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Effect of drying methods and extraction solvents on the antioxidant properties of cooked and raw spinach leaves

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Abstract
Owing to substantial loss of nutrients during food processing, there is a dire need to explore various processing techniques to retain maximum antioxidant potential of green leafy vegetables. The current study was aimed to evaluate the effects of various drying techniques on the physicochemical and antioxidant properties of raw and cooked spinach leaves. Extraction was carried out by using different solvents (methanol, ethanol, acetone and water were used for extraction purpose). Oven dried sample were significantly different with sun dried samples in terms of Free Radical Scavenging Activity (90.63±2.52, 83.2±6.55), total phenolic contents (937.04±48.45 mg GAE/100g DW, 1251.78±110.05 mg GAE/100g DW) and ferric reducing antioxidant power (7452.27±245.5 µmol Fe^2/100g, 8780.27±184.4 Fe^2/100g). Similarly, raw and cooked spinach leaves showed distinct extraction rate with significant free radical scavenging activity (1199.8±212.92), total phenolic contents (1199.8±212.92 mg GAE/100g DW, 1034.39±423.55 mg GAE/100g DW) and ferric reducing antioxidant power (8169.47±336.5 Fe^2/100g, 5635.53±452.3 Fe^2/100g).

Keywords: processing, extraction, antioxidants, cooking

1. Introduction
Antioxidants are plant substances occurring naturally which protect the body from injury or any damage triggered by destructive molecules referred as free radicals. Antioxidants improve the body's immune system and thereby lowers the risk for many infections and diseases. Inhibition of the enzymes responsible for antioxidant activity or low levels of antioxidants may cause oxidative stress that damage the body cells [1]. To moderate and prevent the oxidation related diseases, it is obligatory to sequester the free radicals from the body [2]. Spinach (Spinacia oleracea) is a vital dietary vegetable, cultivated worldwide and is a common raw material in food processing industry, also it consumed as fresh in salads, canned leaves or frozen [3]. Spinach leaves are recognized source of antioxidants hence, the purpose of this study is to evaluate the potential of spinach leaves under various processing techniques such as sun drying, oven drying, processed or cooked and fresh/raw for their possible antioxidant activity. Thereby, creating an awareness for consumers to retain better and healthy lifestyle. Current approach will strengthen the food industry in terms of diverse food processing techniques to attain the maximum antioxidant potential of green leafy vegetables like spinach. Hence, this study was conducted by keeping in view the objectives of estimating the effect of various processing techniques on the antioxidant activity of spinach leaves. To check the influence of applied processing techniques in comparison with different solvent extractions such as ethanol, methanol, acetone and water.

2. Materials and methods
2.1 Procurement of Raw Material
Fresh spinach samples were procured randomly from the selected fields around the peri urban area of Multan as well as from markets.

2.2 Treatment of raw material
Raw material was subjected to different preparatory operations i.e. washing, cleaning, chopping and size reduction. Washing of leaves was done under tap water to remove dust and dirt particles. Chopping and size reduction was conducted by using household stainless steel knife and choppers.

2.3 Drying of spinach leaves
Different drying methods were adopted to evaluate their effects on the estimation of antioxidants.

2.3.1 Oven drying
Fresh spinach leaves were chopped into small pieces and dried in hot air oven at 40±1°C temperature [4].

2.3.2 Sun drying
Chopped pieces of spinach leaves were dried under sunlight.

2.4 Preparation of cooked and uncooked spinach sample
Processing techniques such as traditional cooking at constant temperature of 75±5°C were employed to cook the spinach sample. On the other hand, raw or uncooked

spinach leaves were taken for the comparative study of antioxidants. The raw/fresh samples were blended in a laboratory scale blender.

2.5 Preparation of leaf extracts

Leaf extracts were prepared both for oven and sun-dried samples by using four different solvents i.e. ethanol, methanol, acetone and water. For making extracts the solvents were used in the ratio of 70:30 (solvent: water). For cooked and uncooked (raw/fresh) samples the extracts were taken as filtrate. Extraction was performed at 40ºC with constant shaking for 3 h. Extracts were filtered through filter paper (whatman no. 41). Filtrates were concentrated by rotary evaporator at 25°C. Concentrated samples were stored at 4ºC.

2.6 Antioxidants profiling of spinach leaves

Antioxidants were determined by using spectrophotometer at specific wavelengths for TPC, Total Flavonoids, Radical Scavenging Activity (RSA) and Ferric reducing antioxidant power (FRAP).

