Phytoconstituents, antioxidant and inhibitory activity against α-amylase and α-glucosidase of *Opuntia ficus-indica*[†]

المكونات النباتية ومضادات الأكسدة والنشاط المثبط لأنزيمات الفا- اميلاز والفا -جلوكوسيداز لنبات الصبر

Lamees Rabi[*], Mohammad Altamimi[*#] & Nidal Jaradat[**]

لميس رابي، ومحمد التميمي، ونضال جرادات

*Department of Nutrition and Food Technology, Faculty of Agriculture and Veterinary Medicine, An-Najah National University, Nablus Palestine.

**Department of Pharmacy, Faculty of Medicine and Health Sciences. An-Najah National University, Nablus Palestine.

*#corresponding author: m.altamimi@najah.edu

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Abstract

Hyperglycemia and oxidative stress are associated with type 2 diabetes mellitus. The aim of this study is to evaluate the antioxidant, anti α-amylase and α-glucosidase activities of *Opuntia ficus-indica* cladodes and fruit juice. Four extracts of *Opuntia ficus-indica* cladodes were used, while fruit juice was only freeze-dried. Phytochemicals were determined of each extract and each extract was *in-vitro* evaluated for free radical scavenging, α-amylase and α-glucosidase inhibitory activities. *Opuntia ficus-indica* cladodes methanol extract has the highest contentin flavonoid, phenols and alkaloids and it also has the highest antioxidant capacity with IC_{50} value 6.16±0.59 µg/ml; compared to Trolox's IC_{50}value 2.09±1.7µg/ml. For α-amylase inhibitory effect aqueous extract was the most potent with IC_{50} value 16.98±0.77µg/ml followed by acetone extract with IC_{50} value 25.11±0.89µg/ml. All extracts showed inhibitory activity with different extent against α-glucosidase enzyme, the aqueous extract showed the most potent activity followed by acetone extract.

Keywords: Bioactive Ingredients, *Opuntia Ficus-Indica*, Antioxidant, α -Amylase and α -Glucosidase

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ملخص

ان تحلون الدم والاجهاد المؤكسد عوامل مرتبطة بمرض السكري النوع الثاني. ان هدف هذه الدراسة هو تقييم النشاط المضاد للأكسدة والمضاد لأنزيمات الفا-اميلازالفا- جلوكوسيداز لألواح وعصير ثمار الصبر. تم استخدام أربع مستخلصات مجفده من الواح الصبر وواحد من عصير الثمار. تم تحديد الكيماويات النباتية لكل مستخلص ومن ثم تقييمها من حيث نشاطها كمضادات اكسده او مثبطه لأنزيمات الفا -اميلاز والفا -جلوكوسيداز. كان مستخلص الالواح الميثانولي يحتوي على اعلى محتوي من الفلافونويدات والفينولات والالكلويدات وكذلك الأعلى كمضاد اكسدة بمقدار اقل تركيز $_{50}$ = 6.16±0.59 مايكر غ/مل مقارنة بقيمته لترولوكس0.17± 2.09 مايكروغ/مل. بينما في تثبيط انزيم الفا-اميلاز كان المستخلص المائي هو الأقوى حيث كان قيمة التركيز الأقل $_{50}$= 16.98 ±0.77 مايكروغ/مل متبوعاً بمستخلص الاستون بقيمة اقل تركيز $_{50}$= 25.11 ± 0.89مايكروغ/مل. كل المستخلصات أظهرت نشاطاً مضاداً لأنزيم الفا-جلوكوسيداز بدرجات متفاوتة ولكن المستخلص المائي هو الأقوى متبوعا بمستخلص الاستون.

الكلمات المفتاحية: مكونات نباتيه نشطه، الصبر، مضادات اكسده، مثبط انزيم اميلاز، والجلوكوسيداز.

Introduction

These days the consumption of fruits and vegetables is gaining more attention worldwide since their health benefits as cardio protective, anti-cancer, anti-diabetic and anti-obesity substance. The most important bio-active compounds include polyphenols such as flavonoids, tannins, cate-chins, β-carotene and several others were confirmed to have health bene-fits (Bazzano, Serdula & Liu, 2003). The antioxidant ability of phenolic compounds in fruits and vegetables could be attributed to their properties as reducing agents, hydrogen donors and singlet hydrogen quenchers (Zhang *et al*., 2013).

