6 min of incubation, the remaining absorbance was measured at 734 nm. The percentage of radical inhibition was calculated, and the IC_{50} value (concentration required to scavenge 50% of the initial free radicals) was computed. Ascorbic acid served as the positive reference standard.

Results and Discussion

Anatomical Characteristics of the Nopal Cactus Cladode

Microscopic analysis revealed distinctive xerophytic adaptations in the cladode tissue of the Phu Yen Nopal cactus. The cross-sections exhibited a well-defined epidermis coated with a thick protective cuticle layer and populated with functional stomatal complexes. Beneath the dermal layer lay an exceptionally expansive water-storage parenchyma [3]. This tissue was dominated by large, thin-walled, vacuolated parenchymal cells optimized for rapid water retention during brief rainy periods to sustain the plant through extended droughts.

Distributed uniformly within this ground tissue were vascular bundles arranged with prominent phloem elements and spiral xylem vessels. Furthermore, a high density of star-shaped calcium oxalate druses (sphaeraphides) was observed embedded inside the parenchymal cells [3]. These crystal formations participate in intracellular ionic regulation and calcium homeostasis, while functioning as a structural defense mechanism against herbivorous predation.

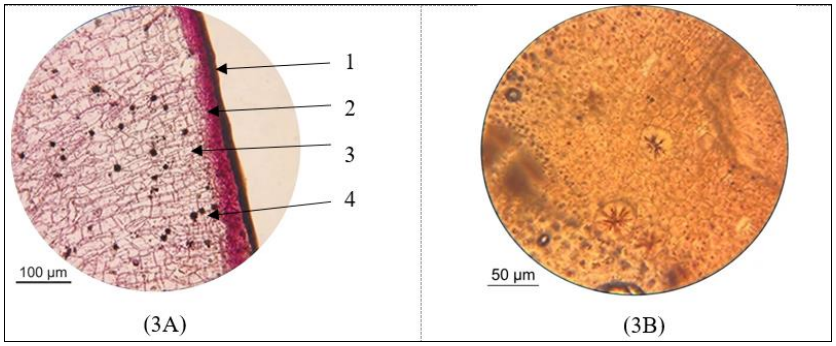

Fig 3: Transverse microsections of the *Opuntia ficus-indica* cladode

(3A) General anatomical view (1: Epidermis, 2: Collenchyma, 3: Parenchyma ground tissue, 4: Calcium oxalate druse)
(3B) High-magnification view of a star-shaped calcium oxalate druse (sphaeraphide).

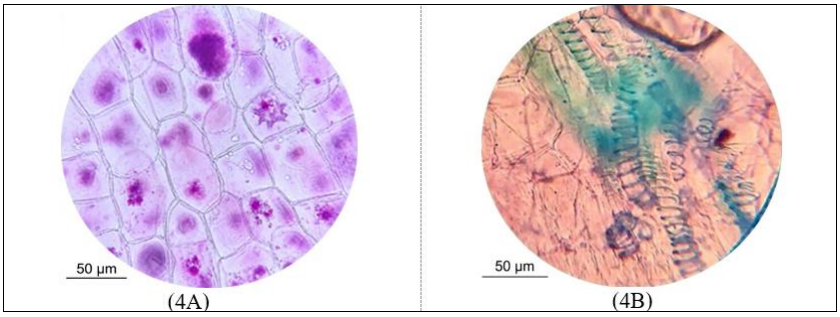

Fig 4: Detailed cellular elements of the Nopal cladode ground tissue

(4A) Magnified water-storage parenchyma cells; (4B) Isolated spiral xylem vessel element showing typical lignified wall thickening.

Drying Kinetics and Thermal Repeatability

The experimental drying data highlighted an inverse relationship between the processing temperature and the time required to achieve moisture equilibrium. However, at the highest investigated temperature (60°C), the thermal energy input exceeded the activation energy required to destabilize the covalent bonds within the native pigments. This caused the conjugated double-bond system of the betalain chromophore to undergo rapid thermal cleavage via decarboxylation and hydrolytic degradation, resulting in a visible loss of pigment intensity [4]. Conversely, operations conducted at the lowest threshold (30°C) led to prolonged drying cycles. This extended moisture retention activated endogenous polyphenol oxidases (PPO), driving the enzymatic oxidation of native phenolic compounds into quinones, which caused undesirable tissue browning.

