



## The Effect of Chemical Composition of Wild *Opuntia Ficus Indica* Byproducts on its Nutritional Quality, Antioxidant and Antifungal Efficacy



Adel G. Abdel-Razek<sup>1</sup>, Mohamed G. Shehata<sup>2</sup>, Ahmed N. Badr<sup>\*3</sup>, Karolina Gromadzka<sup>4</sup>, Lukasz Stepień<sup>5</sup>

<sup>1</sup>Fats and Oils Dept., National Research Centre, Dokki 12622, Cairo, Egypt.

<sup>2</sup>Food Technology Dept., Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application, Alexandria, Egypt.

<sup>3</sup>Food Toxicology and Contaminants Dept., National Research Centre, Dokki 12622, Cairo, Egypt.

<sup>4</sup>Chemistry Dept., Poznan University of Life Science, ul. Wojska Polskiego 75, 60-625 Poznań, Poland.

<sup>5</sup>Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska, 60-479, Poznań, Poland.

**W**ILD *Opuntia ficus-indica* is a plant commonly grown in the desert. This study aimed to evaluate the chemical composition; fatty acids, macro and micro-elements, vitamin C, total phenolic and flavonoid contents of *Opuntia Ficus indica* byproducts (cladodes and fruit peels) on its nutritional quality, as well as, their antimicrobial, and antioxidant efficacy. Dietary fiber was  $30.68 \pm 5.28$  in peels and  $25.65 \pm 3.07$  in cladodes. The total phenolic content for each byproduct was estimated as  $520 \pm 2.64$  and  $788 \pm 1.35$  mg GAE/g for fruit peels and cladodes, respectively. Whereas flavonoids were estimated as  $65.7 \pm 3.41$  and  $25.36 \pm 1.55$  mg Cat., /g for fruit peels and cladodes respectively. Results reflected considerable content of microelements and omega fatty acids. Eicosadionic was the major fatty acid component and it recorded 32.1 and 38.4% for fruit peel and cladodes, respectively. Byproducts extract demonstrated an efficiency against bacteria and toxigenic fungi. Inhibition impact of byproducts was very clear on *Fusarium* fungi using diffusion assay on agar media. The unique chemical composition and distinctive minor components of cladodes and peels reflected distinguishing bioactivity against harmful food-microorganisms. This could benefit for food safety particularly against toxigenic fungi. It could be concluded that *Opuntia Ficus indica* byproducts are promising for the application in the food industry.

**Keywords:** *Opuntia ficus indica* byproducts, Chemical composition, Nutritional components, Antifungal activity, Antioxidant activity.

### Introduction

*Opuntia ficus-indica* (OPF) is a wild plant, grows in sub-Saharan and North Africa. The OPF plant originated from the American area was inserted into the Mediterranean countries [1]. The wild-OPF reported an ability to grow widely, particularly in arid and semi-arid areas [2]. In North Africa, the cultivation of OPF is practiced to prevent soil corrosion in arid lands and as a forage replacement during drought periods. The

OPF, also called prickly pear, was principally utilized for its fruits which consists of a thick peel and an edible juicy pulp with many hard seeds [3].

The OPF has two types of byproducts cladodes and peels which frequently not used for nutrition. These OFP byproducts (OFPBs) need advanced research as it could contain multiple active molecules. The previous studies recorded the existence of various active molecules and minor components in prickly pear fruits. These

\*Corresponding author e-mail: [noohbadr@gmail.com](mailto:noohbadr@gmail.com); Tel: +2 01000327640;

ORCID: <https://orcid.org/0000-0002-5588-6545> Scopus ID: 57194519147

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fruits reported affluence toward vitamins [4], Tocopherols [5], poly and oligosaccharides [6], enzymes such as peroxidase [7]. Few studies were addressed the bioactive components of prickly pear byproducts, its applications, and benefits [8-10]. These could contain several minor components with significant function, particularly in the living cell system. Peels could contain pigments (purple, orange, and green), but cladodes mainly contain mucilage with high polysaccharide amount [6].

In the previous study by El-Mostafa *et al.* [10] referred to the utilization of the OFB for health, nutritional, and cosmetic applications. It employed in food manufacturing including juices, tea, jams, and bakeries. Further, it utilized for herbal therapy of various public health issues and consumed as food whether fresh or dry. Also, the study pointed to the OFB significant contents of pectin, fatty acids, minerals, poly & oligosaccharides, dietary fibers, and antioxidants.

The byproducts of food process became very significant, it is still extended and developed. By the development of analytical capability, the researcher turns into more knowledgeable of the biochemical structure and bioactive compounds functionality in foods, food byproducts, and their effects on the human systems [11]. This led to the rise in popularity of various health-promoting food byproducts. Through the progress in medical and nutrition sciences, bioactive molecules showed a very important for health maintenance by lowering its incidence and reduce the common and chronic disease hazard [12-16]. Amongst all bioactive components includes dietary fibers (DF) have acquired massive importance due to its availability in various food byproducts, it is easy to use in prevent and treat common diseases including colon cancer [17].

The existence of bioactive components in food materials represents the main line of defense against contaminates and food-harmful. Antioxidant potency achieves the suppression of several compounds which could be causing oxidative stress in live-systems [13]. As mentioned by Sharma *et al.* [15] several bioactive materials from agricultural byproducts show nutritional and health benefits. Earlier studies reported the rich of food byproduct in minerals, phenolic components, flavonoid compounds, and vitamins [12, 18, 19]. Previous studies were dealings partially with these byproducts. Otherwise, several food byproducts reported as a source of DF which has a great role in gastrointestinal tract health [14].

