Effect of Metal Salts on Antibacterial Activity of *Zingiber Officinale* Roscoe Extract

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(Received on 17th March 2012, accepted in revised form 21st January 2013)

Summary: The antibacterial activity of ethanol extract of *Zingiber officinale* Roscoe (ginger) and its combination with different salts like $CuSO_4$, $ZnSO_4$ and $MnCl_2$ was investigated. Both Gram positive and Gram negative bacteria were tested by agar diffusion method. The results showed that ethanol extract of *Zingiber officinale* gave the maximum zone of inhibition at 50mg/ml concentrations against *Escherichia coli* among Gram negative bacteria and against *Staphylococcus aureus* in Gram positive bacteria. However antibacterial activity of the ginger and metal salts combination was greater than activity of ethanol extract. These investigations indicate that though ethanol extract has antibacterial activity against Gram positive and Gram negative bacteria, ginger and metal salts complex has more inhibitory effect on microorganisms. Antibacterial activity was also compared with standard drug ampicillin.The minimum inhibitory concentration (MIC) of ginger extract and metal salts complexes against all test organisms ranged from 0.3125 to 2.5 mg/ml.

Keywords: antibacterial activity, Gram positive, Gram negative, inhibition, extract.

Introduction

Antimicrobial drug resistance in bacterial pathogens is a world wide problem and as consequences control of these pathogenic organisms and its effective treatment remains as an important challenge. In every major class of antibiotic, bacterial resistance has existed [1]. The emergence of resistance to antibiotics has been increasing for important pathogens such as *Echerichia coli, Salmonella* spp, *Staphylococcus* spp and *Enterococcus* spp. [2, 3]. Many bacteria have advance protective mechanisms for the detoxification of heavy metal ions [4]. Despite of this many literature reports indicate the development of metal compounds as antimicrobial agents. Metal compounds having low molecular mass exhibited bactericidal and bacteriostatic activities. In many studies solutions of metal salts i.e. $CuSO_4$, $NiCl_2$, $ZnSO_4$ and $CoCl_2$ were used to determine their antibacterial activity against *Staphylococcus* strains [5]. In addition, all strains were sensitive to $AgNO_3$ over the concentration range studied. Ionic silver salts have been particularly useful as a bactericide at minute concentrations. These apparent antimicrobial effects of metal ions coupled to a lack of significant toxicity in human cells have led to their incorporation into a wide range of healthcare products from catheters to wound dressings [6, 7]. In an other study metal salts, $FeSO_4$, CuSO4, MnO_2 (4.8mMol) has been used against wide range of Gram positive and Gram negative bacteria [8]. New metallo-antibiotic agents include a range of ligands that have been chelated to metal ions and to date, antimicrobial activities have been demonstrated for metal complexes of imidazoles, phenanthrolines, quinolones, aminoquinolines and benzoylhydrazones [9-12]. Despite the growing need for new antimicrobial therapies, the mechanisms of action of many metal binding antibiotics are not fully understood [13].

Zingiber officinale (Ginger) is as important medicinally as it is culinary. It is best known as spice plant, but has also used in medicines for thousand of years. Its constituents are aromatic volatile oils and pungent chemicals such as borneol, cineol, gingerol. They have been found invitro to be effective antimicrobial substance against a wide range of microorganisms. The gingerols have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects [14-16]. *Zingiber officinale* has the capacity to eliminate harmful bacteria, such as *Escherichia coli*, which is responsible for most of the diarrhea [17].Ginger has been reported effective for treating nausea due to morning sickness and chemotherapy, rheumatism, cold, heat cramps and diabetes [18-20].

The aim of this study was to enhance the antibacterial activity of the ginger with different metal salts such as manganese, zinc and copper. The novel combination was assessed for antimicrobial effects against a range of Gram positive and negative bacteria to develop novel stable formulations to combat drug resistant bacterial infections.

Results and Discussion

Preliminary screening was utilized to compare the antimicrobial activity of ethanol extract of ginger alone and its complexes with metal salts following incubation for 24 hours against a group of bacteria, employing the agar plate diffusion assay results are presented in Table-1, 2 and 3.

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Table-1: Antibacterial activity of *Zingiber officinale* against Gram positive and Gram negative bacteria.

Name of organisms	5mg	10mg	20mg	30mg	40mg	50mg	DMF-ve control	Standard Ampicillin
S. aureus	**-ve**	**10.83±1.04***	**14.5**±18.5****	**16.06±0.40****	**17.5±0.5****	**18.83±1.04****	**7.8±0.3**	**28.16±0.76**
B. subtilis	**8.5±1.5***	**12.5±0.5****	**18±0.5****	**19.16±0.76****	**20.33±0.76****	**21.16±1.04****	**8.06±0.25**	**29.8±0.3**
B. pumilis	**8.1±1.04***	**15.43±0.81****	**20.16±0.76****	**20.66±0.28****	**21.16±0.76****	**21.83±0.76****	**7.7±0.41**	**30.26±0.64**
E. coli	**8.4±0.60***	**16.16±1.04****	**19.4±0.65****	**15.44±0.76****	**21.4±0.65****	**25.66±0.28****	**8±0.5**	**28.5±0.5**
Ps. aurogenosa	**8.5±0.5**	**16.06±0.40****	**18.63±0.70****	**20.8±0.26****	**22.16±1.04****	**24.16±0.75****	**7.7±0.32**	**31±0.77**
K. pneumoniae	**11.3±0.76***	**12.26±0.68****	**14.06±0.92****	**19.2±0.60****	**20.16±0.75****	**21.4±1****	**7.8±0.8**	**27.66±0.5**

