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Study of free radical scavenging activity and phytochemicals of the methanol extract of broccoli (Brassica oleracea)

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ABSTRACT

Consumption of fruits and vegetables has been inversely associated with morbidity and mortality from degenerative diseases. Oxidative stress is an important part of many human diseases. The use of antioxidants in pharmacology is extensively studied as a treatment for stroke, cancer and neurodegenerative diseases. Antioxidants play an important role of protecting the human body against damage by the free radicals. In present study the antioxidant properties and phenolic contents of the methanol extracts of the broccoli (Brassica oleracea) floret were evaluated using in vitro standard procedures. Spectrophotometry was the basis for the determination of total phenolics, flavonoids and antioxidant capacity. The antioxidant activities of broccoli floret were determined by ferric reducing antioxidant property (FRAP), DPPH radical scavenging assay. In our study IC_{50} values of broccoli floret was 30 mg ml^{-1}. The total phenolic and flavonoid content was found to be 10.55±2.98 mg GAE / g dry weight and 2.85±0.32 mg QE /g dry weight respectively. Thus, the present study indicates that broccoli could serve as a free radical scavenger acting possibly as a primary antioxidant. It may be served as a dietary supplement to minimize oxidative stress.

Keywords: Antioxidant, Broccoli, Phenolics, FRAP, DPPH.

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INTRODUCTION

Epidemiologic studies have indicated that diet has major role in the development and pathogenesis of most the chronic diseases like cancer, coronary heart diseases, obesity, type 2 diabetes (non-insulin dependent diabetes), hypertension and cataract. These studies indicate that predominantly plant based diet rich in fruits and vegetables, pulses & minimally processed starchy staple foods reduce the risk for the development of these diseases significantly. Fruits & vegetable provide best protection against the development of diseases & there is no need to take vitamin or other micronutrient supplements for disease prevention [1].

The common factor involved in the pathogenesis of most chronic diseases is the oxidative stress. Oxidative stress is excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. Oxidative stress occurs when there is an imbalance between free radical reaction and the scavenging capacity of antioxidative defense mechanism of the organism. Oxidative stress is related to the production of ROS & RNS including free radicals, by all aerobic organisms. Dietary antioxidants form an important defense mechanism against the damage by free radicals [1]. Plants contain a wide range of free radical scavenging molecules such as phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity. Many dietary polyphenolic constituents derived from plants are more effective antioxidants in vitro than vitamin E or C & thus might contribute significantly to the protective effects in vivo [3]. It is estimated that one-third of all cancer deaths in the U. S. could be avoided through appropriate dietary modification [4].

Consumption of fruits & vegetables has been inversely associated with morbidity & mortality from degenerative diseases [9]. Research indicates that diet rich in cruciferous family vegetables helps to decrease the risk of developing cancer & other degenerative diseases. In this family broccoli is distinguished by the presence of numerous bioactive substances like glucosinolates, phenolics, vitamin C, B_1, E, carotenoids & selenium which have health promoting properties [8, 10].

Phenolics have antioxidant capacity & may protect the cells against oxidative damage. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic & oxidative enzymes & anti-inflammatory action [5]. To prevent or slow down the oxidative stress induced by free radicals, sufficient amount of phenols as antioxidants need to be consumed in the diet.

In the present study methanolic extract of broccoli floret was used for the evaluation of free radical scavenging activity by DPPH & FRAP assay [7] & phytochemicals like phenolics & flavonoids by in vitro standard procedures. These assays are based on electron transfer reactions & spectrophotometry.

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MATERIALS AND METHODS

Chemicals:

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Aldrich, Germany. 2, 4, 6- tri pyridyl-s-triazine (TPTZ) gallic acid and L-ascorbic acid were purchased from Sisco Research laboratories, Mumbai, India.

Plant collection and extract preparation

Broccoli sample was purchased from the local market. Broccoli florets were shade-dried to get consistent weight. 2 g of dried florets were mixed with 200 ml methanol and kept overnight at room temperature. Then soxhlet extraction was carried out. The extract was concentrated by using rotary vacuum evaporator. The residue was dissolved in methanol and used for determination of antioxidant capacity [15].

FRAP (Ferric reducing-antioxidant power) assay:

A modified method of Benzie & Strain [6] was used for the FRAP assay. The fresh working solution was prepared by mixing 25 ml of 300mM acetate buffer (pH 3.6), 2.5 ml (10 m M in 40 mM HCl) of TPTZ and 2.5 ml (20 mM) FeCl3. Plant extract (20 µl) was allowed to react with 2850 µl of FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 to 1000 µM $FeSO_4$. Results were expressed in µM Fe (Π)/g of dry mass.

$DPPH^{\bullet}$ radical scavenging activity determination:

The stable 1,1-diphenyl-2- picryl hydrazyl radical (DPPH) was used for the determination of the free radical scavenging activity of the extract. Different concentrations of each extract were mixed with an equal volume of methanolic solution of DPPH (0.135mM). After 15 min of incubation at room temperature the absorbance was recorded at 517 nm. L-ascorbic acid was used as standard control. The experiment was repeated three times. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

Phenolics determination:

Total phenolics were determined by Folin-Ciocalteu reagent [5]. A dilute plant extract or gallic acid ()standard phenolic compound) was mixed with FC reagent (5 ml, 1:10 diluted with distilled water) & aqueous Na_2CO_3 (4 ml, 1 M). This mixture was allowed to stand for 15 min & absorbance was taken at 765 nm. The standard curve was prepared by using 0, 10, 20, 30, 40, 50 µg L^{-1} solution of gallic acid in methanol: water (50:50 v/v). Total phenols were expressed in mg gallic acid equivalent/ g of floret.

