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Antioxidant and Antimutagenic Potential of Seeds and Pods of Green Cardamom (*Elettaria cardamomum*)

¹Asma Saeed, ¹Bushra Sultana, ^{2,3}Farooq Anwar, ¹Muhammad Mushtaq,
⁴Khalid M. Alkharfy and ^{5,6}Anwarul-Hassan Gilani

¹Department of Chemistry, University of Agriculture, Faisalabad, 38040, Pakistan

²Department of Chemistry, University of Sargodha, Sargodha, 40100, Pakistan

³Department of Pharmaceutical Chemistry, College of Pharmacy,

Salman Bin Abdulaziz University, P.O. Box 173, Al-Kharj, 11942, Saudi Arabia

⁴Department of Clinical Pharmacy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

⁵Department of Biological and Biomedical Science, Aga Khan University, Karachi, Pakistan

⁶Department of Pharmacy, College of Health Sciences, Mekelle University, Ethiopia

Abstract: The present study reports the antioxidant and antimutagenic potential of the aqueous (30 and 70%) and acidified (0.5 and 1 N) methanolic extracts from seeds and pods of green cardamom (*Elettaria cardamomum*). The Total Phenolic Compounds (TPC) of extracts were determined by Follin-Ciocalteu reagent. Antioxidant activity of the extracts produced was evaluated by the measurements of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and percentage inhibition of linoleic acid peroxidation method, whereas mutagenic and antimutagenic activities were assessed using Ames bacterial test. The TPC, scavenging of DPPH^o and inhibition of linoleic acid were found to be 27.75-126.35 mg gallic acid equivalent g⁻¹ DW, 46-91% at 5 mg L⁻¹ of extract concentration and 34-83%, respectively. Furthermore, the extracts of seeds and pods of green cardamom showed strong antimutagenic potential against mutant strains *S. typhimurium* TA98 and *S. typhimurium* TA100 while none of the extract showed mutagenicity. The results of this study support that the extracts from cardamom seeds and pods can be explored as potential chemotherapeutic agents against cancer as well as for the pharmaceutical and nutraceutical applications.

Key words: *E. cardamomum*, extraction media, antioxidant activity, phenolic compounds, antimutagenic attributes

INTRODUCTION

The natural process of oxidation in living organisms is accomplished by the reaction of molecular oxygen with other biomolecules and thus leads to generation of free radicals and Reactive Oxygen Species (ROS). These ROS and free radicals along with other secondary oxidation products are strongly linked with the incidence of variety of degenerative diseases like atherosclerosis, heart diseases, cancer and neurodegenerative disorders (Saeed *et al.*, 2005; Saminathan *et al.*, 2013; Adebayo *et al.*, 2014). Recent literature reports indicate that many plants contain biologically active secondary metabolites (antioxidants) which render high capacity of scavenging free radicals and provide first line defense against oxidation related damages thus reducing

prevalence of various diseases. Now it is widely accepted that there is an inverse relationship between the dietary intake of antioxidant rich foods and the incidence of diseases (Pourmorad *et al.*, 2006; Saminathan *et al.*, 2013).

Elettaria cardamomum (family, Scitamineaceae), commonly known as queen of spices, green cardamom, true cardamom, ceylon cardamom, Elachi (in Pakistan and Ellaykka (in Malayalam), is indigenous to tropical rainforests of India, Pakistan, Burma, Sri Lanka, Malaysia and western Indonesia. Cardamoms are most extensively investigated spices due to their uses in traditional medicine for the treatment of teeth and gums infections, kidney and gall stones, throat troubles, congestion of the lungs and pulmonary tuberculosis, inflammation of eyelids, digestive disorders and snake and scorpion bites. (Jamal *et al.*, 2006; Gilani *et al.*, 2008; Namazi *et al.*, 2012).

Corresponding Authors: Anwarul-Hassan Gilani, Department of Biological and Biomedical Sciences, Aga Khan University, Karachi, Pakistan
Farooq Anwar, Department of Chemistry, University of Sargodha, Sargodha, 40100, Pakistan
Tel: +92-48-923-0546 Fax: +92-48-322-2121

The seeds of cardamom have been used to stimulate the appetite in people with anorexia, for coughs, cold, bronchitis, asthma and indigestion (Singh *et al.*, 2008; Khan *et al.*, 2011).

