Research Article

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SCREENING OF SOME EDIBLE PLANTS FOR ANTIOXIDANT POTENTIAL

Tripathi Parul, Srivastava Apeksha, Rekhi Lavie, Singh Aditi *
Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow Campus, Malhaur, Gomti Nagar Extension, Lucknow, India

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***Corresponding author**
E-mail: asingh3@lko.amity.edu

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ABSTRACT

Oxygen- free radical can induce damage to DNA, which in turn can lead to tissue damage. Antioxidants are substances that neutralize the effect caused by free radicals and reactive oxygen species (ROS). Synthetic antioxidants have been seen to cause harm to human health. Hence, the search for non-toxic and effective antioxidants is increasing. Plants have an innate ability to biosynthesize various non-enzymatic antioxidants that are capable of attenuating oxidative damage. The aim of this study was to monitor antioxidant potential of some fruits and vegetables commonly found in India. The aqueous extracts of nineteen fruits and vegetables were screened for their antioxidant potential and ethanolic extract for phenolics and flavonoid levels. The results have reflected that of all plants studied *Chenopodium album, Coriandrum sativum* and *Anethum graveolens* were found to have most promising results with significantly high levels of total antioxidant, phenolic as well as flavonoid levels. However, highest total antioxidant content was demonstrated by *Spinacia oleracea* whereas *Anethum graveolens* and *Citrus sinensis* gave maximum amount of phenolics and flavonoids. When statistically analyzed by *one way ANOVA* it showed significant variation amongst antioxidants potential of studied plants with $p < 0.05$. When correlation was established between total phenolics and total antioxidants, a positive correlation of 0.43 was observed and between total flavonoid and total antioxidants a positive correlation of 0.66 was observed. Our results indicate flavonoids and phenolics account for major antioxidant activity. This antioxidant profile would be advantageous for providing support for dietary guidelines.

Key words: Antioxidants, Flavonoids, Free radicals, Phenolics, *Chenopodium album*, *Anethum graveolens*.

INTRODUCTION

'Reactive oxygen species' (abbreviated as 'ROS') include hydroxyl radicals, superoxide radicals, hydrogen peroxide and singlet oxygen. Often, a biological reaction or certain exogenous factors result in the generation of these 'free radicals' as by-products[1]. The human body is known for generating ROS continuously. Some of these free radicals play a crucial role in various metabolic processes inside the cell. These include 'phagocytosis', energy-production and intercellular signaling[2]. But, ROS generated by means of UV radiations, sunlight, chemical reactions, ionizing radiations and various other metabolic processes exhibit a large amount of deleterious effects like carcinogenesis, neuro-degenerative diseases, cardiovascular diseases, aging, DNA damage etc.[3].

These ROS mediated diseases and cellular damage can be prevented by means of a potential 'scavenger' of the free radicals[4]. Antioxidants have been found to detoxify these free radicals present in the body. Various antioxidant-based formulations and drugs have been developed in order to prevent and treat these complex and harmful diseases[5]. Apart from the numerous health benefits, these marvelous 'scavengers' are also known to prevent or/and delay oxidation of food materials which is initiated by ROS during the exposure of food items to various environmental factors like light, air, moisture, temperature, etc[6]. Hence, antioxidants are of great use in the food industry. Studies reveal that several commercially available synthetic antioxidants, like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) discovered in the past thirty years have proven to be quite unsafe due to their toxic nature[7]. Therefore, their application has faced restrictions and a trend has been started which involves their substitution with naturally-occurring antioxidants.

Besides, the plants contain wide range and levels of phenolics, flavonoids, flavones, anthocyanin, isoflavones, catechins, isocatechins and coumarin lignans within them. According to several studies, these compounds have antioxidant, anti-inflammatory, anti-carcinogenic activities[8] and various other positive biological effects. Crude-extracts of spices, herbs, fruits, vegetables and other plant materials, rich in phenolic content are of increasing interest in the present scenario. Apart from providing protection and treatment, they also contribute in retardation of oxidative-degradation of lipids and thus improve and enhance the nutritive value and quality of food items. On the other hand, flavonoids come under phenolics and can be described as a group of polyphenolic compounds possessing known properties which include providing vibrant colours to food items apart from their inhibitory actions on hydrolytic and oxidative enzymes, ROS scavenging activity and anti-inflammatory nature[3]. Antioxidant-based drug formulations have been used for the treatment and prevention of many diseases like diabetes, cancer, stroke, atherosclerosis, and Alzheimer's disease[9]. This has attracted a great deal of research interest in the natural antioxidants.

