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Physicochemical properties and bio-functional attributes of *Opuntia ficus-indica* (Prickly Pear) fruit juice

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Abstract
Opuntia ficus-indica fruit is recognized for its nutritional and functional significance due to its rich bioactive profile. This study aimed to evaluate the physicochemical characteristics and bio-functional properties of *O. ficus-indica* fruit juice, with emphasis on antioxidant potential. Fresh fruits were processed to obtain juice, which was analysed for proximate composition, pH, titratable acidity, and viscosity using standard methods. Bio-functional attributes were assessed by determining total phenolic content (TPC), total flavonoid content (TFC), betalain concentration, and antioxidant activity using DPPH and ABTS assays.
The fruit juice exhibited high moisture content (90.6±0.21%), low fat (0.06±0.008%), moderate carbohydrate levels (7.9±0.25%), and high viscosity (5067±230.9 cP). Strong antioxidant activity was observed, with DPPH and ABTS radical scavenging capacities of 658.8±25.10 and 587.3±17.41 mM Trolox equivalents per 100 g, respectively. The juice contained appreciable levels of phenolics (99.8±1.76 mg GAE/100 g), flavonoids (39.6±1.07 mg RE/100 g), and betalains. Overall, *O. ficus-indica* fruit juice represents a promising natural source of antioxidants with potential application in functional food formulations.

Keywords: *Opuntia ficus-indica*, prickly pear, antioxidant activity, phenolic compounds, betalains, bio functional properties

Introduction
Opuntia ficus-indica (L.) Mill, commonly known as prickly pear cactus, is a xerophytic plant widely distributed in Mediterranean regions, the southwestern United States, northern Mexico, and several arid and semi-arid parts of the world. Owing to its remarkable adaptability to harsh climatic conditions and minimal agronomic requirements, *O. ficus-indica* has gained increasing attention as a sustainable crop with nutritional, functional, and economic significance. The fruit is consumed fresh and processed into juices, jams, and other value-added products, while different parts of the plant, including cladodes, fruits, peels, seeds, and flowers, have been extensively investigated for their bioactive composition and health-promoting properties.
Prickly pear fruits are characterized by wide variation in colour, flavour, and biochemical composition depending on species, cultivar, and maturity stage. They are a rich source of bioactive compounds such as ascorbic acid, phenolic compounds, flavonoids, carotenoids, and betalains, which contribute to their strong antioxidant capacity and functional attributes (Fernández-López *et al.*, 2010) [12]. The presence of these phytochemicals has been associated with a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory effects (Moore *et al.*, 2016) [22].
Among the various bio-functional properties, the antioxidant potential of *O. ficus-indica* has been extensively documented. The fruit pulp and cladodes contain significant levels of betalains, particularly betacyanins and betaxanthins, which are recognized as potent free radical scavengers (Boutakiout *et al.*, 2017) [4]. Regular consumption of prickly pear fruit has been reported to enhance systemic antioxidant status and improve metabolic parameters, including serum insulin levels (Tesoriere *et al.*, 2004) [30].

Comparative studies have also demonstrated variability in antioxidant activity among different *Opuntia* species, attributed to differences in phytochemical composition (Marhri *et al.*, 2024) [20]. In addition to antioxidant activity, *O. ficus-indica* exhibits notable antimicrobial properties. Extracts derived from the fruit, juice, and cladodes have shown inhibitory effects against a range of pathogenic microorganisms, including *Salmonella enterica* (Iftikhar *et al.*, 2023) [18]. These antimicrobial effects have been largely attributed to the synergistic action of polyphenols, betalains, and organic acids present in the fruit pulp (El-Mostafa *et al.*, 2014; Addai *et al.*, 2022) [10, 1].

The anti-inflammatory and chemopreventive properties of prickly pear have also been reported in several in vivo studies. Phytosterols and betalain pigments, particularly indicaxanthin, have been shown to modulate inflammatory pathways and exhibit antiproliferative effects against various cancer cell lines (Reddy *et al.*, 2005; Naselli *et al.*, 2014) [27, 25]. Furthermore, regular consumption of *O. ficus-indica* has been associated with improved lipid metabolism, including reductions in plasma cholesterol and low-density lipoproteins, without adversely affecting high-density lipoprotein levels (Galati *et al.*, 2003) [14].

