PHYTOCHEMICALS, ANTI-OXIDANT AND VOLATILE COMPOUNDS
EVALUATION OF EGYPTIAN PURSLANE LEAVES

Aliaa A. Almashad¹*, Ibrahim G.E. and Rabab H. Salem¹
1- Food Science and Technology Dept., Fac. of Home Economic, Al-Azhar Univ., Tanta, Egypt
2- Chemistry of Flavour and Aroma Dept., National Research Center, Dokki, 12622, Giza, Egypt

*Corresponding author: aliaa.akr2002@yahoo.com

ABSTRACT

Portulaca oleracea (PO) has several applications as vegetable and can be used in medicine and food industry. Therefore, the current study aimed to compare the phytochemical constituents, phenolic compounds and antioxidant activity in ethanol and aqueous extract of Egyptian purslane fresh leaves. Also, the fatty acids and volatile compounds in fresh leaves had been determined. Concentrations of saponin and alkaloid were the highest phytochemical present in both extracts of the purslane leaves. Percentage of tannins was highest in the ethanol extract of the leaves (3.15%) compared to aqueous extract (2.65%). Saponin level was highest in the ethanol extract (4.98%) compared to aqueous extract (4.58%). While, the ethanol extract was more effective in phytochemical including tannins and saponins; whereas both of the two extracts were nearly similar in the extract of phenolic and flavonoids. HPLC analysis showed that pyrogallol was the main phenolic compound in ethanol and aqueous extracts with concentration of 24.85 and 23.65%, respectively. The main volatile compounds of Headspace (HS) were: (E)-2-Hexenal (15.64%), (E)-2-Nonenol (12.03%), Hexanal (10.92%), and Ethyl linoleate (8.02%). On the other hand, the main volatile compounds obtained by water distillation (WD) were; (E)-2-Hexenal (12.46%), (E)-2-Nonenol (9.52%), Hexanal (9.32%), and Menthol (8.04%). Portulaca oleracea plant extract could be used as a source of phytochemical and antioxidants, especially for functional food.

Keywords: Portulaca oleracea, Phytochemicals, Antioxidant, Volatile compounds.

1. INTRODUCTION

Nowadays, Purslane (Portulaca oleracea L.) had great attention from food and medicinal industries as well agriculturalists. It can be found in the field crops or weed in turfgrass areas (Kamal-Uddin et al 2014). Several species more than 120 of purslane belonging to the family “Portulacaceae” under many names grow in a wide range of climates and regions throughout the world from Asia, Mediterranean areas of central Europe to America and is commonly called “Rejlah” in Egypt (Shehata and Soltan, 2012). Due to its nutritive value and biological activity such as antioxidant and antidiabetic, many researchers consider it as ‘Power Food’ of the future (Hussein and Abdel-Gawad, 2010). The World Health Organization (WHO) had
listed *P. oleracea* as one of the most application medicinal plant and gave it the name of ‘Global Panacea’ (Chu et al 2002; Katalinic et al 2006). A wide spectrum of medical and pharmacological properties of *P. oleracea* like antidiabetic, anti-obesity, antiinflammatory, and anticancer activities had mentioned (Lingchao et al 2019).

The phytochemical screening of purslane has suggested that the plant has a higher content of β-carotene and ascorbic acid than some of the traditional nutritive plant crops. Furthermore, the plant has been reported to be a storehouse of omega-3 fatty acids such as linolenic acid (Liu et al 2000).

The presence of several phytochemicals in purslane like essential fatty acids, alkaloids, flavonoids, vitamins and sterols gave it possesses a number of nutritional benefits (Petropoulos et al 2016).

Polyunsaturated fatty acids (PUFAs) (ω-6 and ω-3) are essential lipids for human health. Actually, people from developed countries include large amounts of saturated and ω-6 PUFAs in their diets which are detrimental to their health leading to increasing the risk of cardio vascular diseases CVD and other chronic diseases. Alpha-Linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential PUFAs because mammals cannot synthesize them de novo (Whelan and Rust, 2006).