It has been found that individuals having a diet rich in vegetables and fruits show a decreased risk of certain forms of cancer and cardiovascular diseases[10,11]. Antioxidants from plant sources, especially phenolics and flavonoids from spices, wine, tea, fruits and vegetables have been commercially exploited either in the form of nutritional-supplements or as antioxidant additives[12,13]. Still there is a great demand to retrieve more information regarding the free radical scavenging property of plant species since they are safe as well as bioactive.

Therefore a study was planned with the aim to assess the antioxidant potential of edible plants viz., *Spinacia oleracea* (spinach), *Trigonella foenum-graecum* (fenugreek), *Anethum graveolens* (dill), *Chenopodium album* (bathua), *Coriandrum sativum* (coriander), *Brassica oleracea* (cabbage), *Capsicum annuum* (capsicum), *Capsicum frutescents* (green chilli), *Lagenaria siceraria* (bottle gourd), *Momordica charantia* (bitter gourd), *Raphanus sativus* (radish), *Pisum sativum* (pea), *Citrus limon* (lemon), *Citrus sinensis* (orange), *Malus domestica* (apple), *Musa paradisiaca* (banana), *Psidium guajava* (guava), *Ziziphus mauritiana* (Indian plum) and *Vitis vinifera* (grapes).

MATERIALS AND METHODS

Collection of Plant Material

Samples of the nineteen plants were collected from a local marketplace of Lucknow, Uttar Pradesh. The various plant parts used for the study are given in the Table below. For extraction and analysis, fresh leaves of *Anethum graveolens, Brassica oleracea, Coriandrum sativum, Chenopodium album, Spinacia oleracea* and pulp of *Citrus limon, Malus domestica, Musa paradisiaca, Vitis vinifera, Momordica charantia, Capsicum annuum, Lagenaria siceraria, Psidium guajava, Ziziphus mauritiana and Trigonella foenum-graecum, Raphanus sativus, Capsicum frutescens* (fruits), *Citrus sinensis* (peel), *Pisum sativum* (seeds) were taken in the study.

Plant part used	Plant name
Leaves	*Anethum graveolens, Brassica oleracea, Coriandrum sativum, Chenopodium album, Spinacia oleracea*
Pulp	*Citrus limon, Malus domestica, Musa paradisiaca, Vitis vinifera, Momordica charantia, Capsicum annuum, Lagenaria siceraria, Psidium guajava, Ziziphus mauritiana and Trigonella foenum-graecum, Raphanus sativus*
Fruit	*Capsicum frutescens*
Peel	*Citrus sinensis*
Seeds	*Pisum sativum*

All collected samples were washed with detergent followed by sterile water. They were blot dried and then used in the study. The plant parts were homogenized and extracted differently for different purposes as mentioned below.

Total Antioxidant Activity

Total antioxidant activity was estimated by the protocol of Cacig et al., (2005)[14] with some modifications. One gm of tissue was homogenized in 4 ml of distilled water and incubated for 24 hours at 4°C. It was then filtered twice with Whatman no. 1 filter paper and the collected filtrate was stored at 4°C. 100 µl of sample was taken in a 3 ml glass cuvette containing the oxidative mixture of 0.18 ml potassium permanganate (0.01 M); 0.42 ml sulfuric acid (2 M) and 2.3 ml distilled water. The decrease in absorbance was measured at 535nm, with ascorbic acid as standard.