Neuroprotective and anticlastogenic effects of *O. ficus-indica* have also been documented. The flavonoid-rich fruit pulp and seeds have demonstrated protective effects against oxidative neuronal damage, while betalain pigments from coloured fruits have shown the ability to reduce chromosomal damage induced by toxic agents, indicating potential chemoprotective properties (El-Hawary *et al.*, 2020) [9]. Traditionally, *O. ficus-indica* has been used in folk medicine, particularly in Mexico and Mediterranean countries, for the management of metabolic disorders. The hypoglycaemic and antidiabetic effects of cactus stems and fruit extracts have been validated through experimental studies, which demonstrated improved glycaemic control and enhanced insulin activity in animal models and human subjects (Cicero *et al.*, 2004) [5].

Despite extensive evidence supporting the functional properties of *O. ficus-indica*, variations in physicochemical composition and bioactive content due to cultivar, geographical origin, processing, and extraction methods necessitate further investigation. Therefore, the present study aimed to evaluate the physicochemical characteristics, antioxidant potential, and key bio-functional attributes of *O. ficus-indica* fruit juice, with the objective of assessing its suitability as a natural functional ingredient for value-added food applications.

Materials and Methods

Materials

Fresh, mature, and fully ripened *Opuntia ficus-indica* fruits were procured from the local market of Rajkot, Gujarat, India. Fruits of uniform medium size and free from physical damage, microbial spoilage, or over-ripening were selected for the study. All chemicals and reagents used for analysis were of analytical grade. Folin-Ciocalteu reagent, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS [2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)], Trolox, gallic acid, rutin, and other standards were obtained from standard commercial suppliers.

Preparation of *O. ficus-indica* fruit juice

The fruits were initially sorted based on external appearance, and damaged or diseased fruits were discarded. Surface thorns were removed by brief exposure to direct flame. The fruits were then washed with a 100 ppm chlorine solution at room temperature, followed by rinsing with potable water. After washing, the fruits were manually peeled using a stainless-steel knife and cut into small pieces. The cut fruit pieces were passed through a wire mesh to separate seeds and mucilage from the juice. The retained mixture of seeds and mucilage was further pressed through a muslin cloth to recover residual juice. The extracted juice was blanched at 90 °C for 1-2 min and immediately cooled to room temperature. The juice was then stored at -20 °C until further analysis. The process of prickly pear fruit juice preparation is shown in Figure 1.

Physicochemical Analysis

The physicochemical properties of *O. ficus-indica* fruit juice were determined using standard AOAC (2019) [2] methods. Moisture content, total solids, crude fat, protein, and total ash were analysed. Total carbohydrate content was calculated by difference. Titratable acidity was determined and expressed as percent citric acid, while pH was measured using a digital pH meter. Viscosity of the fruit juice was measured at 25 °C using a Brookfield viscometer (spindle no. 63) at a rotational speed of 0.3 rpm. All the analysis was done in triplicates.

Preparation of Fruit Juice Extract

The aqueous extract of *O. ficus-indica* fruit juice was prepared according to the method described by Boutakiout *et al.* (2017) [4], with minor modifications. The juice was centrifuged at 16,000 rpm for 30 min at 4 °C. The supernatant was filtered through Whatman No 4 filter paper. An aliquot (2.5 mL) of the filtrate was diluted to 25 mL with distilled water and used for further bio-functional analyses.

Determination of Total Phenolic Content (TPC)

Total phenolic content of the fruit juice extract was determined using the Folin-Ciocalteu method as described by Lugo-Zárate *et al.* (2021). Gallic acid was used as the standard, and results were expressed as mg gallic acid equivalents (GAE) per 100 g of fruit juice.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was measured spectro-photometrically following the method reported by Iftikhar *et al.* (2023) [18], with minor modifications. Rutin was used as the reference standard, and results were expressed as mg rutin equivalents (RE) per 100 g of fruit juice.