Since detailed analysis of volatile constituents for Egyptian purslane were not found to be report-ed earlier, this study might help investigate this aspect. Therefore, our study aimed to investigate and compare the phytochemicals, antioxidant and phenolic compounds present in aqueous and ethanolic extracts of fresh sample of *P. oleracea* leaves collected from the local fields of El-Sharqia government, Egypt. Also, the comparison between volatile constituents from water distillation and headspace had carried out.

2. MATERIALS AND METHODS

2.1. Plant materials

Fresh Purslane (*Portulaca oleracea* L.) plants were harvested from a private fields (El-Sharqia Governorates, Egypt) prior to flowering period during November 2018.

2.2. Preparation of extracts

2.2.1. Ethanol extract of purslane

Purslane leaves were soaked with ethanol 80%, for 24 hrs at room temperature, and the ratio between the material and the solvent was 1:6 (4kg \*24L). The extract was first filtered through Whatman No.1 filter paper. The solvent was removed at 40°C using a rotary evaporator (Stuart, ST150SA, UK). The resulting extract was concentrated to a dry mass by freeze dryer (Model Snijders scientific, No Netherlands), stored at 4°C until analyzed.

2.2.2. Aqueous extract of Purslane

Purslane leaves were soaked for 72 hrs in 1000 ml of sterilized distilled water with occasional shaking. The extract was then filtered through a Whatman filter paper (No. 42) into different conical flasks. The water was evaporated using a water bath at 100°C for 1 hour, then left overnight at laboratory temperature for evaporation of the remaining water and stored at 4°C until analyzed.

2.3. Phytochemical constituents estimation

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu (FC) colorimetric method of Shahidi and Naczk (2004). Flavonoid determination by the method of Boham and Kocipai-abyzan (1994); tannin determination by Atanassova and Christova-Bagdassarian (2009). method; alkaloid determination using Harborne (1973) method; saponin determination was carried out as mentioned by Obadoni and Ochuko (2001).

2.4. Extraction and determination of phenolic compounds

The analysis of extracts were identified and determined using an HPLC apparatus following the method of Goupy et al (1999). Phenolic compounds were extracted by mixing 1 g of sample with 10 ml of methanol, then centrifuged at 1000 rpm for 10 min., and filtered through a 0.2 μm Milli-pore membrane filter. About 1-3 ml was collected in a vial for the analysis. A 5 μl aliquot of the
extract was injected into an Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with UV-10A Shimadzu detector, LC-16ADVP binary pump, DCou-14 A degasser and Zorbax 300SB C18 column (4.5 X 250 mm). The solvents (methanol and acetonitrile) were degassed, the ultraviolet (UV) detector was set at 280 nm, and the column temperature was maintained at 35°C. A gradient separation was performed using the mobile phases (methanol and acetonitrile) at flow rate of 1 ml/min. The solvent system consisted of methanol with 0.1% formic acid (solvent A); and acetonitrile with 0.1% of formic acid (solvent B). The gradient system was programmed as follows: starting at 30% solvent B, increasing to 60% over 10min, increasing to 100%over 5min and then returning to 30%over 5min. Retention time and peak area was used to calculate the phenolic acid concentration.

2.5. Antioxidant activity assays
2.5.1. Determination of DPPH radical–scavenging activity

The antioxidant activity of the extracts were measured by DPPH assay described by Ravichandran et al (2013) as follows: 0.1, 0.3 and 0.5 μg/mL of the extracts was mixed for 30s with 3.9 ml of DPPH solution (6 x 10^-5 M). The solution was incubated at room temperature for 30 min, and the decrease in absorbance at 515 nm was measured at the end of incubation period with a UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The DPPH solution without extract was analyzed as control. The antioxidant activity was calculated as follows:

Scavenging activity (% ) = [(A control – A sample ) / A control] x 100

Where A is the absorbance at 515 nm.