Keeping in view the potential folk medicinal uses, green cardamom (*E. cardamomum*) can be explored as a fascinating source of novel pharmaceutically important compounds and antioxidants for developments of natural chemopreventive pharmaceuticals and nutraceuticals. Hence, the present study was aimed to quantify polyphenols as well as appraise antioxidant and antimutagenic potential of extractable components from seeds and pods of cardamom using different extraction media.

MATERIALS AND METHODS

Sample collection: Samples of cardamom (*E. cardamomum*) were purchased from a local market in Faisalabad, Pakistan. Seeds and pods were manually separated, air-dried, ground into fine powder and preserved in air tight zipper bags for further analysis.

Chemicals and reagents: All the chemicals, reagents and standards including 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH^o), Butylatedhydroxytoluene (BHT), Gallic acid, Folin-Ciocalteu reagent and linoleic acid were purchased from Sigma-Aldrich (Chemie GmbH, Germany), whereas, potassium dihydrogen phosphate, ferrous chloride, ammonium thiocyanate, dipotassium hydrogen phosphate and sodium bicarbonate from Merck (Darmstadt, Germany). Two mutant strains *S. typhimurium* TA98 and *S. typhimurium* TA100 and sterile chemical mutagens i.e., sodium azide and potassium dichromate were purchased from TRINOVA (Biochem GmbH, Germany).

Extraction of bioactive components: The extractable bioactive components from seeds and pods of cardamom were extracted/recovered using different media including 70% methanol (70:30, v/v methanol:water), 30% methanol (30:70, v/v, methanol:water), 1 N acidified methanol (9:91, v/v, HCl:Methanol) and 0.5 N acidified (4.5:95.5, HCl:Methanol) methanol. Accurately weighed 10 g of each of ground seeds and pods of cardamom were separately extracted with 100 mL of extraction media (70, 30%, 1 N acidified and 0.5 N acidified methanol, respectively) by shaking at 120 rpm for 24 h at room temperature in an orbital shaker (Gallenkamp, UK). The extracts were then made free of solvent by evaporating at 45°C under vacuum using a rotary evaporator (EYELA, S B-651, Rikakikai Company Ltd., Tokyo, Japan). The yields of bioactive extracts (BE) from the seeds and pods were calculated gravimetrically.

Mutagenic and anti-mutagenic assay: The mutagenic and antimutagenic potential of BE from seeds and pods of cardamom was evaluated by using the Ames bacterial reverse mutation test with some modification as purposed by Razak and Aidoo (2011). The strains *S. typhimurium* TA98 and *S. typhimurium* TA100 were incubated at 37°C in a liquid nutrient broth. The reagent mixture was prepared by mixing Davis Mingioli salt (21.62 mL, 5.5 times concentrated), D-glucose (4.5 mL, 40% w/v), bromocresol purple (2.3 mL, 0.2% w/v), D-biotin (1.19 mL, 0.01% w/v) and L-histidine (0.01% w/v) aseptically, in a sterile vial. The reaction mixture (2.5 mL) was taken separately in five sterile vials labeled as Blank, Negative control, Positive control, Mutagen and Antimutagen, diluted with 17.5 mL deionized water and inoculated with 5 µL of bacterial strain (except blank). Into the positive control vials, 0.1 mL of standard mutagen (0.5% sodium azide and 30% potassium dichromate for *S. typhimurium* TA100 and *S. typhimurium* TA98, respectively), whereas, in mutagen and antimutagen assay vials, 5 µL of 1.0 mg plant extract mL⁻¹ in dimethyl sulfoxide (DMSO) were added.

