Original Article:

Antimicrobial effects of Zingiber officinale extracts against multi-drug resistant Acinetobacter baumannii clinical isolates recovered from hospitalized patients in ICU

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ABSTRACT

Zingiber officinale as an herbal medicinal plant is used for its potential antimicrobial activity against different microbial pathogens. Multi-drug resistant Acinetobacter baumannii as an important nosocomial pathogen especially in intensive care units is responsible for a wide range of serious infections in humans. The purpose of this study was to investigate the antimicrobial activity of Z. officinale extracts on growth of A. baumannii strains isolated from hospitalized patients in intensive care units in Tehran, Iran. During a 9 month study, 50 multi-drug resistant A. baumannii isolates were recovered from patients in ICU. The Kirby-Bauer disk diffusion method was used to determine resistance patterns of Multi-drug resistant A. baumannii isolates to antimicrobial agents. Micro-broth dilution method was used to determine the antimicrobial activity of methanol, acetone, and chloroform extracts of Z. officinale against multi-drug resistant A. baumannii isolates. The results of susceptibility testing showed that all the isolates were resistant to ceftriaxone, ciprofloxacin, ceftazidime, cefotaxime, cefepime and piperacillin. Resistance to colistin was found to be low (4%) and exhibited good antibacterial activity against tested isolates. This study's findings revealed that methanol, acetone, and chloroform extracts of Z. officinale have anti-bacterial activity against tested bacterial isolates. Based on the results, the chloroform extracted fraction showed the highest level of activity at a minimal inhibitory concentration of 25 mg/ml on multi-drug resistant A. baumannii (64%). The minimal inhibitory concentration of ginger extract was as low as 3.2 mg/ml. The present study indicated that Z. officinale extracts, at various concentrations could be used as an antibacterial agent for treatment of patients in ICUs.

Key words: Acinetobacter baumannii; Zingiber officinale; Ginger; MDR

INTRODUCTION

Acinetobacter baumannii (A. baumannii) as an important nosocomial pathogen especially in Intensive Care Units (ICUs) is responsible for a wide range of infections that can be ranged from surgical wounds, urinary tract infections, meningitis, and bacteremia to ventilator-associated pneumonia and other life threatening infections [1, 2]. According to the previous published data, resistance to antimicrobial agents among A. baumannii and emergence of multidrug resistant A. baumannii (MDRAB) isolates are increasing which could be a serious concern in global public health. A survey in 2008, conservatively estimated that in the US, more than 12000 people every year are affected by A.

baumannii infections among which 7300 isolates (63%) are confirmed as MDRAB strains with at least 500 dying as a result of the infection [3, 4]. Antibiotic resistance as a global multifaceted phenomenon has become a major threat to global health which highlights the need for heightened awareness among clinicians, veterinarians, and policymakers [5, 6]. In fact, infection with A. baumannii clinical isolates are increasingly becoming extremely difficult, and sometimes impossible to treat which is one of the main factors influencing morbidity and mortality among patients undergoing these infections [7]. With this background, over the past several decades, many investigators have paid attention to antibacterial activities of natural plants to combat antibiotic-resistant bacteria. According to data reported by World Health Organization (WHO), the use of traditional folk medicine plant is common in more than 80% of the world's population [8, 9]. During recent years, extracts, essence and oils of herbal plants with antibacterial and anti-fungal effects have been recruited for treatment of many human infectious diseases [10]. In many of the cultures of Asia, Africa, and some areas of America, traditional medicine has been used for decades to maintain human health and also treat various human diseases [11]. These herbal plants contain ingredients that used for pharmaceutical, food and cosmetic industries [12, 13]. Many studies have displayed several biological activities of herbal medicine including antioxidant, anti-inflammatory, cell proliferation, immune modulating, as well as antibacterial, antiviral, and antifungal properties [14-16]. Zingiber officinale (Z. officinale**),** commonly known as ginger, is one of these well-known herbal plants that used as a popular spice in foods, desserts and drinks all around the world. Ginger is a plant with thick roots and vertical, upright stems. To date, more than 400 active ingredients have been identified in ginger. It has been traditionally used for treatment of allergy, constipation, asthma, diabetes, nervous disorders, gastrointestinal disorders, cardiovascular disease and respiratory tract infections [17]. Furthermore, various studies have confirmed several biological activities of ginger including antioxidant, anticoagulation activities and anti-inflammatory [18-24]. Although antimicrobial activities of ginger has been documented, there is scant data about in vitro antimicrobial activity of ginger against MDR clinical isolates. Considering these points, the present study was carried out in order to investigate antibacterial activity of methanol, acetone and chloroform extracts of ginger against MDRAB strains isolated from hospitalized patients in intensive care units (ICUs).

