In vitro Antibacterial Activity of Methanol Seed Extract of *Elettaria cardamomum* (L.) Maton

Soriful ISLAM [1, 2]
Atikur RAHMAN [2]
Mominul Islam SHEIKH [2, 3]
Mostafizur RAHMAN [2]
Abu Hena Mostofa JAMAL [4]
Firoz ALAM [2] (✉)

Summary

Antibacterial activity of methanol seed extract of *Elettaria cardamomum* (L.) Maton was tested using agar disc diffusion method against 10 human pathogenic bacteria (Gram positive: *Staphylococcus aureus*, *Streptococcus-β-haemolytica*, *Bacillus subtilis*, *Bacillus megaterium*, and *Sarcina lutea* as well as Gram negative: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, and *Shigella sonnei*). Methanol extract inhibited the growth of all the tested bacteria having various degrees of inhibition zones. Highest inhibitory activity was observed against *Salmonella typhi* (16.83 mm) with lowest MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) values viz. 25 mg/ml and 50 mg/ml, respectively in gram negative bacteria and the same was observed against *Streptococcus-β-haemolytica* (15.5 mm) with the MIC and MBC value of 50 mg/ml in gram positive bacteria. On the basis of this experimental result, it can be concluded that methanol seed extract of *E. cardamomum* could be considered for further isolation and evaluation as therapeutic antimicrobial.

Key words

methanol seed extract, cardamom, antibacterial assay

[1] Department of Molecular Pathology and Innovative Therapies, Anatomy and Cell Biology Polytechnic University of Marche, Via Tronto 10 A, 60020 Ancona, Italy
[2] Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh
✉ e-mail: falambiotech@gmail.com
[3] Department of Environmental Forest Products, Division of Environmental Forest Sciences, College of Agriculture and Life Sciences, Gyeongsang National University, Republic of Korea
[4] Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh

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Introduction

Infectious bacterial diseases are becoming serious threat in developing countries like Bangladesh where peoples are not aware of their primary healthcare. Due to the lack of proper treatment, indiscriminate use of antibiotics and also ignorance are the major problems to control such bacterial diseases. Nowadays, it is a common phenomenon that microorganisms are developing their resistance to many commercial antibiotics that is the major cause of failure to treat various infectious diseases. Therefore, immense clinical problem in the treatment of infectious diseases has been raised (Davies, 1994). Recently, considerable attention has been focused on identifying naturally occurring active compounds, capable of inhibiting and controlling some infectious bacterial diseases. In this view point, already a lot of spices have been tested for their antibacterial properties such as cumin (Iacobellis et al., 2005; Chaudhry and Tariq, 2008; Sheikh et al., 2010), cardamom (El-Malti et al., 2007; Singh et al., 2009), nigella (Mashhadian and Rakhshandeh, 2005; Chaudhry and Tariq, 2008), clove (Fu et al., 2009), ginger (Park et al., 2008) etc.

Cardamom is a dried fruit of *Elettaria cardamomum* (L.) Maton (Zingiberaceae) that is commonly known as queen of spices for the versatile use in culinary practice. Cardamom is a perennial shrub with thick, fleshy, lateral roots which can grow to a height of eight feet (Kapoor, 2000). Cardamom is native to South Asia but it is commercially cultivated in Southern India, Sri Lanka, Tanzania, and Guatemala and recently Morocco also became producer of cardamom (El-Malti et al., 2007). Cardamom has antibacterial, antifungal (Agaoglu et al., 2005; Bansod and Rai, 2008; Singh et al., 2008), anticancer (Sengupta et al., 2005), antioxidant (Singh et al., 2008; Lin et al., 2009; Sultana et al., 2010) and also gastroprotective effect (Jamal et al., 2006).

Recently, some researchers have documented the antibacterial activity of cardamom extracts using different solvents against several bacterial species like *Pseudomonas aeruginosa* (Agaoglu et al., 2005; El-Malti et al., 2007; Arora and Kaur, 2007), *Klebsiella pneumoniae* (Agaoglu et al., 2005; Nanasombat and Lohasupthawee, 2005; El-Malti et al., 2007), *Staphylococcus aureus* (Agaoglu et al., 2005; Arora and Kaur, 2007; Singh et al., 2008; El-Malti et al., 2009), methicillin resistant *Staphylococcus aureus* (Karthy et al., 2009), *Escherichia coli* (Agaoglu et al., 2005; Nanasombat and Lohasupthawee, 2005; Arora and Kaur, 2007; El-Malti et al., 2007; Singh et al., 2008; Zhang et al., 2009), *Salmonella typhi* (Arora and Kaur, 2007; Singh et al., 2009), *Salmonella typhimurium* (Agaoglu et al., 2005; Nanasombat and Lohasupthawee, 2005; Arora and Kaur, 2007), *Bacilus cereus* (El-Malti et al., 2007; Singh et al., 2008) and *Shigella sonnei* (El-Malti et al., 2007). However, till now some emerging human pathogenic bacteria are remaining to be explored to test their sensitivity especially against methanol seed extract of cardamom. Moreover, it has been demonstrated that use of different solvents may lead to extracts with different chemical profiles and activities (Singh et al., 2008). Therefore, the present investigation was conducted to evaluate the antibacterial activity of methanol seed extract of cardamom against some human pathogenic bacteria.