2.6.1 Total phenolic contents

Total phenolic contents (TPC) of spinach leaves were quantified by using the methods adopted by Mélo [5]. Spinach extracts will be dissolved in methanol – water solution and 0.5 mL of aliquots were pipetted out in a test tube. A volume of 2.5 mL of 10-fold diluted Folin-Ciocalteu Reagent (FCR) was added to the sample tubes followed by the addition of 2 mL of 7.5% Na2CO3. Sample and the reaction mixture could stand for 30 min at 25ºC and the absorbance was subsequently taken by spectrophotometrically (UV-Vis 3000) at 760 nm. Gallic acid was used as standard compound. Series of gallic acid standard with concentration range of 10 – 100 mg/L was run as standard to plot the standard curve. Results obtained were expressed as mg gallic acid equivalent (GAE) per 100g.

2.6.2 Total flavonoid contents

Aluminum chloride colorimetric method with some modifications were used to determine the total flavonoids content [6]. Plant extract (1 mL) in methanol was mixed with 1 mL of methanol, 0.5 mL aluminum chloride (1.2%) and 0.5 mL potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by making quercetin (5-60 µg/mL) solution in methanol. Flavonoid contents were expressed in terms of quercetin equivalent mg/g of extracted compound.

2.6.3 Radical scavenging activity (RSA) of spinach extract

Radical scavenging property of spinach extracts was determined by DPPH method as described by Singh [7]. Multiple concentration of extracts ranging from 50 – 100 µl (50 to 100 mg/L) was prepared and pipetted in labeled test tubes. Volume of the tubes was adjusted to 100 µL by using methanol. Standard DPPH reagent (5 mL of 0.1mM) was prepared with methanol and added to the contents of the test tubes and vortexed. Stay time of 20 min was given to the test tubes at 27ºC. Extract was replaced with methanol in control. Change in absorbance were observed at 517 nm. Free radical scavenging activity of methanolic, ethanolic, acetone and water extracts were calculated by using the following equation:

Radical Scavenging Activity % = (Control OD - Sample OD/Control OD) × 100

2.6.4 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) of spinach extracts was determined by the method adopted by Zahin [8] with some modifications. FRAP reagent was prepared from 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mM), ferric chloride (20 mM) and sodium acetate buffer (pH 3.6) in a ratio of 1:1:10. Reaction mixture was heated for 10 min at a temperature of 37ºC and 300µl of it was pipetted in a test tube. Powdered extracts weighing 25 mg were taken in FRAP reagent tubes and vortexed. Absorbance of samples and control was measured at 593 nm wavelength spectrophotometrically. Ferrous sulphate was used as standard and its multiple concentrations ranging from 100 – 1000 µM was prepared. Absorbance of the standard solution accordingly read at 593 nm. Results of the samples and control were expressed as µM of Fe (ferrous ions to ferric ion conversion).

2.7 Statistical analysis

Samples were measured in triplicates and collected data was analyzed statistically by using standard statistical procedures by following the methods of Mason [9].

3. Results & Discussion

Effect of various processing techniques on the antioxidant activities of spinach leaves was performed in two phases. Phase one included the summer crop whereas second phase was comprised of winter crop. For this purpose, various solvents like methanol, ethanol, acetone and water were used for extraction of spinach leaves. Three different parameters such as DPPH, TPC and FRAP were taken for exploring the antioxidants potential of processed as well as fresh spinach.

3.1 Free radical scavenging activity for oven dried spinach leaves

The free radical scavenging activity of the antioxidants present in plants or their extracts are routinely evaluated (at room temperatures) using synthetic radicals like that of DPPH in the presence of polar organic solvents. DPPH is a stable free radical, which can accept an electron or a hydrogen radical to become a stable diamagnetic molecule. Generally, the scavenging of DPPH radicals is used to evaluate chain-breaking activity in the propagation phase of lipid (and protein) oxidation. The mechanism of action involves the reaction of specific compounds or plant extracts with DPPH· in methanol.

Total antioxidant activity of spinach leaves under the influence of various processing techniques (sun dried, oven dried, cooked and fresh or raw spinach) was carried out in terms of DPPH. The generation of DPPH free radical formed the basis of spectrophotometric method adopted to measure the total antioxidant activity of extracts.

Four different solvents such as methanol, ethanol, acetone and water were used for extraction purpose. Results of oven dried spinach with different solvents vary significantly. Mean square values of oven dried spinach by using acetone, methanol, ethanol and water were 1793.56, 1644.79,

1380.78 and 745.802, respectively. Highly significant results obtained with acetone, whereas least significant values observed in samples extracted with water (Table 1).