Recently phytochemicals derived from natural sources have received much attention in the treatment of diabetes for various reasons and several researchers have focused on isolation of hypoglycemic agents from many medicinal plants (Harborne, 1984 a and b). Plant polyphenols and flavo-noids are some of the naturally occurring anti diabetic agents that showed an inhibitory effect on carbohydrate hydrolyzing enzymes, by their capac-ity to bind with proteins. This phenomenon contributes to lower postpran-dial hyperglycemia in diabetes and so these compounds will form valuable

alternatives for chemical anti diabetic medications (Kokate, 2003 and Kokate, 2005).

Some focus has been done on cladode and seeds of *Opuntia ficus-indica* which contains major phytochemicals in search for new natural antioxidants. Cactus "*Opuntia* spp", belonging to Cactaceae family is native to Mexico. However, this plant is widely distributed in arid and semi-arid regions of Africa, Central America, Mediterranean region and South Africa. *Opuntia* genus has more than 200 species. Moreover, its important economic value, fast grows, less water requirement, and adaptation to nutrient-deficient soils made the cactus pear a prominently cultivated crop all over the world (Galati *et al*., 2002; Mohamed–Y, Barringer & Splittstoesser, 1996).

Diabetes has become a major health problem in the world. It is a metabolic disease characterized by a high blood glucose level and can cause other health complications, such as cardiovascular disease, neuropathy, high blood pressure, weakness, gangrene, retinopathy, nephropathy and other dysfunctions (Bhandari *et al*., 2008). One of the therapeutic approaches aimed to suppress the glucose production from carbohydrates digestion is by inhibiting digestive enzymes, mainly α-amylase and α-glucosidase. Acarbose has been used in the clinical trials as an effective inhibitor of carbohydrate hydrolysis, however, it has some side effects such as abdominal pain, diarrhea and flatulence (Chiasson *et al*., 2002).

Prolonged hyperglycemia state usually leads to the auto-oxidation of glucose and formation of advanced glycated end products which are included in the generation of reactive oxygen species (ROS) that cause lipid per-oxidation and play an important role in the production of secondary complications in T2D. Oxidative stress is considered to be a common pathway linking diverse mechanisms for the pathogenesis of micro-vascular and macro-vascular unwanted complications of diabetes disease (Palanisamy *et al*., 2011). Postprandial hyperglycemia management is an important key in the treatment of diabetes mellitus. α-Glucosidase secreted from intestinal chorionic epithelium is considered to be responsible for the hydrolysis of several carbohydrates. In 1980s, α-glucosidase inhibitors became a new class of the anti-diabetic drugs. α-Glucosidase inhibitors slow

down the process of degradation and absorption of carbohydrates by competitive blocking the activity of this enzyme. As noticed the peak concentration of postprandial blood glucose is reduced and so the blood sugar level can be well controlled. α-Glucosidase inhibitors can offer variety of advantages and has been recommend by the Third Asia--Pacific Region Diabetes Treatment Guidelines as the first-line in diabetes treatment for controlling postprandial hyperglycemia (Yin *et al*., 2014).

Materials and methods

Chemicals

Benedict’s reagent, Ninhydrin solution, Molisch’s reagent and H_2SO_4 were obtained from Alfa-Aesar, England. Di methyl sulfoxide (DMSO), Iodine, and $FeCL_3$ from Riedel-de-haen Germany. Methanol (99.9%), Trolox, DPPH, pNPG, Acarbose, α-glucosidase (Baker’s Yeast alpha glucosidase), α-amylase, DNSA and potassium phosphate from Sigma-Aldrich, USA.

Plant collection

Opuntia ficus-indica cladodes and fruits of were collected randomly from many cactus trees in the north of Palestine during June and July 2017. Taxonomical identifications were established by the pharmacogenomics Dr. Nidal Jaradat at the Pharmacognosy Laboratory at An-Najah National University and the voucher specimen codes were; Pharm-PCT-246, Pharm-PCT-1507, Pharm-PCT-1888, Pharm-PCT-548, Pharm-PCT-617, Pharm-PCT-394 and Pharm-PCT-2808.

The plant cladodes were washed well then, the peels were removed and placed in the oven at 45 ºC for drying. Finally, the cladodes were grounded using a house hold grinder into a fine powder and transferred into airtight containers and kept at 4 ºC for further tests.

Cactus fruits were harvested in the maturity stage (sugar12-14 Brix). Since all of the samples were collected from the same geographical location and were harvested in the same season with similar maturity level, the effect of climate or plant variation were ignored.