In order to quantitatively compare the antioxidant activities, the following formula was used:

$$A50 = \frac{t\ standard}{t\ plant\ sample} \mathbf{x} \frac{C\ standard}{m\ plant} \mathbf{x} \frac{V\ standard}{V\ plant\ sample} \mathbf{x}\ V\ extract$$

where:

*A*50 — antioxidant activity articulated, reflected in the time until the sample induces a decrease of the oxidizing agent ($KMnO_4$) concentration up to one half, compared against a standard [ascorbic acid] (mmol equivalent / g plant tissue)

t plant sample — the time until the sample induces a decrease of the permanganate concentration up to one half (min)

t standard — the time until the standard (ascorbic acid) persuade a decline of the permanganate concentration up to one half (min) [0.66 minutes as seen in standard curve]

C standard — standard (ascorbic acid) concentration [0.01 mmol/ml]

m plant — weight (gm) of the plant sample subjected for extraction [1gm]

V plant sample — volume of the plant extract subjected for the analysis [0.1 ml]

V standard— volume of the standard subjected for the analysis [1 ml]

V extract— volume (ml) of the obtained extracts [4 ml]

Measurement of Total Phenolics

Total phenolic content was determined by the modified protocol of Fatma et al., (2013)[15]. 25 gm of tissue was homogenized with 100 ml of 70% ethanol. The extract was filtered through filter paper. The total phenolic content was determined by using Folin-Ciocalteu reagent. A volume of 0.5 ml of the plant extract was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and then neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated for 30 min. The absorbance of the resulting color was measured at 765 nm. All the sets were prepared in triplicate. The total phenolic content was determined using standard curve of gallic acid (G.A).

Total Flavonoids content

Total flavonoids was extracted by the method of Kevin et al., (2002)[16] with some modifications. 25gm of tissue was submerged in 100 ml of 80% ethanol overnight. The extract was filtered using filter paper. Filtrate was collected and used for estimation of flavonoids. Ethanolic extract was diluted to 1:10 with 80% ethanol to a total of 3 ml. Absorbance was taken at 362 nm in triplicate and total flavonoids were calculated using standard curve of Quercetin (QE).

Statistical Analysis

The data analysis was done using Microsoft Excel 2010 software. One way ANOVA (without replication) was applied for calculation of significant variation in the antioxidant potential among respective plants.

RESULTS AND DISCUSSION

A total of nineteen plants were tested for their antioxidant potential, flavonoids and phenolic content, out of which thirteen were vegetables and six were fruits. A comparison has been made between fruits and vegetables to determine their ability of having total antioxidants, phenolics and flavonoids level.

The total antioxidant activity, which reflected the ability of the vegetable extracts to inhibit the bleaching of β-carotene, was measured and compared with that of the fruits to check the level of antioxidant components in two. As evident from Figure 1, the highest total antioxidant content in case of vegetables was found in *Spinacia oleracea* followed by *Chenopodium album* and *Anethum graveolens*; while the minimum was found to be in *Ziziphus mauritiana.* In another study, antioxidants activity was evaluated and the results indicate that the extracts of *A.graveolens* showed stronger hydroxyl radical scavenging activity[17]. Further, a comparative study by Isbilir et al., (2011)[18] was done in which the possible antioxidant properties of water, ethanol, and acetone extracts of dill leaves were investigated, the water extract of dill leaf showed the most potent antioxidative capacity in each assay.