MATERIALS AND METHODS

The present cross-sectional research was conducted from May 2016 to January 2017 on 50 MDRAB strains recovered from different samples of hospitalized patients in ICUs. One isolate per patient was included in the study and duplicate samples were excluded. Bacterial identification was done based on the conventional microbiological methods and the API 20 NE system (bioMérieux SA, Marcy-1'Etoile, France). To ensure identification, A. baumannii isolates were subjected to polymerase chain reaction (PCR) for detection of blaOXA-51-like gene as previously described (Figure 1) [6]. Confirmed A. baumannii isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for further investigation.

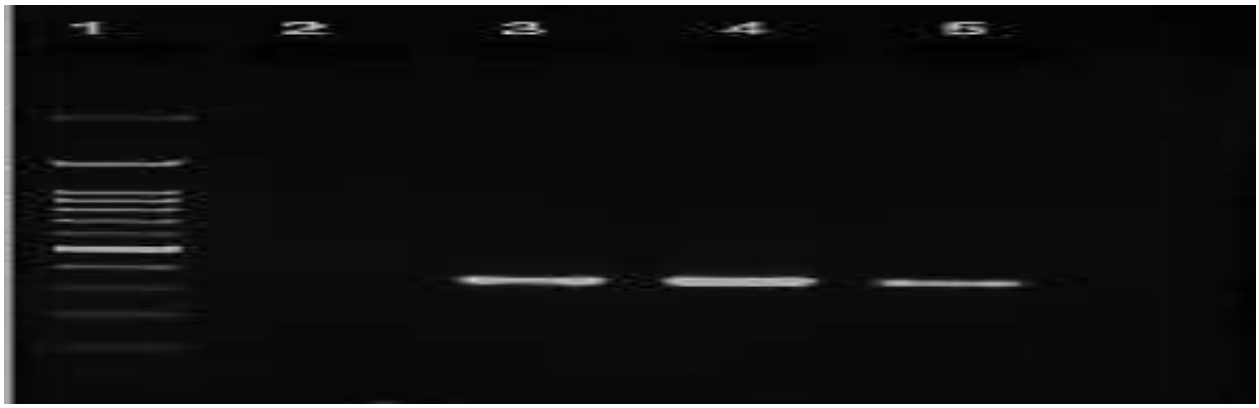

Figure 1. PCR of OXA-51 gene products. Lane 1,100 pb DNA ladder; Lane 2, negative control; Lane 3 positive control; Lane 4 and 5, OXA-51 gen

Antimicrobial Susceptibility Testing (AST)

The susceptibility of MDRAB isolates were assessed using the Kirby-Bauer disk diffusion technique, according to CLSI guidelines for cefotaxime, ceftriaxone, cefepime, ciprofloxacin, ceftazidime, piperacillin tazobactam, imipenem, meropenem, amikacin, gentamycin, trimethoprim/sulfamethoxazole, tetracycline, colistin and piperacillin antibiotics. All the antibiotic disks used in the present study were supplied by Mast Co., UK. Pseudomonas aeruginosa strain ATCC 27853 was used as quality control strains in each run test.

Preparation of methanol, acetone, and chloroform extracts of ginger

Fresh rhizome of the plant was purchased and dried in shadow at room temperature of 25 °C. The rhizome was ground into fine powder using an electrical blender. One hundred grams of the powder was mixed in one liter of methanol, acetone, and chloroform separately and kept at room temperature for 3 days. After three days, suspensions were filtered using a Whatman no. 1 filter paper. The filtered solutions were sterilized using a 0.45 micrometer membrane filter. Then, the solutions became concentrated and vacuum-dried at a low temperature. Dried extract was stored at -20°C.

