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Der Pharmacia Lettre, 2016, 8 (1):181-187
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Antimicrobial effect of Ginger (*Zingiber officinale*) and mallow (*Malva sylvestris*) hydroalcholic extracts on four pathogen bacteria

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ABSTRACT

Antibiotic resistance is growing and has limited ability of physicians to treat some infectious diseases. Discovery of new antibiotics or semi-synthetic derivatives affecting on resistant infections are main objectives for treatment of infectious diseases. The aim of this study was to investigate antimicrobial activity of Hydro-alcoholic extracts of ginger (Zingiber officinale) and Mallow (Malva sylvestris) against Pseudomonas aeroginosa, Staphylococcus aureus, Meticillin resistant Staphylococcus aureus and Listeria monocytogenesis as a number of important factors and infectious microorganisms. Antimicrobial effects of Hydro-alcoholic extracts of ginger (Zingiber officinale) and Mallow (Malva sylvestris) was evaluated by micro-plate dilution and disk diffusion methods. MIC of ginger extract for Staphylococcus aureus, Meticillin resistant Staphylococcus aureus, Pseudomonas aeroginosa and Listeria monocytogenesis were 52, 52, 416 1nd 52 µg/ml and MBC of this extract were 104, 104, 416 and 104 µg/ml, respectively. Growth inhibition zone of ginger extract for these bacteria were 16, 9, 7 and 8 mm, respectively. Mallow extract had no antibacterial effects on studied bacteria. Obtained results showed ginger extract can be introduced as bioactive antibiotic in traditional medicine, microbiology and pharmacology sciences.

Key words: *Zingiber officinale* extract, *Malva sylvestris* extract, antibacterial effect, MIC, MBC.

INTRODUCTION

Infectious diseases are directly responsible for 25% of death throughout the world [1]. Despite advances in the field of infectious diseases, these diseases are debilitating factor and ultimately cause of death for millions people, worldwide [2].

Pseudomonas aeruginosa is an opportunistic pathogen of human which started its infectivity from connective tissues. The organism in patients with severe burns, cancer and AIDS patients who have a suppressed immune system cause infections in urinary tract and respiratory systems, soft tissues, bones and joints, digestive tract and also systemic infections [3]. *Staphylococcus aureus* can cause bacterial infections, Abscesses and sty, osteomyelitis, endocarditis, food poisoning and toxic shock syndrome in human [4]. *Listeria monocytogenes* found in the digestive tract and nature and its bacterial infection can lead to diseases with flu-like symptoms, abortion in women, meningitis and septicemia [5].

Antibiotic resistance is growing and has limited ability of physicians to treat some infectious diseases. Incidence and spread of drug resistance in major infectious diseases such as respiratory infections, diarrhea, tuberculosis and malaria has created major problems [6]. Therefore, deal with drug resistance phenomenon for reducing new resistances or limiting resistant microbial agents confirmed by World Health Organization. Discovery of new antibiotics or semi-synthetic derivatives affecting on resistant infections are main objectives for treatment of infectious diseases [7, 8]. Antibiotic resistance is reported exclusively which caused cost increase for each drug [9]. Medicinal plants have antioxidants and bioactive metabolites and are used for treatment of diseases in traditional medicine and ethnopharmacology [10]. Herbs are used in traditional medicine of Iran as a source of medicines for the treatment of various diseases including parasitic diseases [11], neurological and psychiatric disorders [12], respiratory diseases [13], headache and migraine [14] and many other diseases .

Mallow (*Malva sylvestris*) is a yearling, biennial or rarely perennial plant of Malvaceae family. This plant originated in central Asia and is growing around the world including Iran.

Flowers of this plant contain anthocyanins and mucilage. All parts of Mallow, particularly flowers having an impact on softening of respiratory tract. This effect may be due to its high mucilage content [15].

Ginger (*Zingiber officinalae*) of Zingiberaceae family cultivated in humid tropical regions especially India as the largest producer. Ginger is a beautiful plant with glandular branching and rugged rhizome, long and sharp leaves and flowering stems.

Indian and Chinese used ginger to treat many diseases. For thousands of years it has been used to treat different diseases such as colds, nausea, arthritis, migraine, cancer, asthma, dementia, ulcerative colitis, diabetes and high blood pressure. It also has antioxidant, anti-inflammatory, analgesic properties in animals [16, 17].

Ginger (*Zingiber officinalae*) is used in Iranian traditional medicine for treatment of Colds, Fever, and Menstrual pain, Headaches, joints Pain, Nausea, bloat, Indigestion and vomiting. Mallow (*Malva sylvestris*) is used in Iranian traditional medicine for treatment of chest diseases, cough, sore throat, skin, kidney and bladder infections, constipation and other digestive problems and dysentery and intestinal inflammations [18, 19].