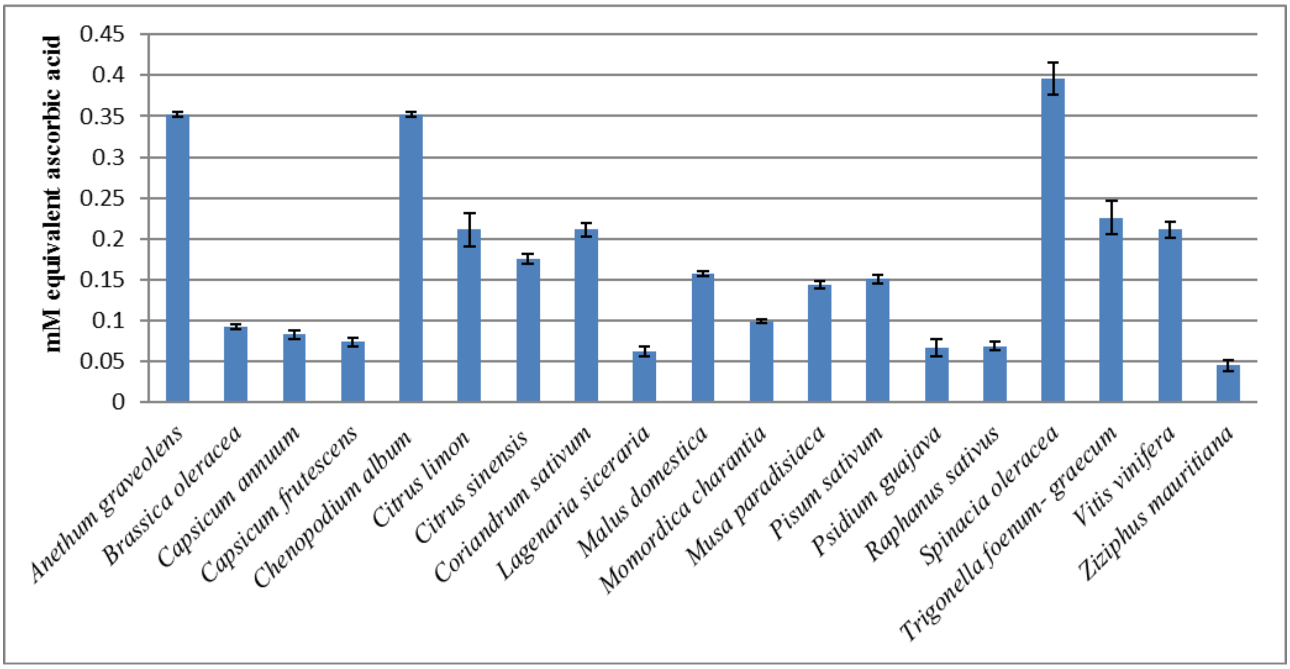

Figure 1: Total Antioxidants (mM equivalent ascorbic acid/ gm tissue + S.D.)