Antioxidant components (included polyphenols, flavonoids...., etc.) that are presenting in food byproduct may play an important function either as pharmaceutical, nutritional, or safety factor by its existence in food materials [20-23].

However, numerous studies were addressed the chemical composition and the proximate analysis of OFP fruit varieties [1, 4, 5], few literatures investigated the OFPBs suggested functions side to the possible applications in food and nutrition [8-10]. But rarely antifungal and antimicrobial impact of OFP components was handled intensively. Moreover, no extensive study was recorded for the effect of cladodes and peels on toxigenic fungi which considered the hazard sources for food contamination. Hence that, this study aimed to evaluate the effect of chemical composition, fatty acids composition, macro and microelements, vitamin C, total phenolic and flavonoid content of *Opuntia ficus indica* byproducts (cladodes and fruit peels) on its nutritional quality, as well as, their antimicrobial, and antioxidant efficacy. The bioactivity of the OFPBs was represented here through the antimicrobial and antifungal evaluation.

## **Materials and Methods**

### *Materials*

#### *Plant materials collection*

Full colored red-purple wild OPF were collected from a region of arid land around Alexandria Governorate, Egypt. The fruit peels and cladodes were stored cool at 4°C in polyethylene bags. The OPFBs were air-oven dried for two days/ 40°C. The dried samples were grounded separately into a fine powder.

#### *Microorganisms*

Four strains of pathogenic bacteria were purchase from *Pseudomonas aeruginosa* ATCC 9027; *Escherichia coli* ATCC11229; *Staphylococcus aureus* NCTC 10788; *Salmonella typhi* ATCC 14028 were purchased from Egypt Microbial Culture Collection (EMCC), Ain Shams, Egypt. These strains were reactivated and inoculated for antimicrobial activity utilizing Tryptic-Soy broth, incubated at 37°C as the method described by Abdel- Fattah *et al.* [20]. The strains of toxigenic fungi used in this experiment were "*Fusarium graminearum* KF841; *Aspergillus niger* ITEM 3856; *Penicillium chrysogenum* ATCC 10106; *Fusarium culmorum* KF191; *Fusarium culmorum* KF846; *Fusarium oxysporum* ITEM 12591; *Fusarium verticillioides* FM19; *Fusarium*

*culmorum* P846. It was isolated and identified in the plant pathology laboratory, Institute of Plant Genetics, Polish Academy of Science, Poznan, Poland. These strains reported having an ability for producing zearalenone and deoxynivalenol toxin. Fungal strains were reactivated and prepared for antifungal evaluation of extracts on Potato dextrose agar media.

#### Methods

##### *Determination of proximate composition*

Crude protein was estimated by Kjeldahl style [24]. Fat content was analyzed by the fat analyzer (Ankom Extractor, Model XT10I O'Neil Road, Macedon, USA) [25]. Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) were estimated due to AOAC enzymatic-gravimetric method [26]. Crude ash content and moisture was estimated due to the methods described by Nielsen [27].

##### *Determination of fatty acid composition*

Methyl esters of fatty acids (FAME) were prepared according to AOCS Official Method Ce1k-07 [28]. Hewlett Packard apparatus with capillary column and FID was utilized for detection with the same condition of Abdel-Razek et al. [29].

##### *Mineral analysis using microwave plasma atomic emission spectrometer (MP-AES)*

Minerals (Na, K, Ca, Mg, Cu, Mn, Zn and Fe) were measured by using microwave plasma atomic emission spectrometer (MP-AES) (Agilent 4100 MP-AES, USA) after the digestion in  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$  and  $\text{HClO}_4$  mixture. Phosphorus was determined by colorimetric method [30]. Results were expressed as mg/100g of dry weight (DW).

##### *Determination of carotenoids and vitamin C contents*

The  $\beta$ -carotene content of OPF was extracted and prepared for determination according to method of Davis et al. [31] with modification. The  $\beta$ -carotene in hexane as the standard and hexane as the blank samples were applied for calibration curve (0–50 ppm). Results were expressed in mg  $\beta$ -carotene/100 g DW. Vitamin C was estimated in byproducts using a titrimetric method by 2, 6-di-chloro-phenol-indophenol reagent [32, 33]. Results were expressed as mg equivalent /100 g fresh weight.

##### *Preparation of raw materials extracts*

The bioactive components were extracted

from the fine powder of each OFPBs types using ten times the volume of aqueous isopropyl (80%). The flask was covered and shaken overnight by shaking incubator (VWR® Incubating Orbital Shaker, Model 3500I), flasks were rest for one day (4°C) then filtered by Whatman filter paper (No.1). The filtrate was evaporated by vacuum rotary evaporator (45°C) till near dried, concentrated solution lyophilized using Dura-Dry MP freeze-dryer (FTS System, USA). Extracted materials by this technique were applied for the following analysis.

##### *Determination of total phenolic and total flavonoid contents*

Total phenolic contents (TPC) were determined by the Folin–Ciocalteu reagent following the method described by Badr et al. [34], and based on a phosphomolybdate– phosphomolybdate complex reduction. 1mg dried extract was dissolved in 1ml methanol. Five hundred microliter of the dissolved sample was added to 0.5 ml deionized double distilled water and a volume of 0.125 ml of Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 5 minutes before the addition of 1.25 ml of 7% sodium carbonate. The solution was adjusted with distilled water to a final volume of 3 ml and mixed thoroughly. Then; it was incubated in darkness area for half an hour, the absorbance was determined at 650 nm versus the blank. A standard curve was plotted using different concentrations of Gallic acid (standard, 0–1000  $\mu\text{g}/\text{mL}$ ). Total phenolic content was estimated as  $\mu\text{g}$  Gallic acid equivalents (GAE)/g of dry weight sample.

