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Comparative study on antioxidant and anti inflammatory properties of three colored varieties of *Capsicum annuum*

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Background &Aim: The current study reviews the correlation between the three Indian, coloured capsicum species, the green, yellow and red varieties (colour depends on time of harvest and degree of ripening) with respect to their antioxidant/anti inflammatory properties. **Methods:**This was achieved by screening of aqueous plant extracts for antioxidant properties like total phenolic content, reducing power assay and 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The anti-inflammatory activity is assessed by inhibiting Soyal ipoxygenase enzyme (LOX). **Results:** the green capsicum extract showed greater phenolic content (3.2985±0.1004), reducing power (0.243 nm), DPPH scavenging effect (92.26%) and LOX % inhibition (46.12 %)compared to yellow and red extracts.**Conclusion:**Result thus suggests that green capsicum is a potential source of useful natural antioxidants and anti-inflammatory agent as well when compared with the other varieties.

Key words: antioxidant, anti inflammation, LOX, pigments, capsicum annuum.

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1. Introduction

Phytochemicals are secondary metabolites of plants which are non-nutritive chemicals that have protective or disease preventive properties. These metabolites are said to exhibit structural similarities to that of the intermediary molecules of animal metabolism. Hence these molecules can interact in a similar mode and may be responsible for the desirable pharmacological properties such as antioxidant and anti-inflammatory effects[1]. Phytochemicals have been considered to be of crucial nutritional importance in the prevention of chronic diseases[2].The major phytochemicals from fruits, vegetables and medicinal plants which are in extensive study for pharmacological actions include polyphenols (derived from carbohydrates), terpenes (a group of lipids), flavonoidsand alkaloids (derived from amino acids)[3]. Antioxidants exert their therapeutic role by scavenging free radicals[4, 5] and thereby could also inhibit the process of lipid peroxidation, thus inhibiting the LOX pathway. Hence, finding a single molecule having both antioxidant and anti-inflammatory activities would be of great therapeutic importance.

These phytonutrients that are good for health also produce bright colours in vegetables and fruits and act as pigments also. Thus colours of fruits and vegetables can help guide the dietary levels of antioxidants as well as anti-inflammatory agents, which is extremely important for good health balance[6].Red fruits and vegetablespredominantly contain phytonutrients such as lycopene, anthocyanins which arealso responsible for their colour. Orange and Yellow fruits and vegetables contain antioxidants like beta-carotene, zeaxanthin, vitamin C etc. Green vegetables contain the pigment chlorophyll and other phytonutrients like lutein, zeaxanthin, calcium, folate, calcium, which are potent antioxidants as well as anti-inflammatory compounds.

Capsicum annuum (Family *Solanaceae)* is a native to Americas but now cultivated worldwide. As in other chili varieties, capsicums too have several cultivar types. These bell peppers range from green, red, yellow and even purple. Green peppers ripen to yellow and red if left on the vine. Most of the differences in colour stem from time of harvest and degree of ripening. In addition to use as spices and food vegetables, capsicum has also found use in medicines. Studies have been reported on the capsicum sps related to antioxidant properties (phenolic compounds), [7, 8,and 9] but not clear with respect to different coloured fresh fruits of Capsicum. As Capsicum is easily cultivated, economically feasible in the Indian market and a prominent ingredient of the Indian cuisine, particularly in the growing fast-food industries, the fresh

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fruit in different colors have been chosen for this study. Green, yellow and red varieties of capsicum are taken on dry weight basis in the present study to compare their antioxidant as well as anti inflammatory activity in vitro. Our results suggest that green capsicum has the most significant antioxidant and anti- inflammatory activity when compared to yellow and red varieties.

2. Materials and methods

2.1 Extraction of vegetables

The three different coloured fresh *Capsicum annum* species (red, green and yellow) were purchased from a local market. 20 gm of each variety were weighed and aqueous extraction was carried out by homogenization, centrifugation followed by lyophilization.

The yield was calculated and expressed as % of w/w. All the experiments were performed in triplicates.Total phenolics, reducing power, DPPH assay and LOX enzyme inhibition were estimated fromaqueous extracts of red, yellow and green varieties of Capsicum at 100µg concentration.

2.2Invitroantioxidant activity of the extracts

2.2.1 Total phenolic estimation

The concentrations of total phenolics in the aqueous extracts of all the varieties were determined spectrophotometrically at 725 nm by the Folin-Ciocalteauassay as per the method of Harbertson and Spayd[10].

2.2.2 Reducing power assay

The reducing powers of all the extracts were determined according to the method of Oyaizu[11].

2.2.3 DPPH scavenging activity

Determination of antioxidant activity by the DPPH method[12] was done for all the aqueous extracts at 100 µg concentrations. The absorbance or radical scavenging activity was expressed using formula:

% of radical scavenging activity = [(Control OD-sample OD)/ (Control OD)] X 100

2.3 Anti-inflammatory effect

2.3.1 Extraction and purification of LOX enzyme from soya bean

Soy LOX-1 was purified from defatted soya flour according to the method of Axelrod[13].

