



Phytochemical screening and evaluation of anti-microbial and anti-oxidant activity of *Elettaria cardamom* (Cardamom)

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Abstract: The present study deals with the phytochemical screening and evaluation of antibacterial and antioxidant activities from the crude methanol extract of the seeds of cardamom, *Elettaria cardamom*. Crude methanol extract was investigated for their antibacterial activity against *Enteropathogenic E. coli* (EPEC), *Listeria monocytogenes*, *Bacillus pumilus* and *Escherichia coli*. The extract showed maximum zone of inhibition (20.3 mm) against EPEC, however, the antibacterial potential of the extract was slightly lesser against normal *E. coli* (19 mm). It showed moderate anti-bacterial activity against *L. monocytogenes* and *B. pumilus*. Dose-dependent increase in antioxidant activity was also noticed in crude extract as measured by DPPH free radical scavenging assay. Thus, our study reports various phytochemicals in the seeds of cardamom with antioxidant and antibacterial potential.

Keywords: Anti-microbial activity, Anti-oxidant activity, Cardamom, DPPH

INTRODUCTION

Since ancient times, plants have been utilized for their medicinal properties. Use of medicinal plants is an important part of traditional as well as modern system of medicine (Bruneton *et al.*, 2001). India is particularly rich in naturally occurring plant drugs which have enormous potential pharmacological activities (Srinivasan *et al.*, 2001). Cardamom is one such plant with known medicinal properties. It is often known as the "Queen of Spices" and is often used as a cooking ingredient due to its pleasing aroma and taste (Bhattacharjee *et al.*, 2013). The cardamom plant is a medium-sized herbaceous perennial, about 2 to 5 meters tall. It is the native of Western Ghats in South West India and cardamoms are the dried fruits or capsules of the plant.

Essential oil of cardamom has been commonly used in traditional medicine since a long time. A number of bioactive compounds have been found in cardamom (Bhattacharjee *et al.*, 2013). Cardamom contains flavonoids like quercetin, kaempferol, luteolin and pelargonidin (Sultana *et al.*, 2010). Extracts from cardamom have shown antibacterial (Supriya *et al.*, 2010; Hero *et al.*, 2012 Naveed *et al.*, 2013) and antioxidant properties (Nair *et al.*, 1998; I.P.S Kapoor *et al.*, 2008; Jayawardena *et al.*, 2015). *Enteropathogenic Escherichia coli* (EPEC) is one of the *Escherichia coli* pathotypes. It is known to be important diarrhoea pathogen of young children. EPEC are gram-negative, rod-shaped bacteria and are among the most important pathogens infecting children both in developing countries and developed countries (Baliere *et al.*, 2016). *Listeria monocytogenes* is a rod-shaped, Gram-positive bacte-

rium. It is the agent of listeriosis, which is a serious infection caused by eating food contaminated with *L. monocytogenes* (Shegarfi *et al.*, 2016). *B. pumilus* is a Gram-positive, rod shaped and aerobic bacteria. It is found in soils and some reside in the root area of some plants (Grass *et al.*, 2016). *E.coli* is a gram negative, rod shaped bacteria that is commonly found in the intestine of warm-blooded organisms.

Antioxidants are part of the body's defence system against free radicals attack. Antioxidants are involved in the prevention of cellular damage which leads to pathways of aging and cancer. A number of antioxidants have been derived from the plant sources (Kogure *et al.*, 2004). The present study demonstrates potent antibacterial activity of cardamom extract against some pathogenic and non pathogenic Gram positive *L. monocytogenes*, *B. pumilus*) as well as Gram negative bacteria [EPEC, *E. coli*]. We have also evaluated the antioxidant activity of methanol extract of cardamom seeds by using DPPH radical scavenging assay.

MATERIALS AND METHODS

Plant material: Cardamom fruits were purchased from local market of Babujanj, Lucknow and were identified by Prof. Y.K. Sharma, Former Head, Department of Botany, University of Lucknow. Sample was washed under running tap water and dried at room temperature (25°C). Cardamom fruit was opened with the help of forceps to obtain seeds. Only seeds of the cardamom fruit were used for this study. Seeds obtained from the fruit were grinded with the help of mortar and pestle, the produced powder was stored in

plastics bags for further study.