The contents of each vial were transferred into a reagent boat and 200 µL of the aliquot mixture was added into each well of 96-well ELISA plate using a multichannel pipette. Plates were tightly packed with aluminum foil and incubated at 37°C for 4 days. The blank plate was checked first and the purple coloration of all wells in this plate showed that no contamination was present. All other plates were scored visually and all yellow, partially yellow or turbid wells were recorded as positive while purple as negative. Background plate represented the spontaneous mutation of the bacterial strain. The extract was considered mutagenic if the number of positive wells is more than twice the number of positive wells in the background plate. The antimutagenic effect was measured as percentage inhibition of mutagenicity by the following Eq. 1:

$$\text{Antimutagenicity (\%)} = 1 - \frac{\text{No. of positive wells in the sample} - \text{spontaneous mutation}}{\text{No. of positive well in positive control} - \text{spontaneous mutation}} \times 100 \quad (1)$$

The antimutagenic effect was considered as strong, moderate and weak when percentage inhibitions of mutagenicity was more than 40, 25 and less than 25%, respectively (Mosovska *et al.*, 2010).

DPPH free radical scavenging assay: The antiradical potential of BE of seeds and pods of cardamom, indicating their capacity to decolorize a purple solution of DPPH in methanol, was evaluated using spectrophotometric

method as described earlier by Yogesh *et al.* (2010). Different concentrations of cardamom extracts were prepared in methanol and 3 mL of each was mixed with 1 mL of 0.1 mM methanolic DPPH solution. The mixture was incubated at room temperature for 30 min and absorbance measured at 517 nm to calculate percentage inhibition of DPPH° as follows:

$$\text{Inhibition (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (2)$$

where, A_{Control} indicates the absorbance of solution containing only the DPPH reagent whereas, A_{Sample} is the absorbance of the sample reaction. The effective dose of 50% inhibition (IC_{50}) was also obtained from a plot of percentage inhibition verses extract concentration. All the experiments were run in triplicate and mean values thus were computed against ascorbic acid and BHT as positive control.

Inhibition of linoleic acid peroxidation: In order to measure the antioxidant activity of different BE of seeds and pods of green cardamom against inhibition of linoleic acid peroxidation, thiocyanide method as reported by Shi *et al.* (2011) was used with slight modification. Briefly, each extract (0.5 mg) was mixed with 0.5 mL linoleic acid (2.51%) in absolute ethanol and 0.5 mL of distilled water at pH 7 using 0.05 M phosphate buffer. The mixture was then incubated at 40°C in dark for autoxidation. Aliquots (0.1 mL) was taken at 12 h intervals and the degree of oxidation was monitored after adding 9.7 mL ethanol (75%), 0.1 mL aqueous ammonium thiocyanate (30%) and 0.1 mL aqueous ferrous chloride (0.02 M in 3.5% HCl) followed by measuring the absorbance at 500 nm, until the absorbance of the control (containing all reagents except the test sample) reached maximum. The antioxidant potential was expressed as percent inhibition of lipid peroxidation relative to control using the following Eq. 3:

$$\text{Lipid peroxidation inhibition (\%)} = \frac{A_{\text{Sample at 72 h}} - A_{\text{Sample at 0 h}}}{A_{\text{Control at 72 h}} - A_{\text{Control at 0 h}}} \times 100 \quad (3)$$

where, A_{Sample} and A_{Control} denotes absorbance of sample and control, respectively.

Determination of Total Phenolic Content (TPC): The total phenol contents in BE of seeds and pods of green cardamom were determined using the Folin-Ciocalteu reagent method while Gallic acid employed as standard following the protocol as described by Albano and Miguel (2011) with minor modification.

Statistical analysis: Data is presented as Mean±SD. Comparison among the extraction media was made by Tukey's multiple-range test to identify optimum treatments ($p < 0.05$).

RESULTS

Percentage yield of extract: The results of percentage yield (g/100 g) of extractable bioactive components from seeds and pods of green cardamom have been incorporated in Table 1. The maximum yield of active compounds from seeds of green cardamom was observed with 1 N acidified methanol whereas, the minimum from pods with 30% methanol. The overall, order of efficacy of the extraction media towards bioactive yields from pods of green cardamom was observed to be: 1 N acidified methanol > 0.5 N acidified methanol > 70% methanol > 30% methanol.