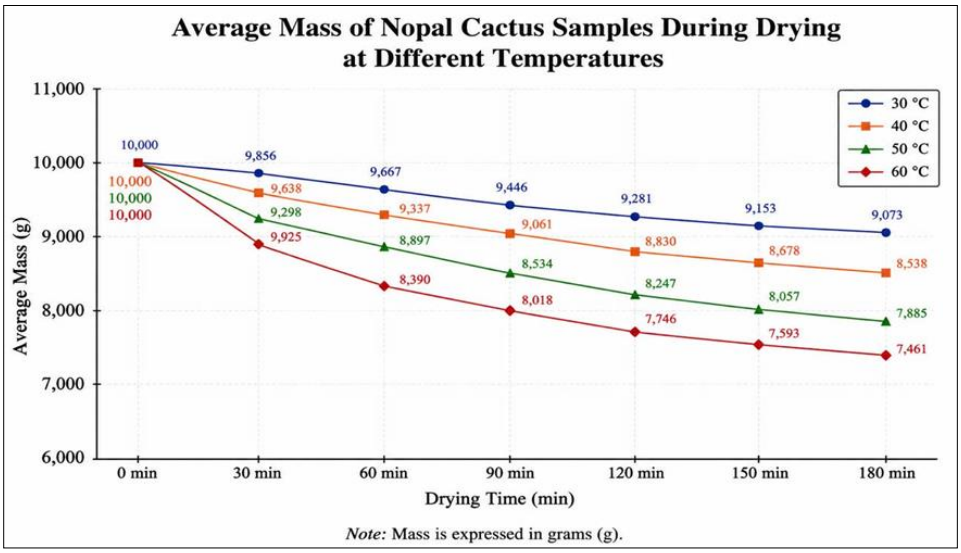

Fig 5: Moisture desorption kinetics representing the mean mass decline of Nopal cactus samples as a function of drying time across different processing temperatures (30°C, 40°C, 50°C, and 60°C)

Based on these physical observations, the moderate temperature band of 40°C - 50°C was identified as the optimal dehydration window. Within this range, water evaporation rates progressed efficiently, the experimental repeatability remained high (indicated by low %RSD values), and the thermal energy stayed below the threshold that triggers the structural degradation of betalains and flavonoids.

Table 1: Mass variation and experimental repeatability (%RSD) of Nopal cactus samples during convection drying across different temperature profiles

Drying time (min)	Drying temperature			
	30 °C	**40 °C**	**50 °C**	**60 °C**
0	10.0032 ± 0.0038 (0.038%)	10.0031 ± 0.0023 (0.023%)	10.0032 ± 0.0013 (0.013%)	10.0026 ± 0.0005 (0.005%)
30	9.8567 ± 0.0143 (0.145%)	9.6179 ± 0.0181 (0.188%)	9.3002 ± 0.0333 (0.358%)	8.9316 ± 0.0286 (0.320%)
60	9.6673 ± 0.0274 (0.283%)	9.3385 ± 0.0292 (0.313%)	8.8986 ± 0.0533 (0.599%)	8.3951 ± 0.0400 (0.476%)
90	9.4484 ± 0.0229 (0.243%)	9.0580 ± 0.0520 (0.574%)	8.5406 ± 0.0348 (0.407%)	8.0193 ± 0.0515 (0.642%)
120	9.2810 ± 0.0338 (0.364%)	8.8327 ± 0.0530 (0.600%)	8.2391 ± 0.0519 (0.630%)	7.7503 ± 0.0602 (0.777%)
180	9.1542 ± 0.0316 (0.345%)	8.6833 ± 0.0389 (0.448%)	8.0501 ± 0.0483 (0.600%)	7.5931 ± 0.0550 (0.724%)
240	9.0732 ± 0.0451 (0.497%)	8.5369 ± 0.0487 (0.570%)	7.8790 ± 0.0504 (0.639%)	7.4494 ± 0.0504 (0.677%)

(**Note:** Experimental values are expressed as Mean ± SD (%RSD). Sample mass unit: grams (g); initial batch size optimized at 10 g, n = 3).

The drying profiles summarized in Table 1 reveal specific thermodynamic patterns:

Moisture Desorption Character

Dehydration at 40°C proceeded via a consistent, predictable linear mass decline. Beginning at 10.0031 ± 0.0023 g (0 min), the mass dropped to 9.3385 ± 0.0292 g at 60 min, reaching a final mass of 8.5369 ± 0.0487 g at the 240 min mark. This indicates that a 240 min (4 hours) drying exposure at 40°C removes approximately 14.66% of the baseline moisture content. Higher temperatures increased the kinetic energy of water molecules, accelerating evaporation rates. This behavior is demonstrated by the descending order of residual sample masses recorded at 240 min: 9.0732 g (at 30°C) > 8.5369 g (at 40°C) > 7.8790 g (at 50°C) > 7.4494 g (at 60°C).

