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Original Research Article

In-Vitro Estimation of Antioxidant Activity in Green Chilli (*Capsicum Annuum*) and Yellow Lantern Chilli (*Capsicum Chinense*)

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ABSTRACT

Chillies are observed as the main commercial vegetables and spice crops at the global level. The interest in its consumption is to a large extent due to its content of bioactive compounds and their importance as dietary antioxidants. Thus the present study was carried out with the aim to investigate antioxidant activity of two *capsicum* species namely *Capsicum annuum* and *Capsicum Chinense*. Three different extracts (Aqueous, ethyl acetate, and ethanol) were prepared from fruit powders of both species. All these extracts were tested for their total phenolic content, β-carotene content, vitamin-C content and antioxidant activity. The result indicated aqueous extract of Green chilli (44.66±0.11mg/100g) had higher concentration of vitamin-C than yellow lantern chilli (37.66±0.20mg/100g). β-carotene content in aqueous extract obtained from capsicum (0.2±0.005) had higher content than that of green one (0.12±0.115). The total phenolic content ranged from 1.45 mg/g to 5.0 mg/g of dry weight extract, expressed as gallic acid equivalents. Antioxidant activity of extracts was expressed as percentage of DPPH (Diphenylpicrylhydrazyl) radical's inhibition and IC_{50} values (mg/ml) which was ranged from 53.88 to 85.61per cent. Ethanolic extract of green chilli had highest antioxidant activity. This study generates base line information about capsicum which will be beneficial to treat various diseases. *Capsicum* has not received sufficient promotion for its medicinal use. Thus this study is important for the food processing and pharmaceutical industries.

Keywords: Antioxidant activity, *Capsicum annuum*, *Capsicum chinense*, phenolic content

1. INTRODUCTION

A treasure of information and scientific proof are swiftly accumulating the beneficial effects of extensive variety of food components on human health. [1] Health concerns are motivating plants breeder and food manufacturers to improve plants and processed products with functional ingredients comprised of nutritive and non- nutritive antioxidants. [2] Fruits and vegetables which are rich in antioxidants are known for their healthful effects against degenerative diseases. Chilli pepper is used as a vegetable and a spice. [3] *Capsicum annuum* is a vegetable known for its rich antioxidants content. [2] Red pepper is highly appreciated for its flavor, color as well as its content of antioxidant compounds. [4] Fresh peppers are an excellent source of vitamin- A and C. [5] Pepper contain moderate to high amount of neutral phenolic or flavonoids, phytochemicals that are valuable antioxidants components of a plant based diet other than traditional nutrients that may lower the risk of degenerative diseases. [6] Capsicum fruit is rich source of antioxidants and also contains high level of vitamin C and E as well as carotenoids & xanthophyll. [3,7] Carotenoids work as antioxidants and free- radical scavengers in our body and reducing the risk of cancers and having a

positive effect on the immune response. In addition, some carotenoids such as β-carotene and β-cryptoxanthin have pro-vitamin activity. [8] Many studies have demonstrated that peppers contain a wide array of phytochemicals. But many species of capsicum have not been evaluated for these vital compounds. Phytochemical changes that occur during maturation and consequence of the antioxidant activity are significant dietary considerations that may affect the consumption of different types of capsicum. Thus the study has been taken up with the objective to investigate the antioxidant activity of two capsicum species.

2. MATERIALS AND METHODS

2.1. Raw material and drying experiments

The fresh fruits of *Capsicum annuum* and *Capsicum chinense* were collected from the surroundings of Delhi, India, during the late rainy season in the month of June-October and identified at Indian Agriculture Research Institute (IARI), Delhi. Both fresh fruits of Capsicum were washed thoroughly with distilled water and dried. The method used for drying both fruits was different. Yellow lantern chilli was sun dried whereas green chilli was oven air dried. After that samples were finely ground to fine powder, which were then used for testing, extract preparation.

2.2. Preparation of extracts:

Three extract were prepared using distilled water, ethanol, and ethyl ether respectively. One gram of fine powder of fruits was extracted with 50 ml of distilled water/ethanol/ethyl acetate at room temperature using a magnetic stirring bar for 30 minutes. The extracts were filtered through a type HA 0.45 µm membrane filter (Millipore, Bedford, MA, USA), concentrated to dryness using a rotary evaporator under reduced pressure at 30ºC and stored at -20ºC until further analysis. As far as possible, all extraction procedures were performed under daylight protection.

