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***Full Length Article***

# Antioxidant Activity of some Extracts from Gamma irradiated Purslane plant (*portulaca oleracea L.*)

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## Abstract

Purslane plant is one of the most used medicinal in listed in the World Health Organization. This paper aims to evaluate the effect of some solvent and different doses of irradiation on antioxidants level and its activity. The extraction of antioxidants was performed by methanol, ethanol and distilled water. Antioxidant activities were determined by Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing power (FRAP) and β-Carotene bleaching (BCB). Results indicated that extraction by methanol (50%) had higher total phenolic contents (TPC) than other extracts. As well as, the total antioxidant contents and its activities increased with increasing the irradiation dose. Extraction of irradiated purslane plant at 9 kGy by methanol (50%) considered the effective one to obtained natural phenolic acid compounds; which were identified by HPLC to twenty four components. Moreover, methanol extracts were tested for their antioxidant activity on sunflower oil. The obtained data showed that all tested parameters had higher in comparison to control. Methanol extracts were also tested for their antimicrobial activity, which showed higher inhibition activity of all micro-organism (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella Pneumonia*, *Bacillus cereus*). 

**Keywords:** Purslane plant; Gamma irradiation; Phenolic extraction; Antioxidant activity; Antimicrobial activity

## Introduction

Food that contains fat is exposed to deterioration by lipid oxidation. Lipid per- oxidation causes cardiovascular and carcinogenesis diseases (Siddhuraju and Becker, 2003; Shahid *et al*., 2008). In food industry, synthetic antioxidants such as tetra-butyl hydroquinone (TBHQ), butyl hydroxyl anisole (BHA) and butyl hydroxyl toluene (BHT) are used to prevent lipid per oxidation, because being less expensive. Synthetic antioxidants are not safe, and harmful to human health. Therefore, natural antioxidants such as phenolic, flavonoids, tannins, xanthons, coumarins, curcumanoids and terpenoids should be used (Jeong *et al*., 2004). Purslane plan contain natural antioxidants, which has also antibacterial properties (Chan *et al*., 2000). Purslane plant is used as a health food for patients with cardiovascular diseases (Liu *et al*., 2000; Abas *et al*., 2006). The content of plant antioxidants and its activity were reported to increase by gamma irradiation (Variyar *et al*., 1998; Alothman *et al*., 2009). Gamma irradiation was applied at various doses to date palm fruit Mazafati, and amount of total phenolic and antioxidant activity were increased significantly (Ghadi *et al*., 2015). (Mali *et al.,* 2011) irradiated pomegranate peel and reported that gamma irradiation led to increase of total phenolic and antioxidant activity. (Sima *et al.,* 2014) observed that methanol extract of Curcuma *alismatifolia* leaves increased total phenolic and antioxidant activity by gamma irradiation.

This study also aimed to extract total phenolic compounds from irradiated purslane plant. Irradiation at doses of 5, 7 and 9 kGy were used and extraction was performed by methanol, ethanol and distilled water. Also, the ability of the best extract obtained was evaluated on the oxidative stability of sunflower oil and antimicrobial activity.

## Materials and Methods

### Plant Material

Purslane plant was collected in September 2014, and identified by Corps unit, Atomic Energy Authority, Nuclear Research Center, Egypt.

### Chemicals

Butylated hydroxyl tuluen, Diphenyl-2-picrylhydrazyl, β-carotene, Gallic acid, Quercetin, iron (III) chloride and aluminum chloride were analytical grade. Water was purified before use in a Milli-Q system (Millipore, Bedford, MA, USA).

## Methods

**Preparation of purslane plant extract:** Samples were washed with distilled water and evaporated residual moisture at room temperature, plant were cut into species then dried in oven at 45ºC for 72 h and ground to fine powder.

**Irradiation of sample:** Purslane plant powder was packed in polyethylene bags then irradiated using a $^{60}$Co Russian gamma chamber; dose rate was 1.3 kGy/h and the radiation doses used were 5, 7 and 9 kGy.

