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Evaluation of Phenolic Contents and Antioxidant Potential of Methanolic Extracts of Green Cardamom (*Elettaria cardamomum*)

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Lipid peroxidation in fats and fatty foods not only deteriorates their quality, but also generates free radical and reactive oxygen species which are implicated in carcinogenesis, mutagenesis, aging and cardio-vascular diseases. The present project is undertaken to measure the antioxidant potential of methanolic extracts of green cardamom (*Elettaria cardamomum*). Green cardamom showed the total phenolic contents from 0.317 ± 00-1.66 ± 0.05 g/100 g; total flavonoids from 11.33 ± 0.03-4.63 ± 0.12 g/100 g. Antioxidant activities of the extracts were determined using thiocyanate method and percentage inhibition of peroxidation was 84.2-90 %. Significant ($p < 0.05$) differences were observed in the antioxidant efficacy of green cardamom extracts within the different solvent concentration. These results showed that green cardamom can be used as a significant source of natural antioxidants.

Key Words: Antioxidant, Phenol, Green cardamom.

INTRODUCTION

Free radicals are constantly formed in the human body which can cause damage to cellular bio-molecules such as proteins, lipids, nucleic acids and carbohydrates. Excess productions of some free radicals in the organisms, particularly reactive oxygen species (ROS) and their high activity leads to oxidative stress and several diseases like ageing, cataract, autoimmune disease, inflammation, atherosclerosis, *etc*.[1]. Antioxidants constitute a range of substances that interfere with the production of free radicals and inactivate them, thus protecting the biological systems against the deleterious effects of oxidative processes[2,3]. Most of these substances are natural products such as carotenoids, tocopherols, ascorbates, polyphenols that contribute to the prevention and treatment of diseases caused due to oxidative stress. This protection is due to the ability of the natural antioxidants to scavenge free radicals[4,5]. There is a growing interest in the use and measurement of antioxidant capacity in plant derived food additives. Many herbs and spices commonly used to flavour foods contain phenolic compounds that are reported to show good antioxidant activity[6]. Consequently, identification of alternative natural and safe sources of food antioxidants from plants has been identified[7].

Mostly spices and herbs are the sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. Fruit of *Elettaria*

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cardamomum is an herbaceous perennial of the ginger family (Zingiberaceae). It is known as "Heel khurd" or "Chhoti ilaichi" in Unani system of medicine[8]. Cardamom seeds are widely used for flavouring of food. Moreover, the seeds were used in the treatment of coughs, colds, bronchitis, asthma and indigestion[9]. In addition to this, cardamom has also carminative, stomachic, diuretic, antibacterial, antiviral and antifungal properties[10]. Keeping in view the significance of this herb the present study is design to investigate the total phenolic contents and antioxidant potential of fruit of the *Elettaria cardamomum* using different solvent concentration of methanol.

EXPERIMENTAL

All the chemicals used in this study were of analytical grade and mainly purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemical Company, USA.

Preparation of extracts: The ground plant material (15 g for each sample) was extracted with 150 mL of each of the solvent system (30 % methanol, 50 % methanol, 70 % methanol and absolute methanol) in 250 mL conical flask and was shaken for 8 h at room temperature in an orbital shaker (Gallenkamp, UK). The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and extracts were combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45 °C, using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan). The dried, crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator (-4 ºC) until used for analyses.

Determination of total phenolic contents: Amount of total phenolic contents in the fruits of cardamom were assessed using Folin-Ciocalteu reagent[11]. Briefly, 50 mg of crude extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 7.5 mL of deionized water. The mixture was kept at room temperature for 10 min and then 1.5 mL of sodium carbonate (2 %) solution was added. The mixture was heated in a water bath at 40 ºC for 20 min and then cooled in an ice bath; absorbance was measured at 755 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Amounts of total phenolic contents were calculated using gallic acid calibration curve within range of 10-100 ppm (R^2 = 0.9952). The results were expressed as gallic acid equivalents (GAE) g/100 g of dry plant matter.

Determination of total flavonoids: The total flavonoids were measured by a spectrophotometric method following a previously reported method[12]. Briefly, plant extract of each material (1 mL containing 0.1 mg/mL) was diluted with 4 mL water in a 10 mL volumetric flask. Initially, 0.3 mL of 5 % $NaNO_2$ was added to each volumetric flask, at 5 min, 0.3 mL of 10 % $AlCl_3$ was added and at 6 min, 2 mL of 1 M NaOH was added. Water (2.4 mL) was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was measured at 510 nm. Total flavonoids were determined as catechin equivalents (g/100 g of dry plant matter).