Radical Scavenging Activity in terms of DPPH of oven dried spinach for different solvent extracts under several concentrations of spinach extract has been presented in Table 1. Different extract concentrations in ppm were taken such as 50, 60, 70, 80, 90 and 100 for spinach samples Outcomes of various extract concentrations (50, 60, 70, 80, 90 and 100 ppm) with respect to their solvent like methanol such as 36.03, 27.57, 51.43, 63.67, 72.2, 90.6; ethanol as 21.93, 25.57, 30.4, 43.9, 54.43, 78.57; acetone as 23.07, 28.8, 41.8, 43.37, 71.63, 85.23; water as 44.83, 60.37, 67.43, 71.4, 82.13 and 89.1 respectively, were recorded in (µmol Trolox eq./g DW). Above given results are clearly pointing out the increasing trend with increasing concentration of extracts irrespective of the solvent used. Maximum radical scavenging activity (90.6) was observed for the concentration of 100 ppm with methanol following water (89.1), acetone (85.23) and ethanol (78.57), separately. The lowest assay (21.93) was observed for 50 ppm concentration with ethanol followed by acetone (23.07), methanol (36.03) and water 44.83 accordingly.

A study on RSA in terms of DPPH activity of spinach leaves was conducted by Sekar [10] with water and methanol used as solvent. Reduction in purple color of solution was observed in radical scavenging activity while using both solvents. Aqueous and methanol extracts revealed 20.7% and 18.3% RSA. So, the result of aqueous was greater than methanol extract which are also related to the outcomes of current research.

Yang [11] had determined superoxide scavenging activity and dry matter (DM) content of various vegetable extracts by using water and methanol. The spinach extract showed the DM of 50 g/kg with TEAC of 206 in methanol and 102 µmol Trolox equivalent/g in water. Whereas SOS in methanol was non-significant and in water, 639 µmol ascorbate equivalent/g respectively. Whereas the results of current study were calculated as 36.03, 27.57, 51.43, 63.67, 72.2 and 90.6 (µmol TE/g DW) with different concentrations of 50, 60, 70, 80, 90 and 100 ppm, respectively.

Alkaloids, carotene and antioxidant contents of shade dried leaves extract of Annona squamosal and spinacia oleracea were investigated by using (98%) methanol. DPPH method was used for screening of antioxidants in both plants. Spinach showed radical scavenging activity 42.574, 28.925 and 15.912 (IC50 values) for 10, 20 and 30 (concentration µg/ml), carotene (0.0392 mg/100g) and alkaloids (0.0392%). So, the outcomes of this study were closely related to current research work [12].

Bhat [13] has conducted a research on various green leafy vegetables in terms of antioxidants potential while using three different solvents. For this purpose, water spinach (Kang Kung) and Indian spinach (Di Huang Miao) were selected for oven drying at 40°C in these solvents. DPPH of water spinach were in methanol (26.1±0.1), acetone (9.0±0.5) and water (20.3±1.4) percent inhibition of extract. DPPH of Indian spinach was in methanol (5.6±0.1), acetone (6.6±0.2) and water (9.4±0.4) percent inhibition of extract. Efficiency of these solvents strongly varied among in line of free radical scavenging activity. Methanol was concluded best among solvents used for extraction. This study also strongly correlates with current work and provides the useful information for health sensible consumers and basis for upcoming assessment of these green vegetables as possible foundation of antioxidants for nutraceutical and food applications.

Table 1: Radical Scavenging Activity (DPPH) of oven dried spinach for different solvent extracts

Extract Concentration (ppm)	ODM	ODE	ODA	ODW
50	36.03±2.31	21.93±3.89	23.07±6.49	44.83±5.37
60	27.57±4.05	25.57±3.52	28.8±6.84	60.37±4.25
70	51.43±3.15	30.4±8.61	41.8±3.16	67.43±5.11
80	63.67±4.45	43.9±7.04	43.37±16.7	71.4±8.4
90	72.12±7.2	54.43±12.46	71.63±5.24	82.13±6.52
100	90.63±2.52	78.57±7.74	85.23±5.86	89.1±13.21

(µmol TE/g DW), (means ± standard deviation), TE= Trolox Equivalent; DW= Dry weight basis; ppm= parts per million; DPPH= 2,2-diphenyl-1-picrylhydrazyl, ODM; Oven Dried Methanol, ODE; Oven Dried Ethanol, ODA; Oven Dried Acetone, ODW; Oven dried Water

3.2 Free radical scavenging activity for sun dried spinach leaves

Radical Scavenging Activity (DPPH) of sun dried spinach for different solvent extracts under several concentrations of spinach extract reported in Table 2. Outcomes of these extract concentrations such as 50, 60, 70, 80, 90 and 100 ppm with respect to their solvent like methanol such as 46.23, 69.03, 69.8, 79.63, 89.2, 89.33; ethanol as 53.33, 48.9, 68.17, 70.2, 78.17, 93.73; acetone as 40.33, 51.83, 69.63, 76.07, 84.5, 88.57; water as 68.5, 72.27, 81.4, 85.67, 88.3 and 100.9 consistently were noted in (µmol Trolox eq./g DW).

