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POLYPHENOLIC AND FLAVONOIDS CONTENT, HPLC PROFILING AND ANTIOXIDANT ACTIVITY OF SOME MEDICINAL PLANTS WITH PANCREATIC HISTOLOGICAL STUDY IN ALLOXAN-INDUCED DIABETIC RATS MODEL

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ABSTRACT

Background: Medicinal plants are considered a very important source of natural crude materials which are used in pharmaceutical industries. Nowadays, many investigators focused their studies for medicinal plants in order to extract components which have effects as antibacterial and antioxidant activities, also, some diseases such as diabetes, cardiovascular, diarrhea, antitumor and anticancer, etc. Our study focused in the biochemical characterization of *Portulaca oleracea* and *Carthamus tinctorius* to study their antidiabetic activity in alloxan induced diabetes in rats.
Methods: Extracts from *Portulaca oleracea* leaves and *Carthamus tinctorius* flowers were prepared then their total phenolic and flavonoid content and identification by HPLC technique were analyzed. Moreover, the antioxidant activity using (FRAP and DPPH radical) were determined. Randomly allocated male white Wistar rats into four groups of five each: non-diabetic control; diabetic control; diabetic treated with *Carthamus tinctorius* extract (200 mg kg-1 BW); diabetic treated with *Portulaca oleracea* extract daily (250 mg/kg), then pancreatic tissues were collected and routinely processed for histopathological examination.
Results indicate that methanolic extract of *Portulaca oleracea* leaves had the largest total polyphenols and flavonoids content, which were 129.03 mg GAE/g and 22.55 mg QE/g, followed by *Carthamus tinctorius* flowers methanolic extract, which were 102.44 mg GAE/g and 13.94 mg QE/g, respectively. Identification of total polyphenols and flavonoids were estimated by HPLC. The methanolic extract of *Portulaca oleracea* leaves, had the highest reducing power which was 1.921 at the concentrations of 80 mg/ml. followed by *Carthamus tinctorius* flowers extract. Also, by using ($DPPH^+$), the highest antioxidants activity was for *Portulaca oleracea* leaves extract. Microscopic examination of pancreatic tissues from rats treated with *Portulaca oleracea and Carthamus tinctorius* revealed their anti-diabetic activity with improved histological tissue changes compared with alloxan induced diabetic group. Moreover, antidiabetic activity of *Portulaca oleracea* recorded more histological improvement than that of *Carthamus tinctorius* compared with the control non diabetic group.

Keywords: *Portulaca oleracea*, *Carthamus tinctorius*, Antioxidants activity, Anti-diabetic activity, rats

INTRODUCTION

It has been stated that nutritional antioxidant compounds etc., (polyphenolic compounds, flavonoids compounds, vitamin E, vitamin C and α-tocopherol) can safeguard the organism from oxidative harm in living structures. When antioxidant protection mechanisms become unbalanced by certain variables, physiological function decline can lead to pathological procedures such as aging, cancer, diabetes, antitumors, coronary heart disease, **Erian *et al.* (2016)**.
Portulaca oleracea belongs to the family of Portulacaceae and consists of large number of various medicinal and pharmacological importance thus representing a priceless tank of fresh bioactive molecules. *Portulaca oleracea* includes numerous pharmacological operations such as antimicrobial, antioxidant, antidiabetic, neuronal and anti-inflammatory activity, **Chowdhary *et al.* (2013)**. *Portulaca oleracea* has a broad range of pharmacological characteristics, including neuroprotective, antimicrobial, antioxidant,antidiabetic, anti-inflammatory, antiulcerogenic and anticancer operations, **Zhou *et al.* (2015)**.
Safflower (*Carthamus tinctorius* L., belongs to the family Compositae), has been used as colors and crude drugs for a long time in many countries. The effects of the plant flower extracts have been described including increased peripheral blood flow, antibacterial activity, inhibition of platelet combination, increased beating largeness of cultured myocardial cell sheet, provoked central sedative activity, anti-inflammatory action and inhibition of tumor raise in mouse skin carcinogenesis. In addition, polysaccharides discovered in safflower petals may activate macrophages and boost inflammatory cytokine production. Reports of antioxidant safflower compounds portray their activity in the scavenging of free radical species like superoxide anion, **Ebadia *et al.* (2014)**.