2.3.2 Precipitation

To the above clarified solution, ammonium sulfate precipitation and dialyzed.

2.3.3 Protein estimation

The concentration of the protein in the dialyzed sample was calculated using the formula as per the method of Layne, E[14]and UV spectrophotometrically.

Protein concentration (mg/ml) = (1.55 x A280 - 0.76 x A260)

2.3.4 Lipoxygenase assay

The bioassay was performed according to the procedure of Axelrod[13].

2.3.5 Inhibition of lipoxygenase activity

The inhibitory assay contains 1mg of the sample extract along with other ingredients as mentioned in the procedure according to Axelrod[13]. The % inhibitions for different samples were determined[15]using the formula,

% inhibition = Control O.D-Test O.D/Control O.D x 100.

2.4 Statistical Analysis

Results were calculated as the mean ± SD (standarddeviation) for each sample. Statistical analysis was done with oneway analysis of variance using SPSS 17. Asignificant difference was judged to exist at a level of $p< 0.01$ (Fig-I, II).

3. Results and discussion

3.1 Percentage yield

The percentage yield (w/w) of aqueous extract of the

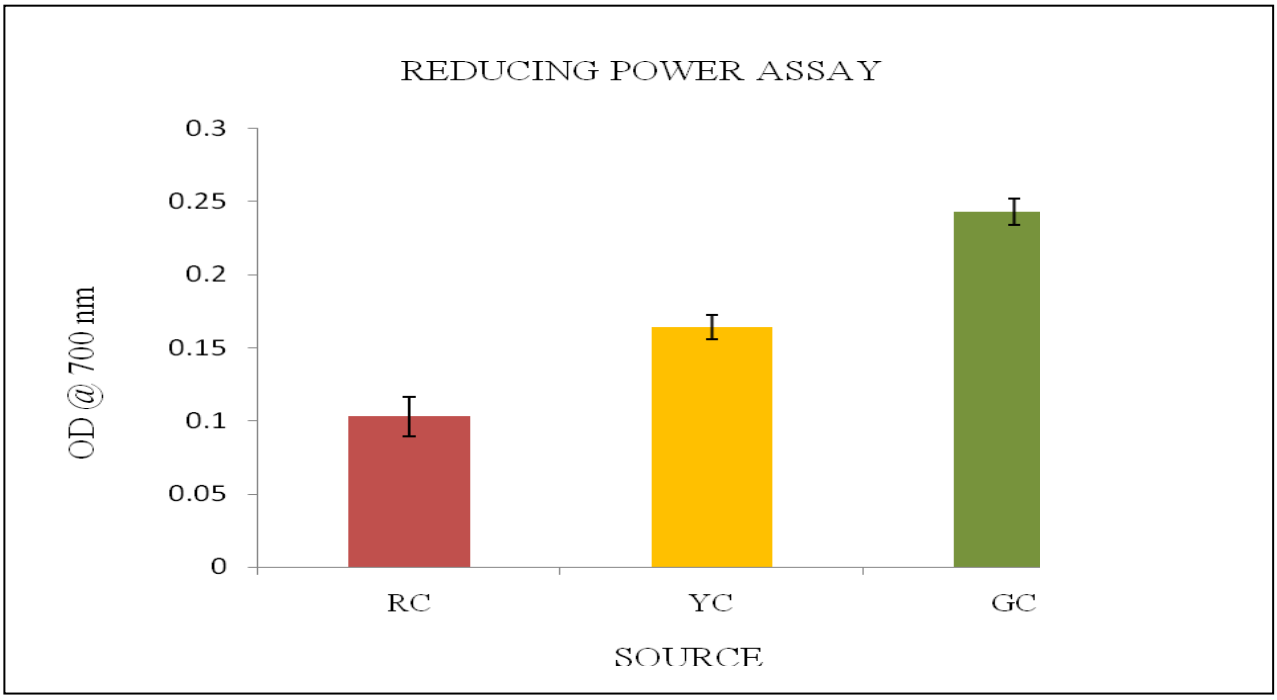

Fig I: Determination of reducing power of three varieties of *Capsicum annuum*

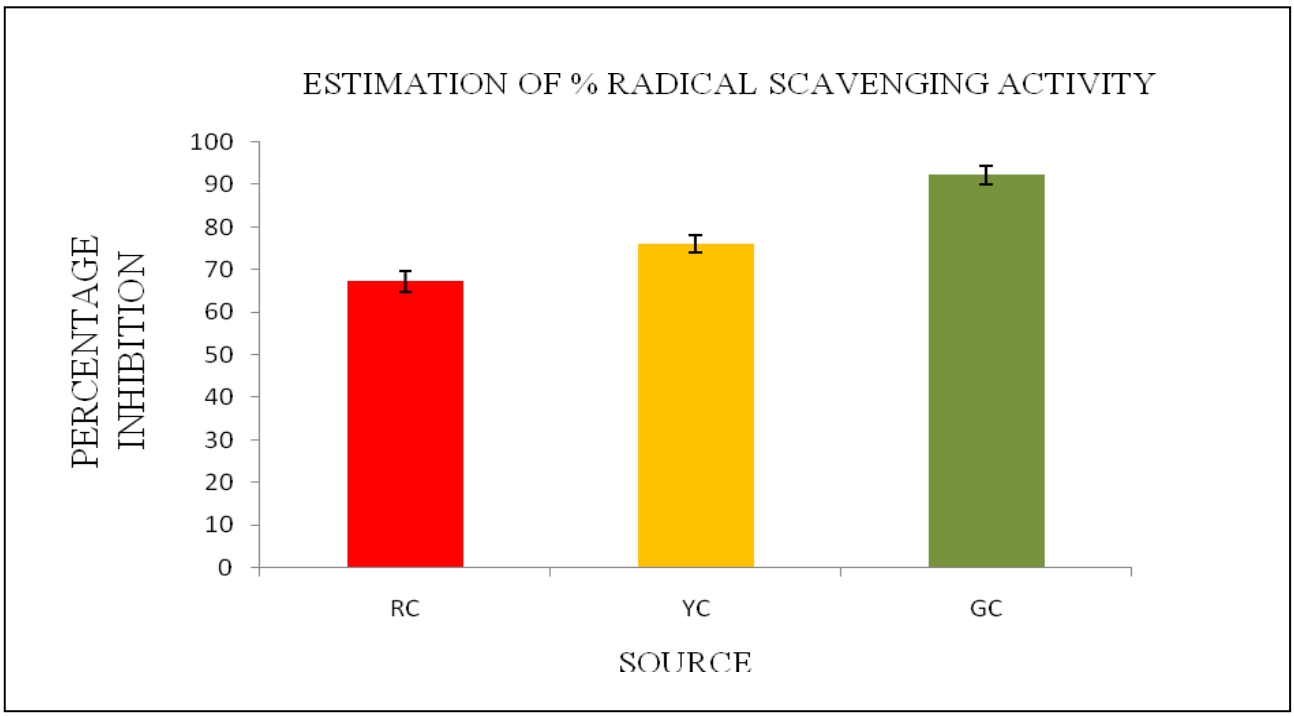

Fig II: Determination of radical scavenging activity of three varieties of *Capsicum annuum*

three *Capsicum annuum* species, red, yellow and green calculated as per the method of Rivillas[16]were 2.55,4.2 and 1.8 respectively.

3.2 Antioxidant activity

3.2.1 Determination of total phenolics by Folin-Ciocalteau assay

The total phenolic content in the aqueous extracts of red,yellow and green capsicum were found to be 1.7913 µgGAEm/µl± 0.0000, 2.5159µg GAEm/µl ± 0.0502 and 3.2985µg GAEm/µl± 0.1004 (Gallic Acid Equivalent mean) respectively. Higher content of phenolics in green Capsicum indicates higher antioxidant property[17].This finding also supports Deepa.N.et.al[7]report, which suggests that all the antioxidant constituents like phenolics, ascorbic acid and carotenoids and AOX, when expressed on the dry weight basis, declined in majority of the genotypes during maturity to red stage.

3.2.2 Determination of reducing power

