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EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF *Opuntia ficus-indica*, SEEDS AND PEELS EXTRACTS

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ABSTRACT: The biologically active compounds isolated from plants are known to be efficient as antibacterial and antioxidants. The antioxidant and antibacterial activities of total phenols and flavonoids drived from the petroleum ether, ethyl acetate and ethanol extracts of *Opuntia ficus-indica* seeds and peels were performed using DPPH and modified Kirby-Bauer disc diffusion technique against gram-positive bacteria (*Bacillus subtilis*) and gram-negative bacteria (*Serratia marcescens*), respectively. In the present study, it is demonstrated that, both alcohol and ethyl acetate extracts show the best antioxidant and antibacterial activities than the petroleum ether extracts. This is consistent with the results of the chemical analyses of both extracts.

Key words: Antioxidant, antimicrobial, seeds, peels, phenols, flavonoids, *Bacillus subtilis*, *Serratia marcescens*, *Opuntia ficus-indica*.

INTRODUCTION

The safety worry, associated with artificial antioxidants and the increasing universal trend in antibacterial resistance. has required investigations into novel, but safe and natural bioactive components of plant extracts with antioxidant (Zrira et al., 2016) and antibacterial properties (Koubaa et al., 2015). The Opuntia ficus-indica plant parts and by-products had recently been attracting a lot of research interest and perhaps integral to the detection of novel and natural bioactive compounds. The prickly pear (Opuntia spp.) has many biologically active compounds and has a good effect in the management of non-communicable diseases (Tesoriere et al., 2005).

Most studies had focused on pulp as a source of bioactive molecules (**Zrira** *et al.*, **2016**). The fruit peels have largely been neglected despite indications that they have significant amounts of bioactive molecules (**Milán-Noris** *et al.*, **2016**).

Opuntia peels makeup 60% of the whole fruit, but not consumed optimal consumption (Milán-Noris *et al.*, 2016). Therefore, Opuntia peel by-products are often eliminated after fruit consumption (**Ramadan and Mörsel, 2003**). The production of nutraceuticals from plant byproducts such as Opuntia peels using food processing techniques will continue to expand as a cheap and cost-effective alternative (**Aruwa et al., 2018**). *Opuntia species* have been used for centuries as food resources and in traditional folk medicine for their nutritional properties and their benefit in chronic diseases, particularly diabetes, obesity, cardiovascular diseases, and cancer (**del Socorro Santos Díaz et al., 2017**).

Polyphenols are an important group of compounds linked with *Opuntia ficus-indica* which possesses antioxidative and antibacterial properties (Khatabi *et al.*, 2011). Many reports have expounded a strong link between the phenol content and the antimicrobial, antioxidant activities of extractable polyphenol extracts from *Opuntia* spp. (Kuti, 2004; Castellanos-Santiago and Yahia, 2008; Khatabi *et al.*, 2011; Anwar and Sallam, 2016; Milán-Noris *et al.*, 2016).

In addition, most studies have shown that extraction solvents and processing methods used could affect biological activity, yield and

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phenolic compound profile of tested extracts (Torres *et al.*, 2010; Abou-Elella and Ali, 2014).

The major objective of this work was to study the antioxidant and antibacterial activities of extracts of *Opuntia ficus-indica* seeds and peels.

MATERIALS AND METHODS

Plant Material

The *Opuntia ficus-indica* were collected and identified from Botany Department, Faculty of Agriculture, Zagazig University. The plant seeds and peels were allowed to dry in airly dark and well- aired place for 14 days, ground to a fine powder using blender and kept for further investigations.

Chemicals

All solvents used throughout the present work were of high analytical grade and obtained from different companies. ABTs, DPPH and Substrates were purchased from (Sigma Chemical Co, St. Louis, USA).

Methods

Chemical composition of plant sample

The ash, crude lipid and crude protein, total carbohydrat determination. The carbohydrat content calculated by. percentages were determined according to the method described in **AOAC (2005)**.

