ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

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Evaluation of antioxidant potential in fresh and boiled juice of purslane leaves

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Article History:

Received on: 14.07.2018
Revised on: 22.12.2018
Accepted on: 24.12.2018

Keywords:

Portulaca oleracea, Phytochemical Screening, Antioxidant activity, Phenolic content, Reactive oxygen species, Oxidative stress

ABSTRACT

Free radical scavenging potentials of fresh and cooked Purslane (*Portulaca oleracea* - Portulacaceae) was estimated by *in vitro* antioxidant assays. Antioxidant activity of fresh aqueous juice and cooked aqueous filtrate of purslane leaves were evaluated against synthetic free radicals such as DPPH, $ABTS^{\cdot+}$ and the results were comparable with that of the reference antioxidant L-Ascorbic acid. The juices showed high antioxidant activity using DPPH and $ABTS^{\cdot+}$ assays with increasing concentration of juice extracts. The total phenolic content was quantified and found to be 55.26±1.04 and 26.15 ±1.25 mg/g evaluated by the gallic acid corresponds to fresh and cooked filtrate respectively. The IC_{50} values of fresh, cooked filtrate and L-ascorbic acid were 145.15 ± 1.85, 85.35 ± 2.17 µg/ml and 65. 40 ± 3.66 µg/ml respectively in DPPH antioxidant assay and likewise the IC_{50} values of fresh, cooked filtrate and L-ascorbic acid found to be 227.35±1.45 µg/ml, 151.15±1.05 and 65.15±2.89 µg/ml, respectively in $ABTS^{\cdot+}$ antioxidant assay. Comparatively the inhibitory concentration of purslane leaves was high in cooked filtrate juice than the fresh aqueous juice.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i1.1906

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INTRODUCTION

The singleton oxygen is formed in the living cells in aerobic organisms for normal cell functions if exceed the ROS level in the living system cause oxidative stress and lead cause oxidative damage. These ROS intermediates threaten to various biomolecules including proteins and enzymes (Stadtman *et al.,* 2000) lipids, DNA (Marnett *et al.,* 2000) and ageing (Cadenas *et al.,* 2000).

Most of the green leafy vegetables contain the carotenoids, which is useful as antioxidants to blocking the early stages of cancer. The green leaves provide various nutritive values; antioxidants include folate, vitamin B, helps to promotes heart health, prevent certain congenital disabilities and to inhibit the development of a variety of tumours and reduce the risk of heart diseases. Green leafy vegetables are used in various aspects such as the low risk of stomach, breast and skin cancer in human beings. Leafy vegetables give minerals, vitamins, pigments with therapeutic potential. *Portulaca oleracea* it is commonly called as purslane. It is an annual succulent plant belonging to the family *Portulacaceae* and it grows up to 40 cm in height.

Portulaca oleracea contains higher omega-3 fatty acids compared to other leafy vegetable and has 0.01 mg/g of eicosapentaenoic acid (EPA) (Simopoulos *et al.,* 1992) along with vitamins, carotenoids, minerals such as magnesium, calcium, potassium and iron, pigments such as betalain, reddish betacyanins, yellow betaxanthins. These pigments are potent antioxidants and antimutagenic properties.

Being part of a healthy diet *Portulaca oleracea* leaves 100 grams of fresh purslane leaves to contain high nutritional benefits. Balanced purslane reduces soluble oxalate; betacyanins improved cognition deficits in aged mice (Wang *et al.,* 2010). And also, it contains anticancer homo isoflavonoids (Yan *et al.,* 2012) and it acts against oral lichen planus (Agha-Hosseini *et al.,* 2010), against insect or snake bites (Bensky *et al.,* 2004) and it arrests diarrhoea, intestinal bleeding (Tiera *et al.,* 1988). The main purpose of this study was to evaluate the antioxidant activities of fresh and cooked purslane leaves.

METHODS AND MATERIALS

Chemicals

2-2-Diphenyl-1-Picryl Hydrazyl, 2, 2'-azinobis-3-ethyl benzothiazoline-6-sulfonic acid, ascorbic acid, gallic acid, and potassium persulfate were obtained from HiMedia, Mumbai and SD Fine Chemicals, India.

Plant Material

Portulaca oleracea were collected from in and around the surrounding areas of Annur, Coimbatore, Tamil Nadu during February 2012 and it was authenticated by Botanical Survey of India (BSI), Coimbatore and the same specimen was deposited at BSI for future reference.

