Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Unveiling The Anti-Alzheimer, Antioxidant, Anti-Inflammatory, Antiviral Therapeutic Functionality Of Polysaccharides Extracted From Opuntia Ficus

Soha A. Murad[a]*, Dina N. Abd-Elshafy[b,c], Doha H. Abou Baker[d], Mahmoud M. Bahgat[b,e] , Eman A. Ibrahim[a] , Alaa A .Gaafar[a], Zeinab A. Salama[a]

[a]*Plant Biochemistry Department, National Research Centre (NRC), 33 EL Bohouth St. (Former El Tahrir St.), Dokki, Giza, Egypt, P.O.1262.*

[b]*Immune and Bio markers for Infection (CEAS), National Research Centre (NRC), 33 EL Bohouth St. (Former EL Tahrir St.), Dokki, Giza, Egypt, P.O.1262.*

[c]*Department of Water Pollution Research, National Research Centre (NRC), 33 EL Bohouth St. (Former EL Tahrir St.), Dokki, Giza, Egypt, P.O.1262.*

[d]*Medicinal and Aromatic Plant Department, National Research Centre (NRC), 33 EL Bohouth St. (Former El Tahrir St.), Dokki, Giza, Egypt, P.O.1262.*

[e]*Therapeutic Chemistry Department, National Research Centre (NRC), 33 EL Bohouth St. (Former El Tahrir St.), Dokki, Giza, Egypt, P.O.1262*

Abstract

Opuntia ficus peels or Prickly pear peel (PPP) is the waste byproduct obtained during fruit processing, the present study aims to extract polysaccharides from PPP and analyze it by HPLC. The biological potential activities as antioxidant, anti-inflammatory, antiviral, and anti Alzheimer of these polysaccharides were examined. Polysaccharides were extracted from PPP using hot water. Its composition was analyzed by HPLC. Profiles of 14 different natural mono sugars were identified. Stachyose, galactose, xylose, and sucrose were the most abundant sugars. Results indicated that the polysaccharides of PPP extract have antioxidant activity against DPPH and ABTS free radical. The scavenging activity of (150 µg /ml) of polysaccharides appeared to be interesting compared to synthetic antioxidants. Polysaccharides of PPP had 77.77% anti-inflammatory activity which is caused by inhibiting the heat-induced albumin denaturation and anti-Alzheimer effect (42.02%) compared to Donepezil drug (59.50%) in a dose-dependent manner. On the other hand polysaccharides of PPP extract showed no activity when tested against HSV-1 and Influenza A by plaque reduction assay. Thus, it could be suggested that polysaccharides PPP demonstrated potential biological activities as an antioxidant, anti-inflammatory, and anti-Alzheimer activities.

Keywords: *Opuntia ficus* peels; polysaccharides bioactivity; HPLC.

1. Introduction

Fruit wastes have increased importance in the quest for natural pharmaceutical by the biological researcher. It is safer, cheaper, and most active. Of particular interest are wastes that have an anti-Alzheimer, anti-inflammatory, hepatoprotective, antihypertensive, and antioxidant [1-3]. The waste products are attractive biomass resources for biorefining, which uses integrated biotechnology techniques to produce numerous chemicals from biomass resources as part of a bioeconomy [3].

Prickly pear belongs to the family Cactaceae. The fruit of prickly pear is a source of many compounds. It contains a diversity of amino acids including proline, taurine, serine, glutamine, and valine. Also, it contains vitamins like ascorbic acid (vitamin C), tocopherols (fat-soluble vitamin E) and minerals like calcium, magnesium, potassium, and beta-carotene. The fruit has a high sugar content of nearly equal amounts of glucose and fructose [4-7]. The prickly pear peel had an antioxidant activity due to the presence of vitamin C, polyphenols, flavonoid compounds (e.g., Isorhamnetin, quercetin, and Kaempferol), taurine as well as pigments (betalains) [8, 9]. Betalains are water-soluble pigments that have two derivatives that are

