

## RESEARCH ARTICLE

### CHANGES IN POLYPHENOLIC CONTENTS AND ANTIOXIDANT POTENTIAL OF THE LEAFY VEGETABLE, *TALINUM PORTULACIFOLIUM* BY MOIST HEAT COOKING

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#### ABSTRACT

In general, most of green leafy vegetables undergo a cooking process prior to consumption. This present study was carried out to investigate the effect of moist heat cooking treatments of the green leafy vegetable, *Talinum portulacifolium* on the total phenolic content and antioxidant potential using phosphomolybdenum reduction, DPPH, and ABTS radical scavenging activities. The total phenol and tannin contents were found maximum in the raw samples of the plant 78.25 and 33.84 mg GAE/g extract, respectively. The boiling and frying treatments significantly reduced the total phenolic and tannin contents in *T. portulacifolium*. In the antioxidant study, the raw sample of ethanol extract exhibited a strong antioxidant potential based on ABTS, DPPH, radical scavenging and phosphomolybdenum reduction assays. Further, the raw and processed powders were subjected to FTIR analysis and the functional groups of the components were separated based on their peaks. It could be concluded that boiling and frying treatments have a determining effect on the levels of phenolic content and antioxidant capacities of vegetables.

**Keywords:** leafy vegetable, moist heat cooking, *Talinum portulacifolium*, antioxidant, phenolics

#### 1. INTRODUCTION

Green leafy vegetables are an important source of dietary micronutrients and antioxidants in the human diet. They are a rich source of carotene, ascorbic acid, riboflavin, folic acids, and minerals like calcium, iron, and phosphorus [1]. Epidemiological studies have shown that the intake of vegetables can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Leafy vegetables are also rich in compounds having antidiabetics, anti-histaminic, anti-carcinogenic, and hypolipidemic properties against cardiovascular disease, aging, obesity, hypertension, and insomnia. Several in vitro, pre-clinical and clinical investigations have revealed an inverse relationship between high consumption of vegetables and a lower incidence of chronic diseases [2].

Most of the green leafy vegetables undergo a cooking process prior to consumption Evidence is emerging that in vivo bioavailability of many protective compounds is enhanced when vegetables

are cooked [3]. It is known that cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds in vegetables. In addition, cooking can make food microbiologically safer to eat as well as to improve the edibility of the food. However, it can also result with some undesired consequences such as the losses of nutrients and the formation of toxic compounds with negative effects on flavor, texture, or color [4,5]. Numerous studies have been focused on the effect of cooking methods on dietary phytochemicals [6-8].

*Talinum portulacifolium* (Forssk.) Asch. ex Schweinf (Portulacaceae) is a succulent subshrub, up to 3 ft tall, growing from tuberous roots. Leaves are elliptic to obovate, up to 12 cm long, much smaller below the inflorescence, slightly fleshy. In traditional Chinese medicine, this plant is used to improve digestion, moistens the lungs and promotes breast milk. This plant is useful for treating headaches, aphrodisiac, pneumonia, diarrhea, polyuria, irregular menstruation, vaginal discharge and little milk [9]. The juice from the leaves is also

used to smooth expenditures, treat ulcers, and increased appetite. It contains high antioxidants level such as ginsenosides, phenol acids, flavonoids, saponins and tannins [10].

The present study aimed to investigate the influence of moist heat cooking methods on the total phenolic content and antioxidant potentials of ethanol extract of *T. portulacifolium*.

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The leaves of *T. portulacifolium* were purchased from local farms at Calicut district in Kerala (Figure 1).



**Fig. 1.** The leafy vegetable, *Talinum portulacifolium* used in this study

### 2.2. Preparation of samples

The leaves of *T. portulacifolium* were washed thoroughly with tap water and drained to remove excess water, then chopped into small pieces. The chopped leaves were divided into three parts: Raw (uncooked served as a control), boiled (100 g of vegetables were boiled in 500 mL of water in a stainless steel pan), and fried (100 g of vegetables were cooked in a frying pan with 100 mL of water). The cooking treatment was carried out under sim conditions using a gas stove for 30 minutes. After cooking treatment, all samples were shade dried and powdered.