Preparation of extracts

Fresh aqueous extract

100g of the leaves were washed thoroughly, chopped and crushed with mortar and pestle with 500 ml of distilled water. After getting a homogenous aqueous plant material, it was passed through a filter using Whatman No.1 filter paper and then freeze-dried (Dkhil *et al.,* 2011).

Cooked filtrate or extract

Boiling: Fresh purslane leaves (100 g) was submerged in the boiling water (0.5L), cooked for 15 - 30 min and the cooked plant materials were drained with a wire mesh filter to separate the filtrates and residues, the filtrate has taken for further studies.

Major phytochemical screening

The major secondary metabolites such as phenolics, alkaloids, flavonoids, terpenoids and tannins were assessed in the purslane leaves (fresh juice and boiled juice) (Trease *et al.,* 1989; Harborne *et al.,* 1973).

Estimation of total phenolic content

The total phenolic content was estimated in the fresh juice and boiled juice of purslane leaves followed by Singleton *et al.* 1999. 1 ml of extract was taken in a test tube mixed with 1 ml of Folin-Ciocalteu reagent and it was kept for 3 minutes. To the solution, 2 ml of sodium carbonate was added (20%) and the reaction mixture was incubated for 30 min at 27°C. The absorbance was read at 700 nm and compared with the gallic acid calibration curve to quantify the total phenolics in the extract.

Free radical scavenging activity by DPPH synthetic radical

DPPH radical scavenging potentials of the extracts followed by the method of Susanta *et al.,* method (2006) (Susanta *et al.,* 2006). Fresh and cooked extract of *Portulaca oleracea* and ascorbic acid dissolved in water and aliquot at different concentration (100-500 µg/ml), 3 ml of freshly prepared methanolic DPPH solution was added and allowed to stand at 27°C in the dark. DPPH alone was used as a control, followed ascorbic acid was used as positive control (reference standard antioxidant). Decreased optical density values of the reaction mixture measured at 517 nm and percentage inhibition calculated using the following formula.

The radical scavenging activity (%)

= (Optical density value of the control - Optical density value of the sample) / (Optical density value of the control) X 100

$ABTS^{\cdot+}$ radical cation decolourisation assay

The radical cation decolourisation assay with ABTS (7mM) using alcohol and potassium persulfate (2.45mM) and formed the dark blue colour after 12-16 hrs at room temperature. The preprepared blue colour formation with ABTS have used to study the radical cation decolourisation assay of samples. Fresh and cooked extract of *Portulaca oleracea* and ascorbic acid of various concentrations (100-500µg/ml) were added to different test tubes and 1 ml of ABTS solution added. Absorbance was read at 734 nm using the spectrophotometer and the percentage of inhibition of ABTS radical scavenging activity was calculated using the above formula.

RESULT AND DISCUSSION

Major phytochemical screening

The phytochemical screening of purslane juice after and before cooking was demonstrated and found that cooked purslane juice contain mode secondary metabolites including alkaloid, terpenoid, flavonoid, steroid, cardio glycosides, and tannin (Table.1).

Table 1: The phytochemical screening of Purslane juice (fresh and cooked)

Extracts	AL	S	TER	FLA	STE	CG	T
fresh juice	+	-	+	+	-	+	+
cooked juice	+	-	+	+	+	+	+

AL-Alkaloids; S-Saponins; TER- Terpenoids; FLA-Flavanoids, STE- Steroids, CG-Cardioglycoside, T-Tannins

Total Phenol Content

Total phenol content of purslane -fresh and cooked extract was shown in figure 1 and was found to be 55.65±2.05 mg/g and 26.15 ±1.25 respectively and expressed in gallic acid equivalents (GAE). The result indicated the presence of high total phenolic content in fresh purslane extracts evidenced from the gallic acid linear curve value obtained with y = 0.1996x-0.277 (R^2=0. 9079).