MATERIAL AND METHODS

Plants

Portulaca oleracea and *Carthamus tinctorius* samples were kindly acquired from the Center for Agricultural Research, Mansoura, Egypt. The samples were dried in the shade and then converted into fine powder. The powdered leaves and flowers, were divided into dry part and methanolic extract. Methanolic Extract: Samples were extracted six times using 30 litters of methanol, then it was concentrated to approximately dryness under vacuum using rotary evaporator at 45ºC, which kept under 4ºC, until use according to **Farid *et al.* (2016).**

Total polyphenols contents

Folin–Ciocalteu reagent was used to determine the total polyphenolic content of air-dried samples according to **(Lin and Tang, 2007)**, Approximately 0.1 g of dried air sample was dissolved in 1 ml of distilled water individually. Aliquots of

0.1 ml from the prior solution were drawn and blended with precisely 2.8 ml of distilled water, 2.0 ml of sodium carbonate (2% w / v) and lastly 0.1 ml of 50% (v / v) of Folin – Ciocalteu reagent. Mixture was incubated at room temperature for 30 minutes and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss -Jena) spectrophotometer. A normal curve using gallic acid (0- 200mg / l) was prepared in the same way for quantitative determination. Total polphenolic contents were expressed as milligram gallic acid equivalent (GAE)/g based on dry weight.

Total flavonoid contents

Total flavonoid content of air dried samples using aluminum chloride was calorimetrically determined as described by **Chang *et al.* (2002)**, About 0.1 g of dried air sample in 1ml of distilled water was dissolved. The resulting solution (0.5 ml) was mixed with 1.5 ml 95% ethyl alcohol, 0.1 ml 10% aluminum chloride (AlCl3), 0.1 ml 1M sodium acetate (CH3COOK) and 2.8 ml distilled water. After 40 minutes of room temperature incubation, the absorbance of the reaction blend was evaluated as blank at 415 nm against distilled water, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was elected as a standard for creating the normal curve (0–50mg / l) of flavonoids. Total flavonoid content levels were displayed as an equal milligram quercetin (QE)/g based on dry weight.

Analysis of phenolic compounds by HPLC technique

The phenolic compounds were determined by HPLC technique according to the method of **Goupy *et al.* (1999)**, as follow; 5 g of plant powder sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membtrane filter then 1-3 ml was composed in a vial for injection into the HPLC Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quarter HP pump (series 1050). The column type was ODS column with measurement of 5µm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml / min. Standard phenolic acid from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by HEWLLET packed software to calculate the concentration of phenolic compounds.

Analysis of flavonoid compounds by HPLC technique

The flavonoid compounds were determined by HPLC technique according to the method of **mattila *et al.* (2000)**, as follow; 5 g of sample was mixed with methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2µm Millipore membtrane filter then 1-3 ml was composed in a vial for injection into the HPLC Agilent 1200 series armed with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 254 nm and quarter HP pump (series 1050). The column type was ODS column with measurement of 5µm x4mm, the column temperature was kept at 35°C. Methanol and acetonitrile gradient separation was performed as a mobile phase at a flow rate of 1 ml/min. Standard Flavonoid from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak zone were used by HEWLLET packed software to calculate the concentration of flavonoid compounds.