### 2.3. Total phenolic and tannin content analysis

The total phenolic content was determined according to the Folin-Ciocalteu method described by Siddhuraju and Becker [11]. Tannins in the extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). The analysis was

performed in triplicate and the results were expressed as the gallic acid equivalents (GAE).

### 2.4. In vitro antioxidant activity

#### 2.4.1. Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex according to the method of Prieto et al. [12]. An aliquot of 100 ml of sample solution was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as g of ascorbic acid equivalents/100 g extract (ascorbic acid equivalent antioxidant activity).

#### 2.4.2. Free radical scavenging activity on DPPH•

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of Blois [13]. Ethanol extracts at various concentrations were taken and the volume was adjusted to 100 ml with methanol. 5 ml of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

$$\% \text{ DPPH radical scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100.$$

IC<sub>50</sub> values of the extract i.e., the concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

#### 2.4.3. ABTS•• radical scavenging activity

The assay was performed by a slightly modified protocol of Re et al. [14]. ABTS solution (7 mM) was reacted with potassium persulphate (2.45 mM) solution and kept for 12–16 h in the dark, to produce a dark-colored solution containing ABTS

radical cations. Prior to the assay, this solution was diluted in ethanol (about 1:89, v/v) and equilibrated at 30 °C to give an absorbance at 734 nm of  $0.700 \pm 0.02$ . Different concentrations of the sample were prepared. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard. Absorbance was measured at 734 nm exactly 30 min after the initial mixing.

% ABTS radical scavenging activity =  $(\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$ . IC<sub>50</sub> values of the extract i.e., concentration of extract necessary to decrease the initial concentration of ABTS by 50% was calculated.

### 2.5. FTIR spectrometric analysis

The infrared spectrum of raw and processed powders from *T. portulacifolium* leaves was determined using an FT-IR (Model/Make: IFS 25, Bruker, Germany) with PC-based software controlled instrument operation and data processing. The data of infrared transmittance was collected over a wave number ranging from 4000 cm<sup>-1</sup> to 500 cm<sup>-1</sup>. The spectral data were compared with a reference to identify the functional groups existing in the sample.

## 3. RESULTS AND DISCUSSION

### 3.1. Recovery percent, total phenolic and tannin contents

Plant phenolics present in the vegetables have received considerable attention because of their potential biological activity. These activities might be related to their antioxidant activity. Therefore, it is necessary to determine the total phenolic content and antioxidant capacity and to make comparisons among vegetables [15]. The yield percent, total phenolic, and tannin contents of the extracts obtained from the leaves of *T. portulacifolium* are shown in Table 1. The maximum extract yield was obtained in the ethanol extract of raw sample *T. portulacifolium* (5.40%). Variations in phenolic and tannin contents were observed in extracts from raw and processed samples. The extractable total phenolics (78.25 mg GAE/g extract) and tannins (33.84 mg GAE/g extract) were found to be higher in the ethanol extract of the raw sample of *T. portulacifolium*. The boiling and frying treatments

significantly reduced the total phenolic and tannin content in *T. portulacifolium* when compared with raw samples.

**Table 1. Extract yield percentage, total phenolic and tannin contents of raw, boiled and fried samples of *Talinum portulacifolium***

Sample	Extract yield (%)	Total phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)
SD	5.40	70.74 ± 4.14	33.84 ± 2.79
SB	3.92	21.23 ± 5.21	10.33 ± 0.97
SC	4.64	55.22 ± 4.81	26.52 ± 1.73

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*.

Total phenolic and tannin contents are expressed as gallic acid equivalent (GAE). Values are mean of three replicate determinations (n =3) ± standard deviation.

Mao et al. [16] observed that the phenolic content and antioxidant capacity were higher after ethanol extraction than in a water extract. It was evident that the loss of total phenolic content increased with prolonged boiling time. In the cases of Thai basil leaf and sweet potato leaf, there was a significant increase in TPC in their extracts at the initial stage of boiling for 1 minute and 5 minutes, respectively. Subsequently, the TPC decreased as the boiling time increased. The initial increase in TPC might have been due to the liberation of phenolics from the intracellular proteins, changes in plant cell structure, matrix modifications, or the inactivation of the polyphenol oxidase [17]. By contrast, as suggested by Migilo et al. [18], water cooking has a detrimental effect on polyphenols in vegetables, resulting in a complete loss of phenolic compounds, likely due to diffusion into the boiling water. In addition, Wachtel-Galor et al. [19] found that with a boiling time of 5-10 minutes, the TPC in vegetable extracts decreased whereas it increased in the cooking water.

