

Original Article

NUTRITIONAL PROFILE, MINERAL CONTENT AND *IN VITRO* ANTIOXIDANT POTENCY OF *CAPSICUM ANNUM L.* CULTIVATED IN 24 PARGANAS [SOUTH], WEST BENGAL, INDIA

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ABSTRACT

Objective: *Capsicum annum L.* is a well-known spice and has long been used in Chinese traditional medicine for the treatment of various ailments. So, the present study was aimed to analyse its nutritional profile and free radical scavenging activity.

Methods: The green *Capsicum annum L.* cultivated in 24 Parganas [South], West Bengal, were studied for the quantitative analysis of proximate composition, mineral and vitamin content. Aqueous and hydroethanolic extracts were screened on a comparative basis for the presence of *in vitro* antioxidant potential including the total phenol and flavonoid content, along with ferric reducing power, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide radical scavenging activity.

Results: The sample was found to contain 89.89% total carbohydrate, 13.48% soluble carbohydrate, 7.95% crude protein, 4.80% fat, 12.9% fibre, 95.42% moisture and 5.12% ash. Besides, the vegetable was also found to be abundant in vitamins and minerals like, sodium (24.4), iron (4.834), zinc (1.632), magnesium (92.612), manganese (0.88), calcium (19.562), copper (1.11), cobalt (0.02), chromium (0.016), boron (0.316), lycopene (2.4) and β -carotene (14.85) expressed in mg/100 g; and potassium (2.236), vitamin C (1.77), vitamin E (6.08), all expressed in g/100 g dry weight of the sample. The free radical scavenging activity, total phenol and flavonoid content of the aqueous extract were significantly higher than the hydroethanolic extract ($p < 0.05$).

Conclusion: It can thus be concluded that capsicum is a nutritionally rich spice and a potential source for antioxidant. The aqueous extract is more potent than the hydro-ethanolic extract in scavenging free radical.

Keywords: *Capsicum annum L.*, Phytoconstituents, Proximate Composition, Free Radical Scavenging Activity, Antioxidant Potential, Oxidative Stress

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INTRODUCTION

A variety of strategies are being applied by the developing countries to continue the fight against malnutrition. In many tropical countries, rural people traditionally harvest a wide range of vegetables and fruits because of its taste, cultural uses, as food supplements or to tide over food shortage [1]. In order to meet the ever increasing food demand, cultivable and wild vegetables are often used as cheap food source for the marginal communities. To apprehend the situation, interests have been centralized on the exploration and quantification of nutrient content of commonly consumed vegetables.

The quality of food depends upon the presence of a relative concentration of various nutrients such as protein, fat, carbohydrate, vitamins and minerals. Vegetables are a rich source of carbohydrate, fat and protein, which forms a major portion of the human diet. Besides, the moisture, fibre, and ash content of vegetables and spices have also been regarded important for human health. Trace elements and heavy metals have certain risks [2], and thus it is important to determine the level of these compounds in widely consumed vegetables. In addition to vitamins, the presence of phytochemicals is also considered to be of crucial nutritional importance in the prevention of chronic diseases [3]. Hence knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources of economical materials. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies [4].

Epidemiological and *in vitro* studies on plants, vegetables and spices strongly support the idea that several plant constituents are capable of exerting protective effects against oxidative stress in biological

systems [5, 6]. Plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as singlet oxygen, superoxide, peroxy radicals, hydroxyl radicals and peroxynitrite which results in oxidative stress leading to cellular damage [7]. Considering the importance of phytoconstituents, in the present study an attempt has been made to analyse the proximate composition and micronutrient content in a green variety of capsicum (*Capsicum annum L.*), and also compare the free radical scavenging activity of the aqueous and hydro-ethanolic extract of green capsicum. In addition to its use as a spice, capsicum has long been used as traditional medicine for the treatment of a sore throat, cough, toothache, rheumatism, etc. [8]. Thus, evaluation of the nutritional profile and *in vitro* antioxidant potential of green capsicum might make it an important component of our daily diet.

MATERIALS AND METHODS

Collection and authentication of plant material

The green variety of *Capsicum annum L.*, cultivated in Ghatakpur, district 24 Parganas (South), West Bengal, and it was authenticated by Botanical Survey of India, Howrah, West Bengal (Specimen No: RMC/PHY/MD/01).

Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), α - α' dipyridyl and dinitro salicylic acid (DNS) were obtained from Sigma, Aldrich. Gallic acid, quercetin, rutin, butylated hydroxytoluene (BHT), 2-deoxy-2-ribose, potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride (FeCl_3), ethylenediaminetetraacetic acid (EDTA), hydrogen peroxide (H_2O_2), ascorbic acid, α -tocopherol, 2-thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), riboflavin were procured from Hi-Media, Mumbai, India. Bromine, Folin ciocalteu, acetone, chloroform,

copper sulphate (CuSO₄), dinitrophenylhydrazine (DNPH), hexane, metaphosphoric acid, xylene, sodium-potassium tartrate, potassium chloride (KCl), sulphuric acid (H₂SO₄), hydrochloric acid (HCl), ethanol, methanol, aluminium chloride (AlCl₃), sodium carbonate (Na₂CO₃), sodium nitrite (NaNO₂), sodium hydroxide (NaOH), disodium hydrogen phosphate (Na₂HPO₄), sodium dihydrogen phosphate (NaH₂PO₄) were procured from Merck India Ltd, Mumbai. All chemicals were of analytical grade.

Determination of nutrient and micronutrient content

Fresh tissue analysis

1g of fresh tissue was smashed and homogenised with 10 ml of 1(M) phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 × g for 30 min at 4°C and the supernatant was used for further analysis.

Proximate composition

The parameters determined for proximate analyses include ash, moisture, crude protein, fat, fibre and carbohydrate. The proximate values were reported in percentage dry weight. Samples of the fresh tissue (5 grams, each) in triplicate were used for determination of moisture content by weighing in a crucible and drying in a hot air oven at 70 °C, until a constant weight was obtained. Determination of ash content was done by ashing at 650 °C in muffle furnace for about 5hours. Crude protein content was estimated using Lowry's method [9] and total and soluble carbohydrate was determined using dinitro-salicylic acid (DNS) method [10]. The crude fibre content of the samples was determined by acid-base digestion according to the method of Maynard [11]. Fat content was estimated according to the method of Itoch and Koneko [12]. The calorific value in, Kcalories per 100 g and the nutritive value in, calories per 100 g were estimated according to Eq. (1) [13] and Eq. (2) [14] respectively.

$$\text{Calorific value} = 16.7x \% \text{ Carbohydrates} + 16.7x \% \text{ Proteins} + 37.7x \% \text{ Fats} \dots\dots\dots (1)$$

$$\text{Nutritive value} = 4x \% \text{ Carbohydrates} + 4x \% \text{ Proteins} + 9x \% \text{ Fats} \dots\dots\dots (2)$$

Mineral content

Mineral content of green capsicum was determined by analysing aliquots of ash solution in 0.1(N) hydrochloric acid (HCl) after proper dilution, using inductively coupled plasma atomic emission spectrophotometer (ICP-AES) (M/s. Ametek Spectro Analytical Instruments GmbH, Germany) along with smart analyser software. A multi-elemental standard solution of 1000 mg/l containing all analysed elements, supplied by National Institute of Standards and Technology (NIST) was used for calibration.

Vitamin content

Determination of lycopene and β-carotene content

β-Carotene and lycopene content were determined according to the method of Nagata and Yamashita (1992) [15]. Contents of β-carotene and lycopene were calculated according to Eq. (3) and Eq. (4). The results were expressed in terms of mg/100 g dry weight of the tissue.

$$\text{Lycopene (mg/100 ml)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \dots\dots\dots (3)$$

$$\beta\text{-carotene (mg/100 ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \dots\dots\dots (4)$$

Determination of ascorbic acid content

Ascorbic acid content was determined by the method of Riemschneider, 1976 [16] using 2, 4-dinitrophenyl hydrazine. Vitamin C concentration was expressed in terms of g/100g dry weight of the tissue from the standard curve with different concentrations of ascorbic acid (10-60 µg/ml).

Determination of α-tocopherol

Vitamin E content was determined using the method of Baker *et al.* [17]. Vitamin E concentration was expressed in terms of g/100g dry

weight of the tissue from the standard curve with different concentrations of α-tocopherol (20-100 µg/ml).