The total flavonoid contents of extracts were determined by a modified colorimetric method described by Gonzalez Aguilar et al. [35] with some modifications, using catechol as a standard. Extracts or standard solutions (250  $\mu\text{l}$ ) were mixed with distilled water (1.25 ml) and 75  $\mu\text{l}$  of 5 % sodium nitrite ( $\text{NaNO}_2$ ) solution followed by the addition of 15  $\mu\text{l}$  of 10% aluminum chloride ( $\text{AlCl}_3$ ) solution 5 min later. After 6 min, 0.5 ml of 1 M sodium hydroxide ( $\text{NaOH}$ ) and 0.6 ml distilled water were added. The solutions were then mixed and absorbance was measured at 510 nm. The results were expressed as mg catechol equivalent /g of dry weight sample. All determinations were performed in triplicate.

##### *Determination of antioxidant activities*

###### *a) Antioxidant activities using DPPH assay*

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the powder extracts

was measured according to the procedure described by Shehata et al. [36] with some modifications. Briefly, 1 mL of solution containing different concentrations of extract was mixed with 1 mL of 0.078 mM DPPH in methanol. The mixture was shaken and allowed to stand at room temperature in dark for 30 min. The absorbance of the solution at 517 nm was measured using a spectrophotometer. All of the tests were carried out in triplicates. The inhibition of DPPH radical was calculated as follows:

$$\% \text{Inhibition} = ((A_{\text{control}}^{517} - A_{\text{sample}}^{517}) / (A_{\text{control}}^{517})) \times 100$$

Where,  $A_{\text{control}}^{517}$  = Absorbance of the control solution at wavelength 517nm.

$A_{\text{sample}}^{517}$  = Absorbance of the test extract at wavelength 517nm.

IC50 value (mg extract/mL) is the inhibition concentration of the test content at which the DPPH radicals were scavenged by 50% and was calculated interpolation from a linear regression analysis.

#### b) Antioxidant activities using scavenging of hydroxyl radicals

Hydroxyl radical scavenging (HRS) assays were conducted by a Fenton reaction method as described in Shehata et al. [23]. Briefly, a reaction mixture containing 1.0 ml of Brilliant Green (0.435 mM), 2.0 ml of  $\text{FeSO}_4$  (0.5 mM), 1.5 ml of  $\text{H}_2\text{O}_2$  (3.0%, w/v), and 1.0 ml of bacterial lysate extract in different concentrations was incubated at room temperature for 20 min, and the absorbance was measured at 624 nm. Changes in the absorbance of the reaction mixture indicated the scavenging ability of the LAB strains for hydroxyl radicals. HRS activity was expressed as follows:

$$\text{Scavenging activity (\%)} = (A_s - A_0) / (A - A_0) \times 100$$

Where:  $A_s$  is the absorbance of the sample

$A_0$  is the absorbance of the control,

$A$  is the absorbance without the sample or Fenton reaction system.

#### c) Antioxidant activities using $\beta$ -carotene bleaching by linoleic acid assay

The antioxidant activity of extracts was performed by the  $\beta$ -carotene linoleic acid assay [37]. The absorbance was measured at 470 nm at the zero time immediately after the addition of the emulsion to each extract ( $\text{Abs}^0$ ) and also after 2h of incubation ( $\text{Abs}^{120}$ ). The antioxidant activity (AA %) values were calculated using the following equation:

$$\text{AA\%} = 1 - (\text{Ab}_{\text{sample}}^0 - \text{Ab}_{\text{sample}}^{120}) / \text{Ab}_{\text{control}}^0$$

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$$\text{Ab}_{\text{control}}^{120}) \times 100$$

The extract concentration providing 50% inhibitor for scavenging of antioxidant activity ( $\text{IC}_{50}$ ) was calculated by interpolation from the graph of  $\beta$ -carotene bleaching inhibition percentage against extract concentration. Ascorbic acid was used as positive control.

#### Preparation of OPF extracts for lethality test

A substitution procedure of dilution was applied for brine shrimp lethality (BSL) assay [38]. The preparation of the different OPFBs extracts dilutions for BSA test, where 20 mg of each dried extract powder was dissolved in 2 mL of the DMSO. The final concentrations were 1, 10, 100, and 1000 ppm. Each concentration was applied in three replicates while the control test existed for the comparing.

#### Determination of brine shrimp lethality (BSL) assay

Brine shrimp eggs were obtained from Demian Aqua-research Laboratory in Hurghada, Red Sea coast, Egypt. Artificial seawater was prepared by dissolving 38 g of salt in one liter distilled water then filtered. Hatching shrimp eggs in glass plates contain seawater as hatching chamber. Two days were allowed for the shrimp to hatch and mature as a larva. About 3 mL of the artificial seawater was pull using pastier glass pipette and 10 brine shrimps were introduced into each pipette. The number of surviving shrimps were counted and recorded after 24 hours in the present and absent of the tested extracts. Using probit analysis, the lethality concentration ( $\text{LC}_{50}$ ) was assessed at 95% confidence intervals.

According to the calculations of the previous studies [38, 39];  $\text{LC}_{50}$  value of (<1 mg/mL) is toxic while  $\text{LC}_{50}$  value of (> 1 mg/mL) is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of the dead larva by the total number and then multiplied by 100%. This is to ensure that the mortality of larvas is attributed to the bioactive compounds present in the extracts.

