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POLYPHENOLS CONTENT AND ANTIOXIDANT PROPERTIES OF GREEN CHILLIES (CAPSICUM ANNUM L.)

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ABSTRACT In the present study, an attempt was made to investigate the efficiency of three solvents viz. acetone, ethanol and water for extraction of total phenolics and flavonoids from green chillies (*Capsicum annum* L.) These extracts were also evaluated for free radical scavenging activity by DPPH method. The results revealed that water extract contained the highest amount of total phenolics (1.04 mg GAE/g fwb) whereas acetone extract contained the highest amount of flavonoids (1.23 mg CE/g fwb) . DPPH free radical scavenging activity of the green chillies extracts varied widely and it increased with increase of concentration levels. Ethanol extract exhibited the highest DPPH free radical scavenging activity with IC_{50} value (1.4 mg/mL) .

Keywords *Capsicum annum*, phenolics, flavonoids ,antioxidant activity.

INTRODUCTION

Plant secondary metabolites are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process[1]. During the extraction of plant material, it is important to minimize interference from compounds that may co-extract with the desired phytochemicals, and to avoid contamination of the extract as well as to prevent decomposition of metabolites or artifact formation as a result of extraction conditions. Extraction of phytochemicals from a plant material can be carried out using different solvents because of diversity of chemical nature and often unique distribution of these compounds in the plant matrix[2-3]. Various solvents are being frequently used for extraction of plant antioxidant compounds. However, the extract yields and antioxidant efficacy of the resulting extracts is strongly affected by polarity of the solvent as well as the chemical nature of the extracted compounds[3-4].

Chilli, a member of family Solanaceae is a major vegetable cum spice crop cultivated in tropical and sub tropical regions of the world[5] . Fruits and vegetables are immensely valuable not only for their nutritional value but also good source of natural antioxidants

containing many bioactive components which play a significant role in prevention of free radical formation by scavenging or pro-motion of their decomposition. Chili is a popular condiment around the world known to contain capsaicin and dihydrocapsaicin as the major pungent ingredients[6].Chili also contain many physiologically active substances, such as ascorbic acid, tocopherol, carotenoids, etc[7]. These substances are important for protection against oxidative damage by free radicals. In the human body, the various defense mechanisms that control free radicals start to decline as a person gets older. Thus antioxidants are crucial in human nutrition in that they can compensate for this decline. In India, Native American and Chinese traditional medicine, Capsicum sp. are used for treatment of arthritis, rheumatism, stomach aches, skin rashes, dog/snake bites etc.[8] Survey of the literature revealed that no systematic work has been done on the effect of solvents on extraction of phenolic , flavonoids content from green chillies and their antioxidant activity. Therefore, the objective of present study was to evaluate the efficacy of solvents towards extracting of phenolic, flavonoids content from green chillies and also to assess their antioxidant activity.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content of green chillies extracts in three solvents varied widely. On fresh weight basis, water extract of green chillies contained the highest total phenolic content i.e. 1.04 mg GAE/g fwb followed by ethanol (0.93 mg GAE/g fwb) and acetone (0.71 mg GAE/g fwb) extracts (Table 1).

Flavonoids content

Flavonoids content of green chillies extracts in three solvents varied widely. On fresh weight basis, acetone extract of green chillies contained the highest flavonoids content i.e. 1.23 mg CE/g fwb followed by ethanol (1.14 mg CE/g fwb) and water (0.45 mg CE/g fwb) extracts (Table 1).

Table 1: Total phenolics and flavonoids of green chillies extracts prepared using different solvents

Solvent	Total phenolics content (mg GAE/g fwb)	Flavonoids content (mg CE/g fwb)
Acetone	0.71 ± 0.02	1.23 ± 0.01
Ethanol	0.93 ± 0.01	1.14 ± 0.02
Water	1.04 ± 0.01	0.45± 0.02

DPPH free radical scavenging activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical (purple colour) and it transforms to non radical form (yellow colour) by abstracting one electron and hence, it is widely used as a measure for the electron donation capacity of antioxidants under assay conditions. In present studies, DPPH free radical scavenging activity (%) of the acetone, methanol and water extracts of green chillies varied widely and it increased with increase of concentration levels (Table 2). It ranged from 11.5 to 83.5% (ethanol extract), from 9.6 to 78.9 % (acetone extract) and from 3.6 to 62.0% (water extract) at different concentration levels ranging from 0.25 to 5.0 mg/mL.

The IC_{50} value of ethanol extract was lowest i.e. 1.4 mg/mL followed by 2.0 mg/mL of acetone extract and 3.4 mg/mL of water extract thereby showing that ethanol extract has highest activity followed by acetone and water extracts.