Material and methods

Spice materials and extraction

Seeds of *Elettaria cardamomum* (L.) Maton were purchased from local market, near the Rajshahi University campus in March, 2009 and taxonomically identified by Dr. A.H.M. Mahbubur Rahman, Assistant Professor, Department of Botany, Rajshahi University, Bangladesh. Voucher specimen of this spice was kept at the Herbarium of the Department of Botany, Rajshahi University, Bangladesh. Seeds were oven dried at 50°C for 24 h and fine powder was made. Fifty gram (50 g) of this powder was dissolved in 300 ml of methanol and left for two days followed by shaking through an orbital shaker *(IKA Labortechnik KS 250 Basic Orbital Shaker,* Staufen, Germany). After filtration through teton cloth and Whatman no. 1 filter paper, the resulted extract was concentrated to dryness (semi-solid) using Water bath (4 holes analogue, Thermostatic water bath, China) under 50-60°C. Semi-solid extract was then dissolved in particular amount of methanol to get a desired concentration and kept into freeze at 4°C until use.

Bacterial species used

Five Gram (+) bacteria with the accession number namely, *Staphylococcus aureus* (BMLRU1002), *Streptococcus-β-haemolytica* (BMLRU1006), *Bacillus subtilis* (BMLRU1008), *Bacillus megaterium* (BMLRU1010), *Sarcina lutea* (BMLRU1012) and five Gram (-) namely *Klebsiella pneumoniae* (BMLRU1005), *Pseudomonas aeruginosa* (BMLRU1007), *Salmonella typhi* (BMLRU1009), *Shigella dysenteriae* (BMLRU1011), *Shigella sonnei* (BMLRU1015) bacteria were used in this investigation. All the strains were collected from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh. The bacterial species were maintained on nutrient agar medium (Hi media, India), and sub cultured in nutrient broth at 37°C, prior to antibacterial activity test.

Antibacterial assay

Disc diffusion method was followed to test antibacterial activity of *E. cardamomum* against studied ten human pathogenic bacteria (Bauer et al., 1966). Tetracycline (30 µg/ml) was used as a positive control. Negative control discs were prepared using only methanol instead of spice extract. Sterilized Whatman No.1 filter paper discs were (6 mm in diameter) soaked with 10 µl of spice extract at 100 mg/ml concentration and dried at room temperature. Discs were placed on the surface of the seeded nutrient agar plates and incubated at 37°C for 24 h. Over night incubated 30 µl bacterial cultures (10^8 cfu/ml) were used for preparing seeded agar plates. After incubation, antibacterial effectiveness of spice extract was evaluated by measuring the diameter (mm) of zone of inhibition around the disc using transparent millimeter scale. Each cycle was carried out in triplicate.

MIC and MBC determination

Standard method was performed as described by Doughari et al. (2007) to determine the MIC and MBC of methanol seed extracts of *E. cardamomum*.

0.5 ml spice extract containing five different concentrations (100, 75, 50, 25 and 12.5 mg/ml) was added into different test tubes with 2 ml nutrient broth, separately and then a loopful bacterial suspension was introduced. After successfully inoculation, all the examine test tubes were then incubated at 37°C for 24 h. Only bacterial suspension containing test tubes with nutrient broth instead of the seed extract were used as a control. After incubation, the lowest concentration of extracts that inhibited visible growth of studied bacteria in test tubes was taken as MIC.

A loopful bacterium was collected from MIC position and sub cultured on to nutrient agar plates. Petri dishes were incubated at 37°C for 24 h. The lowest concentrations of extracts with no visible growth of studied bacteria on agar plates were recorded as MBC.

Statistical analysis

Antibacterial activity of cardamom seed extract and antibiotic were statistically analyzed using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) through MSTAT-C statistical program (Russell D. Freed, Crop and Soil Sciences Department of Michigan State University, USA). All the results are represented as means ± SE of three independent replications. P values < 0.05 were considered as significant.