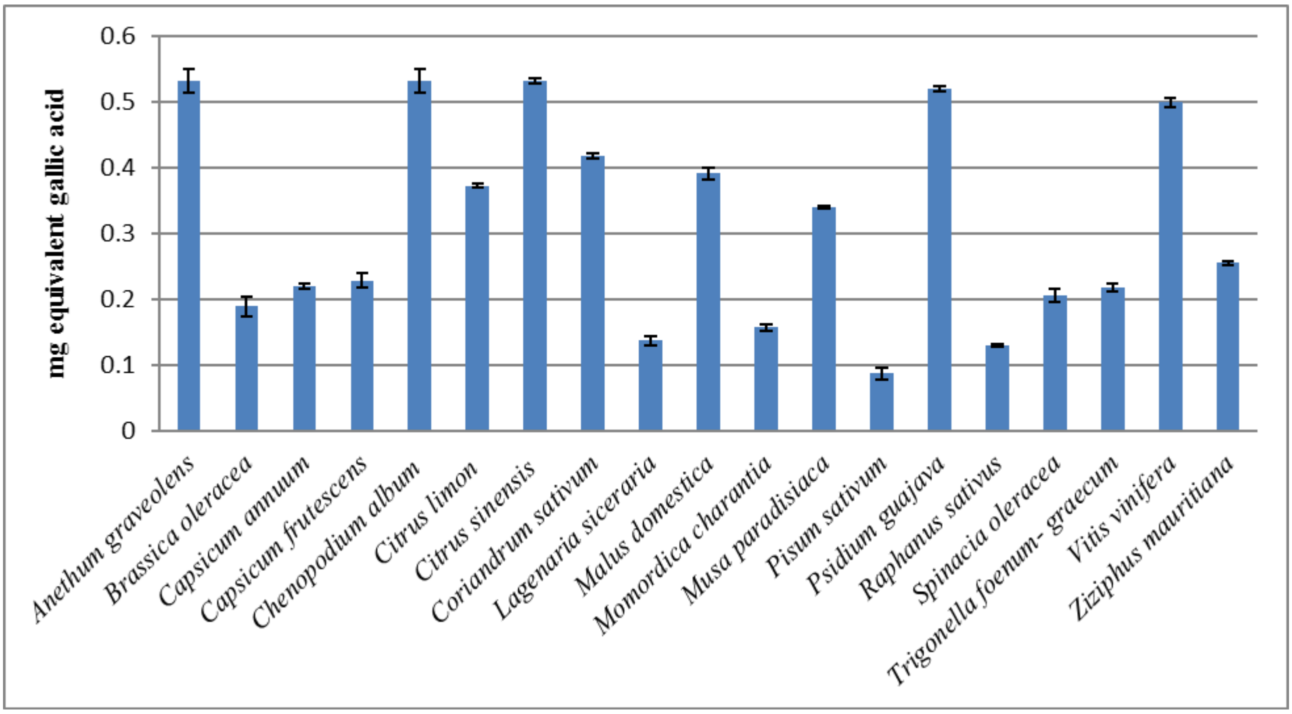

Figure 2: Total Phenolic Content (mg equivalent gallic acid/ gm tissue+ S.D.)

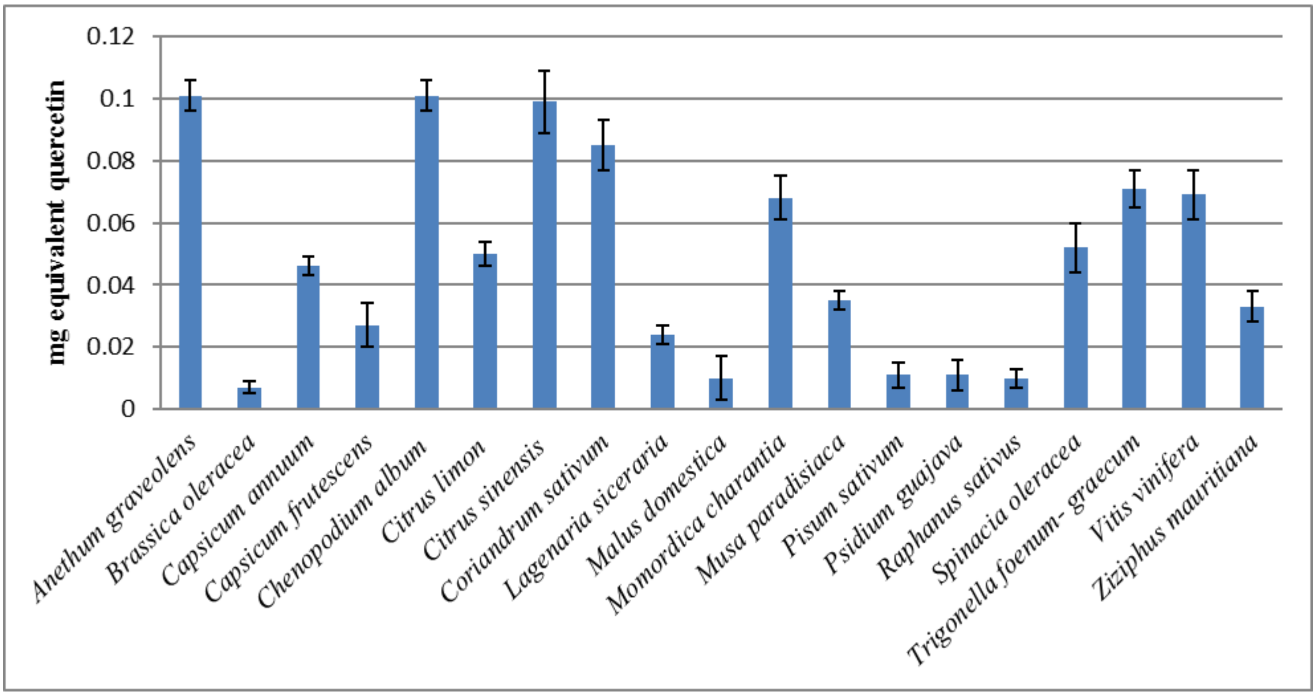

Figure 3: Total Flavonoid Content (mg equivalent quercetin/ gm tissue+ S.D.)