DPPH free radical scavenging assay: The DPPH assay was performed as described by Bozin *et al.*[13]. The samples (from 0.2-500 µg/mL) were mixed with

1 mL of 90 μM DPPH solution and filled up with 95 % methanol, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, Hitachi Instruments Inc., Tokyo, Japan). Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

$$\text{Antiradical activity (\%)} = 100 - (A_{blank} - A_{sample})/A_{blank}$$

where A_{blank} is the absorbance of the control reaction mixture excluding the test compounds and A_{sample} is the absorbance of the test compounds. Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Antioxidant activity determination in linoleic acid system: Antioxidant activity of extracts was determined in terms measurements of % inhibition peroxidation in linoleic acid system following a reported method[14]. Extract solution (0.1 mL) in 95 % ethanol was mixed with 2.5 mL of linoleic acid emulsion (0.2 M, pH 7) and 2 mL of phosphate buffer (0.2 M, pH 7). The reaction mixture was incubated at 37 °C to accelerate the oxidation process and used each 24 h for assessing antioxidant activity. The mixture without added extract was used as control. The mixture (0.1 mL) was taken and mixed with 5 mL of 75 % ethanol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL ferrous chloride ($FeCl_2$) solution (20 mM in 3.5 % HCl) being added sequentially. After 3 min of stirring, the absorbance values of mixtures measured with spectrophotometer (Hitachi U-2001 model 121-0032 Japan) at 500 nm were taken as peroxide contents. Butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) were used as positive control. Per cent inhibition of peroxidation of linoleic acid was calculated by the following formula:

$$IP\ (\%) = 100 - [(\text{Increase in } A_{sample} \text{ at 360 h/Increase in } A_{blank} \text{ at 360 h}) \times 100]$$

where A_{sample} is absorbance of sample and A_{blank} is absorbance of blank.

Statistical analysis: All the experiments were conducted in triplicate and results are reported as mean ± SD. The data was presented as mean values at 95 % confidence interval. Analysis of variance was performed using ANOVA procedures. Significant differences between means ($p < 0.05$) was determined by Minitab Software.

RESULTS AND DISCUSSION

Extraction of antioxidant components from fruits of green cardamom (*Elettaria cardamomum*) was carried out using methanol as solvent. Methanol is a polar solvent and has high solubility. The percentage yields obtained from different methanol concentration are shown are Fig. 1. The percentage yield ranges from 7.9 ± 0.24 to 9.4 ± 0.28 g/100 g. A high value of percentage yield was observed from 70 % methanol and lowest from 30 % methanol. So, 70 % methanol found to be good solvent for the extraction of antioxidant components from green cardamom. The

results revealed that extraction yield of antioxidant components depends on solvent concentration. Effect of solvent concentration on the extract yield is significant ($p < 0.05$). Efficiency of extracts is an important factor for the comparison of antioxidant activity. The plant that has to be extracted depends upon nature, amount and especially the concentration of the solvent. Therefore an efficient, appropriate and adequate concentration of the solvent should be used to extract maximum antioxidant compounds from any plant material. As the antioxidant compounds are organic in nature and organic compounds mostly dissolve in organic solvents. So methanol is very much effective in the extraction of antioxidant compounds due to its organic nature. Methanol is generally employed for the extraction of antioxidant components from plant materials due to their polarity and showed good solubility in these solvents[15]. Previous studies also reported that relatively higher antioxidant activities were observed from methanolic extracts compared to the other solvents including *n*-hexane, diethyl ether, ethyl acetate, acetone and water[16].

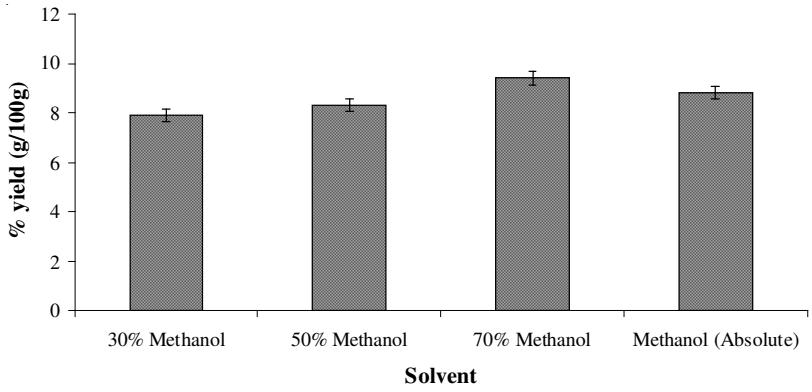

Fig. 1. Percentage yield of green cardamom extracts with different methanol concentration