2.5.2. Reducing power determination

The reducing power of studied samples was determined according to (Nabavi et al 2009). About 2.5 mL of each extracts (0.1, 0.3 and 0.5 mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl3 (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.6. Isolation of fixed oil

Purslane samples were mixed with hexane (1:10, m/V) at (60-80°C) using a Soxhlet apparatus. This process of extraction was repeated for 6 h, the hexane distilled out by distillation assembly, then concentrated by hot plate drying and air-drying at temperature of 40°C.

2.6.1. Fatty acids analysis by gas chromatography GC

Fatty acids composition of purslane leaves oil were determined using gas chromatography (GC Model, Agilent technologies (Palo Alto, CA), equipped with a FID. Detector temperature 240°C, Column temperature 150-180 °C at rate 1 ml/min helium.

2.7. Volatile compounds

2.7.1. Isolation of volatile compounds by water distillation (WD)

The fresh leaves of plant collected were submitted for about 3 hrs to water distillation using Clevenger-type apparatus. The obtained essential oil was dried over sodium sulfate anhydrous and after filtration, stored at 4°C until analyzed.

2.7.2. Isolation of volatile compounds by Headspace (HS)

The analysis was carried out as mentioned by Araujo et al (2008). On Agilent technologies (Palo Alto, CA) HS autosampler (7697 A) was used to monitor the static HS quantitation of volatiles. Samples (10 g) were equilibrated for 60 min at 80°C prior to analysis. The settings of the HSS 7697 A were 5 S for pressurization, equilibration, and filling and 2min for injection. The HS loop (20 mL) temperature was set at 90°C. High-purity helium, filtered through moisture and oxygen traps (Hewlett-Packard), was used for vial pressurization, and an HSS sampler carrier gas at a flow rate of 17.5 mL/min was measured at the splitter outlet.
2.7.3. Gas chromatography—Mass spectrometry (GC-MS) analysis

The analysis of volatile was performed on Agilent 7890 GC coupled to a 5977 MS detector. The MS was run in the scan mode (m/z range from 40 to 400 with a threshold of 100 and a sampling rate of 3 scans/s). Ultrapure helium was passed through moisture and oxygen traps and was used as the carrier gas. The following GC operating conditions were used: a silica capillary column HP-5 (60 m × 0.25-mm × 0.25-μm film thickness); a flow rate of 1 mL/min at 40°C; a split ratio of 1:10; the injection port set at 240°C and the interface line to the MS at 220°C; and the electron energy and electron multiplier voltage at 70 eV and 1647 V. The temperature program began at 50°C for 10 min; increased 4 °C/min up to 240 °C until final time (Dehpour et al 2009).

2.7.4. Identification of volatile compounds

The linear retention index (RI) values for unknowns were determined based on retention time data obtained by analyzing a series of normal alkanes (C₆–C₂₂). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory (Adams, 2007).

2.8. Statistical analysis

Data were expressed as means ± standard deviation (SD) of three replications, and one factor ANOVA was used for the statistical analysis using SPSS program (version 16.0 SPSS Inc). The values were considered to be significantly different when P<0.05 (Steel and Torrie, 1982).

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

The phytochemical analysis of both ethanol and aqueous extracts of purslane leaves are given in (Table 1). The obtained data revealed that concentrations of saponin and alkaloid were the highest phytochemical present in both extracts of the purslane leaves. Percentage of tannins was highest in the ethanol extract (4.98%) compared to aqueous extract (4.58%) (Table 1).

| Phytochemicals | Extract | Water | Ethanol |
|----------------|---------|-------|--------|
| Phenolic       | 0.67±0.27a | 0.69±0.13 |
| Flavonoids     | 1.23±0.19 | 1.26±0.24 |
| Tannins        | 2.65±0.35 | 3.15±0.19 |
| Alkaloids      | 3.92±0.29 | 3.97±0.12 |
| Saponins       | 4.58±0.16 | 4.98±0.26 |

* Values are expressed as Mean ± SD; The same letters within the same raw are not significant (P < 0.05)

These results are agreement with those obtained by Hussein, (2010) who explained that, purslane extract in the form of ethanolic formulation is rich in polyphenols and flavonoids.

