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IN VITRO TOTAL ANTIOXIDANT CAPACITY AND ANTI-INFLAMMATORY ACTIVITY OF VEGETABLES

Nutritional Science

Hinal Patel Assistant Professor, Foods and Nutrition, Children's University, Gandhinagar, Gujarat.

ABSTRACT

Anti-inflammatory and antioxidant activities are frequently used to screen chemopreventive foods. This study evaluated the bitter gourd, bottle gourd, drumstick leaves, and ginger. We estimated total phenolics and flavonoids. Based on the ability of cation radicals to deactivate ORAC, the total antioxidant capacity was calculated. A protein denaturation inhibition experiment was used to assess anti-inflammatory efficacy. Among four vegetables, the methanolic extract of drumstick leaves had the greatest total phenolics and flavonoids. The maximum antioxidant capacity was identified in ginger, while the highest scavenging activity was found in drumstick leaves. Based on the IC50 of the vegetables, bitter gourd>ginger>bottle gourd>drumstick leaves. There was also a link between total phenolic content and antioxidant activity. Because vegetables are so protective, their widespread use and potential health advantages are logical.

KEYWORDS

In vitro anti-inflammation, ORAC, Vegetables

INTRODUCTION

Chronic diseases cause 46% of global disease burden and 59% of world mortality, according to WHO[1]. Inflammation is now recognised as a key player in the pathophysiology of major chronic diseases. Obesity, insulin resistance, type 2 diabetes, metabolic syndrome and several cardiovascular illnesses all have elevated plasma levels of inflammatory markers[2].

In the last two decades, researchers have discovered how chronic oxidative stress can cause chronic inflammation. Oxidative stress causes inflammation, and the pathways that produce inflammation mediators including adhesion molecules and interleukins are all stimulated by oxidative stress. Atherosclerosis and cardiovascular disease commonly combine with inflammation and oxidative stress[2].

Many of these illnesses are treated with anti-inflammatory medicines, such as NSAIDs. Some have dose-dependent negative effects and none are acceptable for primary prevention, limiting their use. The WHO estimates that 80 percent of the world's population uses traditional medicine for primary health care, with much of this therapy involving plant extracts and active components. Studies show a link between a healthy diet rich in fruits and vegetables and a reduced risk of chronic diseases[3]. Fruits and vegetables contain phenolics, flavonoids, anthocyanins, and carotenoids, which may contribute to their physiological roles. Dark coloured fruits, vegetables, and dishes are considered healthier, especially in oriental cultures. The pigments in fruits and vegetables are gaining popularity since they may improve human health or reduce illness risk[3]. Fruits and vegetables are now considered functional foods, able to provide health advantages in addition to meeting physiological needs. Consumption of fruits and vegetables has major health benefits[3]. In vitro and epidemiological data strongly imply that meals rich in antioxidant and anti-inflammatory phytochemicals protect against major disease risks such as cancer and cardiovascular disorders. The anti-inflammatory and antioxidant activities of several vegetables were assessed using a well-known model system.

MATERIALS AND METHODS

Vegetables and Sample Extraction:

Four vegitables were acquired from the Anand market: bitter gourd (Momordica charantia), bottle gourd (Lagenaria siceraria), ginger (Zingiber officinale) and drumstick leaves (Moringa oleifera). An acidified methanol extract was made from 2.0g plant material. The filtered extracts were used for subsequent experiments.

Determination of total phenolic and flavonoid content:

The total phenolic content of the extract was measured by the method give by Singleton[4]. The results were represented as mg of GA equivalent per 100 g of material. The flavonoid concentration was determined using the aluminium chloride colorimetric method with rutin as a standard[5]. Results were expressed in milligrammes of rutin equivalent per 100g of sample.

Ferric reducing antioxidant power assay (FRAP):

The FRAP of the methanolic extract was measured using Benzie and Strain's method[6]. Results were expressed in mg Trolox Equivalent Antioxidant Capacity per 100g Sample.