It has been reported that the antioxidant activity of natural products is derived largely from phenolic compounds. The true antioxidant potential of natural products is expressed in terms of total phenolic contents (TPC)[17]. Total phenolic contents of green cardamom extracts were determined by Folin-Ciocalteu method which was selected due to its sensitivity, lower interference and quickness to quantify the phenolics as compared to other competitive tests. Folin-Ciocalteu reagent reacts nonspecifically with phenolic compounds and forms a complex with phosphomolybdic tungstate and changes color from yellow to blue where absorbance at 755 nm was measured. The total phenolic components of the extracts obtained from different methanolic extract range from 0.317 ± 00 to 1.66 ± 0.05 GAE g/100 g (Fig. 2). Maximum amount of phenolic components (1.66 ± 0.05 GAE g/100 g) was observed with 70 % methanolic extract. The effect of methanol concentration was significant ($p < 0.05$). Chanwitheesuk *et al.*[18] extracted antioxidative compounds from different edible plants of Thailand and these results are very much comparable to the results of the

present study *e.g*., the methanolic extract of *C. siamea* showed phenolic components about 0.384 g/100 g and *C. mimosoides* showed maximum value of 1.7 g/100 g. Similar results were observed in the present investigation as the most affective antioxidant compounds were extracted with 70 % methanolic extract.

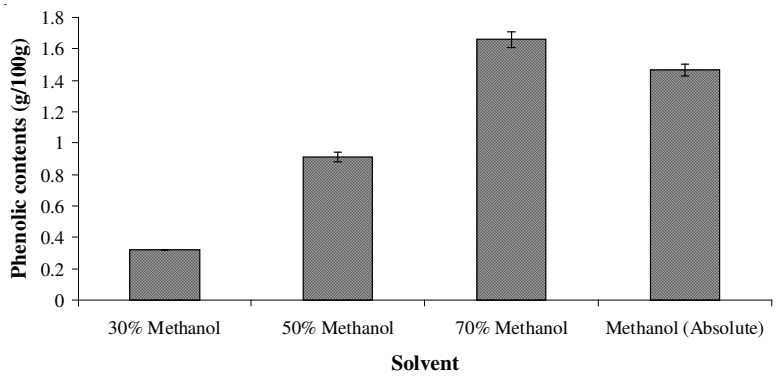

Fig. 2. Total phenolic contents of green cardamom extracts with different methanol concentration

The results of total flavonoids content of different methanolic extracts of green cardamom are shown in the Fig. 3. The flavonoids content of extracts obtained from different methanol concentrations range from 1.33 ± 0.03 to 4.63 ± 0.12 CE g/100 g. The maximum value of flavonoids was 4.63 ± 0.12 CE g/100 g obtained from 70 % methanolic extracts while the minimum (1.33 ± 0.03 CE g/100 g) value recorded for 30 %. The effect of solvent concentration on the amount of flavonoids was significant ($p < 0.05$). The results obtained from the present study were comparable to the earlier reported results[19], in which 80 % methanol was used as solvent to extract total flavonoids contents. The results of total flavonoids content of the *Azadirecthta indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* were comparable to the results obtained from the present study.

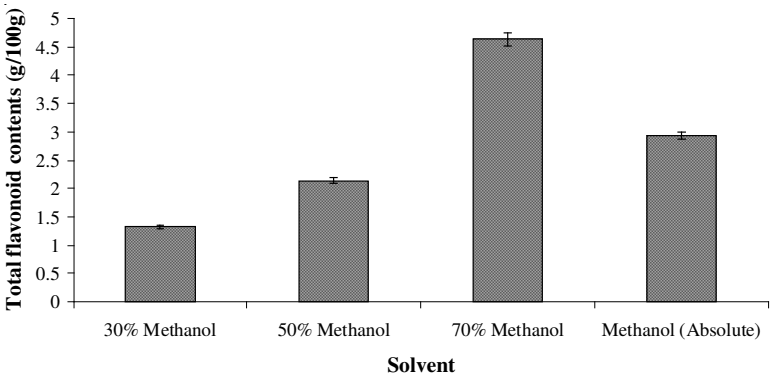

Fig. 3. Total flavonoids of green cardamom extracts with different methanol concentration