The extraction of purslane by ethanol is preferred for saponin, tannins and terpenoids as mentioned by Ezeabara et al (2014). On the other hand, Okafor and Ezejindu, (2014) found high yield of saponin (32%) and alkaloid (26%) but a low content of flavonoid (6%) and tannin (0.03%) in P. oleracea after extraction with ethanol.

3.2. HPLC analysis of phenolic compounds

The phenolic compounds had several biological activities such as anti-inflammation, anti-cancer and anti-atherosclerosis in human health (Arai et al 2000; Havsteen, 2002), the comparison between the aqueous and ethanolic extracts of purslane had carried out and the data are given in (Table 2). The results showed that, the phenolic compounds in purslane ethanol extract had high amount of pyrogallol, chlorogenic, rosmarinic and catechin (24.85, 14.13, 9.86 and, 7.92%) respectively, flowed by salicylic, rutin, apigenen, vanillic, hesperetin , narenginin and quercetin. The data in (Table 2), cleared that, pyrogallol was the main phenolic compound in both extracts of the purslane leaves. On the other hand, the phenolic compounds in purslane water extract had high amount of pyrogallol, chlorogenic and salicylic flowed by, catechin, rosmarinic, vanillic and rutin (Table 2).
Phytochemicals, Antioxidant and Volatile Compounds Evaluation of Egyptian Purslane Leaves

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Table 2. Phenolic compounds concentration (%) as determined by HPLC of ethanol and aqueous extracts of Purslane leaves

| Phenolic compounds | Concentration (%) | Water extract | Ethanol extract |
|--------------------|------------------|---------------|----------------|
| Pyrogallol         | 23.65            | 24.85         |
| Chlorogenic        | 9.87             | 14.13         |
| Vanilllic          | 3.82             | 2.56          |
| Salicylic          | 7.03             | 5.41          |
| Caffeic            | 1.94             | 3.20          |
| Catechin           | 5.32             | 7.92          |
| Rosmarinic         | 4.98             | 9.86          |
| Rutin              | 3.15             | 5.24          |
| Quercetin          | 0.87             | 1.09          |
| Apigenen           | n.d              | 3.26          |
| Hesperetin         | 0.59             | 1.48          |
| Narenginin         | n.d              | 1.55          |

# Values are expressed as relative area percentage; n.d: not detected

In the study carried out by El-Hadidy et al. (2013) reported that purslane powder extract had high amount of hesperidin, catchin, vanillic and caffeic acid (735, 97, 45 and 27 mg/100g) respectively, followed by chlorogenic, catechol, salicylic, procatechic, hesperetin and chrisin. Free phenolic acids like Chlorogenic, caffeic, p-coumaric, ferulic and rosmarinic acids and free flavonoids like quercetin, myricetin, luteolin, apigenin, genistein, genistin and kaempferol were found in P. oleracea plant extract (Yan et al. 2012).

3.3. Antioxidant activity

The antioxidant activity of the purslane aqueous and ethanol extracts as well as BHA and TBHQ standard were assessed on the basis of the radical scavenging effect on the stable DPPH and reducing power and the obtained data are given in Fig. (1). As the concentration of studied extracts increase, an increase in antioxidant activity had occurred.

Fig. (1) shows the antioxidant activities of ethanolic P. oleracea leaves extract at different concentrations (0.1, 0.3 and 0.5 μg ml⁻¹) against DPPH and reducing power. The ethanolic extract of P. oleracea leaves exhibited a significant antioxidant activity in a dose dependent manner and was higher than BHA and comparable to TBHQ Fig. (1).

Various phenolic compounds are present in purslane had several pharmacological and biologically activity such as antioxidant, anti-inflammatory, antiviral and antibacterial (Masoodi et al. 2011). The crude extract and fraction of P. oleracea as carried out by (Naciye, 2012 and Kamal-Uddin et al. 2014) exhibited significant antioxidant activity when the analysis based on lipid peroxidation inhibiting capacity using TBARS. Different phenolic classes such as flavonoids and can be found in P. oleracea leaves. The attached hydroxyl groups to aromatic rings of flavonoids, which enable to undergo a redox reaction that helps them to scavenge the free radicals (Brand-Williams et al. 1995).