Antioxidant Activity Assays

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the fruit juice extract was evaluated according to the method described by El-Said *et al.* (2014) [11]. The antioxidant capacity was expressed as mM Trolox equivalents (TE) per 100 g of fruit juice.

ABTS Radical Scavenging Activity

ABTS radical scavenging activity was determined using the method outlined by El-Said *et al.* (2014) [11]. The results were expressed as mM Trolox equivalents (TE) per 100 g of fruit juice.

Determination of Betalain Content

The betalain content of *O. ficus-indica* fruit juice was determined according to the method described by Lugo-Zárate *et al.* (2021). Betalains were quantified as betacyanins and betaxanthins and expressed as mg Betanin Equivalents (BE) and mg indicaxanthin equivalents (IE) per 100 mL of fruit juice, respectively.

Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean±standard deviation (SD). Data were analysed using appropriate statistical tools to determine consistency and reproducibility of results.

Results and Discussion

O. ficus-indica fruit is recognized as a valuable source of natural bioactive compounds, making it an excellent choice for the development of functional dairy products. They are rich in various nutrients, including sugars, minerals, dietary fiber, and bioactive compounds such as ascorbic acid, betalains, flavonoids, and phenolic compounds (Silva *et al.*, 2021) [29]. Extensive research has been conducted to evaluate the antioxidant properties of *O. ficus-indica* fruit, as documented by studies conducted by Hernández *et al.* (2020) [17], Verón *et al.* (2023) [32], Belkhir *et al.*, (2025) [3], and Touati *et al.* (2025) [31].

Physicochemical Characteristics of *Opuntia ficus-indica* Fruit Juice

The physicochemical properties of *O. ficus-indica* fruit juice are presented in Table 1. The moisture content of the juice was 90.6±0.21%, which is consistent with earlier reports for red varieties of *O. ficus-indica* (Silva *et al.*, 2021; Belkhir *et al.*, 2025) [3, 29]. The high moisture level is a characteristic feature of cactus fruits and contributes to the refreshing nature of the juice, although it may influence storage stability.

The fat content was negligible (0.06±0.008%), confirming that prickly pear juice is a low-lipid food, as also reported by Moraga-Babiano *et al.* (2025) [23]. Protein content was measured at 0.85±0.02%, which falls within the range reported by Duque-Buitrago *et al.* (2024) [7]. The total carbohydrate content (7.9±0.25%) reflects the presence of soluble sugars responsible for the characteristic sweetness of the fruit. Comparable carbohydrate levels have been reported in earlier studies on prickly pear juice and pulp.

The ash content of the juice was 0.62±0.02%, indicating the presence of mineral constituents, in agreement with findings reported by Duque-Buitrago *et al.* (2024) [7]. The pH of the juice was mildly acidic (4.9±0.02), comparable to values reported by Patil *et al.* (2019) [26]. However, higher pH values have also been reported in other studies (Ferreira *et al.*, 2023; Márquez-Montes *et al.*, 2022) [13, 21], which may be attributed to differences in fruit maturity, cultivar, and processing methods.

The titratable acidity of the juice was 0.12±0.02% (citric acid), closely matching the value reported by Belkhir *et al.* (2025) [3]. Variations in acidity reported across studies (Ferreira *et al.*, 2023; Márquez-Montes *et al.*, 2022) [13, 21] may be associated with differences in ripening stage, sugar-acid balance, botanical characteristics, and sample preparation techniques. The viscosity of *O. ficus-indica* fruit juice was relatively high (5067±230.9 cP at 25 °C), which can be attributed to the presence of mucilage, pectin, and other polysaccharides naturally occurring in cactus fruits. Considerable variability in viscosity values has been reported in the literature (Díaz-Lima & Vélez-Ruiz, 2017; Salehi *et al.*, 2019) [6, 28], largely due to differences in spindle geometry, shear rate, temperature, juice concentration, and extraction method.

Bio-functional Attributes of *Opuntia ficus-indica* Fruit Juice

The bio-functional properties of *O. ficus-indica* fruit juice were evaluated through antioxidant activity, total phenolic and flavonoid contents, and betalain concentration (Table 2). The presence of these bioactive compounds has been widely associated with the antioxidant, antimicrobial, antidiabetic, and health-promoting properties of prickly pear fruit (Zenteno-Ramírez *et al.*, 2018; Duque-Buitrago *et al.*, 2024) [34, 7].