*Corresponding author e-mail: sohaahmed77@gmail.com.; (Soha A. Murad).
Receive Date: 04 July 2022, Revise Date: 07 August 2022, Accept Date: 14 August 2022.
DOI: 10.21608/ejchem.2022.148208.6435

present in PPP: betacyanin, which gives the red-purple color, and betaxanthin. These pigments have important antioxidant activities with no toxic effect on humans [9, 10]. Besides, prickly pear as a source of betalain has potency as an anticancer activity [10]. Pheophorbide and pyropheophorbide compounds isolated from Opuntia ficus ethanol extract were tested for their antiviral activities against herpes simplex, poliovirus type 1, and Influenza virus. The result showed significant antiviral activity against both herpes simplex and influenza virus but this hadn't shown any activity against poliovirus type 1[11].

Mucilages are polysaccharides that characteristic of members of the Cactaceae family [12]. Polysaccharides have many bioactivities including immunomodulating, anticancer, antimicrobial, hypocholesterolemic, and hypoglycemic effects [13]. The type of sugar, the chemical composition, the molecular weight, and the degree of branching might be the cause of the activities of the polysaccharide molecule [14].

This study aimed to identify the monosugar of polysaccharides by HPLC and evaluate the antioxidant properties, anti-inflammatory, antiviral, and anti-Alzheimer of *Opuntia ficus* polysaccharides.

2. Materials and Methods

2.1. Plant materials

Peels of red prickly pear fruits were obtained from the local market in Giza, Egypt. The red prickly pear peel fruits were cut into small pieces and stored in a refrigerator until extraction.

2.2. Chemicals and Reagents

High analytical grade chemicals were used in the present study. $ABTS^{\cdot+}$ (2, 2'-azinobis (3-ethyl benzothiazoline - 6 - sulfonic acid)), DPPH: 2, 2-diphenyl-1-picrylhydrazyl, BHT: Butyl Hydroxytoluene and, potassium ferricyanide, (Sigma Chemical Co., St. Louis, MO, USA).

2.3. Preparation of samples

The fresh samples were dried first in the air and then in an oven at $40^{\circ}c$. Dried samples were powdered for the routine analysis of major constituents.

2.4. Chemical assays

2.4.1. Extraction of polysaccharides

Polysaccharides from Prickly pear peels were carried out according to Abd El Baky et al. [15].

2.4.2. Identification of polysaccharide sugars by HPLC

One ml (2N) HCl was used to hydrolyze the polysaccharide (0.4 mg) in a sealed tube at 100 °C for 1 h. The acid was removed by evaporator on a water bath at 40 OC. Then, the hydrolyzed monosaccharides were extracted with petroleum ether plus water. Analysis of the purified hydrolysis monosaccharides was carried out by HPLC (Agilate Pack, serics1200), equipped with Aminex carbohydrate Hp-87c column (300mm × 7.8 mm). Our mobile phase was deionized water at a flow rate of 1 ml/ min. The identification of chromatography peaks was made by comparing the retention times with the respective retention times of known standard reference material. Retention time and peak area were used to calculate the sugar concentration by the data analysis of Agilent Packard [16].

2.5. Biological evaluation of polysaccharide prickly pear extract

2.5.1. Antioxidant activity

2.5.1.1. In vitro DPPH free radical-scavenging assay

The ability of polysaccharides extracted from prickly pear peels to scavenge DPPH. The determination of free radicals was done according to Ye et al. [17].

2.5.1.2. In vitro ABTS free radical scavenging assay

ABTS+ free radical scavenging capacity (%) was measured according to Arnao et al. [18]. Trolox was used as a positive control.

2.5.1.3. In vitro total antioxidant capacity assay

One ml of a polysaccharide extract from prickly pear peel and standard (ascorbic acid) (100 to 400 µg / mL) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate, and 4 mM ammonium molybdate). Tubes were incubated at 95°C for 90 min. After cooling, the absorbance of each sample was measured at 695 nm [19].

2.5.2. In-vitro anti-inflammatory activity

Anti-inflammatory activity of different concentration of polysaccharide prickly pear peel and standard drug diclofenac sodium was measured according to Rahman et al. [20].