In the current study, phenolic compounds such as pyrogallol, chlorogenic acid and rosmarinic acid may be responsible about the antioxidant activity in purslane leaves extracts.

3.4. Fatty acids analysis

The fatty acids composition of purslane leaves fixed oil is given in (Table 3). Some volatile compounds extracted from plants may have antioxidant activity that could mitigate obesity-related complications, including atherosclerosis and some cancers, purslane contain high levels of unsaturated fatty acids and poly-phenols, which are excellent scavengers of reactive oxygen species and represent a promising anti-obesity effects (Ebrahimzadeh et al. 2009). Gonnella et al. (2010) reported that, P. oleracea is the best plant sources of ω-3 fatty acids, α linolenic acid.

Table 3. Fatty acids composition of purslane leaves oils determined by gas chromatography GC

| Fatty acids      | Concentration (%) |
|------------------|-------------------|
| Lauric (C₁₂:0)   | 0.92              |
| Myristic (C₁₄:0)| 3.56              |
| Palmitic (C₁₆:0)| 24.03             |
| Stearic (C₁₈:0)| 2.19              |
| Oleic (C₁₈:1)   | 3.85              |
| Linoleic (C₁₈:2)| 9.46              |
| α-Linolenic acid (C₁₈:3)| 51.72          |

*Values are expressed as relative area percentage
Fig. 1. Scavenging activity of ethanol and water extracts of purslane leaves at different concentrations (ug mL\(^{-1}\)) as determined by DPPH (a) and reducing power (b) assays.
The analysis of fatty acids showed that most abundant fatty acids purslane leaves are α-linolenic acid (51.72%); and palmitic acid (24.03%) and linoleic acid (9.46%). Purslane has highest value of alpha-linolenic acid it is the most abundant one in purslane as shown in (Table 3). Concerning saturated fatty acids, the most predominant fatty acids was Palmitic C16:0 (24.03%) and the others have the lowest values such as Myristic (C14:0), Stearic (C18:0) and Lauric (C12:0) recorded 3.56%, 2.19% and 0.92% respectively.

In comparison with the leafy green vegetables, purslane contain the highest level of α-linolenic acid (ω-3 fatty acids) which play an important role for human nutrition Kamal-Uddin et al (2014). Omega 3 fatty acids improve the immune function, prevention and treatment of hypertension, coronary heart disease, and other inflammatory and autoimmune disorders and had advantage compared to fish oil since they are free from cholesterol (Siriamornpun and Suttajit, 2010).

The cardiovascular and diabetic disease prevention by omega 3 fatty acids in purslane due to its content of the precursor to specific group of hormones and which help in decreasing the thickness of blood (Kamal-Uddin et al 2014; Osman and Hussein, 2015).

3.5. Analysis of volatile compounds

The results of volatile compounds by Headspace (HS) and water distillation (WD) of Purslane leaves and identification with (GC-MS) analysis and RI are presented in (Table 4).