Antioxidant Activity

The antioxidant potential of *O. ficus-indica* fruit juice, evaluated using DPPH and ABTS radical scavenging assays, demonstrated strong antioxidant capacity. The DPPH radical scavenging activity was 658.8±25.10 mM TE/100 g, which is considerably higher than values reported in methanolic extracts (Muñoz-Tebar *et al.*, 2025) [24]. Such differences may be attributed to variations in extraction solvent, fruit variety, maturity, and analytical conditions. Similar variability in DPPH activity has been reported by Gonzalez *et al.* (2021) [15].

The ABTS radical scavenging activity of the juice was 587.3±17.41 mM TE/100 g, confirming its strong antioxidant efficacy. Previous studies have reported a wide range of ABTS values for *O. ficus-indica* fruit, depending on extraction method and expression of results (Zenteno-Ramírez *et al.*, 2018; Márquez-Montes *et al.*, 2022; Duque-Buitrago *et al.*, 2024; Yvonne *et al.*, 2025) [34, 21, 7, 33]. The high antioxidant activity observed in the present study may be attributed to the combined effect of phenolics, flavonoids, betalains, and ascorbic acid present in the juice.

Total Phenolic and Flavonoid Content

The total phenolic content (TPC) and total flavonoid content (TFC) of *O. ficus-indica* fruit juice were 99.8±1.76 mg GAE/100 g and 39.6±1.07 mg RE/100 g, respectively. These values are comparable with those reported by Duque-Buitrago *et al.* (2024) [7] for prickly pear juice. Higher values reported in methanolic extracts Márquez-Montes *et al.*, (2022) [21] further highlight the influence of solvent polarity and extraction efficiency on phenolic recovery.

Phenolic compounds such as gallic acid, catechin, rutin, and ferulic acid derivatives have been identified as major contributors to the antioxidant potential of *O. ficus-indica* fruit (Zenteno-Ramírez *et al.*, 2018) [34]. Variations in TPC and TFC across studies may be attributed to differences in cultivar, geographical origin, agronomic practices, and analytical methodologies.

Betalain Content

Betalains are water-soluble pigments responsible for the characteristic red and yellow colour of *O. ficus-indica* fruit and are known for their antioxidant and health-promoting properties. In the present study, the betacyanin and betaxanthin contents were 29.1±0.44 mg BE/100 mL and 33.3±0.36 mg IE/100 mL, respectively. Comparable

variability in betalain concentration has been reported in previous studies (Gouws *et al.*, 2019; Muñoz-Tebar *et al.*, 2025; Yvonne *et al.*, 2025) [16, 24, 33], which may be attributed to differences in fruit colour, maturity, extraction method, and processing conditions. Despite the reported variability, the betalain levels observed in this study confirm their significant contribution to the antioxidant potential of *O. ficus-indica* fruit juice. Overall, the results demonstrate that *O. ficus-indica* fruit juice is a rich source of bioactive compounds with strong antioxidant activity, supporting its potential application in functional and value-added food products.