Preparation of plant extracts

The airly dried samples of (*Opuntia ficus-indica*) seeds and peels were successively extracted with different organic solvents in increasing polarity order according to **Pathmanathan** *et al.* (2010). Briefly, 100 g of each powder was soaked in 300 ml petroleum ether separately with intermittent shaking for three days. They were first filtered with muslin cloth and then with filter paper.

The residue was further extracted three times by using a fresh solvent. Then all the filtrates were combining together. The resulting residue was airly dried and used for the next extraction with ethyl acetate and followed by ethanol. Finally, solvents were removed from the extracts by treating at 40° C in an oven. After complete drying, the yield of each extraction was measured separately and the extracts were stored at 4° C until used for further study.

Total phenolic determination

Total phenolic contents of *Opuntia ficus- indica* seeds and peels were determined according to the method described by **Ghasemzadeh** *et al.* (2010).

Determination of total flavonoids

Aluminum chloride colorimetric method was used for the determination of total flavonoid compounds of *Opuntia ficus- indica* seeds and peels according to the method described by **Ahn** *et al.* (2007).

Determination of Plant Free Radical Scavenging Activity (RSA)

Different solvents were used to assay the RSA of Opuntia ficus- indica seeds and peels. Therefore, the RSA of Opuntia ficus- indica seeds and peels were assayed using DPPH radical previously dissolved in different solvents. Different solutions of DPPH radicals were freshly prepared at a concentration of 10^{-4} M. The radical, in the absence of antioxidant compounds, was stable for more than 2 hr., of normal kinetic assay. For evaluation, 10 mg of different extracts (in 100 µl different solutions of DPPH) was mixed with 390 µl different solutions of DPPH radicals and the mixture was vortexed for 20 sec. at ambient temperature. Against a blank of pure solvents without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 30 and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of DPPH solutions with or without a sample (control) and the inhibition percent was calculated according to Lee et al. (2002) from the following equation:

Inhibition (%) = [(A of control - A of tested sample) / A of control] x 100.

In vitro determination of antimicrobial activity

Antimicrobial activities of the tested samples were determined using a modified Kirby-Bauer disk diffusion method (Bauer et al., 1996). Plates inoculated with Gram (+) bacteria as Bacillus subtilis; Gram (-) bacteria as Serratia *marcescens* at $35 - 37^{\circ}$ C for 24 - 48 hours., and then the diameters of the inhibition zone were measured in millimeters (Bauer et al., 1996). Standard discs of tetracycline (antibacterial agent) served as a positive control for antimicrobial activity, but filter discs impregnated with 10 µl of solvent (Petroleum ether, ethyl acetate and Ethanol 70%) were used as a negative control.

RESULTS AND DISCUSSION

Proximate Composition of *Opuntia ficus-indica* Fruit Seeds and Peels

The analysis of *Opuntia ficus- indica* seed and peel contents are recorded in Table 1, results showed that crude protein valued 6.81% in peel while amounted 8.52% in seeds, crude fat was as much as 1.82% in peels and 7.02% in seeds, carbohydrate amounted 19.41% in peels and 17.14% in seeds and ash amounted 15.8% in peels and 2.7% in seeds. These results showed that the plants contained a considerably high amount of ash (2.7% in seeds -15.8% in peels). **Saenz-Hernandez (1995)** reported that peels contained higher amount of water (94 and 90%, respectively) than the seeds (18%).

Protein content shows that seeds contain more amount than the peels (8.52% and 6.81%). The same observation was made for lipids where seeds have a greater amount (7.02%) than the peels (1.82%).

Present results demonstrated that the plant contained high amount of ash and carbohydrates in peels than in seeds. On the other hand, it was observed that the protein and the fat contents were considerably high in fruit seeds than in fruit peels.

Active Components in *Opuntia ficus-indica* Seeds and Peels

Rice-Evans *et al.* (1996) and Mattei *et al.* (1998) reported that phenolic compounds have been widely studied; phenolic compounds have at least one aromatic ring which can carry the hydroxyl groups which can work as reducing agents. The natural antioxidants such as phenolic and flavonoid compounds have wide spectrum pharmacological effects like antibacterial, antiallergic, neuroprotective activities, anti-inflammatory and anticancer, also protect plants from the attack of pathogenic microbes.