2.5.3. In vitro antiviral activity

Virus: a) Herpes simplex virus type 1, b) Influenza A virus: The PR8-H1N1 influenza virus was generated in transfected MDCK cells co-cultured with 293T cells with plasmids encoding the eight peptides of the virus [21].

Cells: VERO (African green monkey kidney cells) and MDCK (Marine derby canine kidney cells: both were propagated in DMEM medium containing 10% fetal bovine serum and 1% antibiotic- antimycotic mixture.

2.5.4. Cytotoxicity assays

This test was made to know safe doses of samples that will not harm cells; this was observed by cell morphology technique Aquino [22].

2.5.5. Plaque infectivity count assay

To determine a % reduction in viral count resulting from the effect of samples under test, plaque infectivity count assay was made according to Tebas et al. [23].

2.5.6. Anti-Alzheimer's activity

By using the colorimetric method an assessment of cholinesterase inhibition was carried out. At 412 nm the change in absorbance was measured on a spectrophotometer according to Ellman [24].

2.6. Statistical analysis

All experiments were carried out in triplicates. Statistical analysis was made by analysis of variance (ANOVA) and Duncan's test at $P<0.05$ using Costas-Statistics Software Anonymous [25].

3. Results and Discussion

3.1. Identification of mono sugars of Prickly pear peel by HPLC

Table 1 is showing the mono-sugars in polysaccharide fraction. The most abundant monocular in the prickly pear peel is galactose 3.734%, Xylose 1.679%, stachyose 1.652%, mannose 1.034%, Raffinose 1.016%, galacturonic 0.948%, arabinose 0.608% , L-ramose 0.559% fructose 0.420%, ribose 0.357%, glucose 0.233% , Mannitol 0.08% and sorbitol 0.06%. Whereas; maltose was not detected in prickly pear peel. The prickly pear peel is waste that is rich in dietary fiber, minerals, vitamins, polysaccharide, protein and phenol compound [26].

According to Paulsen & Lund [27] they found that the acidic polysaccharide of O. ficus indica were L-arabinose,D-galactose, L-rhamnnose, D-xylose and D-galacturonic acid. On the other hand, Matsuhiro (28) assayed that the mucilage monosugars of Opuntia ficus indica were galacturonic acid (1.0), rhamnose (1.7), xylose (2.5), and galactose (4.1) in the molar ratio.

3.2. Antioxidant activity of polysaccharides prickly pear peel extract

The antioxidant properties of extracts of polysaccharides prickly pear peel extract were determined using three different methods such as DPPH·, ABTS·, and total antioxidant activity assays

Table 1

Identification of mono-sugar in polysaccharides PPP extract

Sugares	**Concentration %**
Glucuronic	0.527
Stachyose	1.652
Galacturonic	0.948
Raffinose	1.016
Sucrose	1.278
Maltose	-
Lactose	0.166
Glucose	0.233
Xylose	1.679
Galactose	3.734
L-Raminose	0.559
Mannose	1.034
Fructose	0.420
Arabinose	0.608
Mannitol	0.084
Sorbitol	0.06
Ribose	0.3570

3.2.1. DPPH scavenging activity

The results showed significant differences by DPPH· assay in antioxidant activity. The tested extract free radical scavenging was measured (Table 2). The higher activity of polysaccharides PP extract was 44.52% at 150 µg/ml followed by 27.50% at 100 µg / ml and 26.18% at 50 µg/ml compared to synthetic antioxidant BHT.

3.2.2. ABTS·+ scavenging activity

The ABTS·+ discolorations method was used to determine the antioxidant capacities of polysaccharides prickly pear peel. The results were shown in (Table 2). Polysaccharides of prickly pear peel extract showed the highest antioxidant activities at a concentration of 150 µg / ml (77.91% ± 3.16) compared to synthetic substances trolox (122.3±1.13).