The antioxidants activity has also been assessed as ability to prevent from oxidation. Therefore, inhibition of linoleic acid oxidation was also used to asses the antioxidant activity of the methanolic extracts of green cardamom. Antioxidant activity of different extracts was determined by inhibition of peroxidation in linoleic acid. Linoleic acid is a polyunsaturated fatty acid, upon oxidation peroxides are formed which oxidize Fe^{2+}-Fe^{3+}, the later forms complex with SCN^- concentration of which is determined spectrophotometrically by measuring absorbance at 500 nm. Higher the absorbance higher will be the concentration of peroxides formed during reaction, consequently lower will the antioxidant activity. The data regarding the antioxidant activity of different methanolic extracts is depicted in Fig. 4. All the extracts showed high antioxidant activity (above 80 %). However, the 70 % methanolic extract of green cardamom exhibited the highest inhibition 90 ± 0.7 % in linoleic acid system and thus reflected the highest antioxidant activity. While that of 30 % methanolic extract exhibited the lowest (84.2 ± 0.7 %). Al-Mamary[20] determined the antioxidant activity of some of the vegetables commonly consumed in Yemen such as yellow onion, red onion and *C. rotundofolia* and the results were in the range of all the methanolic extracts obtained from present research work.

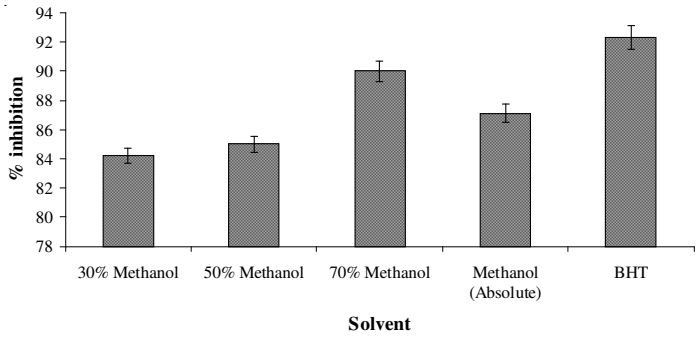

Fig. 4. Percentage inhibition of peroxidation in linoleic acid by green cardamom extracts with different methanol concentration and BHT

DPPH is a very stable organic free radical with deep violet color which give absorption maxima at 515-528 nm, upon receiving proton from any hydrogen donor species mainly, phenolics loses this absorption, resulting in a visually noticeable color change from deep violet to yellow. Free radical-scavenging capacities of the methanolic extracts were measured by the DPPH assay and the results were compared in the form of IC_{50} value (Fig. 5). IC_{50} values of the individual extract were calculated in order to investigate the free radical scavenging activity. IC_{50} of the 30 % methanolic extract was investigated as maximum (22.05 µg/mL) which shows its lowest free radical scavenging activity while 70 % methanolic extract showed the lowest IC_{50} value (17.26 µg/mL) indicating that this fraction has the high free radical scavenging activity. Lower the IC_{50} value of the extract, more effective will be the inhibition of DPPH. The results were compared with BHT as standard (IC_{50} = 8.39 µg/mL).

Yadav and Bhantnagar[21] investigated antioxidant power of some Indian spices such as cloves, licorice, mace and greater cardamom and found that the cloves exhibited the highest DPPH radical scavenging activity followed by licorice, mace and cardamom. Similarly, Kikuzaki *et al*.[22] reported that ethyl acetate soluble fraction of greater cardamom showed a high radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH).

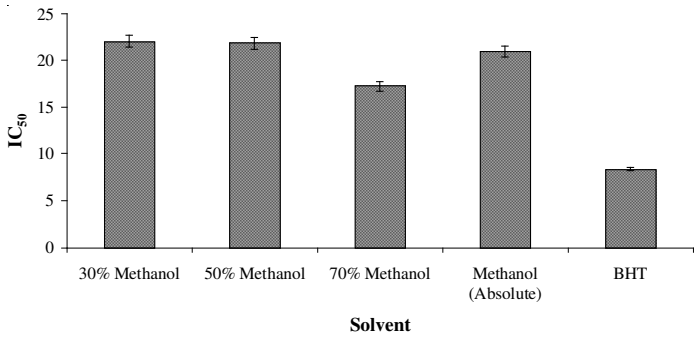

Fig. 5. DPPH free radical scavenging activity (IC_{50} µg/mL) of green cardamom extracts with different methanol concentration and BHT

Conclusion

Spices containing sufficient amount of antioxidants, includes phenolics, flavonoids *etc.*, can inhibit lipid peroxidation due to their antioxidant activity. So the consumption of these spices not only reduces the risk of cancer and various diseases but also prevent the chances of rancidity in fatty foods. In the present study, green cardamom found to be a good source of antioxidants and hence show high antioxidant activity. This study reveals that the use of green cardamom in diet not only provides flavour to the foods but also reduces the chances of their oxidation. This study showed that 70 % methanol is a good solvent for the extraction of antioxidants due to its high polarity. Further work is required to isolate those compounds which showed antioxidant activity.

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