Process Repeatability (%RSD Verification)

The Relative Standard Deviation (%RSD) serves as an indicator of process control and uniformity within the convective drying chamber. Under the optimized 40°C configuration, the %RSD metrics remained within a narrow range, spanning from 0.023% (0 min) to a peak value of only 0.600% (120 min). According to international validation guidelines, an experimental %RSD consistently below 1% provides strong evidence of high technical precision. It confirms that the convective system maintained a uniform temperature and airflow distribution across the sample trays, and validates the homogeneous moisture distribution within the water-storage parenchyma of the Phu Yen Nopal cladodes.

Phytochemical Characterization via Colorimetric and Spectrophotometric Analyses

Upon reaction with concentrated NaOH (pH > 10), the extract rapidly lost its characteristic reddish-purple color and turned pale yellow. This phenomenon confirms the instability of betalain molecules under alkaline conditions and provides biochemical evidence for the abundant presence of this pigment group in the extract [4]. The UV-Vis spectrum further confirmed the presence of betalains through their characteristic absorption bands, particularly the maximum absorption peak at approximately 535 nm corresponding to betacyanins, as well as the characteristic benzene-ring structure associated with flavonoids.

Phytochemical evaluation confirmed the presence of betalains and flavonoids within the plant matrix.

Table 2: Phytochemical screening reactions of the cladode extracts of Phu Yen Nopal cactus

Target Phytochemical Class	Experimental Assay / Reagent	Observable Visual / Spectroscopic Changes	Inferred Density
Betalains	Reaction with strong alkali (NaOH solution, pH > 10)	Rapid loss of the characteristic red-violet coloration, shifting to a pale-yellow shade due to aldimine bond cleavage.	+++
	UV-Vis Spectrophotometric Scanning	Emergence of a prominent, well-defined absorption maximum centered at ~535 nm (characteristic of betacyanins).	+++
Flavonoids	Reaction with dilute alkali (10% NaOH)	Immediate bathochromic shift with an increase in yellow color intensity (deep yellow/orange transition) via phenolate salt formation.	++
	Shinoda Test (Mg turnings + concentrated HCl)	Vigorous gas evolution accompanied by a distinct color change to deep reddish-pink, characteristic of flavone/flavonol core structures.	++

Note: Legend for phytochemical scoring (-): Negative / Absence; (+) Weak positive reaction/ Low concentration; (++): Clearly positive reaction / Moderate concentration; (+++) Strongly positive reaction / High abundance.

The screening results compiled in Table 2 demonstrate an abundance of betalain pigments (+++) characterized by their distinctive color transitions under alkaline conditions and confirmed by the spectrophotometric absorption peak at 535 nm. Concurrently, positive responses to the Shinoda test and dilute alkali treatments verified the presence of moderate concentrations of flavonoids (++). These co-existing phytochemical classes provide the chemical basis responsible for the strong radical scavenging capacity observed in the Phu Yen Nopal extracts.

In Vitro ABTS Radical Scavenging Efficacy

The extracts demonstrated potent, concentration-dependent antioxidant activity against the $ABTS^{\bullet+}$ radical cation. As shown in Table 3, the Fresh Extract 1:2 (M2) displayed strong radical scavenging performance, achieving an IC_{50} value of 48.39 µg/mL. This antioxidant performance is highly comparable to that of the positive control, ascorbic acid (Vitamin C), which exhibited an IC_{50} of 43.43 µg/mL. This provides quantitative evidence of the high antioxidant quality of the fresh plant material harvested in Phu Yen, aligning with international studies on the redox potential of the *Opuntia* genus [2].

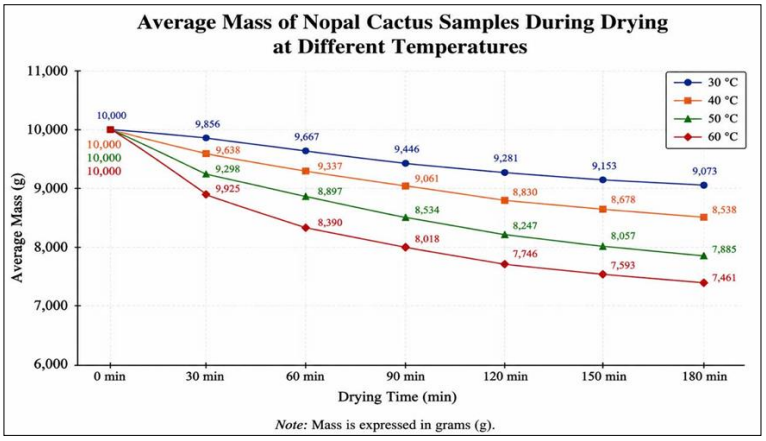

Fig 6: $ABTS^{\bullet+}$ radical scavenging activity of Vitamin C and *Opuntia ficus-indica* stem extracts (M1, M2, and MS) and their corresponding dose–response curves