2.3. Estimation of phenolic content

The concentration of phenolics in fruit extracts were determined using folin ciocalteu reagent. The extract (ethanol, ethyl acetate and aqueous) of concentration 1 mg/ml was used in the analysis and mixed with 80 per cent of ethanol. The extract was centrifuged (10000 rpm) for 20 minutes, and supernatant was collected. Evaporated the supernatant and dissolved the residue with distill water (5-10 ml). Pipette out aliquets (0.2 to 2 ml) and made up to 3 ml with water and added 0.5 folinciocalteu reagent. After 3 minutes added 2 ml of 20 per cent Na_2CO_3 and placed tubes in boiling water bath (1 minute), cooled them and measured the absorbance at 650 nm against a reagent blank. A standard curve was prepared by plotting the absorbance versus the concentration of gallic acid as shown in Figure 1. The standard curve equation Y= aX+b was used.

Where Y=absorbance

X= concentration of gallic acid(mg/ml)

Concentration of phenol in the test sample was expressed as GAE/100g material.

Absorbance was also recorded for test samples using the same procedure. The absorbance value obtained is the Y value. Concentration of galic acid (X) was calculated from the given equation. The amount of gallic acid(mg) in that extract calculated multiplying the X value by the volume of the plant extract (ml). The amount of gallic acid equivalent per gram plant extract was calculated by dividing the value by the amount of plant used for plant extraction (g).

2.4. Estimation of Ascorbic acid

Ascorbic acid content in capsicum extracts was determined by titration method with some modification. One ml of extracted sample mixed with 40 ml of metaphosphoric acid to stabilize vitamin-C content and made up to 100 ml by using 60 ml metaphosphoric acid. Twenty ml of extract was taken, titrated with dye solution (2,6 di-chlorophenol indophenols solution. Faint pink color resisted for at least 15 seconds, Vitamin-C was calculated on

standard solution of L-Ascorbic acid using given formula:

Y/X×20 mg/100ml

2.5. Determination of carotenoids

Total carotenoids were determined by the method of Jensen (1978). [9] One gram sample was extracted with 100 ml of 80 per cent methanol solution and centrifuged at 4000 rpm for 30 minutes. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10 per cent methanolic KOH the mixture was washed with 5 per cent ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for two hours. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

2.5. Estimation of antioxidant activity

The antioxidant action of the capsicum extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity. [10] This method was based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant that displays a strong absorption band at 517 nm and deep violet color appears. The remaining DPPH, measured after a definite time, relates inversely to the radical scavenging activity of the antioxidant. Ethanolic solution of DPPH (0.05 mM) (300 µl) was mixed to 40 µl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken briskly. The mixture was allowed to stand for 5 minutes and absorbance was measured using spectrophotometer at 517 nm. Ethanol was used to set the absorbance zero. A blank sample having the same amount of ethanol and DPPH was also prepared. All experiments were performed in triplicate. Antioxidant activity of tested samples expressed as percentage of inhibition of free radical activity. It was calculated using the following equation. [11]

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Where AA and AB are absorbance values of the test and the blank sample respectively

A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50 per cent inhibition was determined and represented as IC_{50}value for individual test solutions.

3. Statistical Analysis

The data was analyzed with the help of statistical techniques like mean, SD, and ANOVA was used to assess the significance difference between the groups.

4. RESULT AND DISCUSSION

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. They inhibit the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. Antioxidants are effective because they are willing to donates their own electrons to free radicals. When an antioxidant gives the electron to a free radicals then free radicals no longer requires to attack on cell and the chain reaction of oxidation is interrupted. [12] The phenolic compounds are well known to provide quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providing health. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage. [13]

The yield of solid residue obtained after extraction and evaporation of 10g of dry powder found different for each extract as shown in Table 1.

Table1: The yields of solid residue after extraction and evaporation from 10g dried plant parts

Yields(g)	Type of extract
1.98±0.082	Ethanol
1.54±0.094	aqueous
0.49±0.021	Ethyl acetate

4.1. Total phenolic content

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants. [14] The phenols contain hydroxyls that are accountable for

the radical scavenging effect mainly due to redox properties. [15] Thus it was reasonable to determine their total amount in the selected plant extracts. The values found for the concentration of total phenols are expressed as mg of Gallic acid/g of extract. Phenols are highly soluble in polar solvents. Thus high concentration of these compounds in the extracts obtained using polar solvents for the extraction. [16-17] It was found that there was found no significance difference between phenolic content of *Capsicum annum* and *capsicum chinense*. Table 2 shows that the total phenolic content in the evaluated extracts varies from 1.45 to 5.0 mg GAE/g dry weight basis. The content of total phenols in three different extracts (aqueous, ethyl acetate and ethanol) expressed in gallic acid equivalents (GAE) varied between 1.45±0.01 and 2.73±0.15, 1.53±0.03 and 4.74±0.015mg/g in capsicum and green chilli respectively. The highest concentration of phenol was measured in ethyl acetate extracts. Aqueous extract of capsicum and ethyl acetate extract of green chilli contains considerably smaller concentrations of phenols. It was reported in a study that phenolic content expressed as gallic acid equivalent to be 0.55.47 mg/g in aqueous extract of dry powder of *capsicum annuum.* [18] In another study, total phenolic content of capsicum species were in range of 2.02-3.28 g/100g of crude extract and 0.49-1.02g/100g of dry herb powder. [19] Different level of phenolic content reported in the studies could partially associate with method of extraction. [20]