Processing of plant material

The vegetable spice was cut into cubicles after washing with water. It was then shade dried and stored in airtight containers for further analysis.

Preparation of aqueous and hydroethanolic extracts

Shade dried cubicles of green capsicum were extracted using distilled water and 70% hydro-ethanol as solvents at (60-80 °C) with the help of Soxhlet apparatus. 15 gm of the sample was taken in the thimble of the Soxhlet and extracted in 200 ml of the respective solvents, continuously for 72 h. The mixture was then concentrated to dryness by using hot plate and rotary evaporator, respectively. The dried sample was then collected and stored in air tight plastic vials.

Total phenol content (TPC)

The total phenol of all extracts was measured spectrophotometrically using folin-ciocalteu reagent [18]. The dilute aqueous and ethanolic extracts (0.5 ml of 1 mg /ml) and gallic acid (standard) was mixed with folin-ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous sodium carbonate, and the absorbance was measured at 765 nm.

Total flavonoid content (TFC)

Total flavonoid content was measured by aluminium chloride colorimetric assay [19]. Aqueous and ethanolic extracts (1.0 ml of 1 mg /ml) and different dilution of a standard solution of rutin (10-100µg/ml) were added to 10 ml volumetric flask containing 4 ml of water. To the above mixture, 0.3 ml of 5% NaNO₂, 0.3 ml of 10% AlCl₃ and 2 ml of NaOH was added, and the total volume was made up to 10 ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm.

DPPH radical scavenging activity

0.1 mmol solution of DPPH in ethanol (22.2 mg in 1000 ml) was freshly prepared. 1 ml of different concentrations of aqueous (50-500 µg/ml) and ethanolic extracts (100-1000 µg/ml) of green capsicum was added to 2 ml of an ethanolic solution of DPPH. Ascorbic acid (10-100 µg/ml) was used as a standard. The absorbance was recorded at 517 nm [20, 21]. An IC₅₀ value was calculated as the concentration which brought about a 50% reduction in absorbance compared to blank.

Hydroxyl radical scavenging activity

Scavenging of hydroxyl free radical was measured by the method of Halliwell and Chirico [22], with slight modification. 200 µl of 2-deoxy-2-ribose, 1 ml of various concentrations of aqueous and ethanolic extracts (200-1000 µg/ml), 400 µl of 200 µM FeCl₃, 1.04 mmol EDTA (1:1 V/V), 200 µl of H₂O₂ and 200 µl ascorbic acid (1.0 mmol) was mixed to form a reaction mixture. 1.5 ml of 2.8% TCA and 1 ml of 0.336% TBA was added and boiled for 20 min on boiling water bath. After cooling the absorbance was read at 532 nm against a blank. An IC₅₀ value was calculated, and quercetin was used as a standard.

Superoxide radical scavenging activity

The reaction medium contained 2.5 ml of phosphate buffer (pH 7.6), 100 µL riboflavin (20 µg), 200 µL EDTA (12 mmol), 100µL NBT (0.1 mg) and 1 ml of various concentrations of aqueous (100-500 µg/ml) and ethanolic extracts (200-1000 µg/ml). The reaction was started by illuminating the reaction mixture for 5 min. The absorbance was measured at 590 nm. Blank was performed in the same way with 1 ml of methanol instead of test substance [23]. An IC₅₀ value was calculated using Ascorbic acid as standard.

The percentage inhibition activity for the above mentioned free radicals was calculated using Eq. (5):

$$\text{Inhibition (\%)} = 1 - (\text{sample OD} / \text{blank OD}) \times 100 \dots\dots (5)$$

Ferric reducing antioxidant power (FRAP) assay

Various concentrations of aqueous and ethanolic extracts (100-1000 µg/ml) and standard solutions of BHT (100-500 µg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were mixed separately and incubated at 50 °C for 30 min. After incubation, 2.5 ml of 10% TCA was added to each tube and centrifuged. About 2.5 ml of the supernatant was diluted with 2.5 ml water and shaken with 0.5 ml of freshly prepared 0.1% FeCl₃. The absorbance was measured at 700 nm [24].

All tests were performed in triplicate, and the graph was plotted with the average of the three determinations.

Statistical analysis

All results were presented as mean±standard error (S. E.) of at least three individual experimental data, each in triplicate. Student's t-test was carried out to determine the level of significance. All statistical analyses were conducted using SPSS version 20.0.

