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DETERMINATION OF ANTIBACTERIAL ACTIVITY OF COMMONLY USED SPICES IN LOCAL CUISINE OF BANGLADESH

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ABSTRACT

Mondol MAM (2019) Determination of antibacterial activity of commonly used spices in local cuisine of Bangladesh. *Int. J. Expt. Agric.* 9(2), 20-25.

Wide spread development of resistance in human pathogens against common antibiotics due to irrational usages. Such concern necessitates searching for new antibiotics from potentially effective, safer and natural alternative sources. 17 spices used in local cuisine of Bangladesh were selected for the evaluation of their antimicrobial activity. Antibacterial activity of methanol extracts of these spices were tested against one strain of Gram positive (*Staphylococcus aureus*) and three strains of Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus* sp.) multidrug resistant bacteria at a concentration of 1 mg/disc. Black pepper, cinnamon and green cardamom extracts were only moderately active against *S. aureus*. These active spice extracts may be used as a source of natural alternatives of conventional antibiotics to treat multidrug resistant bacteria.

Key words: *antibacterial activity, antibiotics, inhibition zone, methanol extract, multidrug resistant pathogens*

INTRODUCTION

Spices have been used in Asian countries including Bangladesh since ancient time (Lai and Roy, 2004) as natural medicine and food preservatives in recent decades (Nabavi *et al.* 2015 and Zheng *et al.* 2016). Spices could be seeds, fruits, roots, barks, leaves, stems etc which contain natural compounds exhibit antimicrobial properties (Indu *et al.* 2006). Spices are more commonly used in warmer climates, where infectious diseases are common. In addition to these, the spices are also used for the production of cosmetics and perfume and as fresh vegetables.

Traditionally, the plant extracts have been using to treat a wide range of diseases like asthma, urinary tract infection, intestinal disorder, fever etc in the rural areas of Asian and African countries (Tenover *et al.* 2004 and Chang 1995) which are cost effective and eco-friendly. The era of available antibiotics is going to terminate due to rise in drug resistant pathogens (Moellering 2012; Kluytmans and Diederen, 2008 and Rehm 2008). Infectious diseases caused by multidrug resistant pathogens (which are in rise due to irrational uses of antibiotics) increase morbidity, mortality, treatment cost and hospital staying. Currently, at least 0.7 million people die each year due to drug-resistant diseases (Abadi *et al.* 2019) as crucial antibiotics become ineffective. So, now it has become inevitable to search for new antibiotics to control drug resistant pathogens.

Many spices-such as garlic, clove, cinnamon, black cumin seed etc have been used to treat infectious diseases or preserve food as they were experimentally proved to have antimicrobial properties against pathogenic and spoilage fungi and bacteria (Lai and Roy, 2004; De *et al.* 1999, Arora and Kaur, 1999). The unique aroma and flavour are generally derived from phytochemicals or secondary metabolite present in spices (Avato *et al.* 2000). These phytochemicals are capable of fighting against harmful organisms which are recognized as safe with insignificant adverse effects (Nabavi *et al.* 2015). Therefore, spices could be candidates to discover and develop new antimicrobial agents against foodborne and human pathogens.

The objectives of this research were to study antimicrobial activity of methanol extracts of some spices commonly used in Bangladesh against clinical isolates of four multidrug resistant pathogenic bacteria and evaluate the antimicrobial activity of spices by comparing with different antibiotics in order know to whether the extracts can be employed as replacement or as an adjuvant to well established chemotherapeutic agents.

MATERIALS AND METHODS

General experimental procedures

Sterile filter paper disk (BioMaxima S.A., Poland), Mueller-Hinton agar (Scharlau, Spain), potato dextrose agar (PDA) (Scharlau, Spain), filter paper (Whatman Int. Ltd, Maid Stone, England), heavy duty blender (Havells, India), methanol (Merck, Germany) and 13 standard antibiotics (HiMedia, India) were bought from local suppliers. Sterilization, aseptic works and solvent evaporation were done using vertical autoclave machine (Model: LVA-202, Labocon, UK), horizontal laminar airflow cabinet (Model: LLFH-204, Labocon, UK) and rotary evaporator (Model: HS-2005S-N, Hahnshin S&T Co., Ltd, Korea).

