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Screening of antioxidant activities of some green leafy vegetables grown in India

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Abstract
In the current study extracts of some common green leafy vegetables grown and consumed in India were assessed for their antioxidant activities. The total phenolic content,total flavonoid content,, total antioxidant capacity and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assay of extracts of *Amaranthus viridis* L., *Raphanus sativus* L., *Chenopodium album* and *Spinacia oleracea* L. were evaluated.The highest amount of TPC (113.33 mg GAE/gm) and TFC (66.0mg QE/gm) were found in Hydroethanol extracts of *Amaranthus viridis*. Maximum percentage of DPPH radical scavenging activity (39.75%) was also found in Hydroethanolic extracts of *Amaranthus viridis*. Total antioxidant capacity was found to be the highest in the Hydroethanol extract of *Spinacia oleracea* (96.330 mg AAE/gm). The findings of the current study indicate that all the four green leafy vegetables undertaken for the study are rich source of natural antioxidants with ample potential in decreasing the harmful effects of various oxidative stress induced conditions in humans.

Keywords: green leafy vegetables, antioxidants, therapeutic potential, hydroehtanol extracts

Introduction
Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function [1]. Diseases linked to oxygen radicals and reactive oxygen species (ROS) include cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers, sunburn, cataracts and aging [2]. In order to prevent or moderate oxidation-related diseases, it is necessary to sequester and eliminate free radicals from the body [3]. Antioxidants may offer some resistance to oxidative stress by scavenging free radicals, inhibiting cell membrane damage, and suppressing lipid peroxidation, thus preventing the onset of chronic disease [4]. Nutrients (found in foods) can scavenge/deactivate these reactive free radicals turning them to harmless particles [5]. Natural antioxidants discovered in plants have attracted some interest due to their widely acclaimed nutritional and therapeutic values. Antioxidant properties stand to be an essential mechanism of beneficial activity of plant-derived compounds and extracts [6]. Plant derived foods are recognized as sources of natural antioxidants such as flavonoids and related phenolic compounds that combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants[7, 8]. Among plant foods, green leafy vegetables and grains are rich sources of antioxidants apart from energy, protein, and selected micronutrients. Epidemiological studies have shown that intake of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species [9]. Healthy lifestyle advocates search for more natural antioxidants with strong therapeutic potential which can eventually replace with some synthetic drugs with unwanted side effects [10]. *Amaranthas viridis*, *Raphanus sativus*, *Chenopodium album* and *Spinacia oleracea* are some common green leafy vegetables grown and consumed throughout India.The present study aims to estimate the total phenol Content (TPC), total favonoid Content (TFC), total antioxidant capacity (TAC) and DPPH free radical scavenging assay of the extracts prepared from the edible part of these four green leafy vegetables (GLVs). The effect of exaction solvent on the TPC, TFC and antioxidant activities of the green leafy vegetables used for the study is also evaluated.

Materials and Methods
Fresh vegetable samples of *Amaranthas viridis*, *Raphanus sativus*, *Chenopodium album* and *Spinacia oleracea* were purchased from local vegetable market of Surat in the month of December-January. Vegetables were botanically identified with the help of local flora and authenticated by experts.

Preparation of extracts
About 20gm of powdered leaf was uniformly packed into a thimble in a soxhlet apparatus and extracted with 200ml Petroleum ether, 200ml Hydroethanol (30:20 water: ethanol), Methanol (200 ml). Constant heat was provided by Mantox heater for recycling of the solvent. The process of extraction continues for 5-6 hours. The excess solvent was evaporated and the dried extract was kept in refrigerator at 4ºC for their future use [11].

Determination of total phenol content
TPC were determined by the Folin-Ciocalteu method [12]. With some modifications. The diluted aqueous solution of each extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 ml). This mixture was allowed to stand

at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2 ml) was added. After 2 hours of incubation, the absorbance was measured at 760 nm against water blank. A standard calibration curve was plotted using Gallic acid.

Determination of total flavonoid content

TFC were estimated by Aluminium trichloride colorimetric method [13]. A diluted methanolic solution (2 ml) of each fruit extract was mixed with a solution (2 ml) of Aluminum trichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 ml) and with $AlCl_3$. A standard calibration curve was plotted using Quercetin.

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity was evaluated by the Phosphomolybdenum method according to the procedure described by [14]. 0.2 ml extract was combined with 2 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after incubation it at 95ºc for 90 minutes and cooling to room temperature. Reagent (2 ml) in the place of extract was used as the blank.