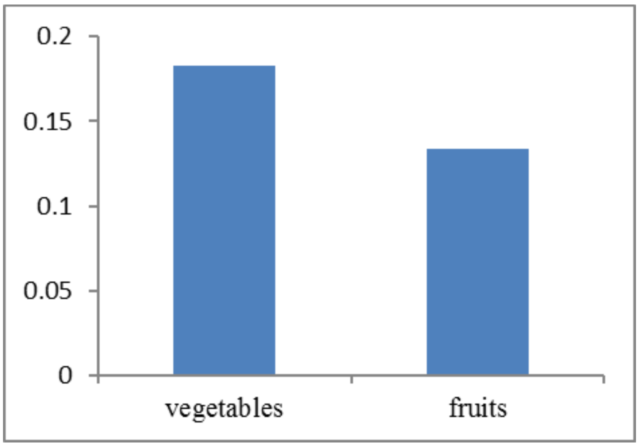

Figure 4: Comparison of total antioxidants between vegetables and fruits

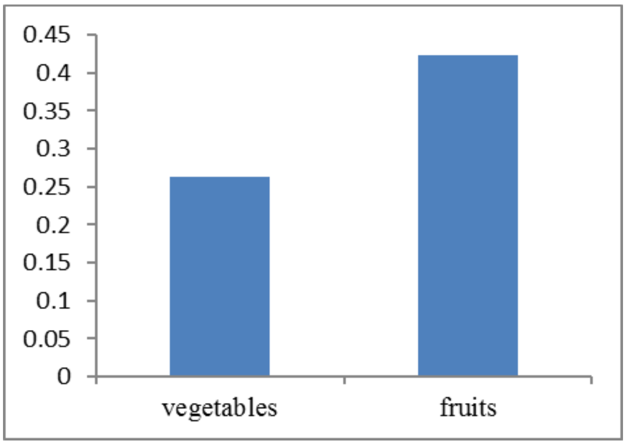

Figure 5: Comparison of phenolics between vegetables and fruits

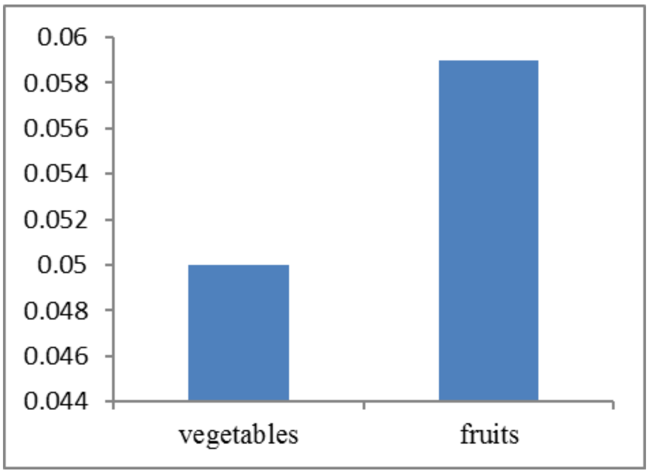

Figure 6: Comparison of flavonoids between vegetables and fruits

When total phenolics were estimated in all the plants of this study, it was observed that *Chenopodium album*, *Citrus sinensis* and *Anethum graveolens* dominated other plants; while minimum was observed in seeds of *Pisum sativum.* Among vegetables, *A. graveolens* and *C. album* and among fruits, *C. sinensis, P. gujava* and *V. vinifera* have the maximum content of phenolics (Figure 2). Lagha-Benamrouche et al., (2013)[19] have reported that phenolic compounds in peels and leaves are different in the seven varieties of *Citrus sinensis* (orange) obtained from Algeria. In another study, high amounts of phenolic compounds were found in the hot water extract and methanolic extract of *Anethum graveolens* also[20].