**Extraction of purslane plant:** Purslane plant powder was soaked in hexane to remove fatty matter and hexane extract was discarded. Samples of 25 g each were individually extracted twice by shacking using 250 mL in each one then filtrated. Solvents were removed using rotary evaporator at 45ºC under reduced pressure. The obtained extracts were stored at -18ºC until analysis. Three extracts were prepared using methanol 50%, ethanol 50% and distilled water.

**Determination of total phenolic and flavonoids:** Determination of total phenolic (TPC) and flavonoids (TFC) was done according to (Saeedeh and Asna, 2007; Ordon *et al*., 2006).

**Determination of antioxidant activity:** Ferric reducing antioxidant power (FRAP), radical-scavenging activity (DPPH) and β-Carotene/linoleic acid bleaching (βCB) of each extract were determined according to the procedures described by (Oyaizu, 1986; Su and Silva, 2006; Keyvan *et al*., 2007) respectively.

**Fractionation and identification of phenolic compounds:** High performance liquid chromatography (HPLC) was used for fractionation and identification of phenolic compounds according to (Goupy *et al*., 1999).

**Oxidative stability:** Antioxidant activity of condensed methanol 50% extract of non- irradiated and irradiated at 0, 5, 7 and 9 kGy purslane plant mixed well with sunflower oil by the rancimat method as described by (Hadorn and Zurcher, 1974; Hasenhuttle and Wan, 1992). The antioxidant activity and increasing index were calculated using the following equations:

$$\text{Antioxidant activity} = \frac{\text{Induction period of sample}}{\text{Induction period of control}} \quad (1)$$

$$\text{Increasing index} = \frac{\text{Induction period of sample} - \text{Induction period of control}}{\text{Induction period of control}} \times 100 \quad (2)$$

**Measurement of antibacterial activity:** Antibacterial activity was determined according to (Baydar *et al*., 2004).

## Statistical Analysis

The ANOVA test was performed for statistical analysis using the HDSS (McClave and Benson, 1991) and assist computer programs (Silva and Azevedo, 2006). A value of $p \leq 0.05$ was considered to be statistically significant.

## Results

### Total Phenolic and Flavonoids Content in Purslane Plant

Total phenolic contents (TPC) were 201.1±0.3, 178.1±0.3 and 100.0±0.8 mg GAE/100g and total flavonoids content (TFC) were 50.1±0.2, 42.0±0.0 and 25.2±0.3 mg QE/100 g, in control sample extracted by methanol 50%, ethanol 50% and distilled water, respectively. (Table 1) Also, TPC ranged between 175.1±0.6 to 273.1±0.3, 186.2±0.5 to 282.3±0.3 and190.2±0.5 to 315.0±0.4 mg GAE/100 g at doses 5, 7 and 9 kGy, respectively according to extract solvent used. While the lowest TPC and TFC were found for distilled water extract being 100.0±0.8 mg GAE/100 g and 25.2±0.3 mg QE/100 g in control sample. The highest TPC and TFC were observed with methanol extract of irradiated purslane plant at dose 9 kGy (315.0±0.4 mg GAE/100 g and 65.0±0.10 mg QE/100 g, respectively).

### Antioxidant Activity from Purslane Plant Extracts

**DPPH radical-scavenging activity:** DPPH radical-scavenging activity with different solvents ranged between 20.2±0.2 to 32.7±0.20% for control sample (Tables 2). Methanol extract showed the highest DPPH radical-scavenging activity of irradiated purslane plant at 9 kGy compared to doses 5 and 7 kGy. Distilled water had the less DPPH at different doses ranged between 20.2 to 28.0%.

**B-Carotene/linoleic acid bleaching (BCB):** Methanol extract had highest scavenging ability compared with ethanol and distilled water in control sample, β-carotene/linoleic acid bleaching (βCB) increased by increasing dose rate (Table 3). Gamma irradiation at 9 kGy enhanced the scavenging ability of all extracts by methanol, ethanol and distilled water extracts, respectively. Also, the methanol extract of irradiated purslane plant at 9 kGy had highest extract in βCB system than other extracts and BHT.