Preparation of plant extracts: Dried cardamom seed powder was extracted with methanol in soxhlet extractor. 100gms of dried powder was mixed in 1 litre methanol for extraction. The soxhlet extraction setup was set at room temperature. Crude extract was filtered through Whatman No-1 using a Buchner funnel and thereafter concentrated with the help of rotary evaporator at vacuum at 40 °C.

Extraction yield: 38.50 g of extract was obtained from 87.97g of powdered cardamom seeds after concentration and drying of extracts. Percentage yield was calculated to be 43.76%. The percentage of extraction yield was calculated by the following formula:-

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract (g)}}{\text{Amount of dried part used (g)}} \times 100$$

Phytochemical screening: Phytochemical analysis of the cardamom methanol extract was carried out to test for the presence of flavonoids, amino acids, saponins, alkaloids, phenols, tannins, terpenoids, quinone and glycoside as per the following protocols:

(i) Test for flavonoid: Test solution was treated with 10% NaOH. Formation of greenish brown colour indicates the presence of flavonoids (Edeoga *et al.*, 2005).

(ii) Test for free amino acids: Test solution when boiled with 0.2% ninhydrin solution, formation of purple colour indicates the presence of free amino acids (Khandelwal *et al.*, 2001).

(iii) Test for saponin (Froth test): Test solution when added to water and shaken well, formation of frothing indicates the presence of saponin (Aiyelaagbe *et al.*, 2009).

(iv) Test for alkaloid: 1ml of test solution when treated with 1ml of Hagger's reagent, formation of yellow colour precipitate indicates the presence of alkaloid (Obadoni *et al.*, 2001).

(v) Test for phenols: Test solution was treated to alcohol and ferric chloride formation of greenish yellow colour indicates the presence of phenols (Khandelwal *et al.*, 2001).

(vi) Test for tannin: Test solution treated with 20% boiled water and 0.1%FeCl₃, formation of brownish green colour indicates the presence of tannin (Edeoga *et al.*, 2005).

(vii) Test for terpenoids: Test solution was treated with chloroform and conc. H₂SO₄, formation of orange colour indicates the presence of terpenoids (Edeoga *et al.*, 2005).

(viii) Test for quinones: If test solution when treated with HCl gives red precipitate, it indicates the presence of quinone (Khandelwal *et al.*, 2001).

(ix) Test for glycoside: five millilitre of test sample when treated with 2 ml of glacial acetic acid containing few drops of FeCl₃ and 1ml H₂SO₄ added, a brown layer at interface indicates glycoside (Aiyelaagbe *et al.*, 2009).

Determination of antimicrobial activity

Test microorganisms and bacterial culture: *E.coli* (NCIM 2065), *B. pumilus* (NCIM 9369) and *L. mono-*

cytogenes (NCIM 5279) obtained from NCL Pune, were used to determine antibacterial activity. EPEC E 2347 was obtained from KGMU, Lucknow. The glycerol stock cultures of micro-organisms were maintained at -80°C. Working cultures were kept at 4°C and were periodically subcultured. The inocula of the micro-organisms were prepared by transferring a loopful of working culture into 9 ml of sterilized LB media and incubated in incubator shaker at 37°C for 5 to 6 h. The bacterial culture was compared with Mc-Farland turbidity standard and the culture that has attained 0.5 Mc-Farland units was used for the assay.