Results

Antibacterial assay

The antibacterial activity of methanol seed extract of cardamom as well as tetracycline (broad spectrum antibiotic as positive control) obtained by disc diffusion method against 10 human pathogenic bacteria is shown in Fig.1. Methanol extract and tetracycline showed various degrees of zone of inhibitions depending on the bacterial species. Highest zone of inhibition was recorded by methanol extract as 16.83 mm against *S. typhi* whereas, tetracycline showed comparatively lower activity (11 mm). On the other hand, highest zone of inhibition was recorded by tetracycline as 17.17 mm against *S. haemolytica* whereas, methanol extract showed comparatively contiguous activity (15.5 mm) (Fig.1). However, *S. typhi*, *S. dysenteriae*, *P. aeruginosa and S. haemolytica* are not significantly different from each other based on the DMRT result obtained by methanol extract (Fig. 2). In addition, no significant differences were observed among the other bacterial species (*S. haemolytica*, *S. aureus* and *K. pneumoniae*). The lowest zone of inhibition was recorded against *B. subtilis* (12 mm) whereas, tetracycline showed comparatively better activity (14.33 mm) (Fig.1). Statistical result indicated that *B. subtilis* and *S. sonnei* were not significantly different from each other (Fig. 2). In addition, *S. aureus*, *K. pneumoniae*, *S. lutea* and *B. megaterium* were not statistically different from each other (Fig. 2).

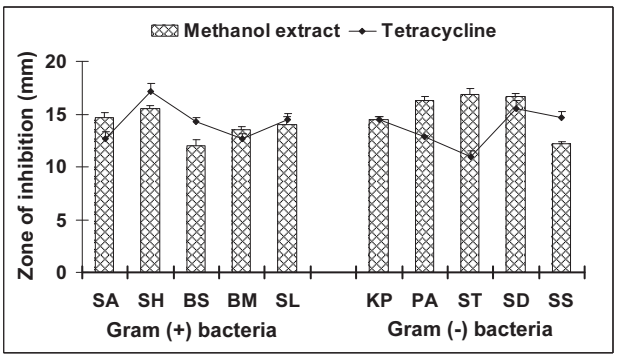

Figure 1. Inhibition of bacterial growth by methanol seed extract (100 mg/ml) of cardamom and 30 µg/ml tetracycline (positive control) in the agar disc diffusion assay. SA, *Staphylococcus aureus*; SH, *Streptococcus-β-haemolytica*; BS, *Bacillus subtilis*: BM, *Bacillus megaterium*; SL, *Sarcina lutea*; KP, *Klebsiella pneumoniae*; PA, *Pseudomonas aeruginosa*; ST, *Salmonella typhi*; SD, *Shigella dysenteriae*; SS, *Shigella sonnei*.

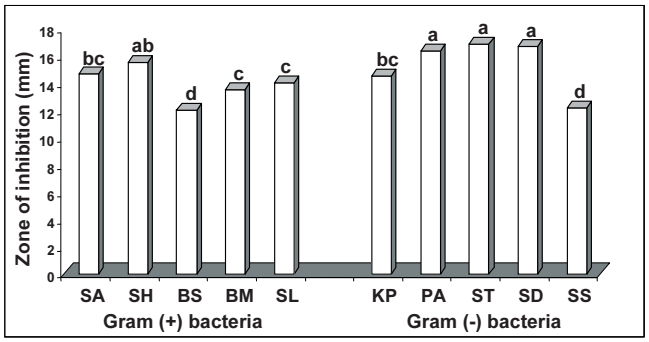

Figure 2. Relationship among the bacterial species on the basis of their sensitivity to methanol seed extract of cardamom by DMRT. Data represent the mean values of three replications. Different letters at the bars indicate statistically significant difference (P<0.05).

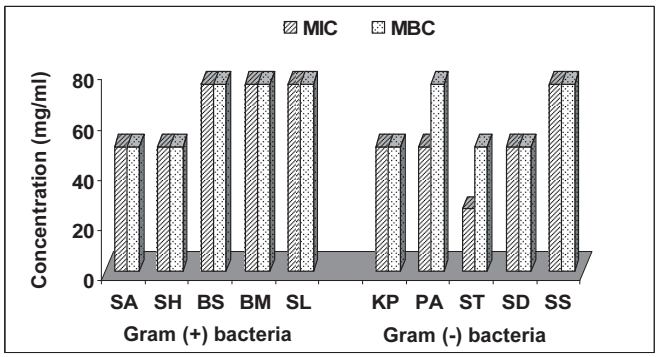

Figure 3. MIC and MBC of methanol seed extract of cardamom against Gram positive and Gram negative bacteria.