Preparation of spice extracts

The fresh spice samples used in the present study were collected from the local market. Spices were dried in hot air oven at 40°C for two days and were powdered using a blender. In order to obtain extract of the spices, 5 g fine powder of each species were soaked in 100 ml of methanol, left for overnight and filtered through Whatman filter paper No. 1 to obtain a clear filtrate. The filtrates were evaporated and dried at 40°C under reduced pressure using rotatory vacuum evaporator. The extract yields were weighted and yield percentages were calculated using the following formula: Extract yield (g/100 g) = $(W_2 \times 100)/W_1$ where W_2 is the weight of the extract residue obtained after solvent removal and W_1 is the powder weight of spices.

Antibacterial activity of the spice extracts

Collection of test pathogens

The antibacterial potency of each spice extract was evaluated against four multidrug resistant bacterial strains causing infectious diseases: one strain of Gram positive (*Staphylococcus aureus*) and three strains of Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Proteus* sp.). Multidrug-resistant test pathogens were collected from the Microbiology Laboratory, Rajshahi Medical College, Rajshahi, Bangladesh. These test pathogens were isolated from the clinical samples of the patients.

Preparation of test pathogen inoculums

To prepare the seed culture, each test pathogen was streaked on the sterilized Mueller-Hinton agar medium (prepared according to the manufacturer instruction) from stock culture and then incubated at 37°C for 24 hours. Inoculum of each test pathogenic bacterium was prepared by suspending colonies from fresh 24 hours culture plate into the test tube containing sterilized physiological saline solution (0.9% NaCl w/v). The turbidity of each suspended bacterium was adjusted either by the addition of more colonies or saline water to 0.5 McFarland standard corresponding to 1.5×10^8 CFU/ml (Kiehlbauch *et al.* 2000). These inoculums were used to study activity of spice extracts.

Antibacterial activity test of the spice extracts

The antibacterial activity test of the spice extracts was done by using a disc diffusion method (Jorgensen and Turnidge, 2015). 50 μl of the standardized suspension of bacterial strains of 1.5×10^8 CFU/ml was diffused on the Mueller Hinton agar (MHA) medium with sterilized swabs. Each extract was diluted with an appropriate combination of ethyl acetate and methanol in such way that 10 μl contains 1 mg extract. 10 μl of each extract was soaked in sterile filter paper discs (6 mm diameter), fully dried under laminar air flow cabinet and then placed on swabbed agar and incubated at 37^oC for 24 h. The diameters of zones of inhibition were measured in millimeters using a ruler. 13 standard antibiotics and blank discs were used as positive and negative control, respectively.

RESULTS AND DISCUSSION

The yield of extract obtained from different spices is shown in Table 1. The highest yield of plant extract was obtained from *Syzygium aromaticum* (28.8%) followed by *Myristica fragrans* (20.0%) while *Coriandrum sativum* gave the lowest extract yield (3.6%).

Table 1. Local, common and botanical names of the spices used in activity test against multidrug resistant bacteria with their percentage of methanol extract yield

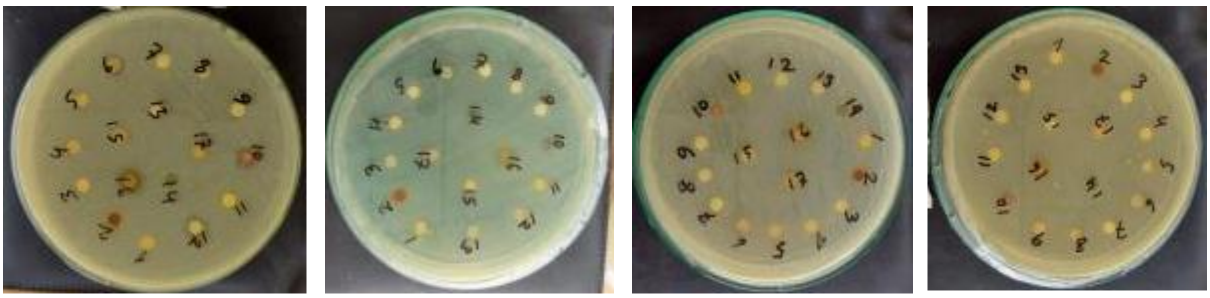
SL	Local Name	Common Name	Botanical Name	Parts used	Yield (%)
1.	Ada	Ginger	*Zingiber officinale*	Rhizome	10.8
2.	Holud	Turmeric	*Curcuma longa*	Rhizome	9.0
3.	Rosun	Garlic	*Allium sativum*	Clove	11.4
4.	Jayatri	Mace	*Myristica fragrans*	Aril of fruit	20.0
5.	Golmorich	Black pepper	*Piper nigrum*	Seed	8.6
6.	Kalozira	Black cumin	*Nigella sativa*	Seed	7.5
7.	Methi	Fenugreek	*Trigonella foenum-graecum*	Fruit	9.8
8.	Donia	Coriander	*Coriandrum sativum*	Fruit	3.6
9.	Mori	Fennel	*Foeniculum vulgare*	Seed	9.4
10.	Daruchini	Cinnamon	*Cinnamomum verum*	Bark	14.3
11.	Zera	Cumin	*Cuminum cyminum*	Seed	11.4
12.	Choto elach	Green cardamom	*Elettaria cardamomum*	Fruit	5.7
13.	Jayfal	Nutmeg	*Myristica fragrans*	Fruit	11.4
14.	Tespata	Bay Leaf	*Laurus nobilis*	Leaf	13.0
15.	Kalo elach	Black cardamom	*Amomum subulatum,*	Fruit	11.2
16.	Lobonggo	Clove	*Syzygium aromaticum*	Flower buds	28.8
17.	Star masla	Star aniseed	*Pimpinella anisum*	Seed pods	18.6

