Opuntia Stricta as a potential source of bioactive compounds: Optimization of polyphenol extraction and evaluation of antioxidant activity

Abdellatif Amnay[1]*, *Taha* Ouriagli[1, 2, 3], *Sidi Mohammed* Raoui[2, 4], *Faouzi* Errachidi[1, 2], *Fouad O.* Chahdi[3], *Bahia* Bennani[1], *Youssef K.* Rodi[3], *Rachida* Chabir[1]

[1]URL CNRST N15, Human Pathology, Biomedicine and Environment Laboratory, Faculty of Medicine, Pharmacy and dentistry, Sidi Mohamed Ben Abdellah University, Fez, Morocco

[2]Laboratory of Functional Ecology and Engineering Environment, Faculty of Sciences and Technologies, Sidi Mohamed Ben Abdellah University, Fez, Morocco

[3]Laboratory of Applied Organic Chemistry, Faculty of Science and Technology, Sidi Mohamed Ben Abdellah University, Fez, Morocco

[4]Higher Institute of Nursing professions and Health Techniques of Fez-Meknes, Morocco

Abstract. *Opuntia Stricta*, a cactus species native to Mexico, has successfully adapted to the arid and semi-arid climates of Morocco. This study aimed to valorize the pulp of this fruit by optimizing the extraction of its bioactive compounds and evaluating its antioxidant potential. The investigation focused on optimizing the solid-liquid extraction process (maceration) by varying two critical parameters: dry matter concentration and extraction time. The results indicated that the optimal conditions were achieved using a 2% dry matter concentration with an extraction duration of 30 minutes, which maximized the overall extraction yield. Furthermore, the FRAP assay confirmed the exceptional bioactivity of this optimal extract, revealing an antioxidant capacity of 3108.2 mg AAE/g DM significantly higher than that of the standard ascorbic acid (1186.5 mg AAE/g DM). These findings demonstrate the high potency of the extracted polyphenols and position *Opuntia Stricta* pulp as a promising natural source of antioxidants for nutraceutical and pharmaceutical applications.

1 Introduction

The genus Opuntia, belonging to the Cactaceae family, occupies a prominent place in arid and semi-arid ecosystems. Among the species of this genus, *Opuntia Stricta* stands out for its remarkable ecological plasticity [1]. Native to Mexico, this plant has adapted to harsh climatic conditions, colonizing various regions of the globe, including the Mediterranean, China, Africa, and particularly Morocco [2]. In Morocco, the prickly pear is not only a food source, but also a strategic crop for combating desertification and promoting socio-economic development in rural areas [3,4].

The fruit of *Opuntia Stricta*, characterized by its intense purple skin and pulp, is attracting growing interest from the scientific and industrial communities. This vibrant coloration is a visual indicator of its phytochemical richness, particularly in nitrogenous pigments called betalains (including betanin, isobetanin, and neobetanin) [5–7]. In addition, this fruit is an important source of diverse phenolic compounds, such as piscidic acid, quercetin-3-O-rhamnosyl-rutinoside, and isorhamnetin glucoxyl-rhamnosyl-pentoside [8–10].

These secondary metabolites offer remarkable biological potential to the fruit. The scientific literature has extensively documented the beneficial properties of these molecules, particularly their antioxidant, anti-inflammatory, and antimicrobial activities [9,10]. Betalains, for example, act as effective free radical scavengers, playing a key role in reducing lipid oxidation and improving cellular redox balance [11–13]. In a global context where the search for natural alternatives to synthetic antioxidants has become a priority for the agri-food and pharmaceutical industries, *Opuntia Stricta* appears to be a promising source of high value-added bioactive molecules.

However, the industrial exploitation of these compounds depends intrinsically on the efficiency of the extraction processes used. The extraction of polyphenols is a critical step, influenced by multiple factors such as the nature of the solvent, the solid/liquid ratio, and the contact time [14,15]. The main objective of this work is to study and optimize the extraction of polyphenolic compounds from *Opuntia Stricta* pulp harvested in the Marrakech-Safi region. More specifically, this study aims to determine the influence of dry matter percentage and extraction time on overall yield, and to evaluate the in vitro antioxidant activity of the extracts obtained using the Ferric Reducing Antioxidant Power (FRAP) assay.