RESULTS

Proximate analysis

The mean values of the proximate composition are represented in fig. 1. The Calorific value is calculated to be 1822.89±3.18 Kcal/100 g and the Nutritive value is found to be 445.27±1.49 Cal/100 gm.

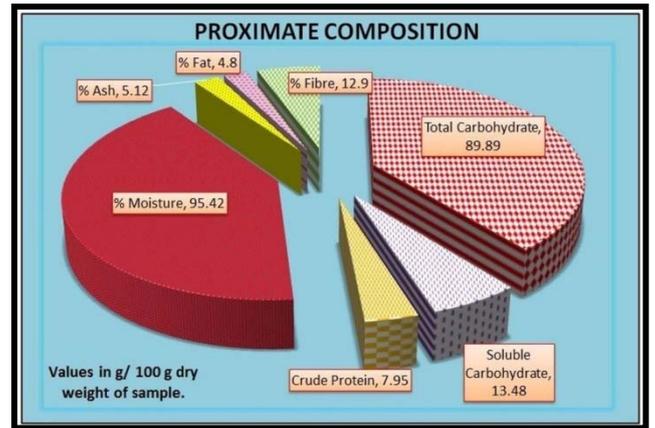


Fig. 1: Proximate Composition of *Capsicum annum*. Values are mean of three three sets of data (n=3); each in triplicate

Vitamin and mineral content

Fig. 2 represents the amount of non-enzymatic antioxidants which include lycopene and β-carotene expressed in mg/100 g dry weight of the sample, and ascorbic acid and α-tocopherol expressed in g/100 g dry weight of the sample, along with standard curves for ascorbic acid and α-tocopherol.