Table 2

DPPH and ABTS scavenging activity of polysaccharide PPP extract

Prickly pear peel	Concentration	ATBS	DPPH
polysaccharide	50µg	49.44±0.34d	26.18± 1.49d
	100 µg	61.03±0.65c	27.50± 1.10d
	15 0µg	77.91±3.16 b	44.52± 1.10c
control		Trolox (st)	BHT
	50 µg	40.76±0.37d	45.86± 4.64c
	100µg	81.53±0.75b	84.2± 4.39b
	150 µg	122.3±1.13a	113.87± 10.83a
LSD		3.68	13.32

Mean ±SE (n=3). Different letters indicate significant differences at $P < 0.05$.

3.2.3. Total antioxidant activity

The total antioxidant activity of polysaccharides extract was followed by the reduction of phosphomolybdic acid (Table 3). The obtained results showed the antioxidant activity increases relative to the concentration of polysaccharides 161.50 µg / ml and were equivalent to 243.46 µg / ml ascorbic acid.

Table 3

Total Antioxidant activity of polysaccharide from PPP extract

Prickly pear	Concentration µg/ml			
	100	200	300	400
polysaccharide	93.01±0.27f	113.09±1.19e	139.28±8.24cd	161.50±0.68b
Control (vit.c)	127.97±8.93ghi	147.37±12.15f	230.14±6.2ab	243.46±3.57a
LSD		15.74		

Yang et al. [29] found that polysaccharides play important roles in immunity, digestive function, and detoxification. Reactive oxygen species (ROS) perform a substantial role in oxidative stress-related to the pathogenesis of diverse important diseases. The production of free radicals is balanced by the antioxidative defense system [30].Water-soluble polysaccharides from *Opuntia stricta* have strong antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl free radical and inhibition reducing power capacity with 94.9% [31]. Polysaccharides PPP extract represents thus a strong electron donor that could react with free radicals and convert them to more stable products and terminate the radical chain reaction. According to Gaafar et al. [32, 33] they found that the antioxidant properties of plant wastes extracts have been attributed to the presence of bioactive compounds, which have great potential as antioxidant agents [34, 35].

3.3. In vitro anti-inflammatory activity of polysaccharides PPP extract

It is reported that inflammation is caused due to the denaturation of proteins. The ability of polysaccharides of prickly pear peel extract in inhibiting heat-induced albumin denaturation was

studied (Figure 1). Significant changes in the anti-inflammatory activity of polysaccharides PPP extract at different concentrations were observed (50-150 ug / ml).The Maximum inhibition (77.77%) was observed from polysaccharides PPP extract at 150 ug / ml. A significant high percentage of anti-inflammatory inhibition was recorded compared to Diclofenac Sodium, a standard anti-inflammation drug 85.5 ug / ml. Hence, the presence of bioactive compounds in the extract of polysaccharides made it effective as antioxidant and anti-inflammatory activity.

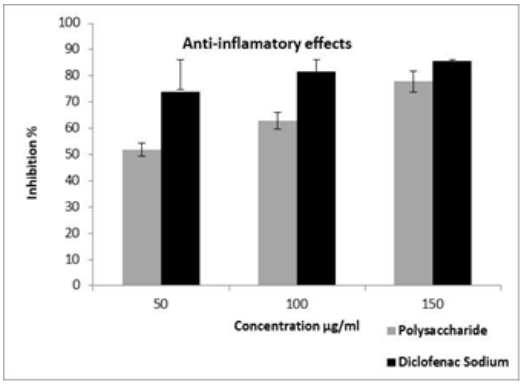

Figure 1:Anti-inflammatory activity of polysaccharide prickly pear peels

These high molecular weight polymers may exhibit therapeutic properties as anti-tumor [33, 34] anti-inflammatory [36, 37] and anti-microbial activities [36]. This all was attributed to the physicochemical properties of each polysaccharide molecule a well-known as the constitution of the lactose residues. Numerous consider groups have been affected in extracting polysaccharides from concerning plants and indus¬trial by-products for their valorization [37,38].The high percentage of mono-sugars of the prickly pear peel extract showed that it has some nutritional advantages; different reports showed that these polysaccharides have multiple biological activities such as antioxidant, antitumor and anti-inflammatory effects as formerly reported by Chavez-Santoscoy [11] due to the existence of not only phenolics but also other agents such as polysaccharides.