* Corresponding author: abdellatif.amnay@usmba.ac.ma

2 Materials and Methods

2.1 Biological Material and Sample Preparation

Opuntia Stricta fruits were harvested at full maturity in November 2023 from the Marrakech-Safi region of Morocco. After harvest, the fruit pulp was carefully separated from the seeds and peel. The pulp was subsequently lyophilized (freeze-dried) and stored at -40 °C in the dark to prevent the degradation of light-sensitive bioactive compounds. he peel was separated, oven-dried, and ground using an electric grinder until a fine powder was obtained. This preparation ensured optimal preservation of the plant material for subsequent extraction.

2.2 Extraction Protocol and Experimental Design

The extraction of polyphenolic compounds from the pulp was carried out using solid-liquid maceration. The solvent system selected was a mixture of ethanol and distilled water (50:50, v/v), used at room temperature (25 °C). This solvent ratio was chosen for its effectiveness in extracting compounds of medium polarity. To optimize the extraction yield, an experimental design was implemented by varying two independent variables:

Factor 1: Dry Matter Concentration (Solid/Liquid ratio), tested at three levels: 1%, 2%, and 3%.

Factor 2: Extraction Time, tested at three levels: 10, 20, and 30 minutes.

The protocol was adapted from previous work on the extraction of saponins from *Marrubium vulgare* [16], with modifications tailored to optimize the yield of polyphenols from *Opuntia* [17,18]. All experiments were performed in triplicate (n=3). Following maceration, the mixtures were filtered to obtain the crude liquid extracts.

2.3 Antioxidant Activity Evaluation (FRAP Assay)

The Ferric Reducing Antioxidant Power (FRAP) of the extracts was determined according to the protocol described by Amnay *et al.* (2023). This assay evaluates the ability of antioxidants to reduce the ferric-tripyridyltriazine complex (Fe^{3+}) to the ferrous form (Fe^{2+}) [16].

The analytical procedure was as follows: 0.5 ml of the extract was mixed with 1.25 mL of phosphate buffer and 1.25 ml of diluted potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 minutes. The reaction was stopped by adding 1.25 mL of 10% trichloroacetic acid (TCA) after cooling. The solution was centrifuged at 3000 rpm for 10 minutes. Finally, 1.25 ml of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of 0.1% ferric chloride ($FeCl_3$) solution.

Absorbance was measured at 700 nm. Ascorbic acid was used as the reference standard, and results were expressed as milligrams of Ascorbic Acid Equivalent per gram of Dry Matter (mg AAE/g DM).

2.4 Statistical Analysis

All experiments were conducted in triplicate (n=3), and the results are expressed as the mean ± standard deviation (SD). The statistical significance of the differences among mean values was determined using a one-way analysis of variance (ANOVA) performed with IBM SPSS Statistics for Windows, version 26.0 (IBM Corp., Armonk, N.Y., USA). To identify specific significant differences between the treatment groups, Tukey's Honest Significant Difference (HSD) post-hoc test was employed. A probability value of $p < 0.05$ was considered statistically significant. In the figures and tables, means indicated by different letters indicate a significant difference between the treatment groups.

3 Results and Discussion

3.1 Optimization of Extraction Yield

The extraction process was evaluated based on the overall extraction yield, a critical parameter for assessing the economic feasibility of the process. The experimental design and the corresponding yields obtained from the nine trials are summarized in Table 1.

Table 1. Experimental design and global extraction yields of *Opuntia Stricta* pulp.