#### Determination of fungal growth inhibition using agar diffusion assay

Extracts for OFP raw materials of cladodes and peels were prepared using aqueous isopropyl as described by Shehata et al. [36], the extracted materials were concentrated to near dried then freeze-dried, 5 mg of each extract was dissolved in 0.5 mL in dimethyl-sulphoxide (DMSO), this value used to inject in the plate wells as a detoxification application against pathogenic

bacteria and toxigenic fungi. The inhibition effect of extracts was also evaluated against two *Fusarium* strains (*F. verticillioides* FM19; *F. culmorum* P846) as strains has ability for zearalenone and deoxynivalenol toxin producer.

#### Statistical analysis

All analyses were carried out in triplicate and the experimental data were expressed as means  $\pm$  SD. The software SPSS V16 was used to compare the results by the analysis of variance with one factor (ANOVA). Differences between the means at 5% levels ( $p < 0.05$ ) was considered statistically significant.

### Result and Discussion

#### Proximate analysis of OPFBs

Chemical composition of OPFBs included cladodes and peels were estimated, a level of variation was noted. Ash, dietary fibers, and fats content looked approximately close in both. Ash contents were recorded at  $26.33 \pm 4.18$  and  $20.5 \pm 2.25$  g/100 g of dry weight (DW) for peels

and cladodes, respectively. The protein content of Peels ( $6.88 \pm 2.26$ g/100g DW) was four times more compared to cladodes ( $1.23 \pm 0.91$  g/100g DW). The cladodes content of total carbohydrates ( $48.79 \pm 3.65$  g/100g DW) and the acidity ( $1.66 \pm 0.19$  determined as % citric acid) were more than carbohydrates ( $30.72 \pm 2.40$ g/100g DW) and the acidity ( $1.7 \pm 0.4$  %) in peels. The insoluble fiber content was more than soluble fiber content of two types of byproducts (Table 1). The carbohydrate is correlated to poly and oligosaccharides content [6], which reported with bioactive and antimicrobial activities, also it has nutritional functions.

Dietary fiber has a great function for health and malnutrition. It could play a preventive action against numerous diseases [19, 40]. The total dietary fiber in wild OPFBs recorded higher than previously reported [41, 42]; and slightly lower compared to the results of Ayadi et al. [43], also insoluble dietary fiber was higher than soluble like that reported before. Nevertheless, the detected amounts might be substantially different using other plant types since this is one of the major

TABLE 1. Chemical composition of OPFBs (Peels & cladodes).

Chemical analysis	OPFBs	
	Peels	cladodes
Protein <sup>1</sup>	$6.88 \pm 2.26^a$	$1.23 \pm 0.91^b$
Total carbohydrates <sup>2</sup>	$30.72 \pm 2.40^a$	$48.79 \pm 3.65^b$
Fat	$5.36 \pm 1.69^a$	$3.82 \pm 1.66^b$
Ash	$26.33 \pm 4.18^a$	$20.5 \pm 2.25^b$
Acidity (as citric acid)	$1.7 \pm 0.4^a$	$1.66 \pm 0.19^a$
Total Dietary fiber (TDF)	$30.68 \pm 5.28^a$	$25.65 \pm 3.07^b$
Insoluble Dietary Fiber (IDF)	$27.21 \pm 2.21^a$	$21.11 \pm 1.67^b$
Soluble Dietary Fiber (SDF)	$2.91 \pm 1.01^a$	$4.40 \pm 1.71^a$

- OPFBs is *Opuntia ficus-indica* byproducts
- Data expressed as means  $\pm$ SD; n=3.
- Within each raw different letters are significantly different  $P < 0.05$ .
- Acidity was determined as citric acid (%).

<sup>1</sup> Results were expressed as g/100 g of dry weight (DW).

<sup>2</sup> Total available carbohydrates were calculated as the difference of moisture, protein, fat, ash and fiber values.

influencing factors of fiber profile [44].

#### *Fatty acids in cladodes and peels*

Fatty acid analysis regarding cladodes and peel content of fats exhibited notable omega fatty acids in both. The majority content was recorded for eicosadienoic acid (C 20:2,  $\omega$ 6) followed by oleic acid then palmitic acid. The results represented in Table 2 showed a content of  $\omega$ 3,  $\omega$ 6, and  $\omega$ 9 - fatty acids that support its application in food chain supplementation. The existence of eicosadienoic acid also recommends the utilizing of OFPBs in baby-foods where it naturally presents in human milk. Eicosadienoic acid can modulate the metabolism of polyunsaturated fatty acids and alter the responsiveness of macrophages to inflammatory stimulations. Accompany with the other mono and polyunsaturated fatty acids; cis-11,14-eicosadienoic acid inhibits the binding of leukotriene B4 to pig neutrophil membranes, which may account in part for its

anti-inflammatory activities [45].

It is worth to mention that;  $\gamma$ -Linolenic acid is existing in a considerable ratio for cladodes and peel of OPF, this ratio make OPFBs in the same line with evening primrose oil as a source of  $\gamma$ -Linolenic acid, this fatty acid reported having function in repair nerve damage, support the immune system, and purported to prevent cancer and heart disease [46]. This results showed the importance of OFPBs as a source of omega and essential fatty acids which reported having nutraceutical and pharmaceutical effects. The fatty acid composition of OFP cladodes missed acids of lauric, docosadienoic, and cerotic. Concerning the peels; arachidic and tetracosadienoic acids were recorded as not detected. In peels; saturated fatty acids (SFA): unsaturated fatty acids (MUFA): polyunsaturated fatty acids (PUFA) are equal to 0.86: 0.91: 1.23, this is moderately close to the WHO of fatty acid balance recommendation in food [47, 48]. In cladodes, the SFA: MUFA:

**TABLE 2. Fatty acid composition of cladodes and peels of *Opuntia ficus-indica*.**

Fatty acid composition	Opuntia ficus-indica	
	Peels	cladodes
Luric Acid (C12:0)	0.69± 0.17	nd
Myristic acid (C14:0)	1.03 ± 0.21	2.36 ± 1.55
Palmitic acid (C16:0)	22.8 ± 1.56	17.97 ± 0.21
Palmitoleic acid (C16:1)	2.14±0.31	1.93±0.24
Stearic acid (C18:0)	2.87±0.26	2.38±0.31
Oleic acid (C18:1 $\omega$ 9)	26.4±0.34	19.61±0.47
Homo- $\gamma$ -Linolenic acid (C 18:3 $\omega$ 6)	7.86±0.22	11.6±1.28
$\alpha$ -Linolenic acid (C18:3 $\omega$ 3)	0.47±0.06	1.28±0.36
Arachidic acid (C20:0)	nd	0.37±0.14
Eicosadienoic acid (C20:2 $\omega$ 6)	32.1±0.28	38.4±1.41
Behenic acid (C22:0)	0.63±0.1	0.27±0.04
Docosadienoic acid (C22:2; $\omega$ 6)	0.84±0.06	nd
Lignoceric acid (C24:0)	0.38±0.08	0.54±0.17
Nervonic acid (C24:1 $\omega$ 9)	1.38±0.14	1.92±0.33
Tetracosadienoic acid (C24:2)	nd	0.37±0.11
Cerotic acid (C26:0)	0.41±0.11	nd
SFA	28.72	23.89
MUFA	30.19	23.46
PUFA	41.27	51.56
SFA: MUFA: PUFA	<b>0.86: 0.91: 1.23</b>	<b>0.72: 0.7: 1.55</b>

• Data expressed as means  $\pm$ SD; n=3.

• nd: means not detected

• SFA: saturated fatty acids; MUFA: mono-unsaturated fatty acids; PUFA: poly unsaturated fatty acids.

PUFA ratio recorded as 0.72: 0.7: 1.55, which has a differentiation compared to peels.

#### *Mineral composition (macro and microelements)*

The microelements (Fe, Cu, Mn, and Zn) and macro elements (Ca, Mg, Na, and K) profiles shown in Table 3. The cladodes and peels proved the most suitable source of microelements, particularly regarding the Fe, Mn, and Zn levels. These microelements are crucial for important biochemical and physiological functions and essential for maintaining health throughout life [49]. The OPFBs content of microelements supports its nutritional utilization in food materials. This result explains the significance of these byproducts as a source of micronutrients for human nutrition. The present results for the OPF-cladodes are significantly different from those reported by Abdel-Hameed et al. [50]. These discrepancies could be due to genotypic factors and environmental culture conditions as our samples represent the natural and wild-type of the plant. Potassium presented the principal element of macro elements composition in OPFBs samples in terms. Potassium is a highly significant ingredient for human health; it decreases blood pressure and reduces cardiovascular disease morbidity and mortality [51], lowering the excretion of calcium in the urine and decreases the osteoporosis risk [52].

#### *Total phenolic and flavonoid content*

It is necessary to determine and quantify the phenolic content of plant extracts. The phenolic and flavonoid content of extracts of peels and cladodes summarized in Table 4. In peels extract; the total phenolic (TPC) and flavonoid contents (TFC) represent  $520 \pm 2.64$  mg Gallic equivalent/g and  $65.7 \pm 3.41$  mg Catechol equivalent/g of

extract, respectively. The cladode extract provided  $788 \pm 1.35$  mg of Gallic equivalent/g and  $25.36 \pm 1.55$  mg Catechol equivalent/g. It is well known that antioxidant activity correlated directly with the quantity of phenolic compounds [53]. Polyphenols are a group of nutrients or minor components considered which an efficient source of bioactive compounds [54].

#### *Vitamin C content of the OPFBs*

The vitamin C content in OFP byproducts was measured. Peels contained the highest vitamin C content more than those recorded in cladodes (Table 4). Vitamin C has been reported possessing beneficial effects on health. It reduces the risk of chronic and cardiovascular diseases side to the function in stimulating the immune system [55]. The yellowish-orange type of Egyptian OPF-fruits reported having a vitamin C content equal to 20.07 mg/100 g fresh weight [56]. The variations among the results could be due to genotypic factors and environmental effects. The concentration of vitamin C was higher than the total carotenoids. This result was consistent with the findings by Kuti [57], and El Kharrassi et al. [58].

#### *The OPFBs impact for BSL assay*

The result of OFPBs toxicity evaluate using BSL assay expressed the availability of peels and cladodes extracts for possible utilization in food application. The result exposed values of  $LC_{50}$  for cladodes at (3.17 mg/mL) and peels at (3.94 mg/mL). The mortality ratio of 1mg/mL for each of cladodes and peel extracts were 34% and 23%, respectively. These results prove the safety characteristics of cladodes and peel extracts, so they could be applied in food application [59].

**TABLE 3. Macro-elements and microelements of OPFBs (peels and cladodes).**

Mineral	Peels	cladodes
Fe	108	57.21
Cu	4.78	9.71
Mn	31.51	36.7
Zn	11.5	23.11
Ca	4.78	9.71
Mg	21.66	24.21
Na	81.23	31.21
k	241	91.22

OPFBs is *Opuntia ficus-indica* byproducts.

- Macro-elements are Ca, Mg, Na and K (expressed in mg/100 g dry weight).
- Microelements are Fe, Cu, Mn and Zn (expressed in  $\mu$ g/100 g dry weight).