Determination of Reducing power, (FRAP)

It was determined the reducing power of methanolic extracts was reduced according to the method of **(Oyaizu, 1986)**, Extract (0–100 mg) from each sample was added to 2.5ml potassium ferricyanide (10mg / ml) in a 0.20mol phosphate buffer, pH 6.6 (2.5ml), blend incubated at 50oC for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture after 10 minutes of centrifugation at 650g. The supernatant (2.5ml) was blended with distilled water (2.5ml) and 0.5ml (1mg / ml) ferric chloride solution. and the resulting color absorption was measured using a 700 nm spectrophotometer from Spekol 11 (Carl Zeiss -Jena). Higher absorbance of the reaction mixture showed greater reduce.

Determination of ($DPPH^+$) radical scavenging activity

The $DPPH^+$ free radical scavenging activity of leaf and flower methanolic extracts at various concentrations was measured by lightening the purple color of (2.2 Diphenyl -1-picryl hydrazyl) using the procedure **Pratap *et al.* (2013)**, Exactly 0.1 ml solution of different concentration of extract was added to 1.4 ml of $DPPH^+$ 0.1mM and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss -Jena) spectrophotometer. And the inhibition coefficient was calculated using the current equation.
Inhibition (%) = (A Blank – A Test) / A Blank) × 100

Animals

Twenty males Wistar rats weighing approximately 150-200 g collected from the Faculty of Pharmacy's animal house, Mansoura University, Egypt. All mice were placed in microlon boxes with normal laboratory diet and water ad libitum in a controlled setting (temperature 25±20ºC and 12 h dark / light cycle).

Induction of Diabetes

At a dose of 120 mg kg^{-1} body weight, the mice were injected with alloxan monohydrate dissolved in sterile ordinary saline, intraperitoneally for two weeks before beginning therapy. A digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Mannheim, Germany) was used to collect fasting blood samples from the rats ' tail vein. And blood glucose was measured to understand diabetes induction, The samples collected with blood sugar concentrations > 200mg / dl from the tail vein Rats have been regarded diabetic.

Experimental Design

Following the induction of alloxan diabetes , the rats were split into four groups and each group included 5 rats.
Group 1: Control negative rats (Non-diabetic) given normal saline intraperitoneally daily to equalize stress induced by injections in all groups for 4 weeks.
Group 2: Control positive rats (Untreated diabetic).
Group 3: Diabetic rats with treated *Carthamus tinctorius* flowers extract (200mg/kg) intra peritoneally daily
Group 4: Diabetic rats with treated *Portulaca oleracea* leaves extract daily (250 mg/kg) daily.

Histological Studies

At the end of the experiment (after 4 weeks), entire pancreas was removed after the animal was sacrificed and gathered in 10 percent formalin solution and processed by the paraffin method instantly. Hematoxylin and eosin (H&E) sections were trimmed and stained for histological examination, **(Bancroft and Turner, 1996)**.

Statistical analysis

Using the statistical software package, information collected were evaluated **(CoStat, 2005)**. All comparisons were first subjected to one-way ANOVA and important distinctions between treatment means were determined using the multi-range test of Duncan at $p<0.05$ as the meaning point **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Total polyphenols and total flavonoids content

Total polyphenolic compounds include several classes of secondary plant metabolites that form part of the diets of humans and animals. Flavonoids are a large group of phenolic compounds that primarily consist of flavonols, flavanols and anthocyanins. Phenolic compounds can play a major role in stopping hydrogen peroxide from injury to body cells and organs, harming lipid peroxides and scavenging free radicals, **(Sroka and Cisowski, 2003)**.
Table (1) demonstrations the total polyphenols and flavonoids content of *Portulaca oleracea* and *Carthamus tinctorius*. Data in table (1) shows that, *Portulaca oleracea* leaves contained highest values of total polyphenol and flavonoid contents, which were (129.0 mg GAE/g) and (22.55mg QE/g), respectively. Moreover, *Carthamus tinctorius* flowers had the average concentration of total polyphenol and flavonoid contents, which were 102.44mg GAE/g and 13.94mg QE/g, respectively. Data confirms to **(Sallam and Anwar, 2017)**, who found that total phenolic contents of methanol 50%, ethanol 50% and distilled water of *Portulaca oleracea*, were 201, 178 and 100 mg GAE/g, respectively. While, the total flavonoid content of the same extracts, of *Portulaca oleracea*, were 50, 42 and 25 mg QE/g, respectively. While **Gallo *et al.* (2017)**, showed that mix extraction was the most efficient compared to other methods. In fact, it obtained amount of polyphenols amounting to (237.8 mg GAE/100g), of *Portulaca oleracea* fresh weight, while in other methods, the range varied from 60–160 mg GAE/100 g *Portulaca oleracea* fresh weight. Though, total polyphenol of *Portulaca oleracea* amounting to (172.9mg (GAE)/100g), of dry weight. While in other methods, the range varied from (44.6 to 115.5 mg GAE/100g) of *Portulaca oleracea* dry weight.
El-Kashef *et al.* (2018), who revealed that the highest amount of phenolic content was found in methanol extract (326.80mg/g), of *Portulaca oleracea* leaves. While the highest amount of flavonoid content was found in cold water extract (60.70mg/g) of the same plant leaves.