Trial	Dry Matter (%)	Time (min)	Yield (%) ± SD
1	1	10	3.575 ± 0.10^{e}
2	1	20	3.665 ± 0.50^{de}
3	1	30	3.810 ± 0.04^{d}

4	2	10	4.165 ± 0.06^{c}
5	2	20	4.325 ± 0.07^{b}
6	2	30	4.485 ± 0.06^{a}
7	3	10	2.595 ± 0.05^{h}
8	3	20	2.750 ± 0.07^{gh}
9	3	30	2.850 ± 0.02^{f}

Data were derived from experimental trials. Results are expressed as mean ± SD (n=3). Means in the same column followed by different superscript letters (a–h) are significantly different ($p < 0.05$) according to Tukey's HSD test.
The results indicate a significant variation in extraction yield depending on the extraction conditions, ranging from a minimum of 2.595% (Trial 7) to a maximum of 4.485% (Trial 6). To better visualize the interaction between the extraction time and dry matter concentration, the data are plotted in Figure 1.

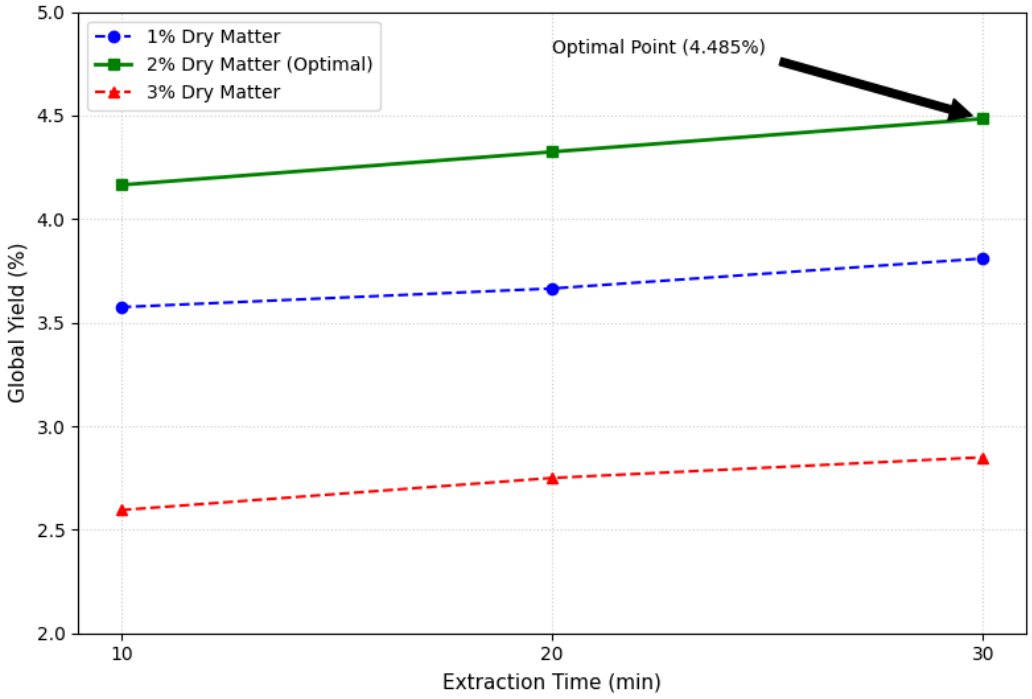

Fig. 1. Effect of extraction time (10, 20, 30 min) and dry matter concentration (1%, 2%, 3%) on the global extraction yield. Note the distinct peak at 2% concentration followed by a sharp decline at 3%.

As illustrated in Figure 1, extraction time had a consistently positive effect on the yield across all concentrations. Increasing the maceration time from 10 to 30 minutes allowed for a more complete diffusion of soluble compounds from the plant matrix into the solvent, as observed with maceration times extending from 10 to 30 minutes [19,20]. However, the concentration of dry matter played a limiting role. While increasing the concentration from 1% to 2% improved the yield significantly, a further increase to 3% resulted in a drastic decline (dropping from ~4.5% to <3%). This phenomenon can be attributed to solvent saturation and increased viscosity, which limit solvent penetration and reduces the concentration gradient driving mass transfer [20,21]. At a 3% concentration, the solvent becomes rapidly saturated, reducing the concentration gradient required for mass transfer (Fick's law), while the higher solid content may hinder the effective penetration of the solvent into the cellular structures. Therefore, Trial 6 (2% dry matter, 30 minutes) was identified as the optimal condition, providing the highest recovery of bioactive material.