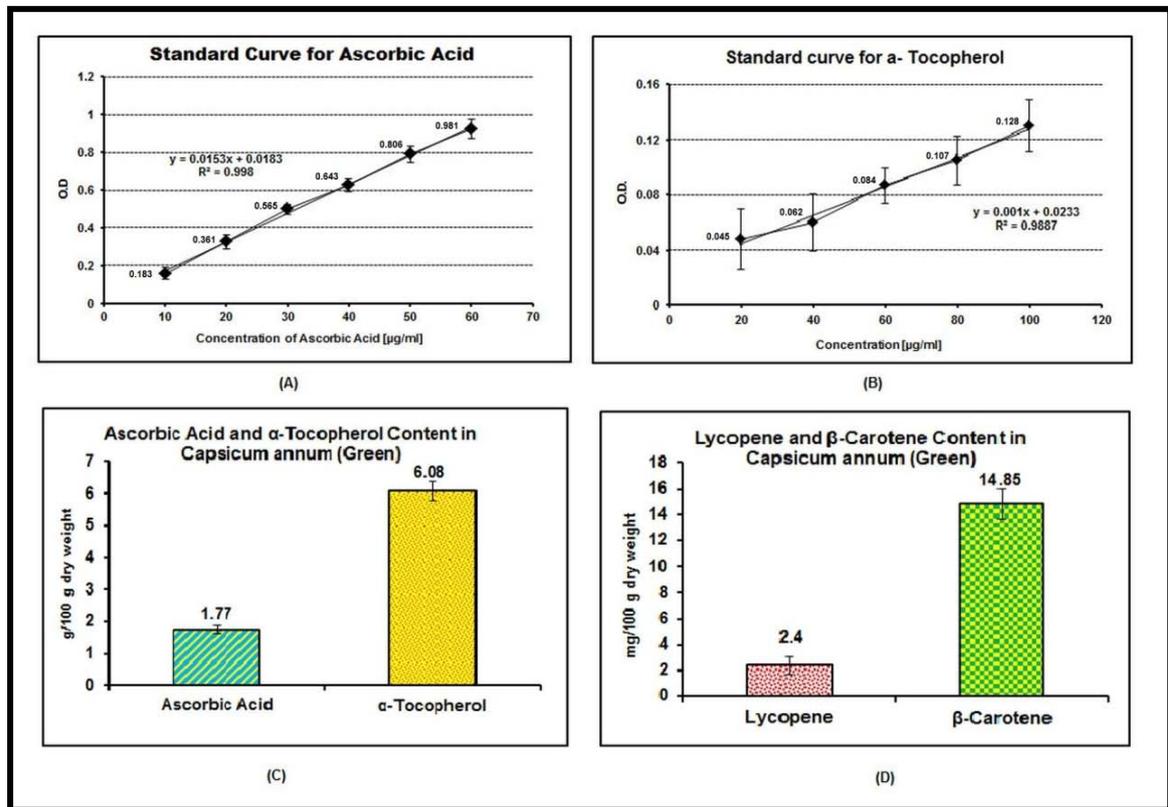


Fig. 2: Vitamin content of green *Capsicum annum*. (A) and (B): Standard curve for Ascorbic acid and α-tocopherol respectively. (C): Ascorbic acid and α-tocopherol content. (D): Lycopene and β-Carotene content. All data are expressed as MEAN±S. E., of triplicate set of values (n=3)

Table 1 represents the essential mineral content of green capsicum along with the dietary reference intakes (DRI) values based on older recommended dietary allowance (RDA) [25]. All

values are expressed in mg/100 g dry weight of the sample, except potassium which is expressed in g/100 g dry weight of the sample.

Table 1: Mineral content of green *Capsicum annum*,

Name of the mineral	Content [mg/100 gm dry weight]	DRI [mg]
Sodium	24.4±2.1	2400
Potassium	2.236±0.27 ^a	4700
Calcium	19.56±0.18	1300
Copper	1.11±0.03	0.9
Iron	4.834±0.05	18
Magnesium	92.612±4.57	420
Manganese	0.88±0.02	2.3
Zinc	1.632±0.19	11
Chromium	0.016±0.06	0.035
Cobalt	0.02±0.012	0.008
Boron	0.316±0.09	20

All data are expressed as MEAN±SE, of triplicate set of values. ag/100 g dry weight of the sample. (n=3)

Total phenol and flavonoid content

The TPC of both the extracts is estimated as mg gallic acid equivalent/g dry weight of the sample. The TPC of the aqueous extract is found to be significantly higher ($p<0.01$) than the hydro-ethanolic extract.

The TFC of the extracts is expressed as mg of rutin equivalent/g dry weight of the sample. The TFC of the aqueous extract is found to be significantly higher ($p<0.05$) than the hydro-ethanolic extract. Fig. 3 below represents the TPC and TFC of green *C. annum* along with standard curves of gallic acid and rutin respectively.