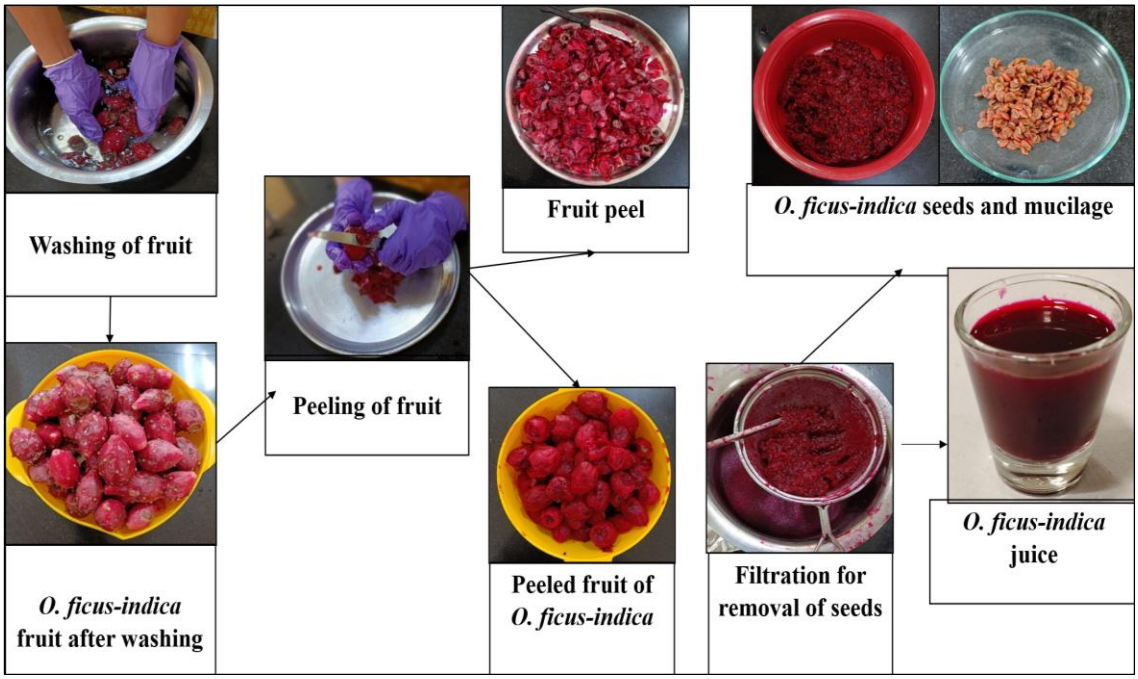

Fig 1: Process of preparation of *O. ficus-indica* fruit juice

Table 1: Proximate composition of *O. ficus-indica* fruit juice

Parameter	Values
Moisture (g/100g)	90.6±0.21
Fat (g/100g)	0.06±0.008
Total solids (g/100g)	9.4±0.22
Total carbohydrate (g/100g)	7.9±0.25
Total Protein (g/100g)	0.85±0.02
Total Ash (g/100g)	0.62±0.02
Acidity (% citric acid)	0.12±0.02
pH	4.9±0.02
Viscosity (25°C), cP	5067±230.9

Data are expressed as mean±SD (N=3).

Table 2: Bio-functional attributes of *O. ficus-indica* fruit juice

Anti-oxidant activity	*O. ficus-indica* fruit juice
DPPH radical scavenging activity (mM TE/100g)	658.8±25.10
ABTS radical scavenging activity (mM TE/100g)	587.3±17.41
Total Phenolics & Flavonoid content	
Total phenolics (mg GAE/100g)	99.8±1.76
Total flavonoids (mg RE/100g)	39.6±1.07
Betalains content	
Betacyanin (mg BE/100ml)	29.1±0.44
Betaxanthins (mg IE/100ml)	33.3±0.36

Data are expressed as mean±SD (N=3).

Conclusion

This study demonstrates that *Opuntia ficus-indica* fruit juice possesses favourable physicochemical characteristics and a high concentration of bioactive compounds, supporting its potential application as a functional food ingredient. The juice exhibited high moisture content, low fat levels, moderate carbohydrate concentration, and elevated viscosity, likely due to the presence of mucilage and polysaccharides. Variations in compositional parameters relative to previous studies may be attributed to differences in cultivar, maturity stage, geographical origin, and processing conditions.

The bio-functional evaluation revealed strong antioxidant activity, as evidenced by high DPPH and ABTS radical scavenging capacities. The substantial levels of total phenolics, flavonoids, and betalains further confirm the

antioxidant efficacy of *O. ficus-indica* fruit juice. Differences observed in antioxidant capacity and phytochemical content across studies emphasize the influence of extraction methods, analytical approaches, and fruit variability on the recovery of bioactive compounds.

Overall, the results highlight *O. ficus-indica* fruit juice as a promising natural source of antioxidants with significant functional properties. Its rich phytochemical profile supports its incorporation into value-added food formulations, including functional and nutraceutical products, with potential benefits for enhancing dietary antioxidant intake.

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