MIC and MBC

MIC and MBC values of methanol seed extract of *E. cardamomum* against the studied 10 bacterial species are represented in the Fig. 3. The obtained MIC values are ranged from 25 to 75 mg/ml. The lowest MIC value was recorded against *S. typhi*. On the other hand, MBC values, obtained are ranged from 50 to 75 mg/ml. The lowest MBC value was recorded against *S. typhi* along with *S. dysenteriae*, *K. pneumoniae*, *S. aureus* and *S. haemolytica*.

On the basis of the entire experimental results like zone of inhibition and corresponding MIC and MBC determination, it can be suggested that Gram (-) bacteria such as *S. typhi*, *S. dys-*

enteriae, and *K. pneumoniae* and Gram (+) bacteria such as *S. aureus* and *S. haemolytica* were aggressively inhibited by the methanol seed extract of cardamom.

Discussion

Antibiotics are valuable drugs for the treatment of several human diseases; however, no doubt their overuse has made worldwide antimicrobial resistance. Therefore, scientists are giving top most priority in search of alternative antimicrobial drugs from different parts of medicinal plants. Diverse usage of *E. cardamomum* seeds such as in culinary practice like flavoring agent in food and drinks and as medicine for centuries as well have proved its non-toxicity feature for human health. Recently, several authors have reported that it has antibacterial, antifungal, anticancer, antioxidant, and also gastroprotective effect (Agaoglu et al., 2005; Sengupta et al., 2005; Jamal et al., 2006; Bansod and Rai, 2008; Singh et al., 2008; Lin et al., 2009; Sultana et al., 2010). The present study also reported that it has potent antibacterial activity against some human pathogenic Gram (+) and Gram (-) bacteria. According to the zone of inhibition, highest activity of methanol seed extract was observed against *S. typhi* whereas Arora and Kaur (2007) have demonstrated comparatively lower activity using hot water extract. Moreover, relatively higher activity was seen at high concentration of essential oil of cardamom against *S. typhi* (Singh et al., 2008). Interesting site of the present study is that, all the studied bacteria were inhibited by the methanol extract and in some cases methanol extract gave better antibacterial activity than tetracycline. This result indicates that the active compounds of seed extract can readily be dissolved or extracted in methanol that could be responsible for growth inhibition of these bacteria. It has been demonstrated that different phytoconstituents have different degree of solubility in different types of solvents depending on their polarity (El-Mahmood and Doughari, 2008). Moreover, several authors have conducted antibacterial study using methanol as an extraction solvent and shown comparatively better activity than others (Mashhadian and Rakhshandeh, 2005; El-Mahmood and Amey, 2007; El-Mahmood and Doughari, 2008; Singh et al., 2008). In the present study, bacterial species including Gram (+) and Gram (-) bacteria exhibited different degrees of sensitivity to the test extract that may occur due to the differences in the chemical composition and structure of cell wall of both types of microorganisms (Goyal et al., 2009). Present study showed fairly better activity of methanol extract against *S. aureus* while, other scientists have shown antibacterial activity using diethyl ether extract (Agaoglu et al., 2005), aqueous extract (Arora et al., 2007), essential oil (Singh et al., 2008), and ethanol extract (El-Malti et al., 2009). In addition, El-Malti et al. (2009) have presented growth inhibition ability of ethanol extract of cardamom against *S. sonnei* while in the present study, methanol extract exhibited comparatively better activity. Furthermore, this extract has also shown potential inhibitory effect against *S. haemolytica*, *B. subtilis*, *B. megaterium*, *S. lutea*, *K. pneumoniae* and *S. dysenteriae* that clearly indicates the presence of broad spectrum active compounds.

The lowest MIC and MBC values obtained by methanol seed extract against *S. typhi* is a good indication of potential growth inhibition ability of this extract, while highest MIC and MBC value obtained against *Bacillus subtilis*, *Bacillus megaterium*, *Sarcina lutea*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Shigella sonnei* indicate that spice extract has less growth inhibition ability against those bacteria. During the present study, tetracycline was used as reference drug for positive control. Remarkable point of our findings is that tetracycline failed to show satisfactory inhibition of some bacteria, even in some cases poorer than our test extract despite being a broad spectrum antibiotic with refined and purified state. From this observation it can be suggested that crude methanol seed extract with a complex mixture of substances might be a potential source of antibiotic like compound that could be further explored.

Conclusion

On the basis of the experimental results and discussion, it can be postulated that the methanol seed extract of cardamom possesses the potent antibacterial properties. Specifically, this extract was more active against *S. typhi*. Not only *S. typhi* but also rest of the studied bacteria was susceptible to the methanol extract that indicates the presence of active compounds. Therefore, further studies will be needed on the methanol seed extract for the isolation of respective pure compounds.

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