**Ferric reducing antioxidant power (FRAP):** FRAP value of methanol extract was the highest one compared with the other investigated extracts of non-irradiated purslane plant (Table 3). Irradiated sample at 9 kGy enhanced FRAP value of all extracts.

**Identification of phenolic compounds:** This analysis was carried out on the best treatment of investigated extracts i.e. methanol 50% and dose 9 kGy. The phenolic acids were identified according to their retention time in comparison with authentic samples; 24 phenolic acids were identified (Table 4). It seems that quantitatively some phenolic compounds increased and others, such as catechein, coumarin, and cinnamic, found absent by irradiation treatment.

**Table 1:** Phenolic and Flavonoid contents of irradiated purslane plant extracts

| γ -irradiation (kGy) | Solvents | | | | | |
|---|---|---|---|---|---|---|
| | Methyl alcohol 50% | | Ethyl alcohol 50% | | Distilled water | |
| | TPC (mg GAE/100 g) | *TFC mgQE/100 g)* | TPC(mg GAE/100 g) | *TFC(mgQE/100g)* | TPC (mg GAE/100g) | *TFC (mgQE/100g)* |
| 0 | 201.10±0.30$^{a1}$ | 50.10±0.20$^{a1}$ | 178.10±0.30$^{b1}$ | 42.00±0.0$^{b1}$ | 100.00±0.80$^{c1}$ | 25.20±0.30$^{c1}$ |
| 5 | 273.11±0.30$^{a2}$ | 57.15±0.30$^{a2}$ | 250.13±0.30$^{b2}$ | 52.08±0.10$^{b2}$ | 175.01±0.60$^{c2}$ | 27.05±0.30$^{c2}$ |
| 7 | 282.03±0.30$^{a3}$ | 63.08±0.20$^{a3}$ | 255.10±0.50$^{b3}$ | 57.71±0.20$^{b3}$ | 186.24±0.50$^{c3}$ | 28.12±0.60$^{c3}$ |
| 9 | 315.00±0.40$^{a4}$ | 65.00±0.10$^{a4}$ | 265.05±0.30$^{b4}$ | 60.11±0.30$^{b4}$ | 190.22±0.50$^{c4}$ | 30.15±0.40$^{c4}$ |

**Table 2:** DPPH radical-scavenging activity of irradiated purslane plant extracts

| γ -irradiation (kGy) | Methyl alcohol 50% | Ethyl alcohol 50% | Distillated water |
|---|---|---|---|
| 0 | 32.70±0.20$^{a1}$ | 29.00±0.10$^{b1}$ | 20.20±0.20$^{c1}$ |
| 5 | 34.00±0.40$^{a2}$ | 31.00±0.20$^{b2}$ | 23.10±0.40$^{c2}$ |
| 7 | 35.20±0.30$^{a3}$ | 33.00±0.10$^{b3}$ | 26.80±0.10$^{c3}$ |
| 9 | 39.00±0.40$^{a4}$ | 35.87±0.30$^{b4}$ | 28.00±0.00$^{c4}$ |
| BHT(200ppm) | 24.21±0.20 | 24.21±0.20 | 24.21±0.20 |

**Table 3:** β-carotene/ linoleic acid bleaching (βCB) system and absorbance of ferric reducing power (FRAP) of irradiated purslane plant extracts

| γ -irradiation (kGy) | Solvents | | | | | |
|---|---|---|---|---|---|---|
| | Methyl alcohol 50% | | Ethyl alcohol 50% | | Distilled water | |
| | β-carotene | FRAP | β-carotene | FRAP | β-carotene | FRAP |
| 0 | 62.30±0.20$^{a1}$ | 3.60±0.00$^{a1}$ | 50.20±0.10$^{b1}$ | 2.88±0.10$^{b1}$ | 43.00±0.20$^{c1}$ | 2.56±0.10$^{c1}$ |
| 5 | 64.71±0.30$^{a2}$ | 3.81±0.20$^{a2}$ | 52.28±0.20$^{b2}$ | 2.89±0.10$^{b1}$ | 43.56±0.10$^{c2}$ | 2.57±0.10$^{c1}$ |
| 7 | 65.22±0.20$^{a3}$ | 3.82±0.00$^{a2}$ | 55.30±0.20$^{b3}$ | 2.89±0.010$^{b1}$ | 44.71±0.50$^{c3}$ | 2.58±0.10$^{c1}$ |
| 9 | 68.21±0.20$^{a4}$ | 3.93±0.20$^{a3}$ | 56.71±0.50$^{b4}$ | 3.00±0.10$^{b2}$ | 46.00±0.30$^{c4}$ | 2.78±0.10$^{c2}$ |
| BHT(200ppm) | 55.62±0.40 | 1.05±0.30 | 55.62±0.40 | 1.05±0.30 | 55.62±0.40 | 1.05±0.30 |