In this study, the samples were sequentially extracted using three different polarities of solvents in order to determine the recovery of total phenolic content (TPC) and total flavonoid by the solvents. The TPC of three different extracts (Petroleum ether, ethyl acetate and ethanol) for *opuntia ficus*- indica seeds and peels are shown in Table 2. The highest value of TPC was exhibited by the ethanol extract of the seeds followed by peel [2.9 and 1.9 mg galic acid equivalent (GAE) per ml extract] while the lowest value of TPC was exhibited by the ethyl acetate extract of seeds (0.632 mg/ml).

The total flavonoid content (TFC) of three different extracts (Petroleum ether, ethyl acetate and ethanol) for *Opuntia ficus- indica* seeds and peels are shown in Table 2. The highest value of TFC was exhibited by the ethanol extract of peel followed by ethyl acetate extract of peels and seeds (574 and 453 μ g GAE 100gm extract) while the lowest value of TFC was exhibited by the Petroleum ether extract of seeds.

Analysis of Opuntia seeds has shown that the presence of phenol valued $-268.4 \ \mu g /100 \ g$ (**Tlili** *et al.*, **2011**) and 48–94 μg GAE/100g (**Chougui** *et al.*, **2013**), as well as flavonoid (1.5–2.8 μg QE/ 100 g) and tannin amounted 4.1–6.7 μg CE/100 g (**Chougui** *et al.*, **2013**). Phenol composition of defatted extract from Opuntia seed correlated significantly with their antioxidant capacity. (**Khoo** *et al.*, **2012**). More than twenty compounds were detected with varying complexities at 330 nm after liquid chromatographic (LC) separation. Significant

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Parameter	Seed	Peel		
Dried weight	14%	17%		
Total proteins	8.52%	6.81%		
Total fats	7.02 %	1.82%		
Total fiber	17.14%	19.41%		
Total ash	2.7 %	15.8%		
Total carbohydrates	50.62%	39.16%		

 Table 1. The proximate compositions of Opuntia ficus- indica seeds and peels (g/100 g aira dried weight)

Table 2. Total phenol and total flavonoids of Opuntia ficus-indica seed and peel extracts

	Peels			Seeds		
	Petroleum	Ethyl acetate	Ethanol	Petroleum	Ethyl acetate	Ethanol
TPC (mg/ml)	1.5	1.03	1.9	1.31	0.632	2.9
TFC (µg /ml)	280	453	574	141	362	423

differences in antioxidant activity have also been recorded for ground seeds compared to whole prickly pear seeds which were attributed to their high total phenol composition (Chaalal *et al.*, 2013; Morales *et al.*, 2014).

Opuntia ficus-indica (L.) Mill] peels contain considerable amounts of neutral glycolipids and phospholipids (Ramadan and Mörsel, 2003). 17- Decarboxy betanin and betanin (Abou-Elella and Ali, 2014); xanthophylls[(all-E)-(all-E)-violaxanthin lutein. and (all-E)zeaxanthin], hydrocarbon carotenes (belonging to two types of oxygenated carotenoid derivatives); and chlorophyll (Yahia et al., 2010; Cano et al., 2017), have also been identified. Flavonoid glycosides dominate the flavonoid profile of cactus peels (Moussa-Ayoub et al., 2011a,b). Spineless cultivars contain more flavonols than the spiny/prickly varieties, and prickly pear peels contain a higher level of flavonoids when compared to the pulp (Yeddes et al., 2013). The components and bioactivities of peel extracts may depend on the extraction method (Koubaa et al., 2016).

Radical Scavenging Activity (RSA) of Extracts

Many studies in the last ten years interested in the theory of free radical disease causation, especially in certain forms of cancer and vascular diseases. Because of the developments in the free radical field have guided us to the consideration on dietary agents, the natural antioxidant (especially vitamins E, A and C), in a possible prophylactic and the role of the disease process. A free radical is a chemical species that has unpaired electrons (Prvor et al., 2006). These electrons, which made free radicals very reactive and take a section in chemical reactions with other components in cell such as proteins, complex carbohydrates, nucleic acids and lipids in the body (Kohen and Nyska, 2002). In the biological systems, free radicals are referred to reactive oxygen species (ROS), as the most biologically significant free radicals. ROS produced in cells include hydroxyl radical (OH), hydrogen peroxide (H_2O_2) , and superoxide anion (O_2) (Pryor *et al.*, 2006).