Scavenging effect on DPPH Radicals:

The scavenging properties of vegetable methanolic extract on DPPH were evaluated by Brand-Williams[7]. The extract was added to a methanolic DPPH solution and measured at 517 nm. Results were expressed in mg of Trolox Equivalent Antioxidant Capacity per 100g of sample.

Scavenging effect on ABTS Radicals:

Scavenging effects of Methanolic Extracts on ABTS were assessed using Re et al's modified technique[8]. The results were represented as TEAC per 100g of material.

Oxygen radical absorbance capacity (ORAC):

The ORAC assay measures the ability of azo drugs to suppress peroxyl radical-induced oxidation. The estimation approach was modified by Ou et al[9]. The data were reported as trolox equivalents as micromole per gram using the area under the curve.

In vitro anti-inflammatory effects on protein denaturation inhibition assay:

The protein denaturation inhibition experiment was performed as described[10]. The turbidity of the methanolic extracts was evaluated spectrophotometrically at 660 nm after treatment with bovine albumin. The % suppression of protein denaturation by diclofenac sodium was determined.

STATISTICAL ANALYSIS:

Each experiment was repeated twice and reported as mean±SD. SPSS 20 was used for statistical analysis. ANOVA was used to analyse variance. In this case, the Duncan's test revealed a significant difference ($p<0.05$). The link was studied using Pearson's correlation. The results were further subjected to multivariate data analysis using SPSS 20.

RESULTS AND DISCUSSION

Vitamin C, tocopherol, phenolics, and beta-carotene are antioxidants found in fruits and vegetables. Among these, phenolics are potent antioxidants due to their hydrogen-donating phenolic hydroxyl groups, as well as their electron-donating characteristics.

Phenolic chemicals contribute to functional quality by reducing reactive oxygen species (ROS) and thereby reducing molecular damage. The total phenolic content of vegetables varied substantially ($p<0.05$) from 52.41 to 372.82 mg GAE/100g, almost seven-fold (Figure 1). The order was bottle gourd, bitter gourd, ginger, drumstick leaves. The pharmacological actions of phenolics as natural antioxidants have sparked great interest. It has been linked to a lower risk of cardiovascular disease and certain malignancies[11]. To extract total phenolics from bitter gourd, Budrat[12] reported 4. 99 mg GAE/g total phenolics. It includes more total phenolics (105.04 mg GAE/g), total flavonoids (31.28 mg QE/g), and has stronger antioxidant activity, according to Brahma et al (85.77%).

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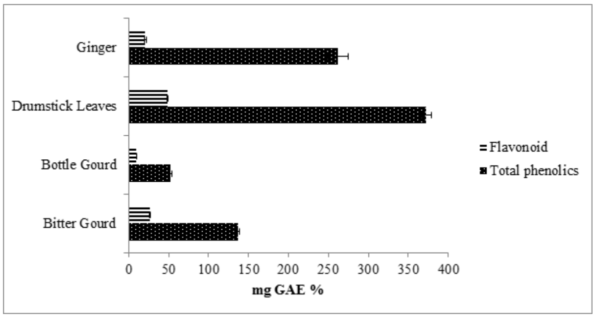

Values are mean±SD;n=4. Total phenolics content in mg gallic acid equivalent per 100g; Flavonoid content in mg rutin equivalent per 100g.

Figure 1: Total phenolics and Flavonoid content of the methanolic extract of vegetables.

Flavonoid content varied considerably ($p<0.05$) from 10.02 to 48.56 mg RT/100g. The contents were: bottle gourds, ginger, bitter gourds, drumstick leaves. Flavonoids are anti-inflammatory, decrease platelet aggregation and inhibit mast cell histamine production[13].