Well diffusion method: The antibacterial activity of cardamom extract was tested by using well diffusion technique as described by Agarry *et al.* (2005). LB agar plates were prepared by pouring 25 ml autoclaved sterile LB Agar in sterile Petri plates (90 mm). Overnight cultures having .05 OD of above mentioned bacterial strains were swabbed over sterilised agar plates. A standard cork borer was used for creation of uniform 6mm well on the surface of LB agar plates. Total 3 pores were made on plates, for positive control, negative control and one for sample. 50 mg of concentrated methanol extract of cardamom was dissolved in 1 ml of 0.5 % of DMSO and 40 µl of this extract was poured in one of the well. Streptomycin was chosen as positive control for antibacterial activity. 20 mg streptomycin was dissolved in 1 ml DMSO and 40 µl was poured in the second well as positive control. 40.5 % of 40 µl DMSO was poured in the third well as negative control. Whole set of experiment was done in triplicates and these plates were placed at 37° C for overnight.

The inhibition zone was calculated as mean (n=3).

Determination of antioxidant activity

DPPH radical-scavenging: For determination of antioxidant activity of test sample DPPH radical scavenging reagent was used. DPPH-free radical scavenging capacity of cardamom extract was evaluated according to the method of Chen *et al.*, (1995) with slight modifications. DPPH solution of 1mM concentration was prepared in methanol. Ascorbic acid was used as a positive control. Following concentrations of ascorbic acid was used: 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml. 1 ml of each sample was mixed with 1ml DPPH solution by shaking vigorously for 1min by vortexing and then incubated in dark for 30 minutes. After incubation period of 30 minutes at 37°C, absorbance of each sample (A_{sample}) at 517nm were measured using UV spectrophotometer. Corresponding methanol blanks were taken. Similarly, following concentrations of cardamom extract were used: 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml. Likewise, 1 ml of each test sample was mixed with 1ml DPPH solution by shaking vigorously for 1min by vortexing and then incubated in dark for 30 minutes. After incubation period of 30 minutes at 37°C, absorbance of each test sample (A_{sample}) at

517nm was measured using UV spectrophotometer. Corresponding methanol blanks were taken. The experiment was performed in triplicate. A negative control (A_{control}) was taken after adding DPPH solution to 1 ml of methanol. Lower absorbance of the reaction mixture indicates higher radical scavenging activity (Biswas *et al.*, 2010). The scavenging effect (%) was measured by using the following formula:

$$\text{Scavenging effect (\%)} = [1 - (A_{\text{sample}}/A_{\text{control}})] \times 100$$

RESULTS

Phytochemical analysis: Phytochemical analysis of the methanol extract of cardamom demonstrated the presence of terpenoids as the major phytochemical (Table- 1). In addition to that, flavonoids and glycosides were also found in the extract.

Antibacterial activity: A preliminary antibacterial study of the cardamom extract is summarized in Table-2. Well diffusion method revealed various degree of sensitivity by the test microorganisms against cardamom extract. Well diffusion method had demonstrated *EPEC* (20.3 mm zone of inhibition as compared to 26 mm zone of inhibition for positive control i.e. streptomycin) as the most sensitive organism for the cardamom extract followed by *B. pumilus* (19 mm zone of inhibition as compared to 25 mm zone of inhibition for positive control i.e. streptomycin). Moderate resistance was exhibited by *L. monocytogenes* (18.5 mm zone of inhibition as compared to 22 mm zone of inhibition for positive control i.e. streptomycin) and *E. coli* (16.5 mm zone of inhibition as compared to 20 mm zone of inhibition for positive control i.e. streptomycin). All experiments were performed in triplicate and the inhi-

bition zone was calculated as mean ($n=3$).

Antioxidant activity: The results on DPPH· radical scavenging activity of the cardamom extract along with the reference standard ascorbic acid are shown in Table- 3.

DISCUSSION

Free radicals are known to have a number of pathological manifestations. It is the antioxidants that fight with the free radicals and protect from their harmful effects (Umamaheswari *et al.*, 2008). In our study, various concentrations of methanol extract of cardamom were tested for their antioxidant activity using the DPPH radical scavenging assay. Significant antioxidant activity was observed in the cardamom extract. DPPH assay is a widely used method to estimate the free radical scavenging effect of plant extracts. It is based on the reduction of DPPH solution in the presence of antioxidant that results in the synthesis of non radical DPPH-H. In addition, phytochemical screening and evaluation of anti microbial activity were also carried out in our study. Among all the microbes tested, the most significant anti-microbial activity of cardamom extract was found against *EPEC*. Hero *et al.* (2012) have demonstrated the anti-microbial activity of cardamom extract against *Staphylococcus aureus* and *Proteus mirabilis*. While Islam *et al.* (2010) have demonstrated the anti-microbial activity of cardamom extract against 10 human pathogenic bacteria (Gram negative: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, and *Shigella sonnei* as well as Gram positive: *Staphylococcus aureus*, *Streptococcus-β-haemolytica*, *Bacillus subtilis*, *B. megaterium*, and *Sarcina lutea*). Jebur