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Fig. 1 shows the antioxidant activity of Opuntia ficus- indica seed and peel extracts. This allowed characterising and comparing the RSA of all samples under the same conditions. Antiradical properties of the different extracts were compared using stable DPPH free radicals. Figure 1 shows that ethyl acetate seed extracts had the highest RSA followed by ethyl acetate peel extracts. After 2 hr., incubation at room temperature, 93% of DPPH radicals was quenched by ethyl acetate seed extracts, while petroleum peel extract was able to quench only 30%. Regarding the composition of different extracts, they have different patterns of bioactive components. Apart from the RSA and oxidative stability of extracts depends on the phenols composition, the presence of minor fat-soluble bioactive and the initial amount of hydroperoxides. It could be said that the RSA of extracts can be interpreted as the combined action of different endogenous antioxidants. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Khaga et al., 2015). Antioxidant activities greatly associated with the presence of phenolic compound (Shahwar et al., 2010).

Antimicrobial Activity

The intensive use of antibiotics is often followed by the presence of resistant strains of microorganisms. In view of the resistance of bacteria to drugs, the search for natural compounds having antibacterial activity is an urgent one in order to cope with the harmful effects of these microorganisms. For these reasons, accordingly, in this research three extracts for seeds and peels *i.e.* petroleum ether, ethyl acetate and ethyl alcohol were tested against different microorganisms grampositive *B. subtilis* (G^+) and gram negative bacteria S. marcescens (G⁻). Inhibition zones are recorded as shown in Table 3. Control was in the same conditions. It was observed that control did not produce any inhibition zones (data not shown). It is shown that all extracts gave effects on the two types of bacteria (gram positive and negative).

The results of the present study showed that the ethanolic extract of *Opuntia ficus- indica* inhibited the growth of tested isolate strongly; this may be due to the presence of the phytochemical groups as mentioned in Table 2.

According to the findings, B. Subtilis was found to be more sensitive to the extract than Serratia marcescens. These results were agreed with (Mishra et al., 2014) they found that the presence of a variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that are found to have effective as antimicrobial properties. The probable mechanism of phenolic compound's activity includes enzyme inhibition by oxidizing compounds, possibly through reaction with sulphedral groups or through more nonspecific interaction with proteins. Their antibacterial activity is probably due to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to disruption of microbial membranes (Tsuchiya et al., 1996). Many plants contain non-toxic glycosides which can get hydrolyzed to release phenolic which are toxic to microbial pathogens (Aboaba and Efuwape, 2001).

Compounds belong in a range of phenolic and non-phenolic classes such as betalains, polyphenols and phenolic acids (caffeic, cinnamic. catechol). quinones, flavones. flavonoids. flavonols. tannins, coumarins, lectins and polypeptides, alkaloids, terpenoids, (spermidine), essential oils, polyamines thiosulfinates, glucosides, isothiocyanates, polvacetvlenes and acetvlene compounds (Tapiero et al., 2002; Ciocan and Bara, 2007).

The antimicrobial activities of *Opuntia species* extracts are attributed to the presence of quite number of these compounds.

Opuntia ficus-indica extracts in a wide array of solvents have shown activity against different bacterial strains such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella Klebsiella pneumoniae, spp., Citrobacter freundii and Streptococcus pneumoniae (Shafiei et al., 2013; Wasnik and Tumane, 2016), and against Bacillus subtilis (Gnanakalai and Gopal, 2016). Terpenoids, glycosides, saponins, alkaloids and flavonoids were identified in the extracts.