Total Phenolic Content (TPC): The total phenolic compounds in the methanol extracts (1 N acidified, 0.5 N acidified, 70 and 30% methanol) of seeds and pods of green cardamom ranged from 27.75-126.35 mg Gallic Acid Equivalents (GAE) g^{-1} of DW (Table 1). The maximum amount of phenolic compounds, 126 mg GAE g^{-1} DW, was recorded in pods of green cardamom extracted by 1 N acidified methanol while the minimum in 30% aqueous methanol extracts (27.75 mg GAE g^{-1} DW). The trends for distribution of amount of extracted phenolics among four solvent systems in a decreasing order were as follows: 1 N acidified methanol > 0.5 N acidified methanol > 70% methanol > 30% methanol.

Inhibition of peroxidation in linoleic acid system: The values for percentage inhibition of linoleic acid oxidation by different extracts of seed and pod of green cardamom varied significantly ($p < 0.05$) in relation to extraction media (Table 1). The results indicated that acidified methanol extracts of green cardamom inhibited peroxidation in linoleic acid system more affectively indicating its greater antioxidant activity (40.12-83.25%) as compared to aqueous methanol extracts (34.46-42.72%).

DPPH° scavenging activity: The antioxidant potential of cardamom seeds and pods extracts measured in terms of their DPPH° radical scavenging at different concentrations (0.001-5 mg mL^{-1} of each extract) has been expressed in Fig. 1. The resulting plot (Fig. 1) indicated that DPPH° radical scavenging of seeds and pods of green cardamom ranged between 69.89-79.27 and 76.04-91.67%, respectively.

Table 1: Extraction yield, Total Phenolic Content (TPC) and inhibition of linoleic acid peroxidation of seed and pod of green cardamom (*E. cardamomum*)

Solvent used	Extract yield ^L		TPC ^M		Inhibition (%) ^N	
	Seed	Pods	Seed	Pods	Seed	Pods
1 N acidified methanol	31.20±0.93 ^a	17.50±0.99 ^a	101.29±3.03 ^a	126.35±3.97 ^a	83.25±1.28 ^a	72.05±2.16 ^a
0.5 N acidified methanol	23.02±0.20 ^b	12.22±0.48 ^{bc}	100.71±3.02 ^a	105.12±0.28 ^b	51.04±1.06 ^{bc}	40.12±1.03 ^{cd}
70% methanol	6.21±0.42 ^d	14.04±0.25 ^c	96.16±2.88 ^a	65.35±1.96 ^c	42.72±0.99 ^{cd}	37.52±1.12 ^d
30% methanol	14.05±0.18 ^c	3.54±0.23 ^d	33.71±1.01 ^b	27.75±0.83 ^d	35.623±0.58 ^d	34.46±1.20 ^d

L: Values are (g/100 g) Mean±SD; M: Values are given as mg gallic acid equivalent per gram of dry matter; N: Results were calculated against BHT (84% inhibition of linoleic acid peroxidation). Different lowercase letters in superscript within the same column indicate significant differences (p<0.05) among extraction media used

Table 2: Mutagenic potential of seed and pod of green cardamom (*E. cardamomum*)

	Test of <i>Salmonella</i> TA 98			Test of <i>Salmonella</i> TA 100		
	No. of positive wells /96 wells			No. of positive wells /96 wells		
	Seed	Pod	Effect	Seed	Pod	Effect
^L Background	8	8		14	14	
^M K ₂ Cr ₂ O ₇	93	95	+	NA	NA	NA
^N NaN ₃	NA	NA	NA	93	92	+
70% methanol	7	6	nm	20	19	nm
1 N acidified methanol	4	3	nm	8	7	nm
0.5 N acidified methanol	9	11	nm	27	26	nm
30% methanol	13	15	nm	5	7	nm
BHT	1	1	nm	1	1	nm

L: Negative control, M: Positive control for *Salmonella* TA 98, N: Positive control for *Salmonella* TA 100, +: Mutagenic, nm: non-mutagenic, NA: Test not applied