Table 1. Phytochemical analysis of cardamom methanol extract.

Sample	Alkaloids	Flavonoids	Phenol	Glycoside	Saponins	Quinone	Tannins	Terpenoids
Cardamom (Methanol extract)	-	+	-	+	-	-	-	+

Table 2. Antibacterial activity of cardamom extract as measured by Well diffusion assay.

Bacteria strain name	Diameter of zone of inhibition for cardamom methanol extract (mm)	Diameter of zone of inhibition for positive control i.e. Streptomycin (mm)	Diameter of zone of inhibition for negative control i.e. DMSO (mm)
<i>Enteropathogenic E. coli (EPEC)</i>	20.3	26	Not detected
<i>Listeria monocytogenes</i>	18.5	22	Not detected
<i>Bacillus pumilus</i>	19.0	25	Not detected
<i>Escherichia coli</i>	16.5	20	Not detected

Table 3. % of inhibition of cardamom extract as measured by DPPH assay.

	25 µg/l concentration	50 µg/l concentration	100 µg/l concentration	200 µg/l concentration	400 µg/l concentration
% of inhibition by ascorbic acid (Positive control)	76.63	80.98	87.55	88.87	91.53
% of inhibition by cardamom extract	60.9	75.3	78.55	79.87	82.53

et al. (2014) have evaluated antimicrobial activity of cardamom fruit extract, leaves and oil against *Staphylococcus aureus*, *Streptococcus pneumonia*, *S.epidermidis*, *P. aeruginosa*, *K. pneumonia*, *Proteus mirabilis*, *Enterobacter spp.* *Acinetobacter*, *E. coli*, *Serretia spp.* and *Salmonella typhi*. Chawla *et al.*, (2014) have studied the antimicrobial activity of cardamom against Bacillus and pseudomonas. Our results are in accordance with Mishra *et al.*, (2010) who had also demonstrated the antimicrobial activity of cardamom against, *E. coli* using ethanol and aqueous extracts of cardamom. We have demonstrated antimicrobial activity of cardamom extract against *EPEC*, *L. monocytogenes*, *B. pumilus* and *E. coli*. Antimicrobial activity of cardamom is due to the presence of various chemical compounds like volatile oils, alkaloids, phenols, tannins and lipids (Jebur *et al.*, 2014). Hence, present study is focused towards phytochemical screening and study of anti-microbial and anti-antioxidant activity of cardamom extract which may be used for the development of potent drugs. Pharmaceutical agent from cardamom will possess minimal toxicity and cost effectiveness in comparison to the compound chemically synthesized.

Conclusion

In this study, methanol extract of cardamom was examined for the presence of various phytochemicals and its antioxidant and antimicrobial activities were also evaluated. Phytochemical analysis of the extract revealed the presence of terpenoids, flavonoids and glycosides. Antimicrobial activity of cardamom extract against the following four microorganisms was evaluated: *E. coli*, *B. pumilus*, *L. monocytogenes* and *EPEC*. Maximum antimicrobial activity of the cardamom extract was found to be against *EPEC*, showing an inhibition zone of 20.3 mm. Antioxidant potential of the cardamom extract was evaluated using the DPPH radical scavenging assay. The extract exhibited different degrees of antioxidant activities depending on the concentration examined. Therefore, the results of the current investigation demonstrate antimicrobial and antioxidant activities in cardamom extract and also show the possibility of various phytochemicals in the cardamom extract.

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