Table 3: Linear regression parameters and IC - 50 values of Vitamin C and different Nopal cactus (*Opuntia ficus-indica*) stem extracts

Sample Group	Linear Regression Equation	Coefficient of Determination (R^2)	IC_{50} (µg/mL)
Vitamin C (Positive Control)	y = 0.831x + 13.911	0.8876	IC_{50}= 43.43 µg/mL
Fresh Extract (M1)	y = 0.4456x + 20.024	0.9614	IC_{50}= 67.27(µg/mL)
Fresh Extract (M2)	y = 0.3111x + 34.945	0.8169	IC_{50}= 48.39 (µg/mL)
Oven-Dried Extract 40°C (MS)	y = 0.3859x + 25.61	0.9351	IC_{50}= 64.02 (µg/mL)

Note: Values represent the Mean ± SD, n=3. Different lowercase superscript letters (a, b, c, d) within the same column indicate statistically significant differences ($p < 0.05$) determined by one-way ANOVA followed by Tukey's post-hoc test. Statistical analysis was performed using SPSS/R software.

The superior antioxidant activity observed in the fresh extract (M2) can be primarily attributed to the preservation of thermolabile bioactive constituents—including phenolic compounds, flavonoids, betalains, and vitamin C—which act as strong electron donors. Thermal drying processes can promote oxidation, degradation, or structural modification of these compounds, thereby reducing their free-radical scavenging efficiency. This observation is consistent with previous studies reporting that fresh cactus cladodes generally possess higher antioxidant activity than their dried counterparts due to better retention of redox-active phytochemicals.

Increasing the drying temperature (e.g., up to 60°C) caused a decline in $ABTS^{\bullet+}$ scavenging activity due to thermal degradation of the primary active compounds. However, the antioxidant capacity did not break down completely. This retention of activity occurs because secondary thermal degradation products of betalains (such as betalamic acid), along with the structurally more stable flavonoids, continue to act as electron donors, maintaining a baseline level of

radical scavenging activity within the system [4]. On the other hand, the Oven-Dried Extract processed at 40°C (MS) showed only a slight decrease in antioxidant efficacy (IC_{50} = 64.02 µg/mL) compared to M2. Overall, the data confirm that controlling the drying temperature around 40°C is a critical processing parameter for preserving the bioactivity and medicinal value of Nopal cactus extracts.

Biochemical Correlation: Drying Profiles, Structural Stability, and ABTS Performance

Correlating moisture desorption kinetics with chemical integrity confirms that the 40-50°C temperature window serves as an optimal processing standard:

- Processing within the 40-50°C range significantly reduces dehydration time compared to lower temperatures. At a drying temperature of 30°C, moisture removal progressed slowly (retaining 9.0732 g of residual mass after 240 min). This prolonged exposure to internal moisture creates conditions that activate endogenous polyphenol oxidases (PPO). Active PPO catalyzes the conversion of native *o*-diphenols into reactive *o*-quinones, causing enzymatic browning and reducing the availability of free antioxidant compounds.
- Although a higher temperature of 60°C accelerated water removal (achieving the lowest residual mass of 7.4494 g), the thermal energy input exceeded the structural stability threshold of the betalain pigments. This thermal stress induces hydrolytic cleavage and irreversible decarboxylation of the conjugated betalain core, resulting in a loss of pigment intensity.

Consequently, maintaining a drying temperature between 40-50°C balances process efficiency with compound preservation. It ensures reliable moisture desorption kinetics (%RSD < 1%) while protecting key antioxidant compounds (betalains and flavonoids) from thermal degradation, thereby sustaining strong radical scavenging activity as reflected by low, stable IC_{50} values.

Conclusion

This study highlights the remarkable anatomical adaptations of the Nopal cactus (*Opuntia ficus-indica*) cultivated in the harsh climate of Phu Yen Province, Vietnam. Histological analyses revealed a highly developed water-storage parenchyma and abundant calcium oxalate druses, which serve as essential survival mechanisms against environmental stressors. Our findings demonstrated that convective drying at a moderate temperature of 40°C represents an optimal processing parameter. This temperature ensures efficient dehydration with high experimental repeatability (%RSD < 1%) while protecting thermolabile betalains against severe thermal degradation.

Furthermore, the strong $ABTS^{\bullet+}$ radical scavenging capacity (IC_{50} = 48.39 µg/mL) demonstrated by the fresh extract highlights the tremendous potential of this native botanical resource. It holds substantial promise for future integration into functional foods, nutraceuticals, and dermo-cosmetic formulations. Future research should focus on utilizing advanced chromatographic techniques, such as high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS), to precisely profile and quantify individual betalain and flavonoid species within the plant matrix.

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