DPPH radical scavenging activity

DPPH assay is one of the well-known methods to find out the antioxidant activity of the plant samples. DPPH radical scavenging activity of fresh and cooked extracts of *Portulaca oleracea* and for the standard ascorbic acid is shown in Figure. 2. The decrease in absorbance value of the reaction mixture indicates the high antioxidant and high ROS scavenging power (Figure. 2A). The decrease absorbance values of extracts and standard were taken for calculating the percentage inhibition against synthetic ROS. DPPH radical scavenging activity increased from 42.47± 2.15 to 86.72 ± 1.27 % for fresh extract, 51.76 ± 2.25 to 95.57 ± 3.10 % for cooked extract at a concentration of 100 to 500µg/ml and for the standard ascorbic acid the value was were 99.02 ± 1.47% at 500 µg/ml (Figure.2B). DPPH radical scavenging activity showed the lowest IC_{50} of purslane -fresh and cooked extract 145.15 ± 1.85, 85.35 ± 2.17 µg/ml respectively and were significant for ascorbic acid it is 65. 40 ± 3.66 µg/ml. The lowest IC_{50} value indicates high DPPH radical scavenging activity. Previously methanolic extracts of several plant species *Amaranthus sp*., *Centella Asiatica* and *Murraya koenigii* were studied for their antioxidant potentials with various concentrations. The extract concentration causing 50% inhibition of DPPH (IC_{50}) was determined (*M. koenigii* <*C.asiatica* < *Amaranthus sp*. < *T. graecum*). The maximum antioxidant potential was observed in *Murraya koenigii* (Gupta *et al.,* 2009). Likewise, other green leafy vegetables such as amaranth, chenopod and spinach contain high antioxidant and used different nutraceutical and pharmacological products (Yadav *et al.,* 2013).

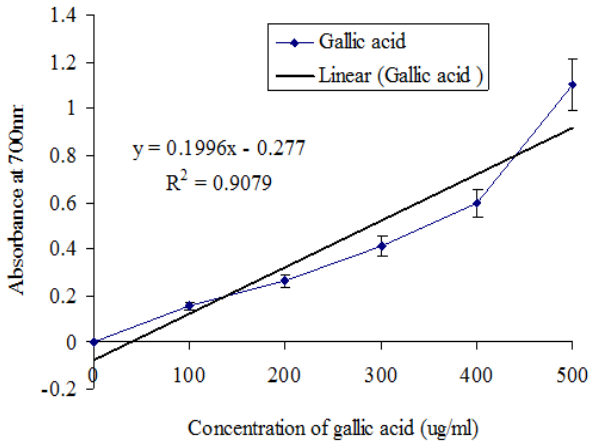

Figure 1: Total phenolic content of fresh and cooked *Portulaca oleracea* leaves

ABTS·+ radical scavenging activity

The fresh and cooked extract of *Portulaca oleracea* exhibited good ABTS·+ radical scavenging activity and the percentage of inhibition was (Figure. 3B) found to be 90.51±1.53, 93.43±1.96 respectively and comparable with standard ascorbic acid 95.51±0.09%. IC_{50} values in scavenging abilities on ABTS· + radicals of a fresh and cooked extract of *Portulaca oleracea* are 227.35±1.45 µg/ml, 151.15±1.05 respectively and for ascorbic acid, it is 65.15±2.89 µg/ml. The decreasing absorbance of ABTS was recorded after treated with samples (Figure. 3A). In the earlier study ABTS·+ Radical cation decolourization assay has been reported. ABTS·+ radical cation is reactive towards most antioxidants including phenolics, thiols and vitamin C. ABTS radical scavenging activity of standard ascorbic acid of leafy green extracts were showed in following order: Ascorbic acid (13.7 µg/ml) > *Tamarindus indica* (35 µg/ml) > *Mentha arvensis* (40 µg/ml) > *Spinacia oleracea* (180 µg/ml) > *Trigonella foenum-graecum* (190 µg/ml) > *Moringa oleifera* (300 µg/ml) > *Amaranthus Viridis* (500 µg/ml) (Raghavendra *et al.,* 2013). It was earlier reported that ethanolic extract of *J. grandiflorum* flower exhibited free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), radical, 2, 2'-azinobis(3-thy-benzothiazoline-6-sulfonic acid) ($ABTS^+$) radical, hydrogen and hydrogen peroxide radical activity compared to L-ascorbic acid with higher inhibition on increasing concentration of the substances (Praveen *et al.,* 2016). Nisha Raj and Arulmozhi, 2013 reported that *Piper nigrum*,