In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of $Fe3^{+}$ to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex formed can then be monitored by measuring the intensity of Perl's blue developed at 700 nm. Increase in absorbance of the reaction mixture would reflect a greater reducing power of the sample. Antioxidant effect often correlates with reductive activity[18].Figure-I shows significant reducing power of the greencapsicum compared to the other varieties which could probably be attributed to the high concentration of antioxidant compounds in the green extract.

3.2.3 Determination of antioxidant activity by DPPH method

DPPH, a free radical was used for assessing antioxidant activity and the reduction of thisradical by an antioxidant results in a loss of absorption at 517 nm. Thus the degree of discoloration of the solution containing the aqueous extract indicates the scavenging efficiency of the added extracts. The radical scavenging activities of the samples were expressed in terms of % inhibition of DPPH**.** Correlation between free radical scavenging activity and the phenolic content have also been reported for various fruits and vegetables[19, 20].Highest free radical scavenging activity was again observed (Fig-II) in the aqueous extract of green capsicum (92 %) followed by yellow capsicum (76 %) and red capsicum (67%).

3.3 Inhibition of lipoxygenase

Lipoxygenases could be inhibited by antioxidants via chelation of its non-heme bound iron[21] or by reduction of its ferric form[22]. Antioxidants are beneficial for chronic inflammatory conditions, antihyperglycemia[23] and thus can act as anti-inflammatory molecules by scavenging the free radicals[24].Our results showed higher % of LOX inhibition bygreen capsicum (46.12 %) followed by yellow (44.09 %) and red (32.18 %),featuring anti-inflammatory property in them.

Conclusion

To conclude, this study suggests that *Capsicum annum species,* contains potential antioxidant and anti-inflammatory compounds which could be tested as drug candidates against oxidative and inflammation-related pathological processes. However, further studies should becontinued to obtain appropriate information about the role ofgreen capsicum to isolate the active principles, elucidate their structures and determine theirpharmacological activities.

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