3.4. In vitro Antiviral activity of prickly pear peel polysaccharides extract

As presented in (Table 4 & 5) the concentrated water extract of the peel of Opuntia ficus indica which isolated as the pure fraction of polysaccharides. Cytotoxicity test showed that it has high safety as it did not show any morphological changes on the cells at all the concentrations used. On testing the effect of this compound on HSV-1 and PR8 - H1N1 influenza virus it showed no antiviral activity at all the concentrations used.

The present work confirmed the strong antioxidant and anti-inflammatory properties of water extract as was previously reported by Barreira (38).The anti-inflammatory activity of polysaccharide in lipo-polysaccharine-primed RAW264.7 cells inhibited nitric oxide by the phosphorylation of the three signal proteins p-38, ERK (extracellular signal-regulated kinase) and JNK(c-JUN N-terminal kinase), and nuclear factor-κB [39].

3.5. In vitro cytotoxicity activity of prickly pear peel polysaccharides extract

The cytotoxicity test showed that it has high safety as it did not show any morphological changes on the cells at all the concentrations used (Table 4). On testing the effect of the PPP extract on HSV-1 and PR8-H1N1 influenza virus it did not lead to any reduction of the induced cytopathic effects by the virus on the MDCK cells in comparison to infected untreated wells at all the used concentrations (Table 5).

Table 4

Toxicity of polysaccharides PPP extract

	Concentrations (µg/100µl)									
	10	20	30	40	50	60	70	80	90	100
safety	-	-	-	-	-	-	-	-	-	-

Table 5

Antiviral activity of polysaccharides PPP extract

Concentration (µg/ml)	**% inhibition PR8-H1N1**	**% inhibition HSV-1**
50	0	0
100	0	0
150	0	0
200	0	0

3.6. Anti-Alzheimer's activity (anticholinesterase inhibitory activity) of PPP polysaccharides extract

Results in (Table 6) revealed a significant increase in anticholinesterase inhibitory activity with increasing the concentration of inhibitor (25-100 µg / ml). Polysaccharides PPP extract showed

anticholinesterase activity (42.02%) at 100 µg /ml compared to standard anticholinesterase drug Donepezil (59.5%) at 100 ug / ml.

It is often many experts have reported that polysaccharides have been utilized for the| prevention of regenerative qualities and function as memory enhancers this may be attributed to that glucose is vital to brain function and is often disturbed in those with depression, anorexia, and bulimia. Galactose or mannose is also polysaccharides that are a strong Al-Alzheimer agent when mixed with glucose. However, when mixed with fructose, mannose becomes of great help to eliminate any inflammation and repair any tissue damage [40, 41].

Table 6

Inhibition Percentage of AChE activity of polysaccharides PPP extract

Concentrations	**25 µg/ml**	**50 µg/ml**	**100µg/ml**
Prickly pear Polysacharides	36.1±0.85e	40.03±1.45d	42.02±0.86d
Donpezil (drug)	49.09±0.9c	56.4±0.3b	59.5±1.2a
LSD	2.23		

4. Conclusion

In conclusion, Prickly pear peels can be viewed as an important source of bioactive polysaccharides. Many polysaccharides have renovated qualities and role as memory enhancers this may be attributed to that glucose is vital to brain function. Galactose or mannose is another polysaccharide that is a predominately powerful anti-inflammatory agent when binary with glucose. Further studies in vivo are needed to prove its efficacy in the treatment of Al-Alzheimer and antiviral activity.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgments

The plasmids used to generate the recombinant influenza virus used in the study were generously provided by Dr. Richard Webby from St.Jude Children Hospital, Memphis, USA through a signed material transferred agreement with Prof. Mahmoud M. Bahgat. This work was supported and funded by the project entitled “Optimization of the agricultural residues of food industries as a source of bioactive compounds” PI: Prof.Dr. ‘Zeinab A. Salama’ and funded by the National Research Centre (NRC), Egypt.