Values represent the mean of triplicates ± standard deviation
Mean with the same letters in the same row is not significantly different ($p \leq 0.05$)
Mean with the same numbers in the same column is not significantly different ($p \leq 0.05$)

**Table 4:** Identified phenolic compounds (mg/100 gm) in Methanol 50% extract of non-irradiated and irradiated Purslane plant samples at dose level of 9 kGy

| Compounds | Control | Irradiated | Compounds | Control | Irradiated |
|---|---|---|---|---|---|
| Gallic acid | 7.26 | 120.66 | Ferulic acid | 8.85 | 17.35 |
| pyrogallo | 58.31 | 80.25 | Iso- Ferulic | 3.63 | 11.34 |
| 3-oH Tyrosol | 52.14 | 86.55 | E-vanillic | 525.94 | 1110.25 |
| 4- Aminobenzoic | 7.11 | | Revesetrol | 13.29 | |
| Protocatechuic | 126.66 | 90.33 | Ellagie | 4.81 | 11.02 |
| Chorogenic | 75.51 | 87.53 | Alohacoumaric | 45.04 | |
| Catechol | 24.72 | 59.99 | Benzioc | 51.06 | |
| Catechin | 98.00 | - | 3,4,5 methyoxycinnamic | 15.54 | |
| caffeine | 14.64 | 14.88 | Salycilic | 36.16 | 12.34 |
| P-oH- Benzoic | 15.94 | 16.014 | Coumarin | 2.14 | -- |
| Caffeic | 14.31 | 14.33 | p- coumaric | 4.97 | -- |
| Vanillic | 2.60 | 14.38 | cinnamic | 1.89 | -- |

**Oxidative stability:** Oxidative stability was used to determine the effect of addition of methanol extracts from gamma irradiated and non-irradiated purslane plant on shelf life of sunflower oil. Data in Table (5) showed that the induction period of control sample was 8.10 h. Also, the addition of BHT (200 ppm) increased induction period to 10.50 h, the induction period was (11.33 h) by addition of methanol extract of irradiated purslane plant at 9 kGy, which increased the shelf life from 12.01 to 16.81 month.

**Antimicrobial activity of purslane plant:** Antibacterial activity of non-irradiated and irradiated pruslane plant methanol extracts was measured against some pathogenic bacteria *Klebsiella Pneumonia, Salmonella Typhimurium, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus cereus*. Non irradiated and irradiated pruslane plant methyl alcohol extracts inhibited both bacterial species (Gram-negative and Gram- positive) (Table 6). The highest inhibition of *Bacillus cereus and Escherichia coli* was achieved with irradiated purslane plant at 9 kGy methanol extracts; zone inhibition diameter was 62 and 57 mm, respectively.