Of the 17 spices tested for their antibacterial effect, only black cumin seed, cinnamon and green cardamom exhibited moderate activity against *S. aureus* (10 mm zone of inhibition) (Table 2 and Fig. 1). Unfortunately, other spice extracts did not show any activity against tested pathogens at applied concentration (1 mg/disc). Out of 13 standard antibiotics, only imipenem (10 μg/disc) showed activity against four tested pathogenic bacteria followed by ciprofloxacin (5 μg/disc) exhibited activity against three tested bacteria (Table 2 and Fig. 2). Test pathogens, *S. aureus* and *Proteus* sp. were sensitive to the most standard antibiotics. All the tested pathogens were completely resistant to the standard antibiotics cloxacillin (5 μg/disc), azithromycin (15 μg/disc) and clindamycin (2 μg/disc) (Table 2 and Fig. 2).

Table 2. Antibacterial activity of spice extracts (1-17) and standard antibiotics (1-13)

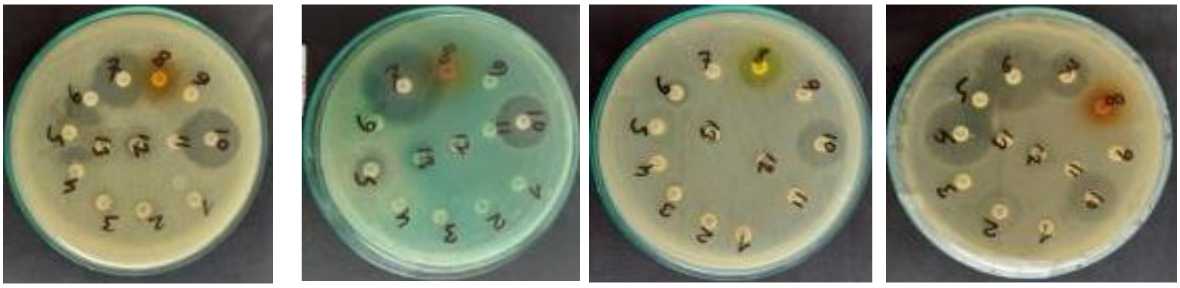
SN	**Spice extract (1 mg/disc)**	**Zone of inhibition (in mm)**			
		S. aureus	***P. aeruginosa***	***E. coli***	***Proteus* sp.**
1.	Ginger	-	-	-	-
2.	Turmeric	-	-	-	-
3.	Garlic	-	-	-	-
4.	Mace	-	-	-	-
5.	Black pepper	-	-	-	-
6.	Black cumin seed	10	-	-	-
7.	Fenugreek	-	-	-	-
8.	Coriander	-	-	-	-
9.	Fennel	-	-	-	-
10.	Cinnamon	10	-	-	-
11.	Cumin	-	-	-	-
12.	Green cardamom	10	-	-	-
13.	Nutmeg	-	-	-	-
14.	Bay Leaf	-	-	-	-
15.	Black cardamom	-	-	-	-
16.	Clove	-	-	-	-
17.	Star aniseed	-	-	-	-
SN	**Standard antibiotics (μg/disc)**				
1.	Cefradine (25 μg)	-	-	-	8
2.	Amoxycilline (30 μg)	12	-	-	20
3.	Doxycycline (30 μg)	15	-	-	-
4.	Cefixime (5 μg)	20	-	-	25
5.	Erythromycin (15 μg)	28	20	-	12
6.	Ceftriaxone (30 μg)	-	-	12	30
7.	Ciprofloxacin (5 μg)	23	25	-	20
8.	Nitrofurantoin (300 μg)	18	-	17	15
9.	Clindamycin (2 μg)	-	-	-	-
10.	Imipenem (10 μg)	25	25	20	20
11.	Cloxacillin (5 μg)	-	-	-	-
12.	Azithromycin (15μg)	-	-	-	-
13.	Vancomycin (30 μg	20	-	10	-
14.	Negative control (Sterile disc)	0	0	0	0