### 3.2. Antioxidant activity

#### 3.2.1. Phosphomolybdenum reduction assay

In the presence of the extracts, Mo (VI) is reduced to Mo (V) and forms a green-colored phosphomolybdenum V complex, which shows maximum absorbance at 695 nm. From the results obtained, it can be seen that ethanol extracts of raw and processed samples exhibited significant antioxidant activity (Table 2). The ethanol extract of the raw sample of *T. portulacifolium* leaves (302.2 mg AAE/g extract) showed the highest phosphomolybdenum reduction activity. The results showed that boiling and frying treatments have a detrimental effect on phosphomolybdenum reduction antioxidant capacity and phenolic concentration of this leafy vegetable.

The differential response of extracts in various antioxidant assay systems may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occur at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants [20]. The variations in antioxidant activity may be due to phenolic and tannin contents. Dasgupta and De [21] investigated the total antioxidant activity of eleven edible leafy vegetables of India in different systems of the assay. The extracts were found to have different levels of antioxidant properties.

**Table 2. Phosphomolybdenum reduction activity of raw, boiled and fried samples of *Talinum portulacifolium***

Sample	Phosphomolybdenum assay (mg AAE/ g extract)
SD	302.2 ± 24.1
SB	212.2 ± 12.7
SC	256.1 ± 14.5

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*.

Values are mean of three replicate determinations (n =3) ± standard deviation.

#### 3.2.2. DPPH radical scavenging assay

DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. DPPH is a stable nitrogen-centered free radical, which produces a violet color in methanol solution. DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up [13]. The results obtained clearly indicate the potential of the plant extracts in scavenging free radicals (Table 3). The ethanol extracts of the raw and processed samples of *T. portulacifolium* leaves exhibited an inhibition ranging from 21.99 to 52.55% at 400 µg/ml.

**Table 3. DPPH· radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium***

Sample	% of activity			
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
SD	27.48 ± 0.95	36.89 ± 0.35	43.00 ± 1.85	52.55 ± 1.51
SB	4.31 ± 0.47	11.97 ± 2.44	17.08 ± 1.02	21.99 ± 0.10
SC	10.90 ± 1.04	21.87 ± 0.88	27.32 ± 1.00	38.44 ± 1.66

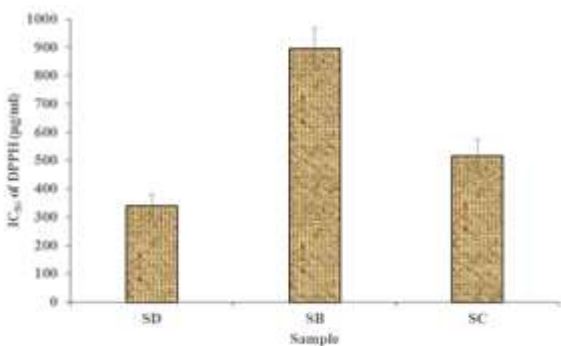
SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*.

Values are mean of three replicate determinations (n =3) ± standard deviation.

As presented in Figure 2, the scavenging abilities of ethanol extracts of raw and processed samples of *T. portulacifolium* were concentration-dependent and

expressed as IC<sub>50</sub> values. All the extracts exhibited appreciable DPPH radical scavenging activity. When compared to processed samples, raw samples of *T.*

*portulacifolium* showed higher DPPH radical scavenging activity with the IC<sub>50</sub> value of 340.5 µg/ml, respectively. The lowest DPPH radical scavenging activity was found in the ethanol extract obtained from the boiled sample of *T. portulacifolium* with the IC<sub>50</sub> value of 896 µg/ml.