Table 3: Antimutagenic potential of extracts from seed and pod of green cardamom (*E. cardamomum*)

	<i>Salmonella</i> TA 98				<i>Salmonella</i> TA 100			
	No. of positive wells /96 wells				No. of positive wells /96 wells			
	Seed	Effect	Pod	Effect	Seed	Effect	Pod	Effect
^L Background	8	NA	8	NA	14	NA	14	NA
^M K ₂ Cr ₂ O ₇	93	--	93	--	NA	NA	NA	NA
^N NaN ₃	NA	NA	NA	NA	93	--	93	--
70% methanol	24	++	24	++	34	++	20	++
1 N acidified methanol	11	+++	12	+++	9	+++	9	+++
0.5 N acidified methanol	11	+++	12	+++	25	++	28	++
30% methanol	13	+++	13	+++	50	+	12	+++
BHT	28	++	28	++	21	++	21	++

^LNegative control, ^MPositive control for *Salmonella* TA 98 ^NPositive control for *Salmonella* TA 100, +: Strong antimutagenic, ++: Moderate antimutagenic, +: Weak antimutagenic, -: Non-mutagenic and --: Mutagenic response

Mutagen activity of plant extracts: The results regarding the mutagenic activities of different extracts of seeds and pods of green cardamom (30, 70, 0.5 N acidified and 1 N acidified methanol) have been incorporated in Table 2.

Anti-mutagenic activity: The percentages of antimutagenic activity of various extracts are given in Table 3. The strongest anti-mutagenic activity (81.01%) was demonstrated by the green cardamom components extracted with 1 N acidified methanol while the lowest (30.58%) was for the 30% aqueous methanol extract. The overall antimutagenic efficacy of the extracts was in the following order: 1 N acidified methanol > 0.5 N acidified methanol > 70% methanol > 30% methanol.

DISCUSSION

Percentage yield of extract: The percentage yield (g/100 g) of extractable bioactive components varied

significantly (p<0.05) due to extent of acidification and aqueous nature (polarity) of the extraction media which was concordant with previous observations by Anwar *et al.* (2013) who applied different extraction techniques and solvents to extract potential bioactives from leaves and flowers of Ghaneri (*Lantana camara* L.).

Plants are excellent source of biologically active compounds including phenolics, flavonoids, phenolic acids, lignins, salicylates, stanols, sterols, glucosinolates among others which possess multiple medicinal and food uses (Ventura-Sobrevilla *et al.*, 2008; Hooper and Cassidy, 2006). The extraction of plant bioactives is a challenging task wherein the availability of plant metabolites is highly dependent upon the nature and composition of extraction media as well as of the plant material. Ideally, extraction should be quantitative and time saving based (Awika *et al.*, 2003; Siddhuraju and Becker, 2003; Cacace and Mazza, 2006). A variety of extraction solvents have been employed including methanol, ethanol, ether,