Active metabolites of medicinal plants have low toxicity and are useful for treatment of infectious diseases [20]. Due to wide spreading of infectious diseases, Side effects of antibiotics and bacterial resistance to antibiotics, identification of medicinal plants and purification of their active compounds are useful in the treatment of diseases. Medicinal plants traditionally are one of the most valuable resources in medicine and can be used as synthetic drugs with plant origin. Medicinal plants not only involved in the treatment of infectious diseases but at the same time reduce a large number of antibiotic side effects [21].

Due to emphasis of Iranian traditional medicine on the antimicrobial activity of ginger and Mallow, the aim of this study was to investigate antimicrobial activity of hydroalcholic extracts of ginger and Mallow against *Pseudomonas aeroginosa*, *Staphylococcus aureus*, Meticillin resistant *Staphylococcus aureus* and *Listeria monocytogenesis* as a number of important factors and infectious microorganisms.

MATERIALS AND METHODS

PLANT MATERIAL

Mallow collected in mountains and ginger provided of market of Khorramabad city in Lorestan province, west of Iran. Genus and species of plants were identified in agricultural research center of Lorestan province of Iran.

PREPARATION OF GINGER EXTRACT

Ginger samples was dried in shade and powdered by a mixer. Ginger extract was prepared by maceration method. 196 grams of ginger powder solved in a mixture of 350 ml of ethanol (70%) and 150 ml of distilled water. suspension was filtered using a whatman filter paper no 3. Dryness of suspension was carried out under vacuum at 40 ◦C with a rotary evaporator (RV-10 digital, IKA, Germany) for 72 hours. Hydro-alcoholic extraction yield of ginger was determined 2.34%.

PREPARATION OF MALLOW EXTRACT

Mallow leaves were dried in shade and powdered by a mixer. Maceration method was used to extraction. 56 g of mallow powder solved in a mixture of 300 ml methanol and 100 ml of distilled water. Suspension bathed for 30 minutes in a water bath using ultrasonic cleaner bath 203H (Rocker inc.) at 40 °C. After that suspension was filtered using whatman filter paper no. 3. Dryness of suspension was carried out under vacuum at 40 ◦C with a rotary evaporator (RV-10 digital, IKA, Germany) for 72 hours. Mallow leaf extract yield was determined 5.4%. Extraction of mallow roots was prepared in the same way.

PREPARATION OF BACTERIAL STRAINS

All used strain of *Staphylococcus aureus* (ATCC:25923), *Pseudomonas aeruginosa* (ATCC: 27853) and *Listeria monocytogenesis* (ATCC: 27853) in this study was supplied of Pasteur Institute of Iran. Meticillin resistant *S.aureus* (MRSA) strain provided of clinical isolates in Ashayer hospital of Khoramabad city, center of Lorestan province, west of Iran.

ANTIMICROBIAL EVALUATION TESTS

Disk diffusion test (Kirby-Bauer method): 30 microliter of each plant extract was added to a blank disc. Discs were dried in an incubator at 40 ° C. Bacterial suspension of each strain was prepared in 0.5 McFarland concentrations. Each suspension streaked on a Mueller Hinton agar (Merck, Germany) plate. Discs of extracts (Ginger and mallow) and antibiotics discs (Mast, UK) were dropped in different zones of the culture on an agar plate for each selected bacteria. Plates were incubated for 24 h at 35 ° C and inhibition zone was measured with a ruler. Antibiotic containing disks (Penicillin, Vancomycin, Gentamycin and ciprofloxacin)(Mast, UK) were used as positive control in disk diffusion test.

Microdilution broth method: Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of each extract were determined using Microdilution broth method according to CLSI recommendation. 12 serial dilution of each extract started in 1664 concentration were prepared and 100 microliter of each dilution was added to wells of a 96-well plates. After that 100 microliter of prepared 0.5 McFarland suspensions in Mueller-Hinton broth (Merk, Germany) of each selected bacteria was added to wells. Microplates incubated at 35◦C for 18-24 hours. Last well with Lake of growth was regarded as MIC. All dilutions with no bacterial growth subcultured in TSA medium (Merk, Germany) and were incubated at 35 °C for 24 hours. All plates that colonies growth not seen on them was known as MBC of plant extracts on selected strains of bacteria [22].

All experiments were repeated triplicate, positive and negative controls were used for both techniques.