TABLE 4. Phytochemical characterization of OPBs freeze-dried (cladodes and peels).

Phytochemical characterization	<i>Opuntia ficus-indica</i>	
	Peels	cladodes
<b>Total phenolic content</b> (mg GAE g <sup>-1</sup> extract)	520± 2.64 <sup>b</sup>	788 ± 1.35 <sup>a</sup>
<b>Total flavonoid content</b> (mg Catechol g <sup>-1</sup> extract)	65.7 ± 3.41 <sup>a</sup>	25.36 ± 1.55 <sup>b</sup>
<b>Vitamin C</b> (mg/100 g DW)	208.86 ± 7.79 <sup>a</sup>	48.06 ± 3.11 <sup>b</sup>

OPFBs is *Opuntia ficus-indica* byproducts

- Data expressed as means ±SD; where n=3.
- The data in each row with same letters are non-significant at P=0.05

#### *Evaluation of OPFBs antioxidant activity*

Cladodes possess a potent antioxidant property than peels (Fig. 1). These results were in agreement with the observed for those botanical components [18, 60]. The higher antioxidant activity might be due to higher level of phenolic compounds, since the influence of an extract phenolic composition in the antioxidant potential is a well-known fact [61]. The high antioxidant activity recorded in OFP byproducts will confirm their bioactivity behavior, particularly scavenging the free radicals that caused oxidative stress.

#### *Antibacterial and antifungal activity*

Four bacterial strains were used to investigate the OPFBs antibacterial inhibition. Also, six fungi strains were used to screen the OPFBs antifungal properties. Cladodes and peels extract exhibited good antioxidant activity. Therefore, it could use to evaluate the antibacterial and antifungal potency. Figure 2 showed the antimicrobial activity wherever the extracts displayed an inhibition zone with microorganisms revealed to peels and cladodes extracts. Antibacterial effect of wild OPFBs against pathogenic strains bacteria shown in Figure 2A. However, antifungal activities were estimated using toxigenic producing fungi strains as recorded in Figure 2B. From these four microorganisms, *S. aureus* NCTC 10788 and *P. eaeruginosa* ATCC 9027 showed an inhibition zone in the presence of cladodes extracts in diameters of 9.65±0.88 mm and 9.23±0.37 mm, respectively.

These observations demonstrated the antimicrobial activity of OPFBs certain compounds. The most suggested methods applied to inhibit microbial growth in foods has been achieved using food preservative. Total phenolic content shown high in cladodes more than those recorded in peels, this finding could explain the more effective function of cladodes in the antimicrobial activity. Bioactive compounds extracted from OPFBs recorded broad scale as food preservative components [62]. These bioactive components are including phenolic, flavonoids, vitamins, and microelements, which were existing in wild OPFBs extracts. The variations in antimicrobial action could partially associate to the difference of bioactive substitutes in peels and cladodes [63]. Furthermore, the previous investigation was described related marks for the fruit of some OPF varieties [64], also for some different plants like fennel and chamomile [65]. In the present investigation, the antimicrobial activity of OPFBs was more efficient against fungi compared to bacteria strains. The inhibition zone diameters of various fungi strains with extracts are presented in Fig 2B. The OPFBs showed different antifungal potency levels. Partially, the cladodes were displayed high activity against all tested fungi compared to peels extract. These exciting results suggest a link between the phenols, flavonoids, and antimicrobial activity.

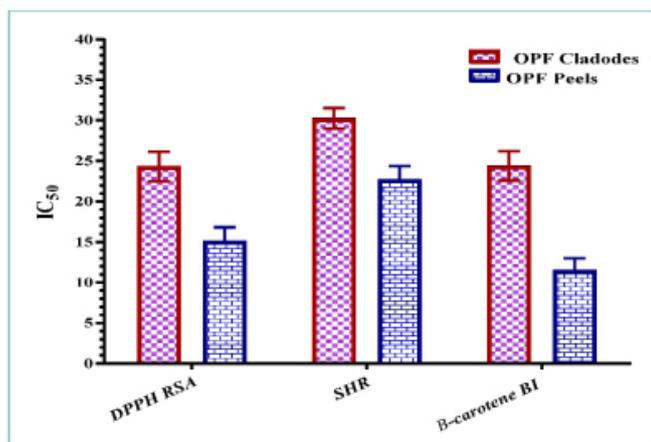


Fig. 1. IC<sub>50</sub> values of antioxidant activities of *Opuntia ficus-indica* bioactive extracts by different in vitro assays.

- OPF: *Opuntia ficus-indica*.
- DPPH RSA: DPPH radical - scavenging activity ( $\mu\text{g/mL}$ );
- SHR: Scavenging of hydroxyl radicals ( $\mu\text{g/mL}$ );
- $\beta$ -carotene BI:  $\beta$ -carotene bleaching inhibition ( $\mu\text{g/mL}$ ).