Opuntia ficus-indica cladodes extract preparation

The dried powder of *O. ficus-indica* cladodes was extracted by fractionation method using solvents sequentially. Solvents were added depending on their polarities starting with non-polar solvent; hexane then acetone (polar aprotic organic solvent) after that methanol (polar alcohol) and finally distilled water (polar protic solvent). Briefly, about 25 g of dried-powdered cladodes has been subjected first to 0.5L hexane for 72 hr in a shaker device at 100 rounds per minute at 25ºC then hexane was replaced by 0.5L acetone. Following same procedure methanol and water were used. Each organic fraction was filtered and concentrated under vacuum on a rotarry evaporator (Heidolph OB2000, VV2000, Germany) while the aqueous fraction was dried using a freeze dryer (Mill rock technology, model BT85, China). Finally, all crude fractions were stored at 4°C in the refrigerator for further use (Jaradat *et al*., 2017).

The yield of each extract fraction was calculated using the following formula:

$$Extract\ fraction\ yield\% = \frac{extract\ weight}{dry\ cladode\ weight} \times\ 100$$

Fruit juice extract preparation

The peeled fruits of nopal cactus squeezed by hand and then juices were filtered using gravity filtration and collected in a separate container to be dried using rotary evaporator and finally the powdered extract was kept in an air-tight container at 4ºC for further use.

The yield of juice extract was calculated using the following formula:

$$Juice\ extract\ yield\% = \frac{juice\ extract\ weight}{fruit\ weight} \times\ 100$$

Qualitative phytochemical screening tests for O. ficus-indica cladodes and fruit juice extracts

Phytochemical analysis was conducted on the nopal cactus cladodes different extracts and fruit juice extract to reveal the presence of phytoconstituents such as phenols, alkaloids, flavonoids, saponins, tannins, and

resins in addition to protein, carbohydrate and fibre. The following tests were conducted; Wagner's test for alkaloids, Keller-Killiani test for glycosides, gelatin test for tannins, foam test for saponin, Lieberman-Burchard's for phyto-steroids, Salkowaski test for terpenoids, ninhydrin test for protein and amino acids, Benedict's test for reducing sugars, Iodine test for starch, Molisch's test for fibre and ferric chloride test for total phenols.

Free radical scavenging assay for Antioxidant activity

Free diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay was the used to measure antioxidant activitiesof the different extract fractions (Cheung, Cheung &Ooi, 2003). Briefly, methanolic stock solution (1mg/ml) was prepared for plant extract fractions and for Trolox as a control with a potent antioxidant activity. Serial dilutions were then prepared from the previous stock solution to make the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 and 100 µg/ml). Plant working solution (1mL) was mixed with 1mL freshly prepared DPPH (0.002 g/mL) methanolic solution and 1mL methanol was then added to the previous mixture. The blank solution contained DPPH with methanol only in a ratio of 1:1. The solutions were incubated at room temperature (25ºC) in a dark place for 30 minutes. Then, their optical densities were determined by the UV/Vis spectrophotometer at 517 nm.

Antioxidant activity was calculated per the following equation:

$$DPPH\ inhibition\% = \frac{Ab - As}{Ab} \times 100$$

A_b is the recorded absorbance of the blank solution

A_S is the recorded absorbance of the sample solution or control.

In-vitro evaluation of α-amylase inhibition activity of extract fraction of Opuntia ficus-indica cladodes and fruit juice

The α-amylase inhibitory activity of each extract fraction was carried out according to the standard method with minor modification. Briefly, each extract fraction was dissolved in few milliliters of 10% DMSO and then further dissolved in a buffer ((Na_2HPO_4/NaH_2PO_4 (0.02 M), NaCl

(0.006 M) at pH 6.9) to give concentrations of 1000 µg/ml. The following dilutions were prepared (10, 50, 70, 100, 500 µg/ml). A volume of 0.2mL of porcine pancreatic α-amylase enzyme solution with concentration of (2 units/ml) was mixed with 0.2mL of the plant fraction then incubated for 10 min at 30 °C. Thereafter, 0.2mL a freshly prepared starch solution (1%) was added and the mixture was incubated for at least 3 min. The reaction was stopped by the addition of 0.2mL di nitro salicylic acid (DNSA) then the mixture was diluted with 5 mL of distilled water and heated for 10 min in a water bath at 90 °C. The mixture was left to cool down to room temperature, then the absorbance was taken at 540 nm. A blank was prepared following the same procedure replacing the plant fraction with 0.2mL of previous buffer(Nyambe-S *et al*., 2015).