Determination of DPPH free radical scavenging assay

The DPPH antioxidant assay was determined as described by [15]. Briefly, 0.5 ml of DPPH radical solution was mixed with an extract of 2 ml. An equal volume of ethanol was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with a UV spectrophotometer. The percentage of scavenging of DPPH was then calculated in the following way:

DPPH scavenging effect (%) = [1- (Test sample absorbance/blank sample absorbance)] ×100(%).

The results of the all these study were presented as the mean of three determinations along with standard deviation. Statistical analysis was done using MS Excel software (CORREL Statistical function).

Results and Discussion

In the present study all the extracts of GLVs were found to be quite rich in phenolic content. Phenolic compounds are the main class of secondary metabolites in plants.

The antioxidant activity of phenolic compounds is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cations [16].

For determination of TPC, Gallic acid was used as a reference compound. The total phenols were expressed as mg/g Gallic acid equivalent (mg GAE/gm) using the standard curve equation: y = 0.0095x + 0.37, R^2= 0.9912, Where y is absorbance at 760 nm and x in total phenolic content of the vegetables (Figure 1). The amount of total flavonoid was determined with the Quercetin reagent used as standard and the total flavonoids were expressed as mg/g Quercetin equivalent (mg QE/gm) using the standard curve equation: y = 0.005250x + 0.123, R^2= 0.998, Where y is absorbance at 415 nm and x is total flavonoid content (Figure 2).

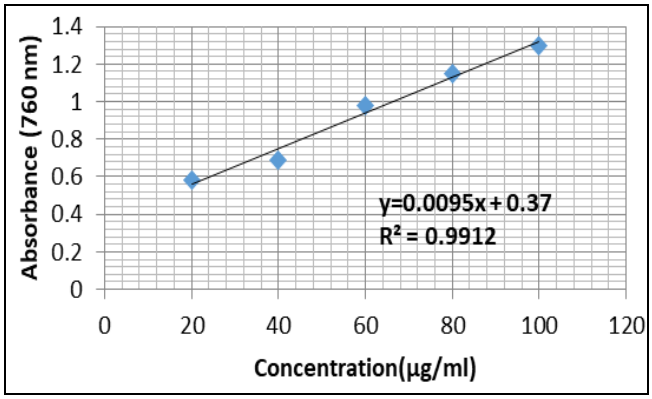

Fig 1: Standard graph of Gallic acid

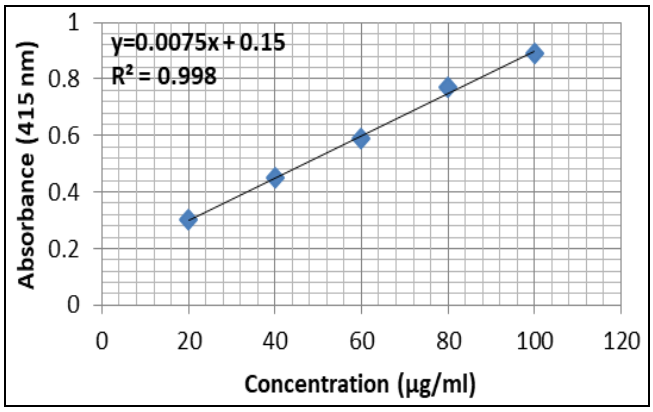

Fig 2: Standard graph of Quercetin

Hydro alcohol extracts of all the GLVs was found to possess highest TPC and TFC with maximum found to be present in *Amaranthus viridis* of TPC of 113.33 ± 0.42 mg GAE/gm (Table 1) and TFC of 66.0 ±0.69 mg QE/gm (Table 1).

Table 1: Total phenol and Total flavonoid Contents of vegetables extracts

GLVs Species	Hydroethnol extract		Petroleum extract		Methanol extract	
	TPCmg GAE/gm	TFC mg QE/gm	TPC mg GAE/gm	TFC mg QE/gm	TPC mg GAE/gm	TFC mg QE/gm
Amaranthus viridis	113.33±.0.42	66.0±0.69	90.11±1.20	56.42±0.64	44.22±.070	30.98±0.25
Raphanus sativus	72.13 ±1.05	41.68 ±0.37	97.24 ±1.26	50.0±0.48	64±0.87	38.72 ±0.27
Chenopodium album	89.52 ±1.2	61.33 ±0.54	102.66 ±1.32	47.33 ±0.45	72.37±1.04	49.92±0.38
Spinacia oleracea	103.73 ±1.33	25.0 ±0.32	107.37±1.36	114.83±1.10	79.27±1.08	60.0±0.39

Each value represents the mean of three replicates ± Standard Deviation

TAC assay by Phosphomolybdenum is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH [14]. The Phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid. The value was expressed as mg/g Ascorbic equivalent using the standard curve equation: y = 0.0056x + 0.03, R^2= 0.9887 (Figure 3).