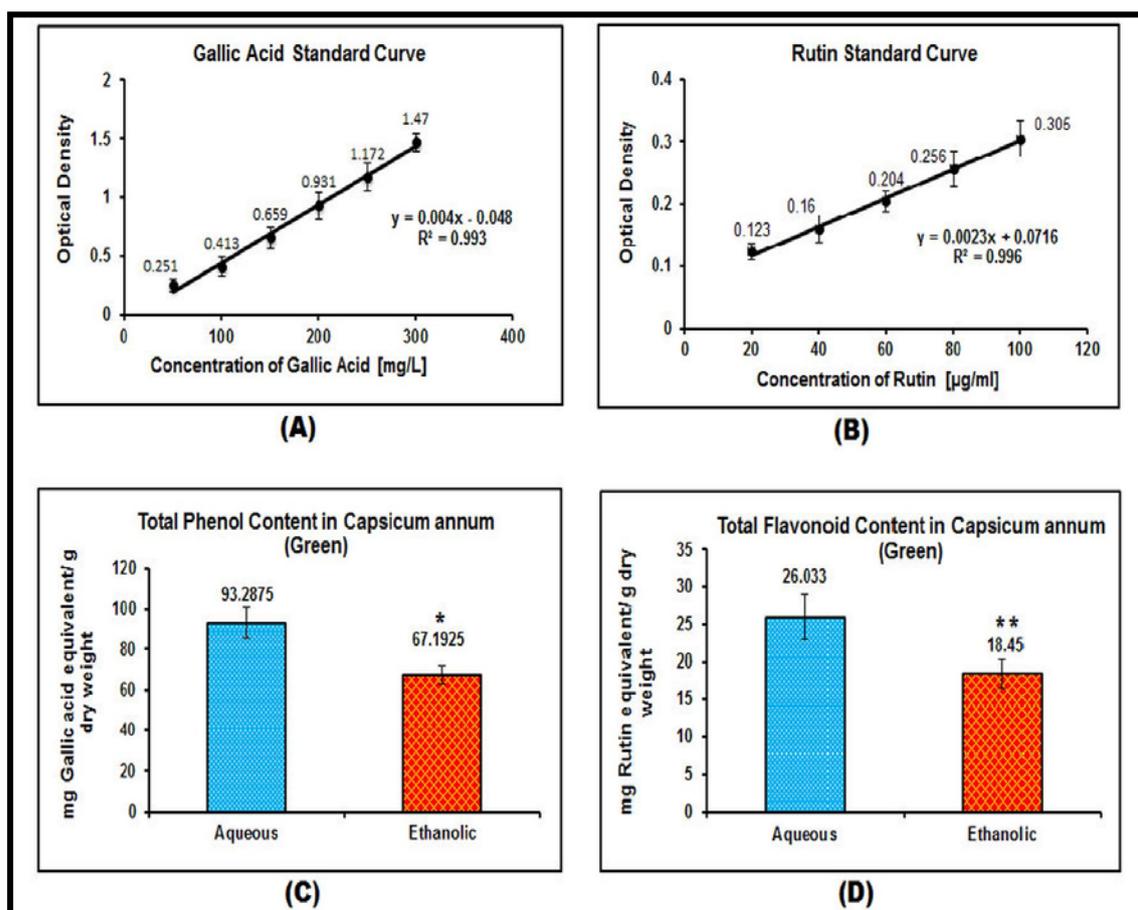


Fig. 3: Total phenol and flavonoid content of green *Capsicum annum*. (A) and (B): standard curve for Gallic acid and rutin respectively. (C) and (D): The total phenol and flavonoid content of green capsicum respectively; * ($p<0.01$), ** ($p<0.05$). All data are expressed as mean±SE, of triplicate set of values (n=3)

DPPH radical scavenging activity

The colour of DPPH was quenched in a concentration-dependent manner by vitamin C and by the aqueous and hydroethanolic extracts of green *C. annum* as well. The % inhibition and IC_{50} values of both the extracts along with its respective standard depicted in fig. 4 (A, B and E) below shows that aqueous extract of a green

variety of *C. annum* possesses significantly higher ($p<0.01$) DPPH radical scavenging activity than the hydro-ethanolic extract.

Hydroxyl radical scavenging activity

Both the aqueous and hydroethanolic extracts of green *C. annum* have the potential to scavenge hydroxyl radical produced *in vitro* by fenton reaction. A significantly lower ($p<0.01$) IC_{50} value of the

aqueous extract thus indicates that the aqueous extract is more potential than the hydro-ethanolic extract. The % inhibition of the aqueous extract is also significantly higher ($p < 0.01$) than the hydro-ethanolic extract. (fig. 4D and 4E)

Superoxide radical scavenging activity

Fig. 4C depicts that the superoxide radical scavenging activity of the aqueous extract is significantly higher than the hydro-ethanolic extract. As relevant from fig. 4E a significantly lower IC_{50} value

($p < 0.01$) of the aqueous extract, makes it more potential in quenching superoxide radical compared to the hydro-ethanolic extract.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is based on its ability of the extracts to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) state. Fig. 4(F) shows an increase in reducing power of both the extracts in a dose-dependent manner. However, the aqueous extract is found to be more potent compared to the hydro-ethanolic extract.