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Flavonoids determination:

Aluminium chloride colorimetric method was used for flavonoids determination [5]. Each plant extracts in methanol were separately mixed with 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1M potassium acetate and 2.85 ml of distilled water. The absorbance of the reaction mixture was measured at 415 nm after 30 min incubation at room temperature.. The calibration curve was prepared by using various concentrations of Quercetin.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three replicates.

RESULT AND DISCUSSION

Table 1: Phenolic contents and Ferric Reducing Antioxidant Power of methanolic extract of broccoli florets

	Mean ± SEM
Total phenolics[a]	10.55 ± 2.98
Flavonoids[b]	2.85 ± 0.32
FRAP[c]	262.67 ± 32.84

(n=3, X ± SEM)
[a]Expressed as mg gallic acid equivalent/g of dry weight
[b]Expressed as mg quercetin equivalent/g of dry weight
[c]Expressed as mM Fe(Ⅱ)/ g of dry weight

In living systems free radicals are constantly generated and they can cause extensive damage to living tissues leading to various disease conditions, especially degenerative diseases. Many synthetic antioxidants exert protective effects against oxidative damage but they have adverse side effects. An alternative solution to this problem is to consume natural antioxidants from food supplements.

In addition to the vitamins and minerals present in broccoli, phytochemicals such flavonoids and other phenolics may contribute to the protective effects. Polyphenols are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[12, 13]. The FCR actually measures a sample's reducing capacity [7]. The total content of phenolic compounds of methanolic extract was determined from regression equation of calibration curve ($y = 0.001x$, $R^2 = 0.997$) and expressed in Gallic Acid Equivalents (GAE). For flavonoid determination regression equation of calibration curve is ($y = 0.003x$, $R^2 = 0.988$) and were expressed as Quercetin Equivalent (QE). In our study the total phenolic and flavonoid compounds were found to be 10.55±2.98 mg GAE / g dry weight and 2.85±0.32 mg QE /g dry weight respectively.

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DPPH is one of a few stable and commercially available organic nitrogen radicals. A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. Upon reduction, the solution color fades and the reaction progress is conveniently monitored by a spectrophotometer. Thus, antioxidant molecules can quench DPPH free radicals either by electron donation or by providing hydrogen atoms resulting in a decrease in absorbance at 517 nm. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time-scale [14]. The main advantage of DPPH is that its reduction is easily measured spectrophotometrically and it gives reliable information on the ability of the tested compounds. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Figure 1 shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} values of our sample and L-ascorbic acid were 30 mg ml^{-1} and 0.45 mg ml^{-1} respectively. Although our results indicate that DPPH radical scavenging activity of broccoli extract is lower than L-ascorbic acid; it can be used as a free radical scavenger, acting possibly as an antioxidant.

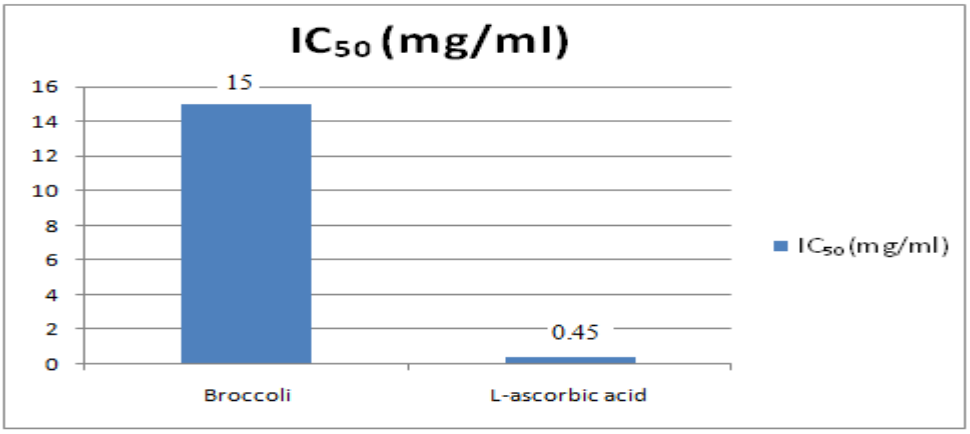

Fig 1: IC_{50} (mg ml^{-1}) values of plant extracts for free radical scavenging activity of DPPH radical. Lower value indicates higher antioxidant activity.

FRAP assay also takes advantage of electron-transfer reactions. Herein a ferric salt, $Fe(III)(TPTZ)_2Cl_3$ (TPTZ) 2,4,6-tripyridyls-triazine), is used as an oxidant. The redox potential of Fe(III) salt (0.70 V) is comparable to that of $ABTS^{-}$ (0.68 V). Therefore, essentially, there is not much difference between TEAC (Trolox equivalent antioxidant capacity assay) and the FRAP assay except TEAC is carried out at neutral pH and FRAP assay under acidic (pH 3.6) conditions. The oxidant in the FRAP assay is prepared by mixing TPTZ (2.5 mL, 10 mM in 40 mM HCl), 25 mL of acetate buffer, and 2.5 mL of $FeCl_3 \cdot H_2O$ (20 mM). The conglomerate is referred to as "FRAP reagent". The final solution has Fe(III) of 1.67 mM and TPTZ of 0.83 mM. In FRAP assay the reducing power of the extract was shown when TPTZ Fe^{3+} complex was transformed into TPTZ-Fe^{2+} complex and blue color was developed The intensity of color depends upon the antioxidant power. This study indicates that broccoli florets have significant antioxidant capacity.

In conclusion the broccoli extract is a significant source of natural antioxidants. Broccoli might be used as a dietary supplement to minimize the oxidative stress which would prevent the risk of developing degenerative diseases.

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