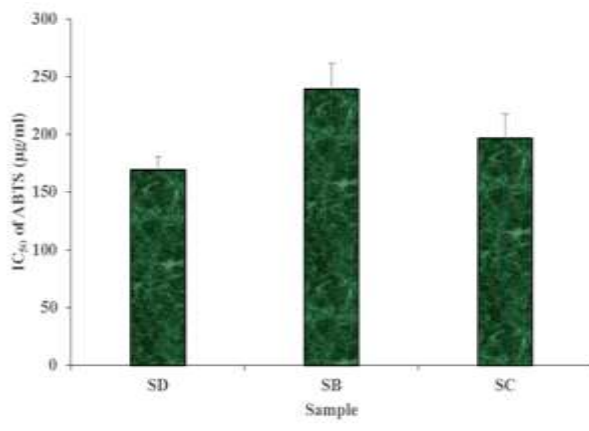
**Fig. 2.** IC<sub>50</sub> of DPPH• radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium*. SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*. Values are mean of three replicate determinations (n =3) ± standard deviation.

Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and form nonradical species, resulting in the inhibition of propagating phase of lipid peroxidation [22]. These results suggested that phenolic compounds were the major contributor to the DPPH radical scavenging activity.

### 3.2.3 ABTS radical scavenging assay

ABTS assay is based on the inhibition of the absorbance of the radical action ABTS<sup>•+</sup>, which has a characteristic long-wavelength absorption spectrum [23]. The results obtained clearly imply that the ethanol extracts of raw and processed samples inhibit the radicals or scavenge the radical in a concentration-dependent manner. The ethanol extracts of the raw and processed samples of *T. portulacifolium* leaves exhibited an inhibition ranging from 66.86 to 87.69% at 300 µg/ml (Table 4). ABTS radical scavenging effect raw and

processed samples of *T. portulacifolium* leaves was in this order: SD> SC> SB (Figure 3). Similar to DPPH radical scavenging activity, the raw sample of *T. portulacifolium* exhibited the highest ABTS radical scavenging activity than processed extracts. The lowest ABTS radical scavenging activity was found in the ethanol extract obtained from the boiled sample of *T. portulacifolium* with the IC<sub>50</sub> value of 239.2 µg/ml.



**Fig. 3.** IC<sub>50</sub> of ABTS radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium*. SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*. Values are mean of three replicate determinations (n =3) ± standard deviation.

Rice-Evans et al. [24] reported that phenolic compounds play a major role in scavenging the free radicals. Hagerman et al. [25] have made a similar observation that the high molecular weight phenolics have more ability to quench free radicals (ABTS<sup>•+</sup>). ABTS radical scavenging activity of these vegetable extracts indicated that the mechanism of antioxidant action of this fraction was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to stable forms [26]. The findings of the present study revealed that boiled or fried leafy vegetables have lower antioxidant capacity than raw ones. Several studies reported that the total phenolic content was decreased after boiling, steaming, microwaving, baking, and frying [27,28]. This decrease may be due to water-soluble phenols leaching into the cooking water and the



structural changes of phenolics that occurs during heat processing [29]. Samples are not in contact with water and the inactivation of oxidative enzymes has prevented the disruption of phenolic

biosynthesis during these cooking methods [30]. Tian et al. [28] suggested that steaming and microwaving methods with lower temperatures may be better for the retention of total phenolic contents.

**Table 4. ABTS radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium***

Sample	% of activity		
	100 µg/ml	200 µg/ml	300 µg/ml
SD	33.85 ± 1.19	67.45 ± 0.86	87.69 ± 0.77
SB	17.93 ± 2.42	39.07 ± 1.14	66.86 ± 1.98
SC	29.03 ± 0.65	53.38 ± 1.70	72.82 ± 0.37