Table 2: DPPH free radical scavenging activity (%) of different extracts of green chillies

	DPPH Free Radical Scavenging Activity (%)					IC_{50}
Extracts Conc. (mg/mL)	5	2.5	1	0.5	0.25	(mg/mL)
Acetone	78.9±0.258 3.5±0.25	58.9±0.35	28.5±0.28	15.8±0.26	9.6±0.25	2
Ethanol	62.0±0.42	72.5±0.35	37.9±0.28	20.5±0.26	11.5±0.25	1.4
Water		38.9±0.30	15.9±0.26	9.6±0.25	3.6±0.29	3.4

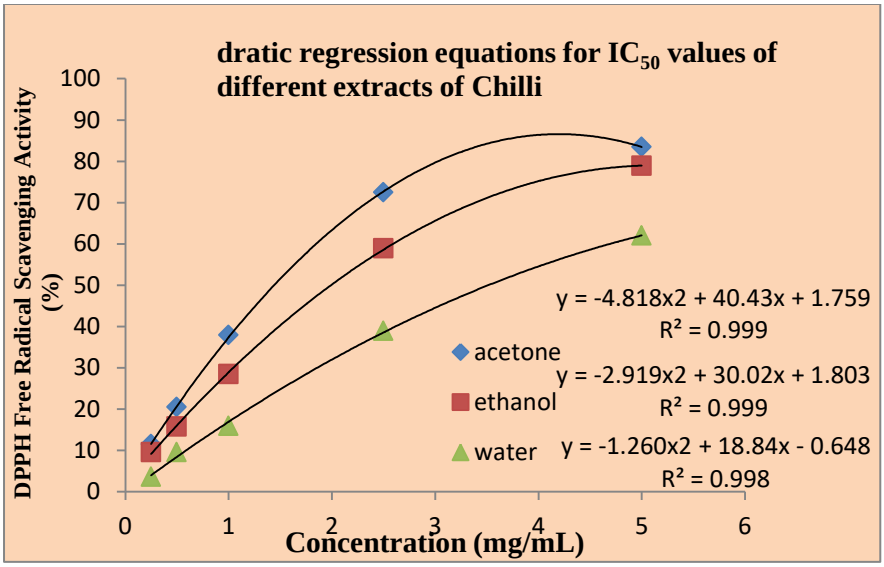

Fig. 1: Quadratic regression equations for IC_{50} values of different extracts of green chillies

EXPERIMENTAL

Plant material and extraction

Fresh and fully mature green chillies (Capsicum annum L.) were procured from the local market of Hisar. Green chillies (cut into pieces) were homogenized in Waring blender to make pulp. Ten gram of homogenized samples were extracted with 60 mL of solvents (acetone, ethanol and water) in conical flasks by shaking on a mechanical shaker for 2 hr. Extracts were filtered and residues were again extracted twice (each shaking time 1 hr) with 40 and 30 mL respective solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted. Extracts were then used for estimation of total phenolics, fiavanoids contents and for evaluation of antioxidant activity.

Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merck and Hi-Media of highest purity, were used for various experimental procedures.

Estimation of total phenolics content

Total phenolics content of extracts was determined using Folin-Ciocalteu method[9]. Aliquots of 0.2 ml of extracts were mixed with 1 ml of 1 mol/L Folin-Ciocalteu reagent. After that, 2.0 ml of 20% (w/v) sodium carbonate solution was added. The solutions were

mixed and volume was made up to 10.0 ml with distilled water. The absorbance was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g on fresh weight.

Estimation of flavonoids content

Flavonoids content of extracts was estimated according to the colorimetric assay[10]. In 1.0 ml of extract, 4.0 ml of double distilled water and 0.3 ml of 5% (w/v) $NaNO_2$ were added. After 5 min., 0.3 ml of 10% (w/v) $AlCl_3$ was added. Immediately, 2.0 ml of 1 M NaOH was added and the volume was made up to 10.0 ml with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using catechin as standard. Results were expressed as mg CE/g on fresh weight as well as dry weight basis.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method[11]. Acetone, ethanol and water extracts were dried up completely and the weight of dry mass was noted. The dry mass of acetone and ethanol extracts was redissolved in appropriate amount of methanol to make the stock solution (50 mg/mL). Since, the dry mass of water extract was not soluble in pure methanol, hence, it was redissolved in 50% (v/v) methanol : water to make the stock solution. From stock solution, different concentrations (0.25-5.0 mg/mL) were made by appropriate dilutions with respective solvents (i.e. methanol for acetone and ethanol extracts and with methanol : water for water extracts). For evaluation of antioxidant activity, in 0.2 mL of extracts (various concentrations), 3.0 mL of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1mM in 100 % methanol) was added and mixed thoroughly for 5 min. For antioxidant activity in water extracts (various concentrations), DPPH stock solution was prepared in 50 % (v/v) methanol : water and remaining procedure was same. A control was also made containing 0.2 mL of each solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after

30 min of incubation in dark at room temperature using the UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. Three replications were carried out for each sample. A graph was drawn by plotting per cent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis). Then using Microsoft Excel Software, quadratic regression equation ($y= ax^2+bx+c$) was obtained and using the quadratic equation IC_{50} was calculated. The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%\,DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample

CONCLUSION

Results of present study shows that solvent play a vital role in the extraction of the plant constituents. Water and ethanol extract of green chillies (Capsicum annum L.) contained high total phenolic whereas flavonoids contents were found to be highest in acetone extract and ethanol extract exhibited highest antioxidant activity. Consumption of green chillies with high antioxidant activity may help in decreasing the incidence of certain types of diseases in humans.

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