Above given results clearly pointing out the increasing trend with increasing concentration of extracts irrespective of solvent used. DPPH assay displayed significant variability for each concentration and solvent used. Maximum radical scavenging activity (100.9) was observed for the concentration of 100 ppm with water following ethanol (93.73), methanol (89.33) and acetone (88.57) independently. The lowest assay (40.33) was observed for 50 ppm concentration with acetone followed by methanol (46.23), ethanol (53.33) and water (68.5) accordingly.

Radical scavenging activity of flowers and leaves of *Moringa oleifera* were taken for study purpose and steps were also taken for comparison of its results with some certain vegetables (peas, cauliflower, broccoli, spinach and cabbage). Analysis of radical scavenging activity by using (DPPH method), total flavonoids content, reducing power and total phenolic contents were taken for determination of antioxidant activity. *Moringa oleifera* showed almost twice phenolic contents and three times total flavonoids contents as compared to other described vegetables. Free radicals remaining percentage was lesser and reducing power of *moringa oleifera* was higher than prescribed vegetables. It also presents the (78.5% inhibition) in 100% methanol, (90.1% inhibition) in 50% methanol, (97.4% inhibition) in 25% methanol of spinach were explored and comparison performed with *Moringa oleifera*. These results strongly provide evidence about antioxidant nature of *Moringa oleifera*. Due to all above described findings, *Moringa oleifera* forms the important component of diet of publics in different emerging countries (especially in the southern

hemisphere) [14].

Table 2: Radical Scavenging Activity (DPPH) of oven dried spinach for different solvent extracts

Extract Concentration (ppm)	ODM	ODE	ODA	ODW
50	46.23±8.16	53.33±4.58	40.33±5.1	68.5±9.76
60	69.03±10.15	48.9±10.34	51.83±6.61	72.27±2.66
70	69.8±4.67	68.17±3.26	69.63±4.53	81.4±4.04
80	79.63±5.76	70.2±21.47	76.07±6.09	85.67±6.74
90	89.2±6.55	78.17±8.36	84.5±5.5	88.3±7.49
100	89.33±8.72	93.73±5.1	88.57±6.87	100.9±6.49

(µmol TE/g DW), (means ± standard deviation), TE= Trolox Equivalent; DW= Dry Weight basis; ppm= parts per million; DPPH= 2, 2-diphenyl-1-picrylhydrazyl, ODM; Oven Dried Methanol, ODE; Oven Dried Ethanol, ODA; Oven Dried Acetone, ODW; Oven dried Water

3.3 Free radical scavenging activity for fresh and cooked spinach leaves

Mean square values for radical scavenging activity (DPPH) of fresh and cooked spinach for water extracts under several concentrations of spinach extract were illustrated in Table 3. Fresh and cooked extracts of spinach with concentrations of 50, 60, 70, 80, 90 and 100 ppm had antioxidant values (µmol Trolox eq./g DW) of 47.97, 53.4, 58.6, 62.43, 66.63, 65.47 and 16.2, 19.33, 19.47, 23.2, 22.53 and 24.87, respectively were observed. The results clearly indicated the increasing trend with increasing concentration of extracts irrespective. DPPH assay displayed significant variability for each concentration. Maximum radical scavenging activity (66.63) was observed in fresh spinach for the concentration of 90 ppm following the (65.47), (62.43), (58.6), (53.4) and (47.97) of 100, 80, 70, 60 and 50 ppm concentrations individually. The lowermost assay (16.2) was detected in cooked spinach for 50 ppm concentration followed by (19.33), (19.47), (23.2), (22.53) and (24.87) of 60, 70, 80, 90 and 100 ppm concentrations accordingly.

Total antioxidant activity (percent inhibition) of spinach leaves in various methods of cooking was investigated as raw (96.1), pressure cooking (100), sautéing (100) and boiling (98.4). Other results of cluster beans and drumstick with raw spinach are about 96% while mushroom and beetroot outcomes are falling between 87-89%. So, on comparison of above various cooking methods had revealed about no significant deviation in antioxidant activity in these vegetables after cooking [15].

In green leafy vegetables, DPPH values normally fluctuates (21-1021mg/100g, Trolox Eq) for curry leaves having the maximum activity, whereas spinach possess the slightest activity. Comparing the antioxidant activity of curry and spinach leaves reported the antioxidant activity in different stages such as regularly consumed raw spinach (21.6), conventional (69), pressure (85) and microwave cooking (104) in (mg/100 g Trolox Eq.). Significant difference in results of above-mentioned cooking methods was noticed and these investigations are also correlated to some extent with present work [16].