RESULTS

Obtained results of microdilution method and disk diffusion tests for hydro-alcoholic extracts of ginger and Mallow showed that antimicrobial effect of ginger extract was very good, while leaves and roots extract of Mallow had no antimicrobial effect. Results and details of the antibacterial effects of extracts and controls are presented in Table 1.

Table 1. Results of microdilution and disk diffusion methods for hydro-alcoholic extracts of ginger (Zingiber officinalae) and Mallow on selected bacteria

	Staphilococus aureus			MRSA			*Pseudomonas aeruginosa*			*Listeria monocytogenesis*		
	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD
Ginger plant	52*	104*	16**	52	104	9	416	416	7	52	104	18
Fairy-cheeses plant (Root)	0	0	0	0	0	0	0	0	0	0	0	0
Fairy-cheeses plant (Leaf)	0	0	0	0	0	0	0	0	0	0	0	0
Penicillin	-	-	19	-	-	-	-	-	-	-	-	-
Vancomycin	-	-	-	-	-	18	-	-	-	-	-	-
Gentamycin	-	-	-	-	-	-	-	-	24	-	-	-
Ciprofluxacin	-	-	-	-	-	-	-	-	-	-	-	24

DD: Disk diffusion (Milimeter)
** Based on Microgram/milliliter*

DISCUSSION

Present study was designed to evaluate effects of hydroalcholic extracts of Mallow (*Malva sylvestris*) and Ginger (*Zingiber officinalae*) on important infectious bacteria. Obtained results showed good antibacterial effects of ginger extract and no antibacterial effect of Mallow extracts. Ginger extract showed strong antibiotic effects on all studied bacterial strains, which indicating antibacterial effects of its ingredients.

Zingerone, Gingirdiol, Zingibrene, and particularly Gingerol and Shagol, Sesquiterpenes such as farnesene, corcomin and beta-Bisabolene are the main active ingredients of giner (Zingiber officinalae) [23].

Phenols and phenolic compounds were found widely in food products. Their significant antioxidant activity has shown in many studies. Phenolic compounds of herbs are known as one of the best sources of natural antioxidan [24].

Results of this study are compatible with results of other studies. Giriraju and Yunus study showed inhibitory effects of 10% ginger extract against *Streptococcus mutans*, *Candida albicans* and *Enterococcus faecalis* [25].

Auta and coworkers showed that ethanolic extract of ginger (*Zingiber officinalae*) with of 20 mg/ml had stronger effect on *Pseudomonas aeruginosa* than Escherichia coli [26].

Unlike, in another study water extract of ginger at the high dose (500 mg) had no antimicrobial effect on Escherichia coli, *Salmonella*, *Shigella*, and *Bacillus cereus* [27]. It seems extraction method and used solvent influence on antibacterial properties of *Z. officinalae*.

In a study conducted by Rosario and coworkers, results showed weak antibacterial effect of Mallow's ethanol extract (*Malva lavatera*) on *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli and Salmonella subtilis* [28]. In another study, effects of methanol extract of Mallow were demonstrated on *Bacillus pumilus* [29]. Dulgar B and Gonuz was studied effective antimicrobial effects of ethanol extract of Mallow on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus, Klebsiella pneumoniae* and *Escherichia coli* [30]. Shoot and root systems of Mallow contain tannins, plenty of enamelled materials, sugar, calcium oxalate, resins, pectin and a dye called Malvin, anthocyanins addition to vitamins A, B and C [31, 32]. Type of extract and used solvent influenced antimicrobial effect of extracts. Since previous studies have demonstrated antimicrobial effect of ethanol extract of *Malva sylvestris,* it seems, in this study alcoholic solvents such as ethanol was more capable for extraction of bioactive materials of mallow but presence of water was one of the reasons for lack of antimicrobial effect of hydro-alcoholic extract. Mallow contains phenolic compounds, anthocyanins, carotenoids and vitamin E, which are antioxidant substances [33, 34].

It is recommended, using of other solvent such as hexane and methanol for extraction of mallow (*Malva sylvestris*) extract and evaluation of their antimicrobial effect on the used bacteria of this study. Other recommendation of this study is isolation of active ingredients of ginger (*Zingiber officinalae*) to introducing bioactive antibiotics for microbiology and pharmacology sciences. The mechanism actions of these plants or their components should be established. It should be noted that a phenolic compounds are substantially possess antimicrobial activities [35-41]. Therefore, these compounds might be involved in antimicrobial properties of these plants. It should be noted that there are a lot of other plants having these goup of compounds which may have antimicrobial activities [42-53]. Many diseases are extremely important and the need for medical treatment to be felt, in particular treatment with herbal medicines [54-118].

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