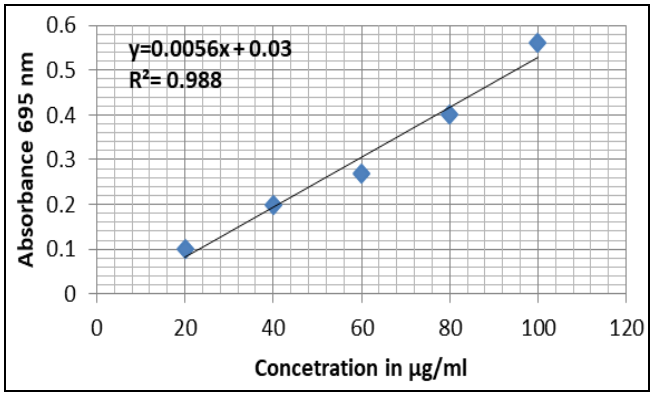

Fig 3: Standard curve of Ascorbic Acid for total antioxidant activity

Table 2: Total antioxidant capacity of studied green leafy vegetables

GLVs	Hydroethnol extract	Petroleum extract	Methanol extract
Amaranthus viridis	92. 0 ± 0.23	75.33 ± 1.2	40.33 ± 0.98
Raphanus sativus	80.0 ± 0.77	23.66 ± 0.92	28.0 ± 1.40
Chenopodium album	82.33 ± 0.51	56.0 ± 0.81	31.66 ± 0.83
Spinacia oleracea	96.33 ± 0.75	42.33 ± 0.97	79. 33 ± 0.62

Each value represents the mean of three replicates ± Standard Deviation

Among the extracts of the different vegetables, total antioxidant capacity was found to be highest in the Hydroethanol extract of *Spinacia oleracea* (96.330 ± 0.75 mg AAE/gm) followed by *Amaranthus viridis* (92.0 ± 0.23mg AAE/gm). The least value of 23.66 ± 0.92 mg AAE /gm was found in Petroleum extract of *Raphanus sativus* (Table 2)

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [17]. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and convert them into a colourless product i.e. 2,2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or electron [18].

Table 3: DPPH radical scavenging activity of studied green leafy vegetables

GLVs Species	DPPH %scavenging activity		
	Hydroethnol extract	Petroleum extract	Methanol extract
Amaranthus viridis	39.75±0.61	11.76±0.20	2.33±0.05
Raphanus sativus	9.42±0.17	4.16±0.08	5.91±0.11
Chenopodium album	0.42±0.02	9.56±0.17	7.88±0.14
Spinacia oleracea	5.26±0.10	18.33±0.31	1.94±0.04

Each value represents the mean of three replicates ± Standard Deviation

In the present study all extracts showed free radical scavenging properties of different levels. Maximum percentage of DPPH radical scavenging activity was shown by hydroethanolic extracts of *Amaranthus viridis* (39.75%) followed by petroleum ether extracts of *Spinacia oleracea* at 18.33 % (Table 3).

In the current study *Amaranthus viridis* was found to possess maximum phenolic and flavonoid contents with significant antioxidant activity. The findings are in agreement with previous report about the species of being excellent source of phenolics, flavonoids, and antioxidants [19].

Conclusion

Identification and development of natural antioxidants not only play an essential role in the prevention or therapy of human diseases but also interrupt any adversity that disrupts normal human health [10].

The present study shows that all the four green leafy vegetables have fairly high phenol and flavonoid content with appreciable antioxidant activities. The efficacy of hydroethanol as the most appropriate extraction solvent is also established from this study. The results of the present study also indicate Amaranthus *viridis* as a promising source of natural antioxidants that can be found to be useful as therapeutic agent to combat the damage caused by an excess of free radicals. However a detailed study is required to have a better understanding of the mechanism of action of the antioxidants.

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