Table 1 lists antioxidants in vegetables. Compared to other vegetables, bottle gourd had the lowest antioxidant capacity tested by four methods. Among vegetables, ginger showed the highest ferric reduction antioxidant capacity. Surprisingly strong DPPH and ABTS scavenging activity in drumstick leaves were found. ORAC hierarchy: bottle gourd, ginger, bitter gourd, drumstick leaves. Sreelatha[14] found that both mature and tender drumstick leaf extracts showed significant antioxidant activity against free radicals. On the other hand, Siddhuraju tested freeze-dried Moringa oleifera (drumstick leaf) extracts for radical scavenging and antioxidant properties. The researchers concluded that methanol (80%) and ethanol (70%) were the optimum solvents for extracting antioxidant components from drumstick leaves[15].

Table 1: Total Antioxidant Capacity by FRAP, DPPH RSA, ABTS RSA and ORAC of selected vegetables

	FRAP	DPPH RSA	ABTS RSA	ORAC
	(mg TE/100gm)	(mg TE/100gm)	(mg TE/100gm)	(micromole /gm)
Bitter Gourd	173.66±10.47^{b}	454.17±37.80^{b}	249.26±26.09^{b}	54.45±7.23^{c}
Bottle Gourd	65.89±10.76^{a}	173.33±7.22^{a}	39.20±3.22^{a}	28.54±5.86^{a}
Drumstick Leaves	764.31±33.39^{c}	1236.00±25.99^{d}	896.19±79.31^{d}	55.76±1.84^{c}
Ginger	1216.12±41.47^{d}	838.68±32.37^{c}	624.92±15.55^{c}	40.49±0.25^{b}
F-Value	1508.36*	1068.48*	323.90*	29.39*

Values are mean±SD;n=4. Different alphabets within column are significantly different ($p\leq0.05$) from each other. ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power assay; DPPH RSA, DPPH radical scavenging activity; ABTS, ABTS radical scavenging activity; TE, trolox equivalent.

Total phenolic content correlated with flavonoid ($r=0.855$), FRAP ($r=0.764$), DPPH RSA ($r=0.997$), ABTS RSA ($r=0.991$), and ORAC ($r=0.593$). They found a significant ($p<0.05$) correlation between flavonoid concentration in the selected vegetables and ORAC ($r=0.807$). However, FRAP content in vegetables did not correlate with flavonoid ($r=0.336$) or ORAC ($r=0.166$). FRAP detects single electron transfers while ORAC detects hydrogen atom transfers. The ABTS radical is susceptible to single electron and hydrogen atom transfers. Because these three approaches work on independent chemical pathways, they are not connected.

This graph shows the % inhibition and IC50 value of protein denaturation in vegetables (table 2). Bitter gourd>ginger>bottle gourd>drumstick leaves have the capacity to suppress protein denaturation. Bitter gourd has the lowest IC50 value, indicating the most anti-inflammatory efficacy. Studies on other models have found similar outcomes[16]. Sulaiman[17] concluded that aqueous extract of drumstick leaves has significant anti-inflammatory action in a dose dependent manner.

Table 2: % inhibition by protein denaturation of selected vegetables

Extracts	Concentration(μg)	%inhibition	IC50(μg/ml)
Bitter Gourd	400	66.6	280.72
Bottle Gourd	400	35.1	507.31
Drumstick Leaves	400	19.8	726
Ginger	400	63.5	340.38
Diclofenac sodium	50	54.91	50.06

Values are mean±SD;n=4.

Principal component analysis (PCA) is a method for reducing the number of variables that account for most of the variance in the observed variables. PCA was used to find the most relevant variables and traits in each dimension, or principle components (PCs). PC1 (51.84%) and PC2 (95.82%) explained roughly 95.82% of total data variability (43.98%). On the right hand of the component plot between attribute vectors are antioxidant capacities that are more predictable.

CONCLUSION

The study found a wide range of phenolics, flavonoids, and antioxidants in vegetables. Vegetable methanolic extracts had anti-inflammatory effects, presumably due to phenolic components. To prevent or reduce the process of reactive oxygen species and associated oxidative stress related degenerative illnesses, the present investigation demonstrated that several vegetable extracts had substantial antioxidant activity and scavenging effects on free radicals. Thus, increasing vegetable consumption in the human diet is advised.

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