Radical-scavenging activity of cooked spinach was also determined by Yamaguchi [17] in cooked tissues (73.6± 11.3 µmol Trolox eq./100 g) and in cooking water (284.4±25.4 µmol Trolox eq./100 g) separately by using DPPH-HPLC method, while the fresh summer spinach was also calculated as (374.3±26.7 µmol Trolox eq./100 g) by using same method. Significant difference in the antioxidant activities were noticed for all the samples as are related to the present work. Similarly, the antioxidant activity of spinach leaves extract with DPPH method was investigated (16.65%) by using cold water, while 16, 14.2 and 13% were reported with 10, 30 and 60 minutes in 100°C boiling water. The cold water was found more effective for this purpose rather than time and temperature [18]. Effect of various cooking methods on Antioxidant activity (DPPH inhibition) of spinach with 80% methanol extract was (fresh 67.4 ± 7.82, boiling 87.1 ± 0.40, steaming 85.5 ± 0.17 and microwaving 85.8 ± 0.22) investigated. After cooking, total antioxidant activity increased or remained unchanged depending on the type of vegetable but not type of cooking method [19].

Table 3: Radical Scavenging Activity (DPPH) of oven dried spinach for different solvent extracts

Extract Concentration (ppm)	Fresh Spinach	Cooked Spinach
50	47.97±5.13	16.2±3.32
60	53.4±6.85	19.33±6.06
70	58.6±11.52	19.47±6.07
80	62.43±6.05	23.2±2.72
90	66.63±7.75	22.53±2.18
100	65.47±7.41	24.87±2.01

TE= Trolox Equivalent; ppm= parts per million; DPPH= 2,2-diphenyl-1-picrylhydrazyl, (µmol TE/g)

3.4 Total phenolic contents

Total phenolic contents of spinach leaves under the influence of various processing techniques (oven dried, sun dried, cooked and fresh or raw spinach) was carried out in terms of TPC. The generation of TPC free radical formed the basis of spectrophotometric method adopted to measure the total phenolic contents of extracts.

3.4.1 Total phenolic contents for oven dried samples

Mean square values of oven dried spinach by using ethanol, water, acetone and methanol were 611135, 208474, 59747.9 and 28082.5, separately. Highly significant results were attained with ethanol, whereas least significant values observed in samples extracted with methanol as revealed in Table 4. Total Phenolic Contents (TPC) of oven dried spinach for different solvent extracts under several concentrations of spinach extract were offered in Table 5.8. Different extract concentrations in ppm were occupied for spinach samples such as 50, 100, 200, 400 and 800.

Results of various extract concentrations such as 50, 100, 200, 400 and 800µg with respect to their solvent like methanol such as 1097.46, 1201.71, 1037.03, 1041.19, 937.04; ethanol as 2721.21, 2430.54, 2221.07, 1768.06, 1640.94; acetone as 1433.66, 1504.03, 1280.32, 1248.44, 1157.83; water as 1080.35, 1164.26, 791.72, 1297.68 and 1502.47 consistently were verified in (mg Gallic Acid eq./100g DW). Above given outcomes visibly indicated the increasing trend with increasing concentration of extracts.

TPC showed significant variability for each concentration and solvent. Maximum total phenolic contents (2721.21) were observed for the concentration of 50µg with ethanol following the acetone (1433.66), methanol (1097.46) and water (1080.35), separately. The lowest content (791.72) were observed for 200µg concentration with water followed by methanol (1037.03), acetone (1280.32) and ethanol 2221.07 accordingly.

Efforts were taken for exploring total phenolic potential of

spinach leaves by (sequential extraction method) after drying at 40°C for 24 hours. Contents of TPC were calculated by two different solvents such as methanol (17.25µg/g, GAE) and water (16.625µg/g, GAE). Thus, extraction of methanol showed the dominant result for total phenolic contents than water extract of spinach. So current study provides close result to conducted research [10].

Water spinach (Kang Kung) and Indian spinach (Di Huang Miao) were used after oven drying at 40oC in three different solvent for extraction purpose. TPC of water spinach were found to be in mg (GAE)/g for methanol (65.67±1.1), acetone (32.20±0.8) and water (41.99±0.1). TPC of Indian spinach reported with methanol (50.38±0.2), acetone (36.55±0.3) and water (40.02±0.3) in mg (GAE)/g of extract. Efficiency of these solvents strongly differs among in perspective of TPC and antioxidants. Methanol was concluded best among three solvents used for extraction. This study also strongly correlates with current work and provides the useful information for health sensible consumers and basis for upcoming assessment of these green vegetables as possible foundation of antioxidants for nutraceutical and food applications [13].