In our study when total flavonoids were estimated using Quercetin (QE) as standard, *Anethum graveolens* and *Chenopodium album* exhibited highest total flavonoid content; whereas *Brassica oleracea* had the lowest levels (Figure 3). Previously studied by Tanruean et al., (2014)[20] methanolic extracts of *Anethum graveolens* had a good free radical scavenging activity with IC_{50} value of 22.3 µg/ml. Results of a study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage[21]. As reported by Guimarães (2010)[22] the peels polar fractions have the highest contents in flavonoids, phenolics, carotenoids, ascorbic acid, and reducing sugars that contribute to the highest antioxidant potential. Occurrence of the flavonoids is directly associated with human daily dietary intake of the antioxidants, therefore evaluation of flavonoid sources in food is very important. Vegetables and fruits are the main dietary sources of flavonoids[23]. Many flavonoids have been shown to have anticancer activity, free-radical scavenging capacity and anti-oxidative activity. In the present study, *A. graveolens, C. sativum, T. foennum- and S. oleracea* among vegetables and *C. sinensis* and *C. limon* among fruits have been found to have higher content of flavonoids in comparison to other vegetables and fruits.

Comparing the levels of all three parameters, i.e. total antioxidants, phenolics and flavonoids, the present study found *Chenopodium album* and *Anethum graveolens* to be most promising antioxidant sources. Among all the fruits tested, peels of *Citrus sinensis* demonstrated high levels of phenolics and flavonoids. From this, it can be inferred that the peel extract is more efficient than pulp as also reported by Ghasemi et al., (2009)[24]. It was further observed in the study that *S. oleracea, T. foennum-graecum, C. sativum,* and *A. graveolens* among vegetables and *V. vinifera* among fruits had maximum antioxidant potential.

The Pearson correlation was also calculated as a measure of strength for relationship between two variables. When correlation was established between total phenolics and total antioxidants, a positive correlation of 0.43 was observed; whereas correlation between total flavonoid and total antioxidants gave a positive value of 0.66. Significant variation has been observed when two-way ANOVA was applied with ($P< 0.05$). A comparison between vegetables and fruits in the study suggests no significant difference in all three parameters as vegetables have higher total antioxidant potential (Figure 4) where as tested fruits have shown higher phenolics and flavonoids (Figures 5 and 6); so we can say that both supplements are equally important for diet.

Evidence suggests that total antioxidants, phenolics and flavonoids present in fruits and vegetables may be highly beneficial in preventing the negative consequences of oxidative stress[25]. Many of such plants are known to exert positive effect on various chronic diseases[26] and play a crucial role in decreasing the risk of various cancers, cardiovascular diseases and neurological diseases[27,28]. Thus they can be highly useful in cosmetics, food and pharmaceutical industry[29,30]. Postulations have been made that various antioxidants, phenolics and flavonoids with complementary action-mechanisms and several redox-potentials work in ‘synergistic’ interactions[31].

CONCLUSION

There has been an amplified interest worldwide to identify antioxidant compounds that are pharmacologically effective and also have low or no side effects for use in preventive medicine as well as the food industry. The vegetables and fruits taken in this study are easily available in the market and are within the reach of common man. Since many of these plants have shown significant levels of activity, they should be further investigated as good sources of edible antioxidants, phenolics and flavonoids. The increase in intake of these vegetables and fruits in the diet of people will protect them from various harmful diseases. Also, a correlation needs to be established between the antioxidants in these plants and their positive effects on the body. Creating awareness regarding the health-benefits of these plants is very crucial. The findings of this study may be beneficial in allowing the people to make better food choices and thus helping them improve their health and nutritional status. Also, there is a need to identify other such ‘economical’ plants which are rich sources of antioxidants apart from providing nutrition and taste so that they may help mankind fight against major threats of the present time such as cancers, cardiovascular disease and diabetes.

There is a strict regulation for the use of synthetic antioxidants in the food products as they are associated with health risks. Increasing knowledge in antioxidant phytoconstituents and including them in the daily uses and diet can give our body sufficient support to fight the diseases. Vegetables and fruits have been found to be important sources of antioxidant substance. Herbal medicine and phytoconstituents are also significant to manage pathological conditions of those diseases that are caused by the free radicals. Identification and isolation of those phytoconstituents are presenting enormous scope for their therapeutic applications. Exploring and identifying plant sources and our traditional therapeutic knowledge, as well as interpreting it according to the recent advancements will help fight against oxidative stress.

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