MIC of ginger

To determine the MIC of ginger against MDRAB isolates broth, microdilution method was carried out in 96-well cell culture plates. In brief, the bacterial suspensions, adjusted equivalent to a no. 0.5 McFarland standard (approximately 1.0×10^8 CFU/mL) were further diluted 1:100 (10^6 CFU/mL) in the broth media. 100µl of broth medium was added to each well. Fifty mg/ml dilution was considered as stock. Serial dilutions of ginger extract (25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39 and 0.19 mg/ml) were prepared as follow; 100 µl of the extract (50 mg/ml) was added to the first well to achieve 1/2 dilution. Next, 100 µl of the first well was transferred to the second well to achieve 1/4 dilution and concentrations of the extract were prepared and dispensed in 96-well cell culture plates. Twenty microliters of each bacterial suspension was added to each well and incubated at 37˚C overnight. Positive and negative controls were used in each run test [6]. To estimate MIC of ginger, the absorbance of each well was measured at 595 nm.

Data analysis

Statistical analysis was performed using SPSS software for Windows, version 17.0 (SPSS Inc., Chicago, IL).

RESULTS

The average age of the patients was 41 years (range, 1 to 86 years). A. baumannii infection was highest in the 46-60 year age group (40%) and lowest in the 1 to 15 years age group (2%). From 50 isolates included in the study, 35 isolates (70%) were recovered from male patients and 15 isolates (30%) from female patients. According to the AST results, all isolates were resistance to ceftriaxone, ciprofloxacin, ceftazidime, cefotaxime, cefepime and piperacillin. The highest level of resistance among tested isolates was observed to be related to piperacilin

tazobactam (98%), followed by imipenem (96%), meropenem (96%), trimethoprim/sulfamethoxazole (88%), gentamycin (86%), amikacin (80%) and tetracycline (64%).

Antibacterial activity of ginger extracts

The findings obtained of antibacterial activity assessment of ginger revealed that at MIC 25 and 12.5 mg/ml, chloroform extract, at MIC 6.25 mg/ml both methanol and acetone extract and at MIC 3.125 mg/ml acetone and chloroform extract had the most antibacterial activity against MDRAB isolates. These differences were not significant (P>0.05). None of the MDRAB isolates were sensitive to dilutions of ginger equal and less than 0.78 mg/ml. In vitro susceptibility of different concentration of extracts against the MDRAB isolates are summarized in Table 1.

Table 1. Summary of the antibacterial activities of methanol, acetone and chloroform extracts of ginger against MDRAB isolates.

Ginger extract		**MIC (mg/ml) N (%)**							
		25	12.5	6.25	3.125	1.56	0.78	0.39	0.19
Methanol	MIC	30 (60)	11 (22)	6 (12)	3 (6)	0 (0)	0 (0)	0 (0)	0 (0)
	MBC	41 (82)	6 (12)	3 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acetone	MIC	22 (44)	13 (26)	6 (12)	4 (8)	5 (10)	0 (0)	0 (0)	0 (0)
	MBC	22 (44)	13 (26)	6 (12)	4 (8)	5 (10)	0 (0)	0 (0)	0 (0)
Chloroform	MIC	32 (64)	15 (30)	5 (10)	4 (8)	4 (8)	0 (0)	0 (0)	0 (0)
	MBC	32 (64)	15 (30)	5 (10)	4 (8)	4 (8)	0 (0)	0 (0)	0 (0)

DISCUSSION

Although antimicrobial properties of ginger are primarily recognized, there have been no published data on MDRAB isolates in Iran. The results of the present study showed that 98% of MDRAB strains isolated from hospitalized patients in ICU were resistant to at least three antibiotics. The evaluation of pattern of antibiotic resistance of MDRAB isolates also showed that the highest resistance rate was reported to be against ceftriaxone, ceftazidime, cefotaxime, cefepime and ciprofloxacin while the lowest rate of resistance was reported for colistin, tetracycline and amikacin.

In line with the present study's results, Arunava et al. (2013) reported highest resistance rate against gentamycin (100%), ceftazidime (81.1%), amikacin and ciprofloxacin (72.7%). They showed that 22.4% of tested isolates were resistant to imipenem and colistin and polimixin B had good activity against A. baumannii strains. The comparison of the rate of resistance in both studies shows that colistin is considered as the best antibiotic for treatment of A. baumannii infections. However, difference in antibiotic resistance patterns in various studies could be related to factors such as geographical area under study, type of samples, duration of study and also the special therapeutic protocols. In another study form Iran, the rate of resistance to tazobactam, ticarcillin, amikacin and imipenem were 88.2%, 84.7%, 81.7% and 52.9% respectively [25]. In this study, polymixin B (90%) and colistin (65.9%) had the best antibacterial activities against A. baumannii strains.