Optimal extraction conditions often balance solvent-to-solid ratio, extraction time, and solvent composition to maximize recovery; for example, ultrasound-assisted extraction of *Tetraclinis Articulata* residues achieved optimal polyphenol recovery at a liquid-to-solid ratio of 30 mL/g and an extraction time of approximately 30 minutes [22]. Similarly, deep eutectic solvent-based ultrasound extraction of maca leaves found a liquid-solid ratio of 40 mL/g and 30 minutes to be optimal for polyphenol and saponin recovery [23]. Microwave-assisted extraction studies also highlight the importance of optimizing time and solvent ratio, with shorter times (10–17 minutes) and moderate sample-to-solvent ratios yielding high antioxidant activity [24].

3.2 Antioxidant Activity (FRAP Assay)

The antioxidant capacity of all nine *Opuntia Stricta* extracts was evaluated using the FRAP assay and compared with Ascorbic Acid (Vitamin C). The results, expressed in milligrams Ascorbic Acid Equivalent per gram of Dry Matter (mg AAE/g DM), are presented in Figure 2.

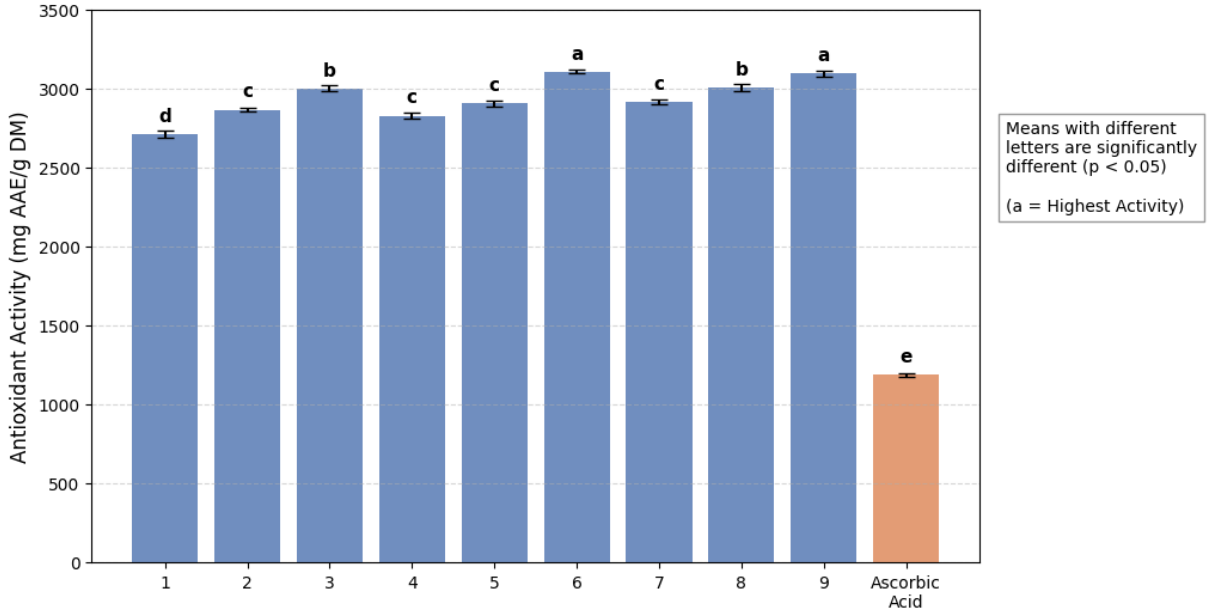

Figure 2: Comparative antioxidant power (FRAP) of the nine *Opuntia Stricta* extracts versus Ascorbic Acid. *Data are expressed as mean ± SD (n=3). Bars marked with different letters (a, b, c, d, e) indicate statistically significant differences at p < 0.05. Trial 6 and Trial 9 share the highest activity group (a).*

Table 2 summarizes the quantitative antioxidant activity (FRAP) results for all nine experimental trials compared with the ascorbic acid standard, including the statistical significance groupings obtained from the analysis.