″-″ Not active at tested concentration



S. aureus *P. aeruginosa* *E. coli* *Proteus* sp.

Fig. 1. Activity test plates of spice extracts against multidrug resistant bacteria



S. aureus *P. aeruginosa* *E. coli* *Proteus* sp.

Fig. 2. Activity test plates of standard antibiotics against multidrug resistant bacteria

Most of the pathogenic bacteria have developed resistance to the currently available antibiotics due to their misuse or overuse. This situation has led to an urgent need to explore different sources for development of efficient, less toxic and cost-effective antimicrobial agents (Russell 1999 and Sheldon 2005).

In this study, 3 (black pepper, cinnamon and green cardamom) out of 17 tested spices displayed same degree of antibacterial activity against Gram-positive multidrug resistant (MDR) strain of *S. aureus* and none of them were active against Gram-negative MDR strains of *P. aeruginosa*, *E. coli* and *Proteus* sp. at applied concentration (1 mg/disc). This result was supported by the many previous studies where Gram-positive bacteria were more susceptible to the tested spice extracts than Gram-negative bacteria (Zhang *et al.* 2019; Benmeziane *et al.* 2018) because of the differences in the organization and components of the cell wall structure.

The antibacterial activity of some spice extracts tested in this study was also reported previously mainly against nondrug resistant bacteria (Zhang *et al.* 2019; Shihabudeen *et al.* 2010 and Ceylan and Fung, 2004). However, it is very difficult to compare the results of this study directly with the results of previous study due to the difference in extraction solvent, extraction method and dosage of samples. More important is that the inhibitory effects of spice extracts on multi-drug resistant bacteria were relatively less reported.

The antimicrobial activity of spice extracts is mainly attributed to phytochemicals present in plants group as glucosides, saponins, tannins, alkaloids, essential oils, organic acids and others (Zhang *et al.* 2019). Methanol extract of black cumin seed showed board spectrum antimicrobial activity (Erdogrul *et al.* 2009). Cinnamon and clove powder showed a strong anti-microbial activity against *E. coli, S. aureus, B. thermosphacta* and *L. rhamnosus* with a MIC ranging from 1.0% to 1.5% (wt/v), while the MIC against *P. fluorescens* was 2.0% of clove and 2.5% of cinnamon, respectively (Kuang *et al*. 2011). Ethanol extract of green cardamom exhibited MICs ranging from < 2.34-18.75 mg/ml against a range of both Gram (+ve) and Gram (-ve) bacteria (Malti *et al*. 2007).

Further analysis of literature data, it has been shown that antimicrobial effects of 3 extracts (ethyl acetate, acetone, and methanol extracts) of clove were tested against eight common pathogenic bacteria (two Gram positive and six Gram negative) by the disc diffusion method (Keskin and Toroglu, 2011). The methanol extract showed zone of inhibition in the range of 8-24 mm against tested pathogens. In another experiment, methanol extract obtained from fennel seeds tested against 3 Gram (+) and 4 Gram (-) bacteria exhibited the best antimicrobial activity with the largest inhibition zone except *E. coli* (Nguyen *et al*. 2014). One thing is clear that the methanol extracts obtained from different spices showed more antimicrobial efficacy than other solvent extracts (Liu *et al.* 2017; Kapilan 2015 and Pandey *et al.* 2014).

Bioassay-guided isolation of bioactive compounds from active spices (black cumin seed, cinnamon and green cardamom) of this work and subsequently optimization of their dosages may lead to the development of novel antimicrobial agents to respond against the growing problem of existence of MDR bacteria.

CONCLUSION

The purpose of this research was to identify active spices against MDR pathogenic bacteria. The present study has identified three spices (black pepper, cinnamon and green cardamom) exhibiting antibacterial activity against drug-resistant human pathogens (Gram positive and Gram negative). They may emerge as a potential therapeutic source to treat hospital-acquired, wound infections. However, there is further need to isolate active compounds from active spices, *in vitro* and *in vivo* experiment to find their mode of action and metabolism in order to develop new antibacterial agents.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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