Table 1 Total polyphenol and flavonoid contents.

Extracts	Total polyphenols (mg GAE/g)	Total flavonoids (mg QE/g)
Portulaca oleracea	129.03	22.55
Carthamus tinctorius	102.44	13.94

Identification of polyphenolic fractions of investigated Plants using HPLC technique

High performance liquid chromatography (HPLC) method has been used to analyze *Portulaca oleracea* and *Carthamus tinctorius* polyphenolic compounds qualitatively and quantitatively. Eighteen polyphenolic compounds as authentic samples namely: Gallic, Pyrogallol, 4-Amino benzoic, Protocatechuic, Cataehein, Chlorogenic, Catechol, E.picatechen, Caffien, P.oH.benzoic, Caffeic, Vanillic, Ferulic, Ellagic, Benzoic acid, Salicylic acid, Coumarin and Cinnamic acid. To define the respective parts of plant polyphenols, these standard samples were used. From table (2), it could be noticed that Gallic acid was the predominant identified component of *Portulaca oleracea* and *Carthamus tinctorius* in concentrations which were 98.45 and 56.12 ppm respectively. While the pyrogallol compound percentage of *Portulaca Oleracea* and *Carthamus tinctorius* was 4.56 and 2.77 ppm respectively. The 4-Aminobenzoic content of *Portulaca oleracea* and *Carthamus tinctorius* were 17.11 and 18.59 ppm, respectively. The Protocatechuic content of the same line was 59.24 and 71.28, and ppm respectively. Also, the catechin content in plants was 18.68 and 44.51 ppm respectively. From table (2), it could be noticed that chlorogenic was the predominant identified component in both *Portulaca oleracea* and *Carthamus tinctorius* in concentrations, which were 140.32 and 166.35 ppm. While Catechol component content of *Portulaca oleracea* and *Carthamus tinctorius* were 22.16 and 25.94 ppm respectively. While E.picatechen the average content of *Portulaca oleracea* and *Carthamus tinctorius* was 66.20 and 73.51ppm. From table (2), it could be noticed that ellagic was the predominant identified component in both *Portulaca oleracea* and *Carthamus tinctorius,* in concentrations of 54.92 and 159.69 ppm respectively. Also, the highest content of Benzoic compound was 177.15 and 72.31ppm of the same plants respectively. Likewise, Salicylic was the main component of polyphenols of the same plants in concentration of 130.848 and 112.58 ppm respectively. The p-Hydroxy benzoic acid and Ferulic compounds are not found in *Carthamus tinctorius*. While all compounds (Caffien, Caffeic, Vanillic, Coumarin and Cinnamic) are intermediate values.