Acarbose was used as positive control following the same procedure. The α-amylase inhibitory activity was calculated using the following equation:

$$\alpha - amylase\ inhibition\ \% = \frac{Ab - As}{Ab} \times 100$$

where:

A_b: is the absorbance of blank

A_S: is the absorbance of tested sample or control.

In-vitro evaluation of α-glucosidase inhibition activity of extract fraction of O. ficus-indica cladodes and fruit juice

α-glucosidase inhibitory activity of each extract fraction was carried out according to the standard protocol with some modification (Muñoz *et al*., 2002). In each test tube a reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of extract fractions (100,200,300,400and 500 mg/ml) was incubated at 37°C for 15 min. Then preincubated 20µl of (5 mM) PNPG was added as a substrate of the reaction and again incubated at 37°C for further 20 min. The reaction was terminated by adding 50 µl

$Na_2 CO_3$ (0.1M). The absorbance of the released p-nitrophenol was measured by a UV/Vis spectrophotometer at 405 nm. Acarbose with similar concentrations as plant extracts was used as appositive control.

Inhibition percentage was calculated using the following equation:

$$\alpha - glucosidase\ inhibition\ \% = \frac{Ab - As}{Ab} \times 100$$

where:

A_b: is the absorbance of blank

A_S: is the absorbance of tested sample or control.

Results

Yield (%) of each fraction extracted from plant is shown in Table 1. The highest yield was achieved in aqueous fraction which was 28.8% and 28% for cladodes and fruits respectively.

Table (1): The yield percentage of *Opuntia ficus-indica*cladodes extract fractions and fruit juice.

Extract Fractions	Plant material (g)	Extract (g)	Yields, %
Hexane	25 g	1.55g	6.2%
Acetone	25 g	1 g	4%
Methanol	25 g	0.94 g	3.76%
Aqueous	25 g	7.2g	28.8 %
Fruit juice	1000g	280g	28%

Qualitative content of bioactive compounds in plant extracts

The different extract fractions of *O. ficus-indica* cladodes and fruit juice have contained a variety of phytochemical active ingredients as shown in Table 2. It was observed that aqueous extract fraction was rich in polysaccharides, glycosides, proteins, phenols and saponins. While methanol fraction contained flavonoids, phenols, alkaloids, glycosides and steroids.

Table (2): Phytochemicals content of different extract fractions of *Opuntia ficus-indica* cladodes and fruit.

Phytochemical compound	Aqueous extract	Methanol extract	Acetone extract	Hexane extract	Fruit juice (freeze dried)
Protein & amino acids	+	-	-	-	+
Reducing sugars	-	-	-	-	+
Complex polysaccharides	+	-	-	-	+
Starch	-	-	-	-	-
Phenols	+	+	-	-	+
Tannins	-	-	+	+	+
Flavonoids	-	+	+	-	+
Saponin	+	-	-	-	+
Glycosides	+	+	+	+	+
Steroids	-	+	+	+	-
Terpenoid	-	-	+	-	-
Alkaloids	-	+	+	+	-

On the other hand, tannic compounds have appeared in both acetone and hexane extract fractions. Fruit juice extract was rich in polysaccharides but not starch, saponin, flavonoids, phenols and glycosides.

Free radical scavenging activity of extracts

Free radical scavenging activity of the four different plant extract fractions and fruit juice was evaluated by DPPH radical protocol using Trolox as a potent antioxidant. DPPH percentage inhibition results and IC_{50} are shown in Table 3. and Figures 1 and 2.

Table (3): The DPPH inhibition percentage of *Opuntia ficus-indica*cladodes extract fractions and fruit juice compared to Trolox (reference compound) and values of IC_{50}.

Conc. g/mL)	Trolox	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction	Fruit juice
			Inhibition (%)			
0	0	0	0	0	0	0
1	32.9±1.23	3.18±0.49	6.05±0.83	53.5±0	28.98±0.3	6.05±0
2	58.12±1.75	10.19±0.75	17.51±1.33	53.82±0.79	28.98±0.52	17.51±1.14
3	69.85±2.23	10.19±0.62	21.65±0	54.14±0.63	35.03±0	25.9±2.22
5	75.8±1.62	10.19±1.77	24.52±0	54.14±0.83	35.98±1.03	35.12±0
7	80.12±2.1	10.9±0.62	28.6±1.03	54.46±0.3	39.8±1.03	37±03
10	83.22±1.52	11.14±1.15	29.93±1.7	56.05±0	43.3±0	40.9±0
20	87.25±1.85	11.14±0	30.25±1.24	59.55±0	43.3±1.24	44±1.14
30	88.5±1.3	13.05±0	37.89±0.75	59.55±1.15	46.17±0.59	44.8±2.22
40	88.65±1.82	13.05±0.57	45.22±1.24	59.55±0.75	49.68±1.7	58.12±0
50	92.3±1.34	21.33±0.63	46.81±0.21	59.55±0.75	53.18±0.49	58.12±1.52
80	96.31±2.25	21.33±0.78	52.22±0.3	62.42±0.49	57.96±0	76.34±2.2
100	97.12±1.4	21.33±0.41	52.22±0	65.92±0.62	60.5±0.21	83.95±1.44
IC_{50} (µg/mL)	2.09±1.7	316227±0.72	74.13±0.53	6.16±0.59	31.62±0.65	19.49±1.08