**Table 5:** Effect of purslane plant methanol extract on oxidative stability of sunflower oil

| γ - irradiation (kGy) | Induction Period | Shelf life at 25˚C (month) | Antioxidant activity | Increasing index (%) |
|---|---|---|---|---|
| control | 8.10$^{e}$ | 12.00$^{e}$ | 0.00$^{e}$ | 0.00$^{e}$ |
| 0 | 10.00$^{d}$ | 14.83$^{d}$ | 1.23$^{d}$ | 23.48$^{d}$ |
| 5 | 10.22$^{c}$ | 15.16$^{c}$ | 1.26$^{c}$ | 26.22$^{c}$ |
| 7 | 10.59$^{b}$ | 15.71$^{b}$ | 1.30$^{b}$ | 30.80$^{b}$ |
| 9 | 11.33$^{a}$ | 16.80$^{a}$ | 1.39$^{a}$ | 39.96$^{a}$ |
| BHT(200 ppm) | 10.50 | 15.58 | 1.29 | 29.72 |

Mean with the same letters in the same column is not significantly different ($p \leq 0.05$)

**Table 6:** Antimicrobial activity of purslane plant methanol extract against some pathogenic bacteria

| γ-irradiation | Methyl alcohol 50% | | | | | |
|---|---|---|---|---|---|---|
| (kGy) | *Pseudomonas aeruginosa* | *Salmonella typhimurium* | *Escherichia coli* | *Staphylococcus aureus* | *Klebsiella Pneumonia* | *Bacillus cereus* |
| 0 | 39 | 35 | 35 | 40 | 35 | 37 |
| 9 | 43 | 44 | 57 | 47 | 42 | 62 |

## Discussion

Methanol was the best solvent extract with total phenolic and flavonoids contents than ethanol and distilled water, the extract of total phenolic and flavonoids contents increased by increasing dose rate of gamma irradiation. Methanol that had higher polarity had ability to inhibit the action of oxidation (Wieland *et al.,* 2006). Also, irradiation treatment at 9 kGy in the same solvent had an increasing in TPC and TFC of all extracts particular compared to other treatments at 0, 5and 7 kGy. It seems that irradiation treatments might cause some chemical changes in components of purslane plant (Variyar *et al.,* 1998; Topuz and Ozdemir, 2004).

DPPH accepts free radical to become a stable molecule (Gulçin *et al.,* 2004). The high scavenging ability of methanol extract of irradiated purslane plant at 9 kGy can be correlated to the highest phenolic content, irradiation increase extractability and improved antioxidant activity (Noda *et al.,* 2002; Poyrazoglu *et al.,* 2002) reported that inhibiting lipid peroxidation by phenolic and flavonoids compounds showing their properties to scavenge free radicals.

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Dorman *et al.,* 2003). Methanol extract of irradiated purslane plant at 9 kGy was the highest in ferric reducing power value compared to ethanol and distilled water extract and synthetic antioxidants (BHT).

### Identification of Phenolic Compounds in Extracts

Some quantitative phenolic compound increased and some phenolic compounds found absent by irradiation treatment in purslane such as catechein, coumarin, and cinnamic. The increase in phenolic contents with degradation of tannins and changes the conformation of the molecules (Variyar *et al.,* 1998; Topu and Ozdemir, 2004).

### Oxidative Stability

Foods containing fat is more exposed to oxidation and deterioration. Therefore we must use some natural antioxidants to increase oxidative stability to fats and increase shelf life of foods (Thomsen *et al.,* 2000). The induction period is the length of time until progressive oxidation exponentially accelerates the generation of oil degradation compounds (Kristott, 2000). Phenolic compounds increase oxidative stability of sunflower oil also extracts of irradiated purslane plant at 9 kGy had higher oxidative stability than control.

Control and irradiated pruslane plant at 9 kGy extracts by methanol inhibit both bacterial species (Gram- negative and Gram- positive). The highest inhibition of *Bacillus cereus* and *Escherichia coli* was achieved with irradiated purslane plant at 9kGy methanol extracts, these results agree with (Shoko *et al.,* 1999; Pimia *et al.,* 2001) that phenolic compounds have properties antimicrobial, because it cause DNA mutagenic.

## Conclusion

This study concluded that methanol 50% is the best solvent for extracting antioxidant compounds from plants than ethanol 50% and distilled water. Also, irradiation treatment at 9 kGy increased extraction and antioxidant activity in the same solvent of all extracts particular compared to other treatments at 0, 5 and 7 kGy. In addition phenolic compounds increase oxidative stability of sun flower oil and showed activity against all gram negative and gram positive test bacteria.

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