Table 2 Identification of phenolic compounds using HPLC technique

Phenolic Compounds	R_{time}	*Portulaca oleracea* (ppm)	R_{time}	*Carthamus tinctorius* (ppm)
Gallic	7.109	70.06	7.122	26.11
Pyrogallol	7.647	4.56	7.470	2.77
4-Aminobenzoic	7.001	17.11	8.224	18.59
Protocatechuic	8.249	59.24	8.440	71. 28
Catechin	8.615	18.68	8.650	44.51
Chlorogenic	9.054	140.32	9.120	166.35
Catechol	9.399	22.16	9.500	25.94
E.picatechen	9.647	66.20	9.700	73.51
Caffien	9.790	22.79	9.780	18.57
p-Hydroxy benzoic acid	10.041	47.54	9.909	39.02
Caffeic	10.277	20.13	10.300	28.61
Vanillic	10.570	38.01	10.420	52.48
Ferulic	12.200	8.06	--	--
Ellagic	13.288	54.92	12.548	159.69
Benzoic	13.730	177.15	13.830	72.31
Salicylic	14.245	130.848	14.200	112.58
Coumarin	14.450	5.14	14.400	8.03
Cinnamic	15.700	4.51	15.640	3.72

Several authors identified some polyphenolic derivatives from the *Portulaca oleracea*, for example **Sicari *et al.* (2018)**, they found that the *Portulaca oleracea* ethanolic extract contains 5.58, 18.77, and 9.27 mg/kg, of caffeic acid, p-coumaric acid and ferulic acid, when analyzed using HPLC-DAD, respectively. **Ai *et al.* (2015)**, found that the contents of the eight analytes in *Portulaca oleracea* from different location its extracts by UPLC-UV, e.g. Caffeic acid, p-coumaric, Scopoletin, Ferulic acid, Quercetin-3-Orhamnoside, Quercetin, Apigenin, and Bergapter, were 17.86, 169.8, 81.89, 26.89, 20.40, 29.49 , 0.476, and 2.710µg/g, of liu'an location. While, of beijing location were 27.20, 572.9, 16.59, 46.13, 320.0, 11.82, 0.895, and 0.00µg/g, of the same plants respectively.

Identification of Flavonoid fractions of investigated plants using HPLC technique

High performance liquid chromatography (HPLC) method has been used to analyze *Portulaca oleracea* and *Carthamus tinctorius* flavonoid compounds qualitatively and quantitatively. Eleven Flavonoids fractions as authentic samples namely: Narengin, Rutin, Hisperdin, Rosmarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Luteolin, Hispertin, and 7-Hydoxyflavon were used different concentrations comparing with standard compounds. Data obtained indicated that 11 compounds with distinct retention times were identified in *Portulaca oleracea* leaves, HPLC chromatogram and 10 compounds with distinct retention times were identified in *Carthamus tinctorius* flowers, HPLC chromatogram respectively.

From table (3), it is clear that all investigated Flowers samples contained, Rutin, Hisperdin, Rosmarinic, Quereitrin, Quereetrin, Narenginin, Kampferol, Hispertin, and 7-Hydoxyflavon with different concentrations comparing with standard compounds. From table (3), it could be noticed that Narengin was the predominant identified component in both *Portulaca oleracea* and *Carthamus tinctorius* in concentrations of 400.15 and 386.01ppm. While rutin was the main component of flavonoids in *Portulaca oleracea and Carthamus tinctorius* in concentration of 240.91 and 307.12 ppm. The highest values of flavonoids content were found to be hesperidin compound in all investigated medicinal plants which were 1169.00 and 2006.14ppm in *Portulaca oleracea and Carthamus tinctorius* respectively.