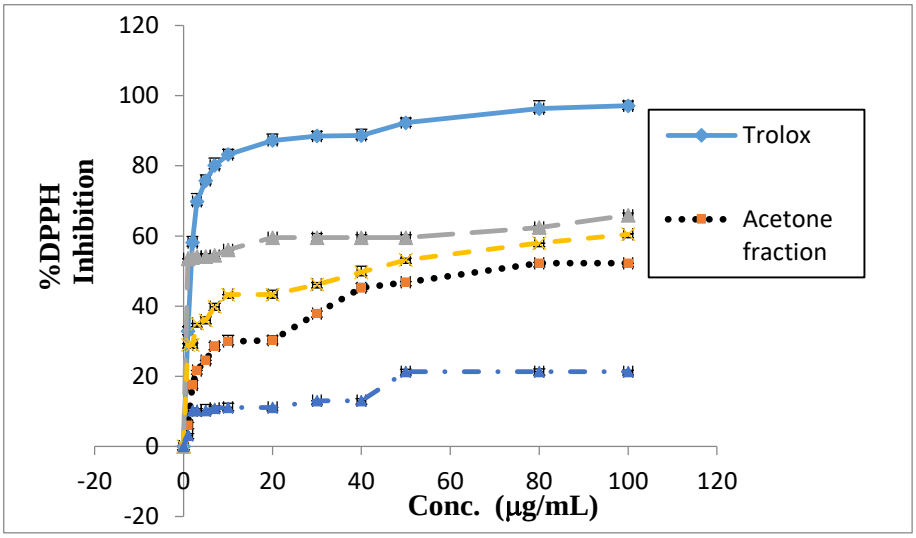

Figure (1): DPPH Inhibition % by Trolox (standard) andcladodes different extract fractions. Plant extract fractions and Trolox (1mg/ml) were serially diluted, then were mixed with 1mL DPPH (0.002 g/mL). The solutions plus blank were incubated at room temperature (25ºC) in a dark place for 30 minutes. Then, their optical densities were determined by the UV/Vis spectrophotometer at 517 nm.

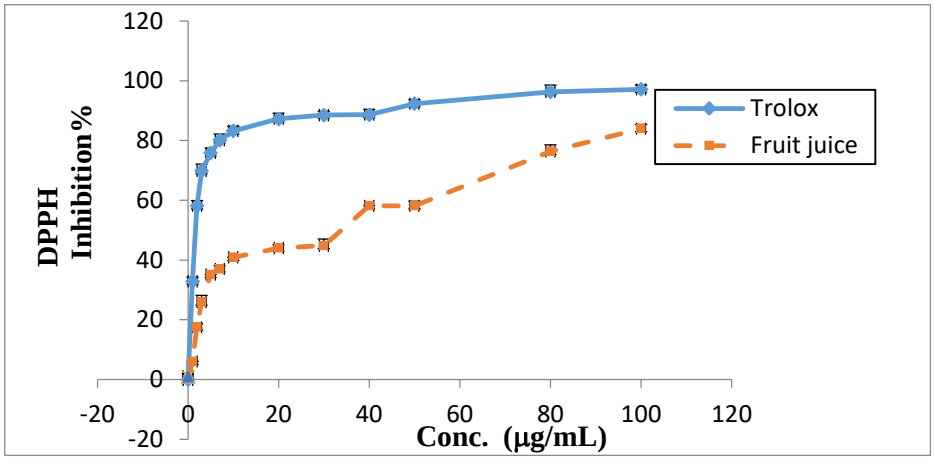

Figure (2): DPPH Inhibition % by Trolox (standard) and fruit juice extract. Fruit juice extract fraction and Trolox (1mg/ml) were serially diluted, then were mixed with 1mL DPPH (0.002 g/mL). The solutions plus blank were incubated at room temperature (25ºC) in a dark place for 30 minutes. Then, their optical densities were determined by the UV/Vis spectrophotometer at 517 nm.