In another study conducted in Iran by Meradji et al. in which 80 A. baumannii strains were investigated, the highest resistance rate was reported for ticarcillin (61.25%), followed by ceftazidime and ticarcillin/clavulanic acid (56.25%). Likewise, colistin and amikacin were determined as the most effective antibiotics in this study with 93.90% and 100% sensitivity respectively [26]. There are discrepancies in the antimicrobial effects of various extracts of ginger. In the present study, the majority of strains were inhibited by methanol, chloroform and acetone extract of ginger at MIC 25 mg/ml. The results of observations exhibited good activity of ginger extracts against MDRAB isolates. However, the acetone extract of ginger showed that its antibacterial activity initiated in lower concentration than other extracts, but there is no significant statistical difference. Ranjit Thakur (2013) evaluated the antibacterial effect of ethanol extract of ginger, cinnamon and turmeric on Escherichia coli and P. aeruginosa and Klebsiella pneumonia strains by disk diffusion method. The results of their study showed that ethanol extract of turmeric inhibited the growth of all the bacteria while ethanol extract of cinnamon did not show any effect on these

bacteria. Ethanol extract of ginger had a high inhibitory effect on growth of E. coli and showed a moderate effect on P. aeruginosa and klebsiella [15]. In a study conducted by Usha et al. (2012) in India, the antibacterial effects of ethanol and acetone extract of cinnamon on strains of P. aeruginosa were evaluated by disk diffusion and MIC methods. The MIC of ethanol and acetone extract of cinnamon on P. aeruginosa was 32 mg/ml and 16.64 mg/ml respectively [27]. Gull et al (2012) evaluated the antibacterial effects of aqueous, methanol and ethanol extracts of garlic and ginger on P. aeruginosa by disk diffusion and MIC methods. They found that aqueous extract of garlic had excellent antibacterial activity against all the test strains while aqueous extract of ginger did not show any antibacterial activity. They also showed that strains were inhibited by aqueous extract of garlic at MIC 0.2-0.5 mg/ml, ethanol extract at MIC 0.05-0.5 mg/ml and methanol extract at MIC 0.09-0.7 mg/ml [28]. In another study conducted in India, antibacterial effect of garlic and ginger extracts were assessed against MDR P. aeruginosa. This study exhibited that ethanol extract of garlic has higher and broader spectrum of antimicrobial activity than ginger on P. aeruginosa [29]. In Adeshina's study who considered the antibacterial activity of aqueous extract of red and white onion on Escherichia coli, P. aeruginosa, Salmonella typhi and Staphylococcus aureus, all the tested isolates were resistant to aqueous extract of ginger and aqueous extract of red and white onion had a strong activity at MIC 3.125-25 with the exception of S. aureus [30]. The study of Aghazadeh et al in 2016 was done in order to evaluate the antibacterial activity of ethanol extract of ginger and its effect on biofilm formation. In this experimental study, the ethanol extract of ginger was evaluated as a mouthwash against fungi and bacteria. Their findings showed that P. aeruginosa, S. aureus, K. pneumonia, Bacillus cereus, A. baumannii, Candida albicans and Candida cruzei strains were inhibited by ethanol extract ginger at MIC 10, 20, 20, 20, 20, 40 and 40 mg/ml, respectively [31]. There are differences in the antimicrobial effects of various extracts of ginger. Wang et al (2010) showed that 6-hydro gingerol, 10-gingerol, 6- shogaol, and 6-gingerol are 4 main compounds present in ginger which have a strong antibacterial effect against extensively drug-resistant A. baumannii. It is well established that the concentration of this compounds in ginger is directly related to its antibacterial effect. Therefore, different organic solvents and applied techniques in preparation of ginger extract can be effective in its antibacterial activity [32].

CONCLUSION

In conclusion, the present study showed that ginger extract could be active against MDRAB strains at various concentrations and the use of it at optimum concentrations can help better therapy of many microbial diseases. However, further studies in order to identify bioactive components ginger are necessary and also help to use the ginger as an alternative option for treatment of many microbial diseases.

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"The authors declare no conflict of interest"

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