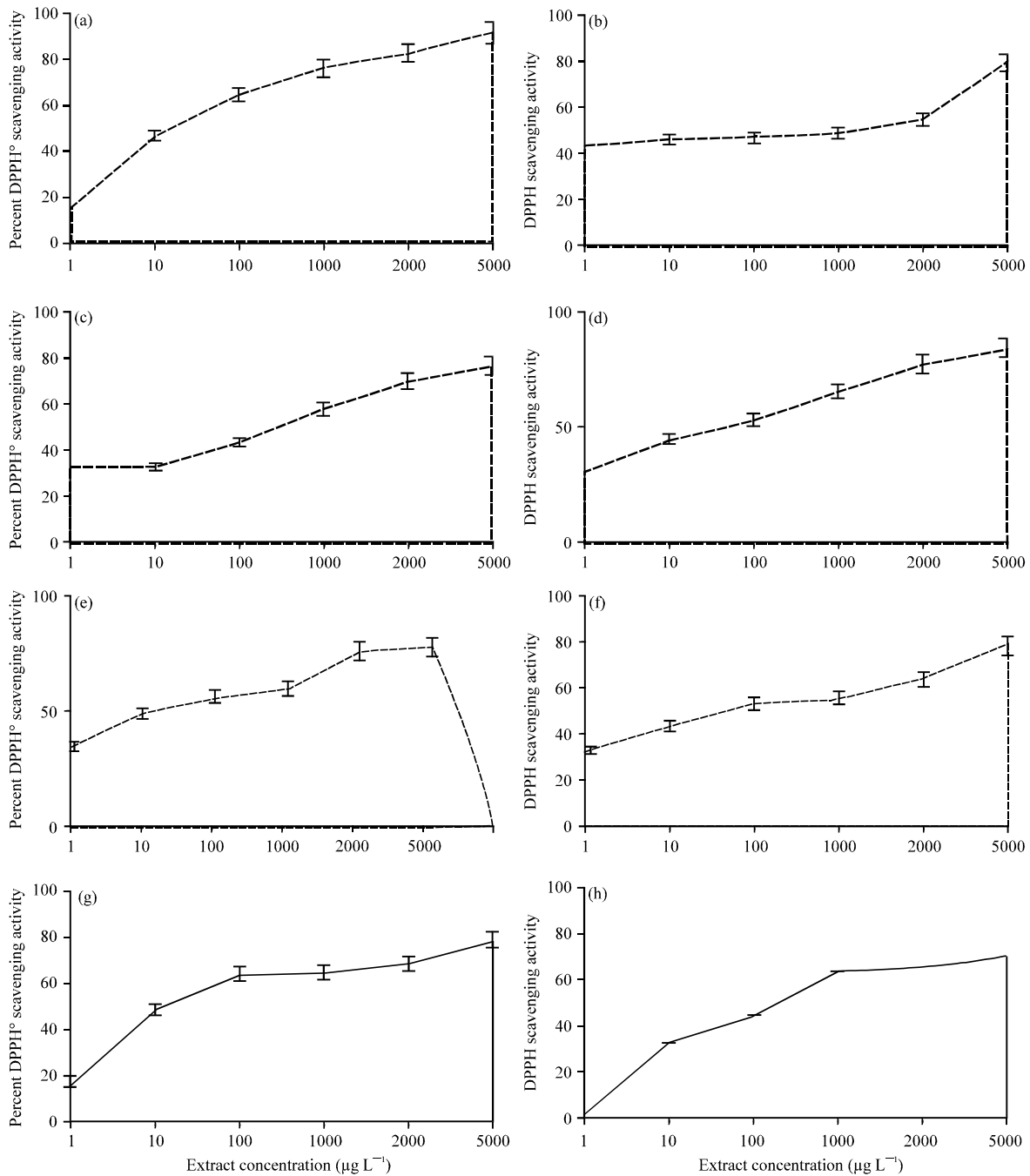


Fig. 1(a-h): DPPH° scavenging activity (%) of green cardamom seed extract in (a) 70%, (b) 30%, (c) 0.5 N acidified, (d) 1 N acidified methanol, (e) pod extract in 70%, (f) 30%, (g) 0.5 N acidified and (h) 1 N acidified methanol, respectively

ethyl acetate, acetone, n-hexane, water and others for the extraction of plant bioactives. Methanol and ethanol in pure forms or as aqueous mixtures are recognized to be more efficient solvents for extraction of plant antioxidants components (Choi *et al.*, 2007; Durling *et al.*, 2007; Sultana *et al.*, 2008; Mushtaq *et al.*, 2012; Anwar *et al.*, 2013).

The enhanced extraction efficiency of 1 N acidified methanol might be attributed to higher cell lysis potential of acidified methanol as compared to its aqueous counterpart. Furthermore, the mean range of extraction yield from seed (6.50-31.25 g/100 g DW) in the present work was higher than that investigated by Amma *et al.* (2010) who reported extract yield of 0.23-5.72 g/100 g DW

from cardmom using different solvents (n-hexane, ethyl acetate, methanol and water).