7. References

[1] El Gengaihi, S. and Baker, H. D. Grape Seeds Extract as Brain Food: A Review. Int. J. Pharm. Clin. Res. 2017; 9: 77-85. https:// doi.org/ 10.25258/ijpcr.v9i1.8270

[2] Salama, A. Z., Aly, F.H., Mohasib, R. M. M., El Baz, K. F. Prickly Pear "Phyto-Chemicals and Potential Health Benefits" in: Pawan, K. (Eds.), Phyto-chemicals and Ethanobotany. ISBN: 978-93-86841-93-3 Edition: Published by: Discovery Publishing House Pvt. Ltd., New Delhi (India). 2019; PP: 6- 40.

[3] Fidelis, M., de Moura, C., Tufy, J. K., Pap, N., Mattila, P., Mäkinen, S., et al. Fruit Seeds as Sources of Bioactive Compounds: Sustainable production of high value-added ingredients from by-products within circular economy. Molecules. 24, 2:54 (2019);.https:// doi.org/10.3390/molecules24213854

[4] El-Mostafa, K., El Kharrassi, Y., Asmaa, B., Andreoletti, P., Vamecq,J., El Kebbaj, M. S., et al. Nopal Cactus (Opuntia ficus-indica) as a source of bioactive compounds for nutrition, health and disease. Molecules.; 19 (9), 14879–14901 (2014).https:// doi.org/ 10.3390/molecules190914879

[5] Butera, D., Tesoriere, L., Di-Gaudio, F., Bongiorno, A., Allegra, M., Pintaudi, M. A., et al. Antioxidant activities of Sicilian prickly pears (Opuntia ficus indica) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J. Agric Food Chem., 50, 6895–6901 (2002). https:// doi. Org/10.1021/jf025696p

[6] Castellar, R., Obón, M. J., Alacid, M., Fernández-López A. J. Color properties and stability of betacyanins from Opuntia fruits. J Agric Food Chem., 51, 2772–2776 (2003). https://doi.org/10.1021/jf021045h

[7] Griffithm P. M. The origins of an important cactus crop, Opuntia ficus-indica (Cactaceae): new molecular evidence. Am. J. Bot., 91(11), 1915-1921 (2004).https:// doi.org/ 10.3732/ajb.91.11.1915

[8] Livrea, A. M.and Tesoriere L. Antioxidative effects of cactus pear [Opuntia ficus-indica (L) Mill] fruits from Sicily and bioavailability of betalain components in healthy humans. Acta Hortic., 811, 197–204 (2009). https://doi.org/10.17660/ActaHortic.2009.811.24

[9] Abou-Elella, M. F. and Ali, M .F. R. Antioxidant and anticancer activities of different constituents

extracted from Egyptian prickly pear cactus (Opuntia ficus Indica) peel. Biochemistry & Analytical Biochemistry., 3 (2), 2-9 (2014).https://doi. Org/10.4172/2161-1009.1000158

[10] Bouslama, L., Hayashi, K., Lee, B. J., Ghorbel, A., Hayashi T. Potent virucidal effect of pheophorbide a and pyropheophorbide a on enveloped viruses. J Nat Med., 65 (1), 229-33. (2011) https:// doi.org/ 10.1007/s11418-010-0468-8

[11] Chavez-Santoscoy, A. R., Gutierrez-Uribe, A. J., Serna-Saldívar, O. S. Phenolic composition, antioxidant capacity and in vitro cancer cell cytotoxicity of nine prickly pear (Opuntia spp.) juices. Plant Foods Hum. Nutr., 64, 146–152 (2009).https:// doi.org/ 10.1007/s11130-009-0117-0

[12] Nobel, S. P., Cavelier, J., Andrade, L. J. Mucilage in Cacti: Its rapoplastic capacitance, associated solutes, and influence on tissue water relations. J. Exp. Bot., 43 (250), 641–648 (1992). https://doi.org/10.1093/jxb/43.5.641

[13] Samee, U., Anees, A. K., Faryal, S., Yuanda, S. Sources extraction and biomedical properties of polysaccharides, Foods., 8 (304), 2-23 (2019).https:// doi.org/ 10.3390/foods8080304