Fig. 1. DPPH scavenging activity of Opuntia ficus-indica seeds and peel extracts

Sample		Inhibition zone diameter (mm/mg sample)					
Control: DMSO		<i>B. subtilis</i> (G+) 0.0			S. marcescens (G-) 0.0		
Seed	2%	16.5	13	9	5.6	8.8	14.3
Seed	1%	0.0	10.4	5.6	0.0	7.8	12
Seed	0.5%	0.0	9	0.0	0.0	6.8	0.0
Seed	0.25%	00	9	0.0	0.0	6	0.0
Peel	2%	6.3	22.5	11	13	12.6	5.6
Peel	1%	4.2	19.5	6.3	4.2	10.8	4.9
Peel	0.5	3.6	5.6	5.6	0.0	8	2.8
Peel	0.25%	4.2	4.2	4.2	0.0	8	0.0

Table 3. Effect of petroleum extract on the diameter inhibition zone (mm) of microorganisms

P: petroleum extract, EA: Ethyl acetate, E. oH : ethanol extract

Acetone extract compared to n-hexane and petroleum ether extracts showed better antimicrobial activity (Wasnik and Tumane, 2016), while aqueous extracts of both stem and fruit (Gnanakalai and Gopal, 2016) showed the least antimicrobial activity which could be attributed to the poor solubility of bioactive components in extraction solvents.

Most important was the antibacterial activity which makes the extracts potentially suitable for

food industry applications, for example, as food additives or preservatives.

The broad-spectrum activity of the extracts was attributed to the adverse effect of bioactive components on microbial cell membrane integrity, function and structure (**Canadanovic-Brunet** *et al.*, 2011).

The extracts of the Opuntia plant, therefore have the potential for commercialization as novel drugs for use in antimicrobial therapy (Moosazadeh *et al.*, 2014).

Conclusion and Recommendations

The result and discussion of this study clearly indicated that Opuntia ficus- indica extracts have ample potential to inhibit two pathogenic bacteria as it was seen from its strong inhibition against tested organisms. Opuntia ficus- indica shows much promise in the development of phytomedicine, having antimicrobial properties and the drug derived from Opuntia ficus- indica may have the possibility of the alternative medicinal source because of their antibacterial activity. This study also indicated that ethyl acetate and ethanol extract of peel has the highest capability to antibacterial activity against Serratia marcescens and Bacillus subtilis. In general, it can be concluded that ethyl acetate and ethanol extract of Opuntia ficus- indica is a strong inhibitor for bacterial growth. Therefore, it is recommended to identify the active ingredients of the antibacterial agent and obtaining a chemotherapist agent in different drug formulations, therefore, be used for enteric and systemic infections caused bv Serratia marcescens and Bacillus subtilis.

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تقدير خصائص مستخلصات قشور وبذور التين الشوكي كمضادت اكسده ومضادات للبكتريا

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من المعروف أن المركبات النشطة بيولوجيا المعزولة من النباتات تعمل كمضادات اكسده ومضادات للبكتيريا، في هذه الدراسة تم استخلاص متتابع للقشرة وللبذور بو اسطة الأثير البترولي، خلات الابيثيل وكحول الايثانول ٧٠% كذلك تم تقدير الفينولات الكلية، الفلافونويدات الكلية، تم اختبار مستخلص البتروليم ايثر والايثيل اسيتات والايثانول ٧٠% الناتج من الاستخلاص المتتابع لبذور وقشور ثمار النين الشوكي وتم قياس نشاط هذه المستخلصات كمضادات للكسدة بطريقة DPPH وكذلك كمضادات للبكتريا الموجبة والسالبة لجرام (الباسلس، والسيرتيا) وقد أظهرت النتائج أن كل من المستخلص الكحولي ومستخلص خلات الايثايل هما الأفضل كمضادات للأكسدة ومضادات للمترابة أو كان الموجبة للجرام وهذا يتقق في نتائج التحليل الكيميائي لكل من المستخلصين، وبالتالي يمكننا استخدام قشور وبذور التين والفعالة كمضادات المحدولة التكلية وقد يكون بمثابة مصدر للعديد من المركبات المعادة للميكروبات والموجبة للجرام وهذا يتقق في نتائج التحليل الكيميائي لكل من المستخلصين، وبالتالي يمكننا استخدام قشور وبذور التين والفعالة كمضادات المحدولة والمكافة وآمنة وقد يكون بمثابة مصدر للعديد من المركبات المعروبات

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