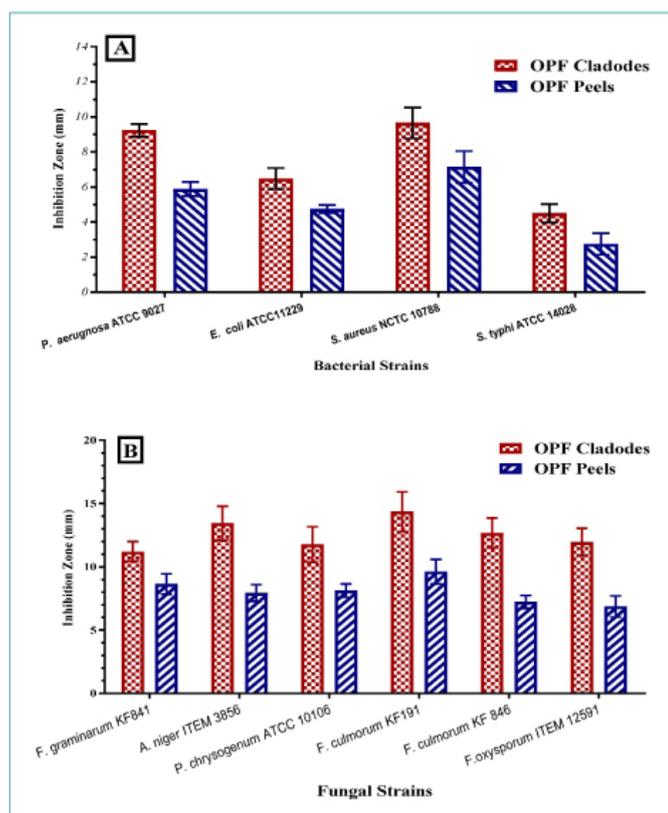


Fig. 2. Anti-bacterial and antifungal properties of *Opuntia ficus-indica* byproducts extracts.

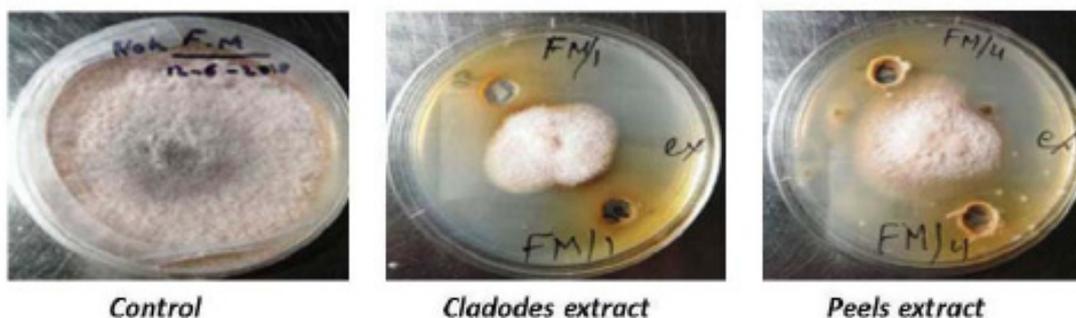
- OPF: *Opuntia ficus-indica*.
- (A) Antibacterial activity against pathogenic bacterial strains
- (B) Antifungal activity against toxigenic fungal strains

Inhibition effect of the OFPBs byproducts was examined against various fungal strains. The result represents the antifungal efficacy of these byproducts for suppressing fungal growth of tested strains (Fig. 2B). The OFPBs capability to limit fungi growth will be correlating to its metabolites secretion including mycotoxins. In this respect; two identified strains of *Fusarium* fungi, which are investigated before and are positively for producing mycotoxin, were utilized to inspect the OFPBs impact on fungi-producing toxins. Toxicogenic fungi represent a source of coupled hazard on food products (contamination by microorganisms and chemical toxins). The impact of antifungal molecules will be serving in the spoilage and mycotoxin contamination restriction. In **Figure 3**; the inhibition impact of cladodes and peel on two identified strains of *Fusarium* fungi (*F. verticillioides* FM19; *F. culmorum* P846) was determined. These strains possess the ability for zearalenone and deoxynivalenol secretion. The cladodes manifested more effective inhibition properties

compared to peels. The two types of byproducts have positive inhibition impacts compared to the control. A further study concerning the effect of these byproducts on mycotoxin reduction will be needed. The positive result will unlock the gate for these byproducts of OFP to apply in the food-safety elevation.

The results of the OPFBs evaluation displayed the good aspects concerning the cladodes antibacterial and antifungal activities. It recorded more than the value shown in the extract of the peels. The better antimicrobial properties of cladodes could be associated with the higher content of total phenolic compounds (Table 4). Besides, the antioxidant activity recorded higher also in cladodes. It was represented nearly 30% increment than the value in peels extract (Figure 1). The higher values of antioxidant activity and total phenolic content connected to the more antimicrobial activities recorded. This antimicrobial was determined concerning several strains of pathogenic bacteria and toxicogenic fungi.

**(A) *Fusarium verticillioides* FM19**



**(B) *Fusarium culmorum* P846**



**Fig. 3. Effect of *Opuntia ficus-indica* on *Fusarium* fungi inhibition.**

## Conclusion

The chemical composition of wild OPFBs reflects a distinctive formation of nutrients, especially dietary fibers. The OPFBs, that contains peels and cladodes, possesses the phytoconstituents as bioactive components. The  $\gamma$ -Linolenic acid is existing in a considerable ratio for cladodes and peel, known to have health and nutritional impacts. The existence ratio of this functional fatty acid seems close to the main source (evening primrose oil). The OPFBs showed balanced of microelements that possess biochemical and physiological functions in the cell systems. The impact of chemical composition on antimicrobial and antifungal properties of OPFBs showed an effective impact due to the high content of phenolic and antioxidant potency. This effect was clearer against two strains of *Fusarium* fungi able to excrete mycotoxin (zearalenone and deoxynivalenol). The OPFBs content supports their food application as nutritional and food safety improver. This study considered a step for investigating the anti-mycotoxigenic impact on mycotoxin and their producing fungi. Further studies focusing on the effect of OPFBs extracts on the production of mycotoxins are needed.