[14] Wang, J., Hu, S., Nie, S., Yu, Q., Xie, M. Reviews on mechanisms of In vitro antioxidant activity of polysaccharides, Oxid. Med. Cell. Longev., 1-13 (2016). https://doi.org/10.1155/2016/5692852

[15] Abd El Baky H., Hanaa El Baz K. F., EL-Latife S. A. (Induction of Sulfated Polysaccharides in Spirulina platensis as Response to Nitrogen Concentration and its Biological Evaluation. J. Aquac. Res. Dev, 5, 206 (2013). doi:10.4172/2155-9546.1000206

[16] Liu, R. J., Chen, J.M., Lin, W. C. Characterization of polysaccharide and volatile compounds produced by kefir grains grown in soymilk. J. Food Sci., 67(1), 104-108 (2002). https://doi.org/10.1111/j.1365-2621.2002.tb11367.x

[17] Ye, H., Zhou, C., Sun, Y., Zhang, X., Liu, J., Hu, Q., Zeng X. Antioxidant activities in vitro of ethanol extract from brown seaweed Sargassum pallidum. Eur. Food Res. Technol., 230 (1), 101-109 (2009).https:// doi. org/10.1007/s00217-009-1147-4

[18] Arnao, B. M., Cano, A., Acosta, M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem., 73, 239–244 (2001). https://doi.org/10.1016/S0308-8146(00)00324-1

[19] Prieto, P., Pineda, M., Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269, 337-341 (1999).https:// doi.org/ 10.1006/abio.1999.4019

[20] Rahman, H., Eswaraiah, C. M., Dutta, M. A. In-vitro anti-inflammatory and anti-arthritic activity of Oryza sativa Var. Joha Rice (An Aromatic Indigenous Rice of Assam). Am Eurasian J Agric Environ Sci., 15(1),115-121 (2015). https://doi.org/ 10.5829/idosi.aejaes.2015.115.121

[21] Hoffmann, R., Lee, G. C., Ramasamy, B. Public policy and olympic success. ApplEconLett., 9,545-548(2002). https://doi.org/10.1080/13504850110102784

[22] Aquino, R., De Simone, F., Pizza, C., Conti, C., Stein, L. M. Plant metabolites; structure and in vitro antiviral activity of Quinovic acid glycosides from UncariaTomentosa and Guettardaplatypoda. J. Nat. Prod., 52, 679-685 (1989). https:// doi. Org/ 10.1021/np50064a002.

[23] Tebas, P., Stabell, C. E., Olivo, D. P. Antiviral susceptibility testing with a cell line which expresses beta-galactosidase after infection with herpes simplex virus. Antimicrob. Agents Chemother., 39 (6),1287- 1291 (1995).https: doi.org/ 10.1128/aac.39.6.1287.

[24] Ellman, L. G.,Lourtney, K. D., Andres, V., Gmelin G. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7, 88-95 (1961)https:// DOI: 10.1016/0006-2952(61)90145-9

[25] Anonymous, A. Cohort Soft Ware Crop. Costat user manual version 1989; 3.03, Berkeley CA, USA.

[26] Anwar M.M., Sallam, M. E. Utilization of prickly pear peels to improve quality of pan bread. Arab J. Nucl. Sci. Appl, 94(2), 151-163 (2016)

[27] Paulsen, S. B., Lund. S. P. Water-soluble polysaccharides of Opuntia ficus indica cv "Burbank's Spineless. Phytochemistry, 18, 569–57 (1979). https://doi.org/10.1016/S0031-9422(00)84262-4Get rights and content

[28] Matsuhirom, B., Lillo, E. L., Saenzb, C., Urzu, C.C., Zarate, O. Chemical characterization of the mucilage from fruits of Opuntia ficus indica. Carbohydrate Polymers, 63(2),263-267 (2006).http://repositorio.uchile.cl/handle/2250/120099