From table (3), it could be noticed that highest value for Rosmarinic of *Portulaca oleracea and Carthamus tinctorius* were 556.73 and 498.63 ppm. The content of Quereitrin and Quereetrin were 655.21 and 69.34 ppm, 352.38 and 55.37 ppm of *Portulaca oleracea* and *Carthamus tinctorius,* respectively. While the values of Quereitrin were higher compared with that of Quereetrin. From table (3), it could be noticed that the low values of Narenginin compound were 17.23 and 18.93 ppm, of the same plants. Moreover, Average values of the Kampferol, Luteolin, and Hispertin compounds were 49.82, 97.46, and 51.03ppm, of *Portulaca oleracea* respectively. The Kampferol content of *Carthamus tinctorius* was 174.54 ppm. While Luteolin compound not found of the same plants. The value of Hispertin compound were 51.03 and 25.40 ppm of *Portulaca oleracea* and *Carthamus tinctorius* respectively. While the lowest value of 7-Hydoxyflavon were 6.00 and 4.76 ppm. While the highest value for 7-Hydoxyflavon of *Carthamus tinctorius was* 178.43ppm compared with *Portulaca Oleracea* which was 6.00ppm.

Table 3 Identification of flavonoid compounds using HPLC technique.

Flavonoid Compounds	R_{time}	*Portulaca oleracea* (ppm)	R_{time}	*Carthamus tinctorius* (ppm)
Narengin	12.500	400.15	12.490	386.01
Rutin	12.570	240.91	12.570	307.12
Hisperdin	12.710	1169.00	12.785	2006.14
Rosmarinic	12.930	556.73	12.901	498.63
Quereitrin	13.650	655.21	13.558	352.38
Quercetin	15.100	69.34	15.220	55.37
Narenginin	15.397	17.23	15.532	18.93
Kampferol	15.671	49.82	15.609	174.54
Luteolin	15.801	97.46	15.750	--
Hispertin	15.855	51.03	15.822	25.40
7-Hydoxyflavon	17.511	6.00	17.660	149.53

Several authors identified some flavonoid derivatives from the *Portulaca Oleracea,* for instance, **Sicari *et al.* (2018)**, found that, the ethanolic extract of *Portulaca oleracea* were analysed using HPLC-DAD, the flavonoids compounds (apigenin, kaempferol, luteolin, quercetin, isorhamnetin, kaempferol-3-O-glucoside and rutin) were 0.09, 1.85, 0.23, 14.14, 0.25, 0.53 and 6.17mg/kg of the same extract respectively. Quercetin and p-coumaric acid were the most profuse compounds.

Reducing power of investigated plant extracts

The efficiency of methanol extract to decrease Fe^{+++} to Fe^{++} was determined by the technique outlined, **(Oyaizu, 1986)**. The obtained data are presented in table (4), the absorption showed the reduction capacity for different concentrations of methanolic and aqueous extracts of *Portulaca oleracea* leaves and *Carthamus tinctorius* flowers, and leaves. The data stated as absorbance at 700nm for creating color as a result for using three levels from concentrations; 20, 40 and 80 mg/ml for each sample.

Some points could be deduced from table (4); By raising the concentration of the methanol extract for all samples, the reduction capacity improved. *Portulaca oleracea* leaves have the maximum percentage of reducing power ranged from 0.995 to 1.921 for methanolic extract at the concentrations of 20 and 80mg/ml respectively. While the average values of reducing power ranged from 0.749 to 1.532 at the concentrations of 20 and 80 mg/ml of *Carthamus tinctorius* flowers, respectively. High levels of reducing power designated the presence of some compounds which could be measured electron donors and could react with free radicals to change them into more steady products, **(Arabshahi and Urooj, 2007)**.
Different studies designated that the electron donation capacity which reflecting the reducing power of bioactive compounds was associated with high antioxidant activity, **Siddhuraju *et al.*, (2002)**. The findings were similar to those reported by **Alam *et al.* (2014)**, who found that reducing power (FRAP) of *Portulaca oleracea* ranged from 7.39±0.08 to 104.2±6.34μmol TE/g d w, respectively.

Table 4 Reducing power of crude methanolic plant extracts.