Table 2. Antioxidant Activity (FRAP) of *Opuntia stricta* Extracts

Trial	Dry Matter (%)	Time (min)	FRAP Value (mg AAE/g DM) ± SD	Significance
1	1	10	2709.9 ± 22.04	d
2	1	20	2866.8 ± 12.54	c
3	1	30	3001.9 ± 18.37	b
4	2	10	2830.1 ± 15.53	c
5	2	20	2908.6 ± 19.02	c
6	2	30	3108.2 ± 10.32	a
7	3	10	2917.1 ± 11.98	c
8	3	20	3008.7 ± 20.32	b
9	3	30	3098.3 ± 18.41	a
Ascorbic Acid	*Ref*	*Ref*	1186.5 ± 14.08	e

Values are expressed as mean ± SD (n=3). Means followed by different letters (a–e) are significantly different ($p < 0.05$) according to Tukey's HSD test. 'a' indicates the highest antioxidant activity.
The analysis revealed significant variability among the trials ($p < 0.05$), with values ranging from 2709.9 to 3108.2 mg AAE/g DM for the extracts. Notably, all *Opuntia* extracts demonstrated significantly higher reducing power compared with the standard Ascorbic Acid (1186.5 mg AAE/g DM

The statistical analysis highlights a compelling nuance between mass yield and biological potency. While Trial 6 (2% DM, 30 min) and Trial 9 (3% DM, 30 min) share the highest statistical grouping (a) for antioxidant activity, showing no significant difference in their cellular reducing power, they differ markedly in extraction efficiency. Trial 6 achieved this high potency (3108.2 ± 10.32 mg AAE/g DM) alongside the maximum global yield. In contrast, Trial 9 exhibited comparable potency but a significantly lower mass yield due to solvent saturation at higher solid concentrations.

This observation suggests that the saturation effect primarily impedes the extraction of bulk structural components (such as sugars or mucilage) rather than the high-potency phenolic compounds. Solvent saturation and increased viscosity reduce mass transfer efficiency, which more strongly affects larger, more abundant molecules that require greater solvent penetration [25,26]. Polyphenols, being smaller and often more soluble, tend to be less impacted by saturation effects [27,28]. Kinetic studies show that polyphenol extraction reaches a saturation concentration but can still proceed efficiently under controlled conditions without significant loss due to saturation [26,28]. Additionally, solvent composition, such as ethanol-water mixtures, plays a crucial role in selectively extracting polyphenols while minimizing co-extraction of bulk components that contribute to saturation [27,29]. Therefore, managing solvent concentration and extraction parameters can mitigate saturation effects on polyphenol yield while bulk structural components are more prone to extraction limitations caused by saturation [27,30,31]. However, from an industrial standpoint, Trial 6 remains the superior candidate. It maximizes the total recovery of the bioactive fraction per unit of solvent, achieving the highest extraction yield without compromising bioactivity.

A major finding of this study is the exceptional performance of the crude extracts compared with pure Ascorbic Acid. The optimal extracts demonstrated a reducing power approximately 2.6 times higher than the vitamin C standard ($p < 0.001$). This result is significant because Ascorbic Acid is a pure, single-molecule antioxidant.