4.2. β-carotene content

Carotenoid plays a positive role in epithelization process and stimulates the cell cycle progression of the fibroblasts. [21] Carotenoid performs as photo protective agents and may lower the risk of sunburn, photo-allergy and even some kinds of skin cancer. [22] Table 2 shows that no significant difference found between β-carotene content of both species. β-carotene content found in the capsicum chinense ranged from 0.20±0.005 to 0.77±0.020mg/100g whereas in the *capsicum annuum* it ranged from 0.12±0.115 to 1.3±0.005mg/100g. Results are in line with a study reported carotenoid content of green chilli varies form 0.81-3.82mg/100g. [23]

4.3. Vitamin-C content

Ascorbic acid performing as a chain breaking antioxidant hinders the generation of free radicals in the process of formation of intracellular substances within the body including collagen, bone matrix and tooth denture. [24-25] *Capsicum* is a vegetable with maximum ascorbic acid content. It has been found that consumption of 100g fresh weight of peppers provides 100-200%of the RDA of ascorbic acid (AA). [22]

Highest ascorbic acid was found to be 44.66±0.11 mg/100g in aqueous extract of Green chilli whereas in ethanol extract of capsicum was noted to have the least value of 32.11±0.03mg/100g. Values of ascorbic acid are reliable with other studies on *Capsicum* varies from 25 to 461per cent of the RDA (10mg-184.4mg) for Vitamin-C. [3,5,26-27] Ascorbic acid content of chilli varieties ranged from 38.59 to 107.52mg/100g on dry powder of fruit. [28] A variation was also observed in ascorbic acid content with 32.86 to 173.6mg/100g. [29] Ascorbic acid content was reported 88.25±2.46mg/100g dry weight of *capsicum annum.* [18] The antioxidant component of the *Capsicum* species can vary with genotypic difference, harvest, climate condition, cultural practices, maturity, harvesting method and post-harvest handling procedure. [30-34] The loss of ascorbic acid depend on several factors including the type of heavy metal (Cu and Fe), light, P^H level, water activity level, dissolved oxygen and drying temperature. [35]

4.4. Free radical scavenging activity and evaluation of IC_{50} value of extracts

Antioxidants, present naturally in many plants, foods and beverages offer health benefits in by combating cellular damage caused by free radicals in the body. [36] However, scientific facts on antioxidant properties of different plants, especially those that are less widely used in culinary

and medicine are still rather limited. Therefore, assessment of such properties remains a fascinating and valuable task, particularly for finding novel sources for natural antioxidants, functional foods and nutraceutical. [37]

Table 2: The total phenolic, β-carotene and Vitamin-C content examined in the fruit extracts (Capsicum and Green chilli)

Ethanol green chilli	Ethyl Acetate green chili	Aqueous green chilli	Ethanol capsicum	Ethyl Acetate Capsicum	Aqueous capsicum	Extracts Antioxidants
1.53±0.03^{ns}	1.66±0.015^{ns}	4.74±0.015	2.73±0.15^{ns}	5.0±0.05^{ns}	1.45±0.01	**Phenols (mg/g)**
0.53±0.005^{ns}	1.3±0.005^{ns}	0.12±0.115	0.77±0.020^{ns}	0.32±0.0115^{ns}	0.20±0.005	**β-carotene (mg/100g)**
40.82±0.02^{ns}	41.13±0.21^{ns}	44.66±0.11	32.11±0.03^{ns}	36.12±0.031^{ns}	37.66±0.20	**Vitamin C(mg/100g)**