[29] Yang, Y., Liu, D., Wu, J., Chen, Y., Wang, S. In vitro antioxidant activities of sulfated polysaccharide fractions extracted from Corallina officinalis. Int. J. Biol. Macromol. 49(5), 1031–1037 (2011). https://doi.org/10.1016/j.ijbiomac.2011.08.026

[31] Nita, M., Grzybowski A. The role of the reactive oxygen species and oxidative stress in the

pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid. Med. Cell. Longev. 2016; 1-23.https:// doi.org/ 10.1155/2016/3164734

[31] Koubaa, M., Ktata, A., Barba, J.F., Grim,N., Mhemdi, H., Bouaziz , F., Driss, D., Chaabouni , E.S. . Water-soluble polysaccharides from Opuntia stricta Haw. Fruit peels: recovery, identification and evaluation of their antioxidant activities. Int. Agrophysics., 29(3),299-306 (2015). https://doi.org/10.1515/intag-2015-0035

[32] Gaafar A. A., Khaled M. Mahmoud and Zeinab A. Salama. Antioxidant potential activity and cytotoxicity effects of different parts of peanuts (arachis hypogaea l.). Int J Pharm Bio Sci., 6(3), (P) 19 – 32 (2015).

[33] Gaafar A. A., Mohsen S. Asker, Zeinab A. Salama, Bagato O., Ali M. A. In-vitro, antiviral, antimicrobial and antioxidant potential activity of Tomato pomace. Int. J. Pharm. Sci. Rev. R., 32(2),262-272 (2015 a).

[34] Riffat, B., Muhammad, R.K., Moniba, S., Saima, A., Zartash Z.. Estimation of phytochemical constituents and in vitro antioxidant potencies of Brachychiton populneus (Schott & Endl.) R. Br. BMC Chemistry., 13, 32 (2019)https:// doi.org/10.1186/s13065-019-0549-z

[35] Gaafar A. A., Zeinab A. Salama, Mohsen S. Askar, Dardiri M. El-Hariri and Bakry A. Bakry. In Vitro antioxidant and antimicrobial activities of Lignan flaxseed extract (Linum usitatissimum, L.). Int. J. Pharm. Sci. Rev. Res., 23(2) nº 47, 291-297 (2013).

[36] Stephane, C., Stephane, S., Monique, L. Evaluation of free radical-scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chem., 95,1-8 (2006).https:/ do.org/ 10.1016/j.foodchem.2004.12.011

[37] Ibrahim, E.A., Gaafar, A.A., Salama, Z.A, El Baz, F.K. Anti-inflammatory and antioxidant activity of Solenostemma argel extract. Int. J. Pharmacogn. Phytochem., 7 (4), 635-641 (2015).

[38] Barreira, M. C. J., Alves, C. R., Casal, S., Ferreira, F. C. I., Oliveira, R. M.B., Pereira, A. J. Vitamin E profile as a reliable authenticity discrimination factor between chestnut (Castanea sativa Mill.) cultivars. Journal of Agricultural and Food Chem., 57,5524–5528 (2009). https://doi.org/10.1021/jf900435y

[39] Wang, Y., Lai, L., Teng, L., Li, Y., Cheng, J., Chen, J., Deng, C.,. Mechanism of the anti-inflammatory activity by a polysaccharide from Dictyophora indusiata in lipopolysaccharide-stimulated macrophagesInt. J. Biol. Macromol., 126, 1158-1166 (2019). https://doi.org/ 10.1016/j.ijbiomac.2019.01.022

[40] Richard J.J., Gomez-Pinilla,F., Nagel M., Nakagawa, T., Rodriguez-Iturbe, B., Sanchez-Lozada, G.L., et al. Cerebral fructose metabolism as a Potential mechanism driving alzheimer's disease. Hypothesis and Thory.; 12:1-15 (2020),https:/doi.org/ 10.3389/fnagi.2020.560865

[41]Rao, V. R., Descamps, O., John, V., Bredesen, E. D. Ayurvedic medicinal plants for Alzheimer's disease: a review. Alzheimer's Research & Therapy., 4,2-9 (2012). https://doi.org/ 10.1186/alzrt125