Table 4: Radical Scavenging Activity (DPPH) of oven dried spinach for different solvent extracts

Extract Concentration (ppm)	ODM	ODE	ODA	ODW
50	1097.46±83.88	2721.21±97.6	1433.66±91.9	1080.35±157.73
60	1201.71±29.08	2430.54±72.48	1504.03±160.15	1164.26±65.96
70	1037.03±57.1	2221.07±401.98	1280.32±184.33	791.72±199.56
80	1041.19±80.37	1768.06±210.31	1248.44±149.62	1297.68±106.59
90	937.04±48.45	1640.94±181.48	1157.83±214.62	1502.47±236.93
100	1097.46±83.88	2721.21±97.6	1433.66±91.9	1080.35±157.73

(means ± standard deviation), DW; Dry Weight Basis; TPC; Total phenolic contents; GAE= Gallic Acid Equivalent; DW= Dry Weight Basis; ODM; Oven Dried Methanol, ODE; Oven Dried Ethanol, ODA; Oven Dried Acetone, ODW; Oven Dried Water

3.4.2 Total phenolic contents for sun dried spinach

Different extract concentrations in µg (50, 100, 200, 400 and 800) were taken for spinach samples. Outcomes of various extract concentrations (50, 100, 200, 400 and 800 µg) for methanol was 1175.53, 1376.78, 1743.12, 844.7 and 1251.78, respectively. For ethanol 2363.79, 1856.49, 2729.34, 2314.86 and 2559.23, respectively. For acetone was 1228.47, 1178.85, 987.89, 1605.02 and1458.42, respectively. For water was 802.16, 1033.46, 1164.75, 914.9 and 1129.56, respectively observed in mg Gallic acid eq./100g DW. Above given results visibly indicated the increasing trend to some extent with increasing concentration of extracts.

TPC presented significant variability for each concentration and solvent. Maximum total phenolic contents (2729.34) were observed for the concentration of 200µg with ethanol following the methanol (1743.12), water (1164.75) and acetone (987.89), noticeably. The lowest content (802.16) was observed for 50µg concentration with water followed by methanol (1175.53), acetone (1228.47) and ethanol 2363.79 consequently.

Influence of time and temperature for extraction on the total polyphenols of spinach leaves with cold water (44.53 mg/100g, GAE on fresh weight basis) and time (10, 30 and 60 minutes) at temperature 100ºC in boiling water (33.70, 32.50 and 32.18 mg/100g, GAE on fresh weight basis) were investigated in which cold water was found more effective for this purpose rather than increasing time and temperature (Shehata *et al*., 2014) [18].

Table 5: Effect of different solvent extracts on TPC of oven dried spinach leaves (mg GAE/100g DW)

Extract Concentration (µg)	SDM	SDE	SDA	SDW
50	1175.53±117.05	2363.79±169.08	1228.47±153.17	802.16±156.91
100	1376.78±91.13	1856.49±101.57	1178.85±175.58	1033.46±139.68
200	1743.12±142.93	2729.34±123.71	987.89±844.78	1164.75±212.71
400	844.7±125.75	2314.86±132.34	1605.02±193.7	914.9±102.65
800	1251.78±110.05	2559.23±149.12	1458.42±207.71	1129.56±138.71

(means ± standard deviation), DW; Dry Weight Basis; TPC; Total Phenolic Contents; GAE= Gallic Acid Equivalent; DW= Dry Weight Basis; SDM; Sun Dried Methanol, SDE; Sun Dried Ethanol, SDA; Sun Dried Acetone, SDW; Sun Dried Water

3.4.3 Total phenolic contents for fresh and cooked spinach

TPC for fresh spinach samples were found to be 1183.02 for 50 µg, 1143.14 for 100 µg, 1163.16 for 200 µg, 1172.83 for 400µg and 1199.8 for 800µg. Whereas, TPC for cooked spinach were reported to be 1076.12 for 50 µg, 986.39 for 100 µg, 908.05 for 200 µg, 788.75 for 400 µg 1105.39 for 800 µg, respectively were noted in (mg Gallic acid eq./g DW). Above given results clearly pointing out the slow increasing trend with increasing concentration of fresh spinach extracts and decreasing trend in cooked spinach extract. TPC displayed non-significant variability for each concentration. Maximum TPC (1199.8) were observed in fresh spinach for the concentration of 800 µg following the 1105.39 mg Gallic acid eq. of cooked spinach. The lowermost TPC (788.75) were reported in cooked spinach for 400 µg concentration followed by 1172.83 mg Gallic acid eq./g DW of fresh spinach accordingly.

Vegetables like drumstick (Moringa), spinach and cluster beans had been originated rich in macronutrients, phytonutrients and antioxidant activity. However, cooking had decreased total phenolic and flavonoid contents and positively increased the antioxidant activity than raw form but their difference was not significant. Total phenolic contents and antioxidant correlation had investigated pressure cooking and sautéing had very encouraging while boiling has adverse impacts. So, open cooking and reasonable heat treatment can be helpful for improving health properties of subscribed vegetables. Study has also introduced the no significant negative impact of cooking on

phytonutrient contents of these vegetables [15].