ns= not significant

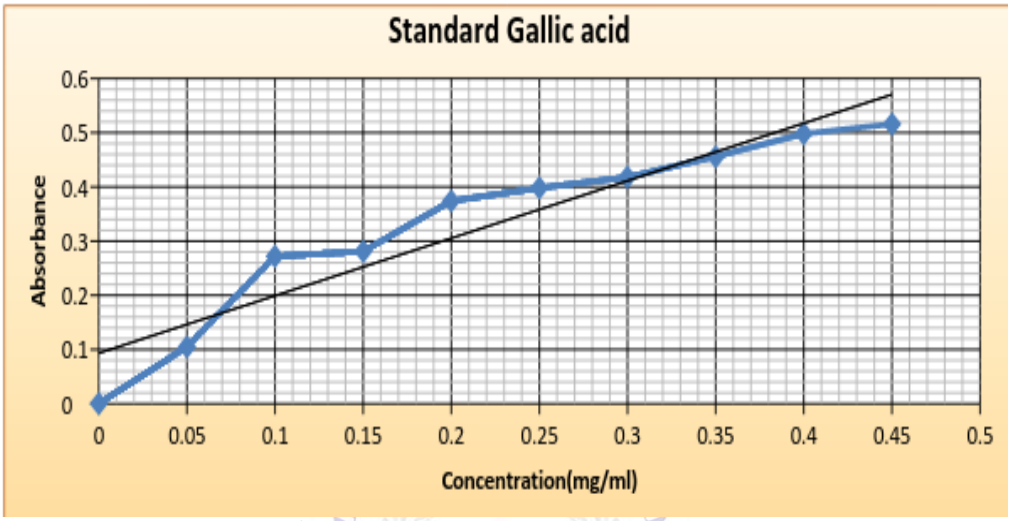

Fig. 1: Curve for standard gallic acid and phenolic content in test sample

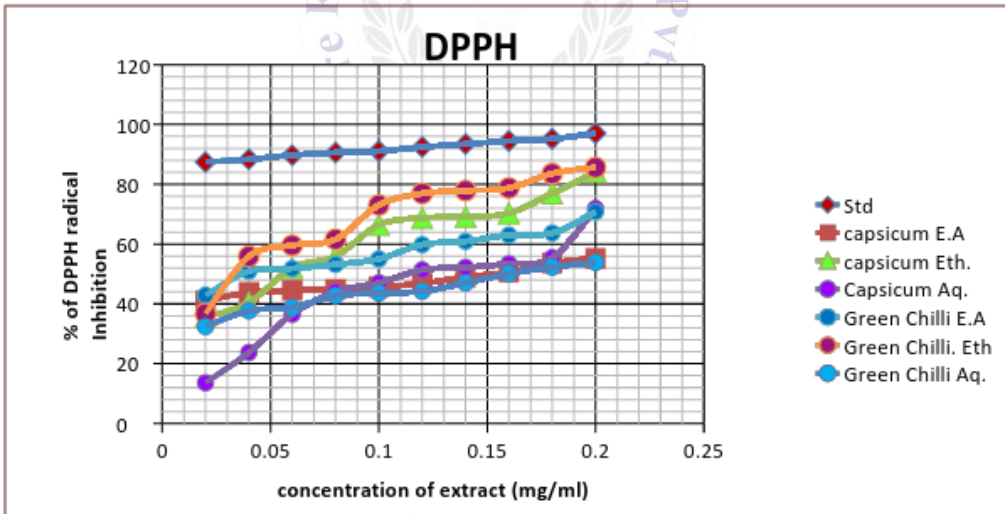

Figure 2: Free radicals inhibition activity of capsicum and green chili extracts and standard ascorbic acid

In this study, the antioxidant properties of samples were identified using DPPH radical scavenging assay. Radical scavenging is the main process by which antioxidants act in food. DPPH is one of the free radical commonly used for testing preliminary radical scavenging activity of a compound or a plant extract. Free radical (DPPH) scavenging activity of fruit extracts was expressed in terms of per cent inhibition of free radical activity against standard (ascorbic acid). Figure 2 shows that ethanolic extract of both species had strong antioxidant activity at concentration of 0.2mg/ml. Figure 2 depicts that IC_{50} of the ethanolic extracts of capsicum and green chilli which were (0.07 mg/ml) and (0.05mg/ml). It indicates remarkable

antioxidant activity of the extracts. Green chilli scavenged the highest amount of radicals (85.61%) followed by yellow lantern chilli (84.02%), while standard ascorbic acid scavenged 97.03 per cent radicals. This finding is in line with the findings of another study. [38]

In overall comparison between all the extracts it can be said that ethanolic extract showed highest free radical activity. High free radical scavenging activity of ethanolic extract of green chilli can be explain by the presence of high phenolic contents. [39] It was reported that among the antioxidants (Vitamin C, E, A, selenium and carotenoids) vitamin-C shows very strong intensity of antioxidant activities. [40] It is extensively acknowledged that the antioxidant activity of a plant extract is correlated to its carotenoid content and also shows that they contribute significantly to the total antioxidant capacity. [37,41-42]

5. CONCLUSIONS

Study concludes green chilli and yellow lantern chilli have significant antioxidant activity and free radical scavenging activity. Present study can be used to explain high free radical scavenging activity of capsicum due to its high phenolic content. It suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present research.

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