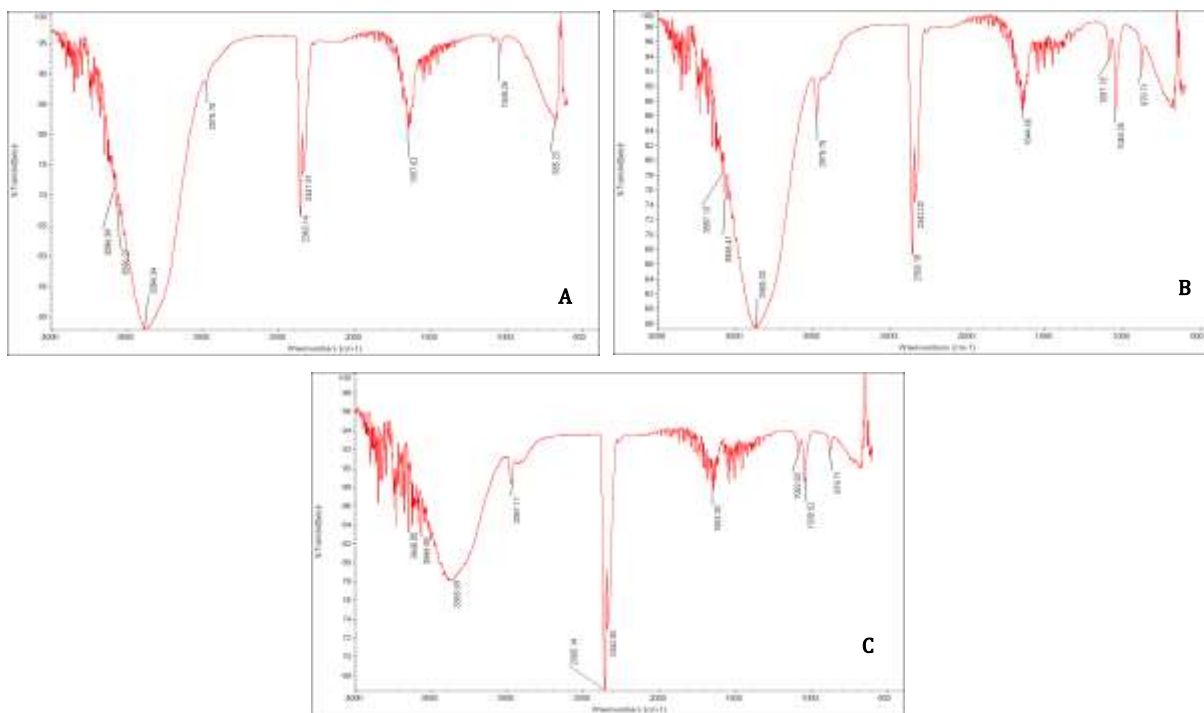
SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium*; SC, fried sample of *Talinum portulacifolium*.

Values are mean of three replicate determinations (n =3) ± standard deviation.

### 3.3. FTIR analysis

The raw and processed powders of *T. portulacifolium* leaves were subjected to FTIR

analysis and the functional groups of the components were separated based on their peaks.



**Fig. 4.** FTIR analysis of raw (A), boiled (B) and fried (C) powders of *Talinum portulacifolium*

The broad band at 3362 - 3366 cm<sup>-1</sup> was attributed to O-H stretching vibration of hydrogen-bonded hydroxyl groups in the sample, and the band at 2975 cm<sup>-1</sup> was attributed to C-H stretching vibration of methylene or methyl groups [31]. The bands at 2360 were attributed to the stretching vibration of N-H/C-O [32]. The band at 1644 -1653 cm<sup>-1</sup> was due to C=O stretching vibration or the HO-H vibration [33,34]. The bands at 1087 cm<sup>-1</sup> and 1048 cm<sup>-1</sup> were attributed to the stretching vibration of the pyran ring [35]. The band in the range of 1000-1200 cm<sup>-1</sup> was also assigned to the bending or stretching vibration of C-O groups. In addition, the band at 879 cm<sup>-1</sup> was attributed to the stretching vibration of aromatic out-of-plane-rings with 2 neighboring C-H groups (Figure 4). FTIR analysis for five selected green leafy vegetables such as *Hibiscus cannabinus*, *H. sabdariffa*, *Basella alba*, *B. rubra* and *Rumex vesicarius* confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol, and amine stretching [31].

#### 4. CONCLUSIONS

The findings of the present study revealed that boiled or fried leafy vegetables possess lower antioxidant capacity than raw ones. The decrease in antioxidant activity may be due to water-soluble phenolic compounds leaching into the cooking water and the structural changes of phenolic compounds that occur during heat processing. Further studies are warranted in relation to other cooking practices like steam cooking, microwave cooking, etc. in order to select the best cooking practice for the improvement of the antioxidant potential of leafy vegetables.

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