Total Phenolic Content (TPC): It is well established that phenolic compounds are responsible for antioxidant and free radical scavenging activities inherited to the herbs and spices, thus making them an efficient chemopreventive and nutraceutical additives (Jayaprakasha *et al.*, 2006; Jirovetz *et al.*, 2006; Mohamad *et al.*, 2009). Amma *et al.* (2010) analysed different varieties of cardmom native to India for their phenolic compounds and found that ethyl acetate was most effective towards extraction of total phenolics (3.7-150 mg GAE g⁻¹ DW), whereas in the present study it was observed that 1 N acidified methanol gave higher yield of phenolic antioxidants (101-126 mg GAE g⁻¹ DW) revealing its greater affectivity as extracting medium. Furthermore, it was observed that the overall amount of extractable phenolics (27-126 mg g⁻¹ DW) in pods of green cardmom were higher as compared to seed samples (33-101 mg g⁻¹ DW). The present results suggest that the pods of cardmom which are often discarded as agrowaste, could be potentially used for the isolation of bioactives for pharmaceutical and/or nutraceutical development.

Inhibition of peroxidation in linoleic acid system:

Antioxidants, either synthetic or natural, can retard lipid oxidation. Therefore, the antioxidant activity of seed and pod extracts of green cardmom measured by the inhibition of linoleic acid oxidation was evaluated. A standard thiocyanate (SCN) method which involves oxidation of Fe⁺² to Fe⁺³, was used. In this method, Fe⁺³ formed complex with SCN and the absorbance of the resultant complex is measured calorimetrically at 500 nm. The values for percentage inhibition of linoleic acid oxidation by different extracts of seed and pod of green cardmom varied significantly (p<0.05) in relation to extraction media (Table 1). In line to above stated TPC data, the results also indicated that acidified methanol extract of green cardmom inhibited peroxidation in linoleic acid system more affectively indicating its greater antioxidant activity (40.12-83.25%) as compared to aqueous methanol extracts (34.46-42.72%).

DPPH° scavenging activity: The DPPH free radical scavenging capacity of the tested extracts increased in a concentration dependent manner (Fig. 1). The antioxidant potential of cardmom seeds and pods extracts (0.001-5 mg mL⁻¹ of each extract) in terms of DPPH° radical scavenging ranged between 69.89-79.27 and 76.04-91.67%, respectively, indicating considerable (p<0.05) variations as function of extraction solvent and plant's tested parts.

The data showed that overall pod extracts exhibited better radical scavenging ability as compared to seed extracts of cardmom. The results further revealed that pod extracts when extracted with 0.5 N acidified methanol showed higher (91.67%) DPPH° scavenging activity whereas extracts from the seeds with 1 N acidified methanol (83.64%) has potent activity. It can be predicted from the results of antioxidant assays in this analysis that the distribution of total phenolics in a specific plant material mainly characterizes its antioxidant properties.

Mutagenic activity: A mutagen causes changes in DNA that can affect the transcription and replication of DNA, resulting in aberrant, impaired or loss of function for a particular gene and in severe cases, it can lead to cell death and cancer. Examples of diseases that are thought to be caused by mutations include Parkinson disease and Alzheimer disease. The results regarding the mutagenic activity of different aqueous (30 and 70%) and acidified (0.5 and 1 N) methanol extracts from seeds and pods indicate that the tested extracts are safe with reference to mutagenesis which may support the development of such herbs as a nutraceutical/ functional food (Table 2).

The present findings on the antimutagenic potential of green cardmom extracts are in a close agreement with earlier data reported by Balaji and Chempakam (2008) and Rahimifard *et al.* (2010). With the increased incidence of diet related cancers, a considerable interest has grown in plant phenolic antioxidants as a potential candidate for chemo-preventive compounds (Langova *et al.*, 2005; Yoo *et al.*, 2006; Aqil *et al.*, 2008).

Anti-mutagenic activity: Based upon potential antioxidant activities measured in terms of DPPH° radical scavenging and inhibition of linoleic acid peroxidation, the extracts produced by different extraction media were tested for their antimutagenic potential against direct mutagens using two strains of *Salmonella* (TA 100 and TA 98). The percentages of antimutagenic activity of various extracts are given in Table 3. The strongest anti-mutagenic activity (81.01%) was demonstrated by the green cardmom components extracted with 1 N acidified methanol while the lowest (30.58%) was for the 30% aqueous methanol extract. The overall efficacy of the extracts was in the following order: 1 N acidified methanol > 0.5 N acidified methanol > 70% methanol > 30% methanol.