It was found that fractions have wide range of antioxidant activities and variable IC_{50} ranged from 6.16±0.59 to 316227±0.72 (µg/mL) for methanol and hexane fractions, respectively. Compared to Trolox activity methanol fraction was almost 30% of the standard activity. Apart from methanol fraction, fruit juice was more active as antioxidant than cladodes materials with IC_{50} = 19.5 ±1.1.

α- amylase inhibitory activity

Table 4 shows the inhibitory activities of cladodes extract fractions and fruit juice for α – amylase. The aqueous and aceton fractions showed better activity compared to Acarbose with IC_{50} 16.98±0.77 and 25.11±0.89, respectively. While other fractions were weaker with methanol fraction has been considered very week with IC_{50} = 309±0.7.

Table (4): α-amylase inhibitory activity of *Opuntia ficus-indica* cladodes 4 fractions and fruit juice extract compared with Acarbse.

Conc. (µg/mL)	Acarbose	Hexane Fraction	Acetone Fraction	Methanol fraction	Aqueous fraction	Fruit Juice
			Inhibition %			
0	0	0	0	0	0	0
10	53.22±1.2	49.7±0.3	54.94±0.83	25±0.75	65.69±1.15	36.37±0.63
50	54.91±0.58	63.66±0.67	65.4±0.79	25±0.75	65.69±1.15	36.37±0.63
70	66.1±1.34	63.95±0.52	65.4±0.79	35.75±0.62	68.31±0.59	55.77±0
100	66.1±1.62	63.95±0.52	67.44±1.03	35.75±0.62	73.54±0.48	55.77±0.83
500	72.54±1.37	64.53±0.74	67.44±1.03	61.43±0.78	77.03±0.49	55.77±0.83
IC_{50} (µg/mL)	**28.18±1.22**	**31.62±0.55**	**25.11±0.89**	**309±0.7**	**16.98±0.77**	**97.72±0.58**

α-Glucosidase inhibitory activity

Table 5 shows the inhibitory activities of cladodes extract fractions and fruit juice for α – glucosidase. The aqueous and aceton fractions showed better activity than other fractions and fruit juice with IC_{50} = 79.43±1.16 and 125.89±1.31, respectively. However, such activities were less than 50% of the standard activity IC_{50} = 38.02 ±0.44.

Table (5): α-glucosidase inhibitory activity of *Opuntia ficus-indica* cladodes 4 fractions and fruit juice compared with Acarbose.

Conc (µg/mL)	Acarbose	Hexane Fraction	Acetone Fraction	Methanol fraction	Aqueous fraction	Fruit Juice
			Inhibitory %			
0	0	0	0	0	0	0
100	65.5±0.5	25.5±0.5	25.75±0.75	16±1	50±0.3	32.25±1.06
200	67.5±0	40.5±2.5	54±2	33±0	54±2	40.5±0
300	73.5±0.33	40.5±2.5	62.21±0.78	44.5±1.5	66.5±1.5	49.75±0.77
400	85.1±0.79	64.33±0.52	66.5±1.5	44.5±1.5	66.5±1.5	62.55±0.63
500	92.15±0.6	65.26±0.74	66.5±1.5	68.47±1.03	77.51±0.49	65.26±0.74
IC_{50} (µg/mL)	**38.02±0.44**	**251.18±1.3**	**125.89±1.31**	**501.18±1.01**	**79.43±1.16**	**199.52±0.64**

Discussion

Variations between plant extracts in their content of active phytochemicals have largely affected their properties as antioxidant and enzyme inhibitors. Products or food products that exert an activity to reduce DPPH with IC_{50}< 50 µg/mL are considered potential antioxidants (Riedl *et al.*,

2009). Therefore, *O. ficus-indica* cladodes could be considered of high antioxidant capacity especially for methanol extract and aqueous extract. Similarly, fruit juice is a high potent antioxidant. While, acetone and hexane extracts showed weak to nil antioxidant activity. This is due to that methanol and aqueous extracts contained phenols, flavonoids, proteins and complex polysaccharides more than other fractions. Phenolic compounds are plant secondary metabolites that constitute one of the most common and wide spread groups of substances in medicinal plants that act as antioxidants, which protect cells and macromolecules against oxidative damage, caused by free radicals and reactive atoms that contribute to tissue damage in the body. It has been reported that these phytochemicals deactivate the substances that promote the growth of tumors (Lattanzio, Veronica, & Cardinali, 2006; Yadav & Agarwala, 2011). In addition, flavonoids are a large group of naturally occurring phenolic compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. These compounds possess antioxidant capacity which have healthy aspect in decreasing the damaging effect of free radicals (Middleton, 1998; Amalesh, Das & Das, 2011).