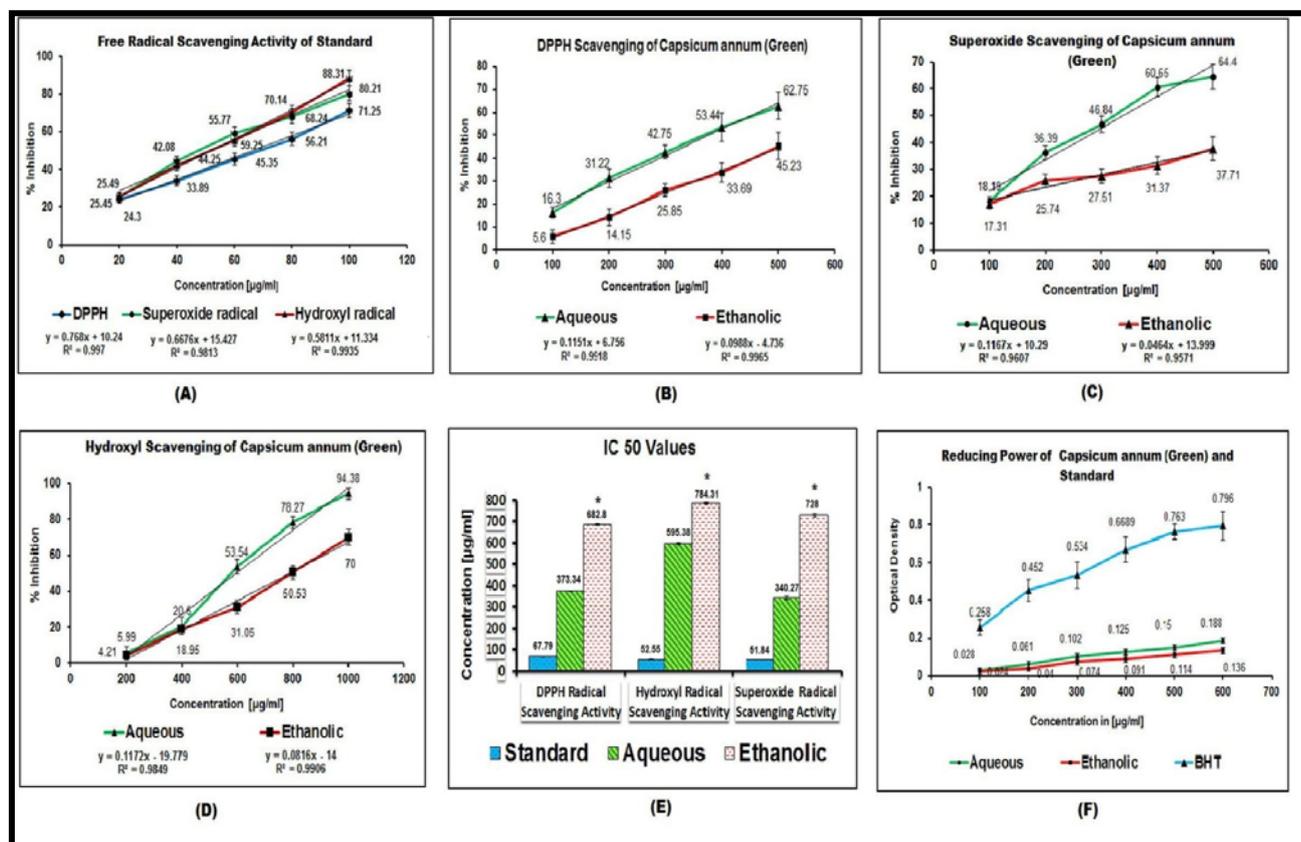


Fig. 4: Free radical scavenging activity of standard and sample in a dose-dependent manner (A): the Free radical scavenging activity of the standards for DPPH, Hydroxyl and Superoxide radicals, and that of the extracts (B), (C), (D). (E): IC_{50} values of extracts and standards. (F): Ferric reducing the power of the extracts and standard. All data are expressed as $MEAN \pm S. E.$, of triplicate set of values ($n=3$). * ($p < 0.01$)

DISCUSSION

The presence of important nutrients like carbohydrate, protein and fat makes the green variety of *C. annum* a nutritionally valuable and healthy ingredient to promote health. Compared to some conventional sources of carbohydrate, such as cereals, *C. annum* can be considered as a potential source of carbohydrate. The percentage ash represents the inorganic content of the vegetable. Moreover, low fat and high dietary fibre content of green capsicum makes it a promising food recommended as part of the weight-reducing diet for obese people. High food fibre aids absorption of trace elements in the gut [26] and reduces absorption of cholesterol [27]. Thus, fibre reduces the risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [28, 29].

In living systems, free radicals are constantly generated, and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases, and extensive lysis. Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [30]. Antioxidant vitamins like vitamin C and vitamin E act synergistically against oxidative stress-related diseases. Vitamin E functions as a chain-breaking

antioxidant which prevents the propagation of free radical reactions whereas; vitamin C is a part of the normal immune mechanism in humans. Lycopene and β -carotene add on to the antioxidant potential of the vegetable.