Table 4. Volatile compounds of Purslane leaves by (GC-MS) analysis

| Volatile compounds | RI^ | Concentration |
|--------------------|-----|---------------|
|                    | HS  | WD            |
| 1-Penten-3-ol      | 976 | 2.36**        |
| Pentanal           | 984 | 4.68          |
| (E)-2-pentalen     | 873 | 3.19          |
| Toluene            | 882 | 5.76          |
| Hexanal            | 916 | 10.92         |
| Butyl acetate      | 926 | 2.01          |
| (E)-2-Hexenal      | 967 | 15.64         |
| Heptanal           | 1015| 0.46          |
| (Z)-4-heptenal     | 1072| 1.33          |
| (E,E)-2,4-heptadienal | 1108 | 2.09 |
| Limonene           | 1143| 1.93          |
| Eucalyptol         | 1148| 1.40          |
| Octanal            | 1119| 2.57          |
| 3-Octen-2-one      | 1153| 0.34          |
| Pheny lacet aldehyde | 1157 | 4.57   |
| (E)-2-Octenal      | 1173| 0.42          |
| o-Cymene           | 1189| 0.46          |
| (E)-2-Nonenol      | 1217| 12.03         |
| (E,Z)-2,6-nonadienal | 1266 | 0.36 |
| Menthol            | 1291| 0.78          |
| Ethyl octanoate    | 1305| 1.60          |
| (E)-2-Decenal      | 1314| 2.66          |
| Benzo thiazole      | 1325| 1.42          |
| Ethyl decanoate    | 1407| 0.98          |
| Phenylethylisothiocyanate | 1483 | 0.52   |
| Ethyl hexadecanoate | 1906 | 2.06 |
| Ethyl linoleate     | 1978| 8.02          |

*: RI retention indices determined on DB-5 capillary column; **: Values are expressed as relative area percentage to the total identified volatile compounds; n.d.: not detected

4. CONCLUSION

Portulaca oleracea plant extract could be used as a source of phytochemical and antioxidants, especially for functional food. Therefore, the study will extend to shade light on the effect of the investigated extracts on some functional food such as yoghurt or bakery products.
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تقييم المرکبات الكيميائية النباتية ومضادات الأكسدة والطیارة لأوراق الرجلة المصرية

علياء عبد الستار المشد1- جمال السيد ابراهيم2- رباب حسن سالم1

1 قسم علوم وتكنولوجيا الأغذية – كلية الاقتصاد المنزلي – جامعة الأزهر- طنطا - مصر
2 قسم كيمياء مكسبات الطعم والرائحة – المركز القومي للبحوث - الدفي- 12622 - الجیزة - مصر

الموجز

Portulaca oleracea يستخدم نبات الرجلة في العديد من التطبيقات الغذائية كنوع من أنواع الخضروات ويمكن استخدامه أيضاً في المجالات الطبية. لذلك فالهدف من الدراسة الحالية هو تقييم المكونات الكيميائية النباتية والمركبات الفينولية ونشاط مضادات الأكسدة في المستخلص الإيثانولي والمائي للأوراق نبات الرجلة. تم تقييم محتوى الأحماض الدهنية والمركبات الطيارة في الأوراق. كانت تركيزات الصابونين والقوليدات هي الأعلى مقارنة بالأحماض الدهنية الأخرى والتي سجلت بحروف أقل، وتعادل Lauric (C12:0) وStearic (C18:0) في 2.19 و 0.92% على التوالي. واستخدام GC-MS و GC تم التعرف على سبعة وعشرين مركب في مستخلصات نبات الرجلة، كانت المكونات الرئيسية (E) Headspace (HS) من الفينول والفلانولونيد (E)-2-Hexenal 31.8%، (E)-2-Nonenol 15.64 Hexenal Ethyl linoleate 10.92 Hexanal، Hexanal 8.02% من ناحية أخرى، كانت المركبات الطيارة الرئيسية الناتجة عن الفينول والأحماض الفينولية في كلا المستخلصات الإيثانولي والمائي (3.15%)، مقارنة بالمستخلص المائي (5.58%). بينما كان مستوى كل من الفينول والفلانولونيد في كلا المستخلصات متشابهين تقريبًا. أظهر تحليل HPLC أن البيروجالول كان المركب الفينولي الرئيسي في كل من المستخلص الإيثانولي والمائي بنسب تقارب 24.85%، على النحو التالي. من ناحية أخرى، كان المحتوى الأعلى للمركبات الفينولية الموجودة في المستخلص المائي،<sub>chlorogenic, pyrogallol</sub> للجزء المكملات النباتية: نبات الرجلة, المواد الكيميائية النباتية, مضادات الأكسدة, المركبات الطيارة

الكلمات الدالة: نبات الرجلة, المواد الكيميائية النباتية, مضادات الأكسدة, المركبات الطيارة

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Corresponding author: aliaa.akr2002@yahoo.com

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