Acarbose activity against saccharidases is well documented. Aqueous and aceton fractions were higher than acarbose in their activity against α-amylase, while other fractions have shown less inhibitory capacity. The main reason behind such differences may be due to presence of saponin in the aqueous and acetone extracts. Saponin compounds which are heterogeneous group of natural products found in many plant-derived foods and medicinal plants; which exerted some biological and pharmacological activities including anti-inflammatory, tonic for liver, wound healing, expectorant and hypoglycemic effects (Shi *et al*., 2004). Traditionally, saponins have been extensively used as detergents, pesticides and molluscicides, in addition to their industrial applications as foaming and surface active agents they have some beneficial health effects (Gong *et al*., 2009). Moreover, the presence of complex polysaccharides in the aqueous fraction may act as competitive inhibitor with the substrate as they are similar in their structure. Researchers have reported the hypoglycemic effect of polysaccharides; for instance, as observed with crude polysaccharides from *purslane* that had hypoglycemic effect in mice (Shi *et al*., 2000).

For acetone and hexane extract fractions the recorded inhibitory activity against α-amylase may be due to the presence of tannin compounds which have some hypoglycemic effects. Since tannins have been identified in other previous researches as active anti-diabetic and anti-adipogenic components in *Lagerstroemiaspeciosa*, a marine member of Family *Lythraceae*, which is rich in tannin that has shown hypoglycemic property (Watson & Dallwitz, 1992; Playford *et al*., 2013).

Carbohydrate digesting-enzyme inhibitors are classified as the third category of oral hypoglycemic agents. Several α-glucosidase inhibitors, such as acarbose and voglibose obtained from natural plant sources, can effectively stabilize blood glucose levels after food intake and have been used clinically in the treatment of diabetes mellitus. Only a few α-glucosidase inhibitors are commercially available. All of them contain sugar moieties and their synthesis involves tedious multistep procedures. Moreover, clinically they have been associated with serious gastrointestinal side effects (Mata *et al*., 2013). All nopal cladode extract fractions showed inhibitory activity against α-glucosidase, presenting different % inhibition activity. Similarly, fractions containing saponins and complex polysaccharides have the highest inhibition percentage, though still less than acarbose inhibition capacity.

Conclusion

Extracts obtained from *O. ficus-indica* cladodes and fruit juice showed high potency as active antioxidants and inhibitors for saccharidases namely; α-amylase and α-glucosidase with some variations among these fractions based on their contents of active phytochemicals. Based on these results *in vivo* studies are recommended to establish the dose effect of each fraction on glycaemia.

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References

- Amalesh S., Das, G. & Das, S. K. (2011). Roles of Flavonoids in Plants. *International Journal of Pharmaceutical Science and Technology, 6*: 12-35.
- Bazzano, L.A., Serdula, M.K., & Liu S. (2003). Dietary intake of fruits and vegetables and risk of cardiovascular disease, *Curr. Atheroscler. Rep. 5*: 492–499.
- Bhandari M.R, Jong-Anurakkun N., Hong G., & Kawabata J. (2008). Alpha-glucosidase and alpha-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergeniaciliata,* Haw.). *Food Chem. 106*: 247–252.
- Cheung, L, Cheung, P.C, & Ooi, V.E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chem. 81*(2): 249-255.
- Chiasson J.L., Josse R.G., Gomis R., Hanefeld M., Karasik A., & Laakso M. (2002). Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet.359*: 2072–2077.
- Galati, E. M., Tripodo, M. M., Trovato, A., Miceli, N., & Monforte, M. T. (2002). Biological effect of *Opuntia ficusindica* (L.) Mill. (Cactaceae) waste matter: Note I: diuretic activity. *J. Ethnopharmacol. 79*: 17–21.
- Gong, F., Li, F., Zhang, L., Li, J., Zhang, Z. & Wang, G. (2009). Hypoglycemic effects of crude polysaccharide from purslane. *Int. J. Mol. Sci. 10* (3): 880-888.
- Harborne J.B. (1984 a). Phytochemical methods - a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall, p. 4-16.
- Harborne J.B. (1984 b). Phytochemical Methods: a guide to modern techniques of plant analysis. 2ed Chapman and Hall Ltd, London, New York, p 1-32.