Plants Extract	Concentration (mg/ml)	Optical density at 700nm
Portulaca oleracea	20	0.995
	40	1.397
	80	1.921
Carthamus tinctorius	20	0.749
	40	1.155
	80	1.532

Previous data of *Portulaca oleracea* leaves agreed with those obtained by **Uddin *et al.* (2012)**, who found that the ferric-reducing antioxidant power (FRAP) for *Portulaca oleracea* at the different growth stages ranged from 1.8 to 4.3 mg GAE/g at 15 and 60 day. **Popova *et al.* (2014),** indicated that the FRAP values of *P. oleracea* plant material were visible in the decoction as well from 8.58 ± 0.05 to 155.75 ± 1.10μM TE/g DW. **(Kuşoğlu and Kahraman, 2015)**, appraised that Reducing power of the *Carthamus tinctorius* extract, was found as 0.885. **Peiretti *et al.* (2018)**, determined that the FRAP content ranged from 0.730 to 1.108 mmol Fe2+/g extract.

Antioxidant activity using (DPPH$^+$) radical

Table (5) shows the antioxidant activity of both methanol extracts ready from the two species of plants studied. The concentration of an antioxidant needed to decrease the initial DPPH$^+$ concentration by 50% (IC_{50}) is a parameter widely adapted to amount the antioxidant activity **Sanchez *et al.* (1998)**. The lower EC_{50} pointed to the higher antioxidant activity. The antioxidant activity of the extracts tested was evaluated using radical of DPPH$^+$ scavenging activity. DPPH$^+$ antioxidants scavenging activities are ascribed to their hydrogen-donating capacity.
Biswas *et al.* (2010). The reference compound was vitamin C. It is evident from table (5) that the scavenging impact (IC_{50}) of *Portulaca oleracea* leaves methanolic extracts has the most efficient inhibition proportion (0.29), followed by the 0.36 Carthamus tinctorius extract.

Table 5 (DPPH$^+$) radical of plant extracts.

Plant Extracts	DPPH$^+$ IC_{50}
Portulaca oleracea	0.29
Carthamus tinctorius	0.36

Gallo *et al.* (2017), determined the antioxidant activity of *Portulaca oleracea* extracts using (DPPH$^+$), It was discovered that the Naviglio extractor (operating at room temperature) showed a high inhibition percentage (69.5%), whereas maceration with ultrasound showed an inhibition percentage of 65.4%, and the mix and maceration showed an inhibition percentage of 54% and 52%, respectively. **Alam *et al.* (2014)**, found that the antioxidant activity using (DPPH$^+$) scavenging (IC_{50}) activity of *Portulaca oleracea* varied between 2.52±0.03 mg/mL and 3.29±0.01 mg/mL respectively. Likewise, **(Sallam and Anwar, 2017)**, found that, the DPPH$^+$ radical scavenging activity of methyl alcohol 50%, ethyl alcohol 50%, and distilled water of *Portulaca oleracea* were 32.70, 29, and 20.20 ppm respectively. Previous data of *Portulaca oleracea* leaves agreed with those obtained by **Uddin *et al.* (2012)**, who found that the DPPH$^+$ free radical scavenging activity (IC_{50} value) for *Portulaca oleracea* at the different growth stages ranged from 1.8 to 4.3 mg GAE/g at 15 and 60 day. **Popova *et al.* (2014)**, established that the free radical scavenging activity (DPPH$^+$) of *Portulaca oleracea* range from 2.56 ± 0.14 to 31.78 ±1.5 μM TE/g DW. **(Golkar and Taghizadeh, 2018)**, established that the antioxidant effects using DPPH$^+$ IC_{50} of *C. tinctorius* was (3.82 ± 0.43μg ml^{-1}). **(Taha and Matthäus, 2018),** showed that the antioxidant activity using DPPH$^+$ IC_{50} of *Carthamus tinctorius* were 0.88, 0.84, and 0.84mg/g of Kharega1, Kharega2, and Giza when uses shaking method respectively.