The superiority of the *Opuntia Stricta* extract can be attributed to the synergistic interaction between its diverse phytochemical constituents. The pulp is rich in both betalains (providing the violet pigment) and phenolic compounds. These classes of molecules likely function through complementary mechanisms, such as free radical scavenging and metal ion chelation, creating a cocktail effect that surpasses the reducing capacity of any single antioxidant acting alone. Research shows that combinations of phytochemicals in whole foods often produce enhanced antioxidant effects beyond those of individual compounds due to such synergistic interactions, which can improve bioactivity and bioavailability [32,33]. Studies demonstrate that mixtures of plant extracts or pure phytochemicals can synergistically inhibit oxidative stress and cell proliferation more effectively than single compounds, often by modulating cellular antioxidant pathways and gene expression [34,35]. For example, combinations of phenolics and flavonoids from different plants have shown synergistic antioxidant and antimicrobial effects, highlighting the importance of phytochemical diversity in enhancing efficacy [36,37]. These findings underscore that the superior reducing power of complex plant extracts arises from the interactive and complementary actions of multiple phytochemicals rather than the effect of any single antioxidant molecule alone [38–40].

The progression of activity from Trial 1 to Trial 6 emphasizes the critical role of extraction time in recovering electron-donating polyphenols. Trials with shorter durations (10 minutes) consistently yielded lower antioxidant values (Group d), indicating incomplete mass transfer. Extending the contact time to 30 minutes provided sufficient duration for the solvent to penetrate the plant matrix and for the phenolic compounds to diffuse into the hydro-alcoholic medium. This confirms that a 30-minute maceration is an essential parameter for maximizing the recovery of the specific molecules responsible for the high FRAP values observed.

The high antioxidant profile may also be attributed to the specific pedoclimatic conditions of the Marrakech-Safi region. the semi-arid climate and intense solar exposure, impose abiotic stress that stimulates the plant's biosynthesis of defense compounds like phenolics and betalains. Abiotic stresses such as drought, high temperature, and strong light increase reactive oxygen species (ROS) production in plants, triggering enhanced antioxidant defenses including enzymatic and non-enzymatic systems to maintain redox balance [41–43]. Some studies show that plants growing under harsher environmental conditions often accumulate higher levels of phenolic compounds and other antioxidants as protective responses to oxidative stress [44–46]. Additionally, Elevated solar radiation and temperature can induce the synthesis of antioxidants that scavenge ROS and protect cellular components, which aligns with findings that stress conditions promote antioxidant compound accumulation [47,48]. The specific pedoclimatic conditions of semi-arid regions like Marrakech-Safi thus likely enhance the nutraceutical potential of *Opuntia Stricta* compared to varieties from milder Mediterranean climates, supporting its value as an underutilized bioresource [49,50]. Consequently, our optimized yield and activity levels are competitive with, and in some cases superior to, those reported for *Opuntia ficus-indica* in the Mediterranean basin. This suggests that *Opuntia Stricta*, often considered an invasive species, represents an undervalued bioresource with comparable nutraceutical potential.

4 Conclusion

This study successfully demonstrated the high nutraceutical potential of *Opuntia Stricta* pulp harvested in the semi-arid region of Marrakech-Safi. Through a targeted optimization process, we established that the extraction parameters are decisive for maximizing both yield and bioactivity.

The results identified the optimal conditions as a 2% dry matter concentration combined with a 30-minute maceration time using a binary hydro-alcoholic solvent. These conditions achieved a maximum extraction yield of 4.485%, overcoming the mass transfer limitations (saturation effects) observed at higher solid concentrations. This finding

demonstrates that a relatively short extraction duration is sufficient for efficient recovery, a critical factor for industrial economic viability.

Most significantly, the biological evaluation revealed that the crude extract possesses exceptional antioxidant capacity. With a reducing power (FRAP) of 3108.2 mg AAE/g DM, the optimal extract significantly outperformed the standard ascorbic acid by nearly threefold. This superior potency is attributed to the synergistic interaction between betalains and phenolic compounds, facilitated by the intermediate polarity of the 50% ethanol solvent, and potentially enhanced by the abiotic stress characteristic of the Marrakech pedoclimatic environment.

In conclusion, this work positions *Opuntia Stricta*, often regarded as an invasive species, as a promising and sustainable bioresource. It offers a competitive natural alternative to synthetic antioxidants for the nutraceutical and pharmaceutical industries. Future investigations will focus on the chromatographic characterization (HPLC-MS) of the specific molecules responsible for this activity and the assessment of their stability and *in vivo* bioavailability.

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