Moreover, green *C. annum* is found to be abundant in potassium content. The high amount of potassium is reported to increase iron utilisation [31] and is also beneficial for people suffering from hypertension [32]. In comparison to the potassium content, the sodium content of green capsicum is low. Thus, a lower sodium/potassium ratio makes green capsicum a recommended nutrient to reduce the risk of elevated blood pressure. Calcium is essential not only for children but also for pregnant, lactating and menopausal women. The presence of calcium in green capsicum might be useful in preventing diseases such as osteoporosis. Besides, minerals are also required for normal growth, skeletal development and contraction of muscles (such as calcium), cellular activity and oxygen transport (copper and iron), chemical reaction in the body and intestinal absorption (magnesium and zinc), fluid balance and nerve transmission (sodium and potassium), as well as for the regulation of acid-base balance (phosphorus). Iron is useful in the prevention of anaemia and other related diseases [33]. Manganese acts as a cofactor in some enzymes and also plays a role in energy production and in supporting the immune system. Zinc is useful for

protein and nucleotide synthesis, normal body development and recovery from illness [34].

Cobalt plays a role in the metabolism of vitamin B-12 and increases its absorption; it also functions as an activating ion in some enzymes [35]. Chromium content of the vegetable is also within the safety limit. Boron assists and improves retention of minerals like calcium, magnesium, and phosphorus; necessary for brain function, memory and alertness, as well as for the activation of vitamin D [36]. The presence of the above-mentioned macro and micro nutrients in green *C. annuum* might be essential in preventing diseases related to malnutrition.

Heavy metal content (arsenic, lead, cadmium and mercury) of the vegetable is also analysed, and interestingly no trace of heavy metal was found. The absence of heavy metals like mercury, lead, cadmium and arsenic in the collected specimens of green capsicum reveals the quality of soil and water used for irrigation. This is in accordance with a recently published data which states the absence of heavy metals in fruits and vegetables collected from Ghatakpur region of 24 Parganas (South), West Bengal [37].

It is said that secondary metabolites such as phenols and flavonoids contribute to the antioxidant potential of the plant material [38]. The aqueous and hydroethanolic extracts of green capsicum are considered as good sources of antioxidants as shown by their total phenolic and flavonoid contents.

Previously it has been reported that the antioxidant activity of green capsicum is more than the red and yellow variety [39]. Interestingly, in the present study, it is observed that using water as a solvent for extraction was better compared to the hydro-ethanolic solvent. The free radical scavenging activity of both the extracts also confirms that the aqueous extract is more potent in quenching free radicals than the hydro-ethanolic extract. Reducing power, a significant indicator of the antioxidant property also suggests that the aqueous extract has a greater ferric to ferrous reducing power.

Although it has been stated that water extraction of plant organs leaves a large amount of residual polyphenols that can be extracted only by an appropriate combination of solvents [40]; literature also suggests that for some herbs and spices like sage, water as a solvent for extraction is more effective [41]. Thus, it can be concluded that polyphenols are either polar or non-polar. Moreover, the temperature for polyphenol extraction, as well as the incubation time, also contributes to the efficacy of the antioxidant activity [42-44]. However, contradicting the above results, few studies also suggested that the total amount of phenolic acids and flavonoids decreased after heat treatment [45, 46]. This indicated that some phenolic acids probably are heat labile, whereas others are converted from insoluble phenolic compounds to soluble phenolics after heat treatment. Thus, in the present study, the higher antioxidant potential of the aqueous extract might be attributed to the higher boiling point of water than 70% hydro-ethanolic solvent.

CONCLUSION

From the present study, it can be concluded that green *Capsicum annuum L.* contains an appreciable amount of carbohydrate, protein, vitamins and minerals, which if properly utilised could assist in combating the problem of malnutrition. Moreover, both the extracts of *Capsicum annuum L.* also possess high antioxidant potential and can thus serve as a good source of natural antioxidant; the aqueous extract being more potent than the hydro-ethanolic extract. Thus *Capsicum annuum L.* can become an important nutraceutical spice and can be used in the formulation of herbal drugs in the years to come. Further research on isolation of bioactive components along with its pharmacological activities is necessary for a novel drug development.

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CONFLICT OF INTERESTS

Declared none

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