Comparison of in-vitro assays: The antioxidant evaluation of seeds and pods of green cardmom was carried out using a number of *in vitro* tests because application of single method does not provide comprehensive information regarding the antioxidant behavior of a substance (Silva *et al.*, 2007; Sultana *et al.*,

Table 4: Pearson's correlation (given as r^2) between antioxidant, antimutagenic activities and total phenolic contents in different parts of green cardamom

Seed	Pod					
	TPC	Inhibition of peroxidation	DPPH ^o	TPC	Inhibition of peroxidation	DPPH ^o
Inhibition of peroxidation	0.782* (0.003)			0.784* (0.003)		
DPPH	0.466 ^{ns} (0.127)	-0.140 ^{ns} (0.664)		0.574 (0.048)	-0.071 ^{ns} (0.827)	
Antimutagenic activity	0.816** (0.000)	0.639* (0.025)	0.587 (0.045)	0.991** (0.000)	0.816** (0.001)	0.517 ^{ns} (0.085)

TPC: Total phenolic contents, DPPH^o: DPPH^o scavenging activity, Inhibition of peroxidation= inhibition of linoleic acid peroxidation ns: Non significant, *Significant at $p < 0.05$, **Significant at $p < 0.01$, Probability values are given within parenthesis

2007). The reason behind this variation might be mechanism and nature of reductant species involved. For example, free radical scavenging potential could be better assessed in terms of DPPH radical hunting potential of extracts. Similarly, antioxidant potential of extracts is measured in terms of their ability to check formation of reactive oxygen species generated during linoleic acid auto-peroxidation. The metal chelating ability and reducing potential can be better understood by reducing potential of extracts. Hence, it would be of practical interest to compare antioxidant potential of seed and pod of green cardamom observed during various *in vitro* testing assays.

The data regarding comparison of antioxidant and anti-mutagenic activities and phenolic contents of seed and pod of green cardamom has been assembled in Table 4. A strong correlation was observed between total phenolics and inhibition of linoleic acid peroxidation ($r^2 = 0.782$; $p < 0.0$) whereas, a non-significant correlation was observed between phenolic contents (TPC) and DPPH radical scavenging potential ($r^2 = 0.466$; $p > 0.05$). This kind of behavior revealed that major antioxidant bioactives in the extracts of green cardamom seed are oxygen and reactive oxygen scavenger rather than free radical killers.

The highly significant correlation coefficient for antimutagenic activity ($r^2 = 0.816$; $p < 0.01$) and total phenolic attributes (TPC) further authenticate that antimutagenic function of seed extract might be due to their potential to cope reactive oxygen species. The antimutagenic potential of extracts of green cardamom pods was more strongly influenced with their TPC ($r^2 = 0.991$; $p < 0.01$) as compared to seeds whereas, a trend for inhibition of peroxidation and TPC was same as observed for seeds. In contradiction to the data for seeds, there was a weak correlation ($r^2 = 0.574$; $p < 0.05$) between DPPH radical scavenging potential and TPC of pod extracts. The stronger correlation observed for antimutagenic potential of green cardamom pod extract might be due to cumulative behavior of free radical scavengers and oxidation inhibitors. Furthermore, the

antimutagenic activity of green cardamom observed in the present work was higher than previously reported by Chughtai *et al.* (1998).

CONCLUSION

The results of the current study showed that the seeds and pods of green cardamom contain substantial amount of total phenolics and antioxidant and antimutagenic activities rendering them as a potential source for the isolation of compounds for development of nutraceuticals. The data regarding solvents extraction efficacy revealed that acidification of methanol enhances both availability of biological components and their antiradical, antioxidant and antimutagenic activities. Further studies are needed to explore the clinical applications of these observations in humans.

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