Histopathological Examination

At the end of the group 1 experiment (Non-diabetic Control adverse rats), microscopic examination of stained pancreatic tissue disclosed normal pancreatic acini,, ducts and islets (Plate 1.1). While group 2 (diabetic control positive group) showed several diabetic pancreatic changes including; pancreatic duct dilatation and pancreatic islets hyperplasia (Plate 1.2.a). Moreover, there was massive necrosis in pancreatic acini together with leucocytic cells infiltration (Plate 1. 2.b). Group 3 (diabetic rats treated with *Carthamus tinctorius* L extract) reported improvement in pancreatic tissue with improved pancreatic acini and regressed pancreatic islets (Plate 1.3). Group 4 (diabetic rats treated with P. oleracea extract) recorded apparently healthy pancreatic parenchyma; acini, islets, and ducts (Plate 1. 4).

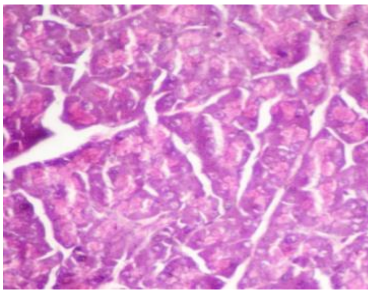

Plate 1.1: Group (1): Normal pancreatic acini, ducts, and islets, (H&E X 400).

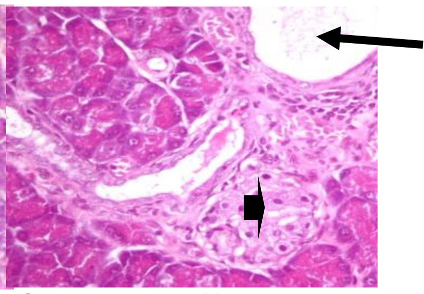

Plate 1.2a: Group (2): Pancreatic duct dilatation (arrow) and pancreatic islets hyperplasia (arrow head), (H&E X 400).

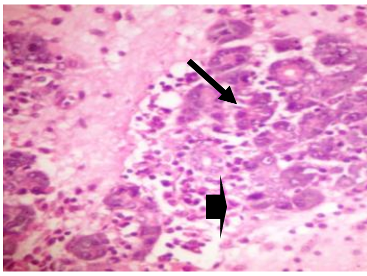

Plate 1.2b: Group (2): Necrotic pancreatic acini (arrow) with leucocytic cells infiltration (arrow head), (H&E X 400).

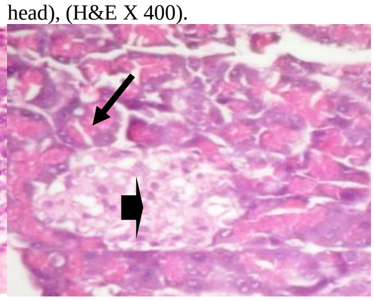

Plate 1.3: Group (3): Improved pancreatic acini (arrow) and regressed pancreatic islets (arrow head), (H&E X 400).

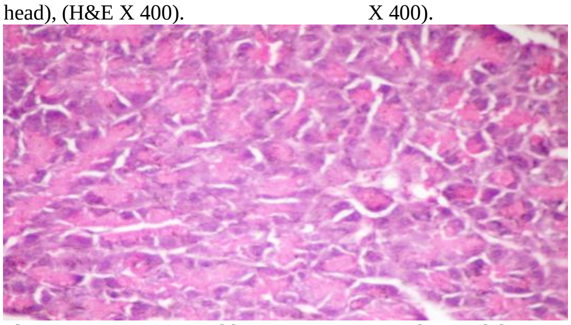

Plate 1.4: Group (4): Healthy pancreatic acini, islets, and ducts, (H&E X 400).

CONCLUSION

A large proportion of active compounds such as polyphenols and flavonoids are found in *Portulaca oleracea* and *Carthamus tinctorius*. The effect of natural extracts as an antioxidant was high of these plant extracts to scavenging the free radicals (FRAP and DPPH$^+$), in laboratory. Moreover, their extract possesses beneficial effect on treatment of diabetes. However, further clinical trials in diabetes are recommended.

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