- Jaradat N.A, Al-Masri M., Zaid A.N, Hussein F., Al-Rimawi F., Mokh A.A., Mokh A. J. & Ghonaim S. (2017). Phytochemical, antimicrobial and antioxidant preliminary screening of a traditional Palestinian medicinal plant, *Ononis pubescens L*. *European Journal of Integrative Medicine. 14*: 46-51.
- Kokate, C. K. (2003). Practical Pharmacognosy. New Delhi: Vallabh Prakashan. Extraction and Phytochemical Screening, pp 157-159.
- Kokate C. K. (2005). A textbook of practical pharmacognosy. Vallabh Prakashan, 5th Edition, 105-111.
- Lattanzio V., Veronica M.T. L, A. Cardinali. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. In Phytochemistry*: Advances in Research*, Imperato F. (Ed). pp 23-67. Research Signpost, Kerala, India.
- Mata R., S. Cristians, S. Escandón-Rivera, S. Juárez-Reyes K, & Rivero-Cruz I. (2013). Mexican anti diabetic herbs: valuable sources of inhibitors of α-glucosidases, *J. Nat. Prod. 76*: 468–483.
- Middleton E. Jr. (1998). Effect of plant flavonoids on immune and inflammatory cell function. Advances in Experimental Medicine and Biology 43**:** 175-182.
- Mohamed–Y, Y., Barringer, S. A., & Splittstoesser, W. E. (1996). A note on the uses of Opuntia spp. in Central/North America. *J Arid Environ 32*: 347–353.
- Muñoz, U., Atha, D.E. Ma, J. Nee, M, H. & Kennelly, E. (2002). Antioxidant capacities of ten edible north american plants. *Phytotherapy Research, 16*: 63-65.
- Nyambe-Silavwe, H., Villa-Rodriguez, J.A., Ifie, I., Holmes, M Aydin, E Jensen, J.M. & Williamson, G. (2015) Inhibition of human α-amylase by dietary polyphenols. Journal of Functional Foods 19: 723-732.
- Palanisamy U.D., Ling L.T., Manaharan T., & Appleton D. (2011). Rapid isolation of geraniin from Nepheliumlappaceum rind waste and its anti-hyperglycemic activity. *Food Chem. 127*(1): 21-27.

- Playford R.J., C. Pither, R. Gao, & Middleton SJ. (2013) Use of the α-glucosidase inhibitor acarbose in patients with 'Middleton syndrome': normal gastric anatomy but with accelerated gastric emptying causing postprandial reactive hypoglycemia and diarrhea, *Can. J. Gastroenterol. 27***:** 403–404.
- Rabi, L. Altamimi, M. & Jaradat, N. (2018). Phytoconstituents, antioxidant and inhibitory activity against α-amylase and α-glucosidase of *Opuntia ficus-indica*. Thesis. An-Najah National University.
- Riedl, K.M., Carando, S., Alessio, H.M., McCarthy, M., & Hagerman, A.E. (2009). Antioxidant activity of tannins and tannin-protein complexes: assessment in vitro and in vivo. Free Radicals in food. In: Chemistry, nutrition, and health effects. Morello M. J, Shahidi F, & Ho Ch. (eds), 14: 188-200.
- Shi, J., Arunasalam, K., Yeung, D., Kakuda, Y., Mittal, G., & Jiang, Y. (2004). Saponins from edible legumes: Chemistry, processing and health benefits. *Journal of Medicinal Food 7*:67-78.
- Shi, S., Huang, Y., Tan, F., He, X. & Boufford, D. E. (2000). Phylogenetic analysis of the Sonneratiaceae and its relationship to Lythraceae based on IT Ssequences of nrDNA. *J Plant Res. 113*: 253-258.
- Watson, L., & Dallwitz, M. J. (1992). The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version 25th.
- Yadav, R.N. & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of Phytology 3*(12): 10-14.
- Yin, Z., Zhang, W, Feng, F., Zhang, Y., & Kang, W. (2014). α-Glucosidase inhibitors isolated from medicinal plants. Food Science and Human Wellness 3 (2014) 136–174
- Zhang R., Zeng, Q., Deng, Y. Zhang M, Wei Z, Zhang Y, & Tang X. (2013). Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. *Food Chem. 136*: 1169–1176.