Impacts of various cooking methods on total phenolic content of several vegetables such as mushroom, drumstick, cluster beans, beetroot and spinach were investigated by Rani [15]. Total phenolic content (mg GAE/100g) of spinach in fresh (433) along with different cooking methods like sauting (354.7,) boiling (104.9) and pressure cooking (84.09) were found. Total phenolic content in uncooked vegetables fluctuated between 46.7-433 mg GAE/100g. Highest TPC was founded in spinach instead of mushroom, drumstick, cluster beans and beetroot. TPC of described vegetables has reduced by above three methods of cooking [19]. Ismail [20] had reported slight increase in TPC of spinach for following cooking methods like microwaving, steaming and boiling compared with the remaining vegetables like swamp cabbage, kale, shallots and cabbage.

Significant changes can be caused in total phenolic contents of vegetables by cooking treatments; on the other hand, damage of phytochemical properties by cooking process is not necessary [21]. Quantification difference may be due to efficiency of cooking methods and extraction. Outcomes of this study have indicated slight phenol losses (due to phenolic collapse) during cooking [19]. Negative relation has been found between TPC and antioxidant activity due to process of boiling. Phenol may not necessary component of antioxidant in aqueous extraction. Some other active components which might be nonphenolic in nature can be extracted by water using as solvent. Uronic acids and amino acids which are non-antioxidant compounds may yield greater antioxidant capacity in test solutions than polyphenols only [22, 23].

Sometimes dietary fibers release the free phenolic compounds by process of cooking from bounded polyphenols with dietary fibers after decomposition which raises their finding [24]. Oxidizing enzymes usually slows the destruction of phenolic with oxidation on contact to environment and polyphenol oxidase usually inactivated with help of heat treatment [17].

Total phenolic content in green leafy vegetables were ranged between 77-1077 mg/100g GAE on fresh weight basis. Curry leaves noticed highest TPC (1077mg/100g, GAE) content and spinach lowermost (77/100g, GAE) content [13]. Above outcomes revealed the close association to the findings of current work. Influence of local processing on polyphenol content of normally consumed spinach raw (77), conventional (96), pressure (125) and microwave cooking (117) in mg/100 g Gallic acid Eq. [16]. This is almost nearby to finding of current homework. Influence of various cooking methods on total phenolic content of spinach (fresh 1274.8 ± 94.09, boiling 1291.8 ± 89.27, steaming 1315.3 ± 14.4, microwaving 1390.7 ± 41.40 mg GAE/100g) was investigated using 80% aqueous methanol. Cooking not effect spinach TPC significantly [19].

Table 6: Total phenolic contents for fresh and cooked spinach

Extract Concentration (µg)	Fresh	Cooked
50	1183.02±232.25	1076.12±188.16
100	1148.14±215.46	986.39±276.46
200	1163.16±191.49	908.05±329.74
400	1172.83±205.32	788.75±124.81
800	1199.8±212.92	1105.39±523.55

mg GAE/100g FW, TPC; Total phenolic contents; FW; Fresh Weight

3.5 Ferric reducing antioxidant power

Ferric Reducing Antioxidant Power of spinach leaves under the impact of various processing techniques (oven dried, sun dried, cooked and fresh or raw spinach) was carried out in terms of FRAP. The generation of FRAP free radical formed the basis of spectrophotometric method adopted to measure the ferric reducing antioxidant power of extracts.

3.5.1 Ferric reducing antioxidant power for oven dried samples

Ferric Reducing Antioxidant Power (FRAP) of oven dried spinach for different solvent extracts under single concentration (300 µg) of spinach extract had been presented in Table 7.

Results of various solvents such as methanol (6279.83), ethanol (5023.37), acetone (3658.83) and water (7452.27) were recorded in (µmol Fe^2/100g, DW) significantly. Above given results clearly indicated significant deviation with changing solvent. Maximum antioxidant power (7452.27) was observed with water and lowest FRAP (3658.83) was noted in acetone.

A research was conducted by Bhat [13] on antioxidants such as DPPH, FRAP and TPC of different green leafy vegetables. Two vegetables like water spinach (Kang Kung) and Indian spinach (Di Huang Miao) were selected for extraction purpose. FRAP of water spinach were observed in methanol (50.02±0.9), acetone (20.65±1.4) and water (72.57±1.0) in (µmol Fe^2/g) of extract. FRAP of Indian spinach reported in methanol (18.63±1.0), acetone (18.31±0.5) and water (54.69±0.1) (µmol Fe^2/g) of extract. Results of these solvents differ among significantly and water was suggested as best solvent for FRAP extraction. This study had also provided the help in correlation to current research.