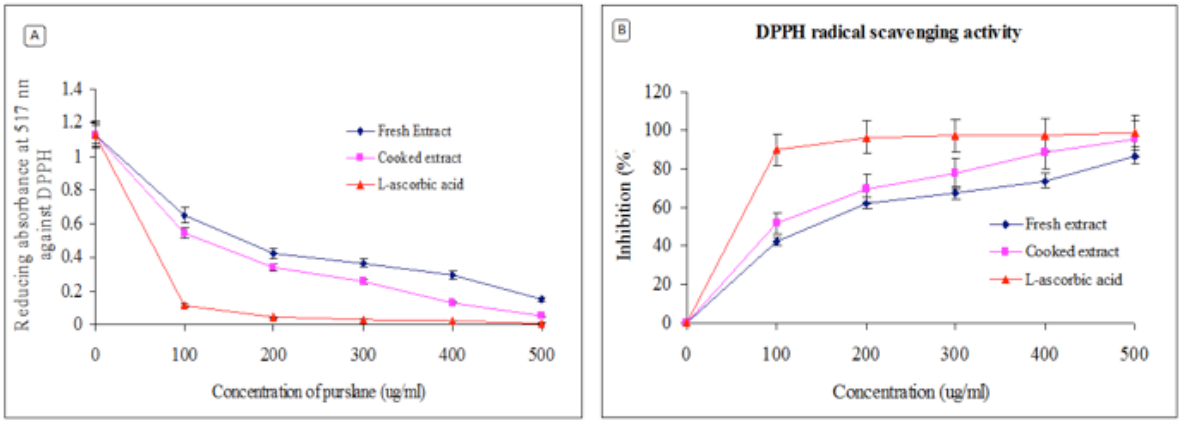

Figure 2: DPPH radical scavenging activity; A. The decreasing absorbance of DPPH treated with Portulaca oleracea extracts, B. Percentage inhibition of fresh and cooked Portulaca oleracea leaves against synthetic radical (DPPH)

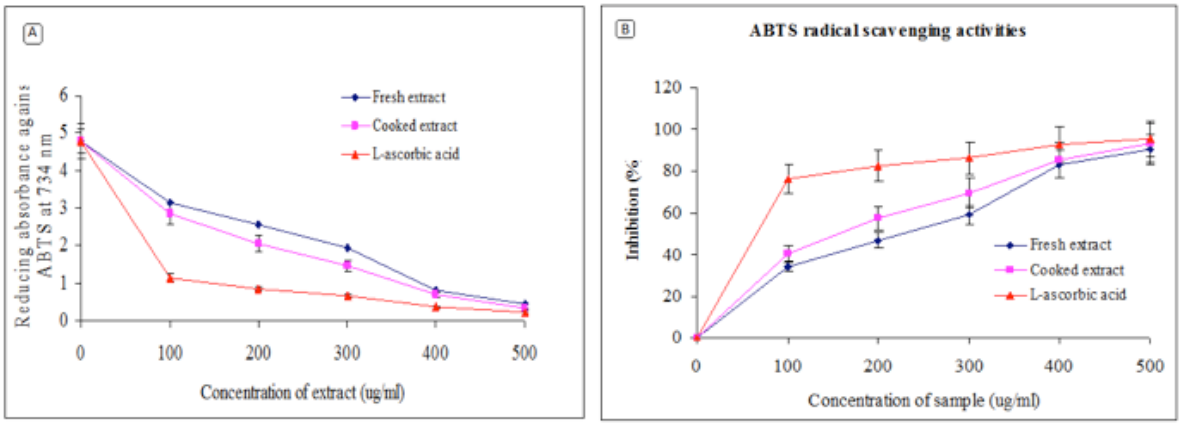

Figure 3: ABTS$^{\cdot+}$ radical scavenging activity; A. The decreasing absorbance of ABTS$^{\cdot+}$ treated with *Portulaca oleracea* extracts, B. Percentage inhibition of fresh and cooked *Portulaca oleracea* leaves against synthetic radical (ABTS$^{\cdot+}$)

Syzygium aromaticum and *Cinnamomum verum* in three different treatments like fresh, heat treatment for 1 hour and 2 hours the antioxidant levels remains the same compared to fresh and heat treatment for 1 and 2 hours.

CONCLUSION

The cooked green leaves possess potent antioxidant activity compared to fresh juice. Thus the present study may contribute to choosing cooked green leafy vegetables as essential nutrients and as phytotherapeutic agents. Hence *Portulaca oleracea* may be considered as a source of natural antioxidant. Moreover, the green leafy vegetables contain low calorie, carbohydrate content and low glycemic index. Further, it helps to regulate the digestive system and weight management with the presence of fibre contents.

Acknowledgement: The authors AJ and SAY gratefully acknowledge the authorities of Karpagam Academy of Higher Education for financial and laboratory (KUIF) support to carry out this research work and author RD acknowledge Prime College of Pharmacy to complete the research work.

Conflict of interests

There is no conflict of interest.

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