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## تأثير التركيب الكيميائي للمنتجات الثانوية لنبات التين الشوكي البرى على إستخدامه كمصدر للمغذيات ومضادات الأكسدة و تقييم الفعالية ضد الفطريات المنتجة للسموم

عادل جبر عبد الرازق سلام<sup>١</sup>، محمد جمال محمد شحاتة<sup>٢</sup>، أحمد نوح بدر<sup>٣</sup>، كارولينا جرومادوزيكا<sup>٤</sup>، ليوكاش ستيبان<sup>٥</sup>  
<sup>١</sup>قسم الزيوت والدهون - شعبية بحوث الصناعات الغذائية والتغذية- المركز القومى للبحوث- القاهرة- مصر.  
<sup>٢</sup>قسم علوم وتكنولوجيا الاغذية- معهد بحوث الاراضى القاحلة- مدينة الابحاث العلمية والتطبيقات التكنولوجية- اسكندرية- مصر.

<sup>٣</sup>قسم سموم وملوثات الغذاء - شعبية بحوث الصناعات الغذائية والتغذية - المركز القومى للبحوث - القاهرة - مصر.

<sup>٤</sup>قسم الكيمياء - كلية بوزنان لعلوم الحياة - بوزنان - بولندا.

<sup>٥</sup>معهد بحوث الهندسة الوراثية - الاكاديمية البولندية للعلوم- بوزنان - بولندا.

التين الشوكي هو نبات برى وقد يزرع في بعض الأراضي الصحراوية وهو من الفواكه الصيفية التي يفضلها الكثير من الأشخاص نظراً لفوائده العديدة لصحة الإنسان. ويهدف هذا البحث لتقييم ودراسة تأثير التركيب الكيميائي للمنتجات الثانوية للتين الشوكي البرى على إستخدامه كمصدر للمغذيات الغنية بمضادات الأكسدة وتقييم الفعالية ضد الفطريات والميكروبات الممرضة. عن طريق تقدير الأحماض الدهنية والمكونات الكبرى والصغرى وفيتامين ج والمحتوى الكلى للمواد الفينولية والفلافونيدات سواء للقشور او الأوراق المسطحة. أظهر التركيب الكيميائي لمنتجات الصنف البرى من التين الشوكي تميزاً واضحاً في محتوى القشور العالى من الألياف الغذائية والفلافونيدات كما تميزت الأوراق المسطحة بمحتوى أعلى من الفينولات الكلية ومضادات الأكسدة وبعض الأحماض الدهنية الأوميغا ذات الأثر الصحى. وقد سجلت قيم الألياف الغذائية في منتجات التين الشوكي البرى الثانوية  $30.68 \pm 5.28$  للقشور و  $25.65 \pm 3.07$  للأوراق المسطحة وكان محتوى المواد الفينولية الكلى  $520 \pm 2.64$  مللى جرام/ جم جاليك للقشور و  $788 \pm 1.35$  مللى جرام/ جم للأوراق المسطحة على الترتيب، في حين كان محتوى الفلافونيدات  $65.7 \pm 3.41$  كاتيكين / جم للقشور و  $25.36 \pm 1.55$  مللى جرام كاتيكين / جم للأوراق المسطحة. أوضحت النتائج أيضاً وجود محتوى كبير من العناصر المعدنية الدقيقة والأحماض الدهنية من نوع الأوميغا خاصة حمض Eicosadionic كحمض دهني رئيسي بنسب بلغت  $32.1\%$  للقشور و  $38.4\%$  للأوراق المسطحة من إجمالي نسب الأحماض الدهنية. وأظهرت أيضاً المستخلصات درجة جيدة من الأمان للإستهلاك الأدمى من خلال تقييمها بإستخدام إختبار الجرعة المميتة على يرقات الجمبرى. على الجانب الأخر أظهر مستخلص التين الشوكي البرى (قشور وأوراق مسطحة) كفاءة عالية ضد البكتيريا الممرضة و الفطريات المنتجة للسموم حيث سجلت القيم الخاصة بالنشاط المضاد للبكتيريا والمضاد للفطريات معدلات أعلى عند إستخدام مستخلصات الأوراق المسطحة عن تلك المسجلة بالنسبة للقشور. والجدير بالذكر أن القيم المرتفعة من مضادات الأكسدة ومحتوى الفينول الكلى ترتبط بالأنشطة المضادة للميكروبات عند تقييم المستخلصات النباتية. وقد سجل المحتوى الكلى من المركبات الفينولية قيم بدت أعلى في الأوراق المسطحة عن مثيلاتها المسجلة لقشور تلك الفاكهة. أيضاً سُجل محتوى مضادات الأكسدة أعلى في الأوراق المسطحة من القيم المقابلة له في القشور بنسبة تصل إلى  $30\%$  تقريباً. كان التأثير المثبط واضح ضد فطر الفيوزاريوم المنتج لنوعين مختلفين من السموم الفطرية بإستخدام طريقة الأجار المنتشر. كذلك فإن المحتوى الفريد من المركبات الكيميائية للمنتجات الثانوية للتين الشوكي البرى قد أعطت نشاطاً حيويًا مميزاً ضد عدد من سلالات البكتيريا الممرضة. وبناءً على نتائج البحث يظهر وبشكل جلى قدرة مستخلصات صنف التين الشوكي البرى في التغلب على مصادر الخطر الميكروبية والفطريات المنتجة للسموم إلى جانب كونه مصدراً واعداً للعديد من المغذيات خاصة الأحماض الدهنية الأساسية ومضادات الاكسدة الطبيعية مما يرشحه لأن يكون أحد المواد الواعدة الصالحة للتطبيق في مجال الصناعات الغذائية.