3.5.2 Ferric reducing antioxidant power for sun dried samples

Ferric Reducing Antioxidant Power (FRAP) of sun-dried spinach for different solvent extracts under single concentration (300µL) of spinach extract had been reported in Table 7. Results of various solvents such as methanol (8678.33), ethanol (6884), acetone (5941.23) and water (8780.27) were noted in (µmol Fe^2/100g, DW) significantly. Above given results clearly demonstrated deviation with varying solvent. FRAP presented significant variability for each solvent used. Maximum ferric reducing antioxidant power (8780.27) was detected with water and lowermost FRAP (5941.23) was distinguished in acetone.

Green leafy vegetables (FRAP activity) varied between 1380–27827mg/100 g (FeSO4 Eq.) conducted by Sreeramulu [16]. Mint leaves were noticed with highest activity and the lowermost detected in spinach. This was given sketch of values of FRAP in green leafy vegetables as whole. Values of current study also correlated to these results.

3.5.3 Ferric reducing antioxidant power for fresh and cooked samples

Mean square values of raw spinach by using methanol, ethanol, acetone and water were 9824.3, 7362.2, 6127.5 and 8169.5 (µmol Fe^2/100g, FW). Whereas for cooked spinach the FRAP was recorded as 4324.6, 3432.0, 2839.4 and 5635.5, respectively for all solvents. Highly significant results were attained with methanol, whereas least

significant values observed in samples extracted with acetone in raw spinach in Table 7. but in cooked spinach water extraction led toward highly significant and acetone extract mean was less significant. Ferric Reducing Antioxidant Power (FRAP) of raw and cooked spinach for different solvent extracts under single concentration (300µg) of spinach extract had been accessible in Table 7.

Results of various solvents of raw spinach with methanol (9824.33), ethanol (7362.17), acetone (6127.5) and water (8169.47) were obtained. On the other hand, FRAP for cooked spinach were reported to 4324.63, 3431.97, 2839.37, 5635.53 with methanol, ethanol, acetone and water, respectively. Data indicated significant variation among all the solvents. Maximum ferric reducing antioxidant power in raw (9824.33) and cooked (5635.53) was detected with methanol and water respectively and lowermost FRAP of above given samples (6127.5) and (2839.37) was distinguished accordingly in acetone.

Effects of native processing on FRAP activity of frequently consumed spinach raw (1380.6), conventional (3196), pressure (3471) and microwave cooking (3502) in (mg/100 g FeSO4 Eq.) was notice by Sreeramulu [16]. Outcomes of these cooking methods varied among significantly and correlated to results of current research to some extent. This study is helpful for future perspective.

Table 7: Effect of different solvent extracts on FRAP of fresh spinach (µmol Fe^2/100g)

Solvents	Oven Dried	Sun Dried	Fresh	Cooked
Methanol	6279.83±453.1b	8678.33±257.8a	9824.33±99.5a	4324.63±249.7b
Ethanol	5023.37±339.2c	6884±90.6b	7362.17±139.7c	3431.97±345.5c
Acetone	3658.83±358.0d	5941.23±174.5c	6127.5±181.5d	2839.37±259.5c
Water	7452.27±245.5a	8780.27±184.4a	8169.47±336.5b	5635.53±452.3a

(means ± standard deviation); means carrying similar letters are statistically non-significant.

4. Conclusion

Leafy vegetables are perishable plants foods which are subjected to postharvest losses. Reactive oxygen species such as singlet superoxide anion, oxygen, hydrogen peroxide and hydroxyl radical are often produced as byproducts from various from exogenous factors or from biological reactions. Reactive species put oxidative damage by interacting with each molecule present in living cells as well as in DNA. Excess of these reactive species, if not removed by antioxidant system, cause high production of lipid peroxides and free radicals which trigger the pathogenesis of deteriorating diseases like carcinogenesis, atherosclerosis, cataract, diabetes and ageing. Epidemiological and experimental evidence proposes a substantial role of diet in the inhibition of degenerative diseases. Plant derived antioxidants, such as phenolic compounds and flavonoids, have numerous biological effects, with antioxidant activity. Drying techniques have provided recommendations for utilization of spinach leaves as a best source of antioxidants. Phenolic extraction with different solvents remained helpful in better understanding of processing techniques applied in food industry. These processing techniques also lead to evaluate the minimal antioxidant losses for regular spinach consumption at domestic as well as commercial level. Comparative study of all the applied techniques was helpful in suggesting a better choice of food consumption. Furthermore, these strategies also highlighted the relationship among different antioxidants. It was concluded that all the solvents (methanol, ethanol, acetone and water) had significant impact on the antioxidant activity of all the samples. However, highest variability was observed for the samples extracted with acetone in comparison with water when used as solvent. Cooking had a drastic effect on the antioxidant activities of spinach leaves as antioxidants are usually heat instable. On the other hand, fresh extracts of the spinach leaves indicated higher antioxidant contents.

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6. References

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