Results express as mean± stdev. * $p< 0.005$, ** $p< 0.005$

The results of antibacterial activity of ethanol extract of ginger and its complexes with different metal salts are presented in Table-1. The screening assay demonstrated that antibacterial activity of ginger extract against *Escherichia coli, Pseudomonas auroginosa* and *Klebsiela. pneumoniae* (Gram negative bacteria) was greater than Gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis, Bacillus pumilus*).A maximum significant inhibition was observed at higher concentration and mild inhibitory effect was seen as concentration decreased. Among Gram negative bacteria, the maximum zone of inhibition 25.66mm±0.28 (p<0.0001) at 50 mg/ml concentration and minimum zone of inhibition 8.4mm±0.60 (p<0.005) at 5mg/ml was obtained against *Escherichia coli.* In case of Gram positive bacteria, at 50mg/ml the maximum zone of inhibition was 18.83mm±1.04 (p<0.0001) against *Staphylococcus aureus* while at 5mg/ml *Staphylococcus aureus* was found resistant. The inhibition rate was greater in Gram negative bacteria as compared to Gram positive bacteria. This difference in activity among Gram positive and Gram negative bacteria may be due to thick cell wall present in Gram positive bacteria which may slightly resist the antibacterial action of ginger extract. These results are in line with other studies which have been reported that ginger has capacity to eliminate harmful bacteria such as *Escherichia coli* responsible for most of the diarrhea [17]. The extract of *Zingiber officinale* and its pungent compounds demonstrate greater antibacterial activity against a variety of bacterial species including *Helicobacter pylori, Pseudomonas auroginosa* and *Esherichia coli* [21].

The inhibitory effect of ginger against microorganisms tested could be due to the presence of many active compounds like zingiberene, zingerone, shagaols, gingerols and geranial. These are terpenoids and have lipophilic properties. Their mode of antibacterial action could be due to their lipophilic nature [22].

Antibacterial activity of metal salts alone was also assessed by agar diffusion method. Results are presented in Table-2. The metal salts alone showed good antibacterial activity against all Gram positive and Gram negative bacteria at all concentrations. Among metal salts, $CuSO_4$ showed greatest activity against all bacterial species at 50mg/ml with average zone of inhibition 23.33mm±1.47 (p<0.0001) while $ZnSO_4$ and $MnCl_2$ exhibited moderate antibacterial activity with average zone of inhibition 19.80mm±3.2 (p<0.005) and 17.94mm±2.5 (p<0.005) respectively.

The antibacterial activity of ginger with metal salts complexes is better than metal and ginger alone results are presented in Table-3. $CuSO_4$, $ZnSO_4$ and $MnCl_2$ complexes with ginger exhibited excellent activity with average zone of inhibition 23.68mm±1.7 (p<0.005), 22.69mm±0.72 (p<0.005) and 22.03mm2.25 (p<0.005) respectively. This study is validated by the work of other scientists who reported antibacterial effect of pomegranate rind extract with metal salts and vitamin C [23]. Other scientists also reported that antimicrobial properties of natural products can be enhanced by the addition of metal ions [24].

The minimum inhibitory concentration was defined as the lowest concentration of extract at which no growth was obtained. The results are presented in Table-4.

The MIC of ethanol extract of *Zingiber officinale* and metal salts complex was tested at concentration ranging from 10mg/ml to 0.1562mg/ml. $CuSO_4$ and $MnCl_2$ complexes showed the lowest MIC of 0.312mg/ml against *Staphylococcus aureus* and *Klebsiela pneumoniae* respectively. The MIC value for $ZnSO_4$ complex against all bacteria varied from 0.625-1.25mg/ml.

In this study, antimicrobial affects of *Zingiber officinale* with metal salts are reported first time while previous studies investigated the antimicrobial affects of ginger alone against wide range of bacteria [14]. From this study it can be concluded safely that antimicrobial affects of ginger can be increased with metal salts.

Table-2: Antibacterial activity of Metal salts against Gram positive and Gram negative bacteria at conc. 5 mmol.

Name of organisms	$CuSO_4$	$ZnSO_4$	$MnCl_2$	DMF -ve control	Standard Ampicillin
S. aureus	25.16±1.04**	21.33±0.76**	18.5±0.5**	7.8±0.3	28.16±0.76**
B. subtilis	23.33±0.76**	15.5±0.5**	15.83±0.76**	8.06±0.25	29.8±0.3**
B. pumilis	22±0.5**	18.66±0.76**	16.5±0.5**	7.7±0.41	30.26±0.64**
E. coli	21.66±0.76**	18.16±1.25**	15±0.5**	8±0.5	28.5±0.5**
Ps. auroginosa	22.83±0.28**	20±0.5**	21.33±0.76**	7.7±0.32	31±0.77**
K. pneumoniae	25±0.5*	25.2±0.51*	20.5±0.5**	7.8±0.8	27.66±0.5**
Average	23.33±1.47**	19.80±3.2*	17.94±2.5*	7.85±0.15	29.23±1.3**

Results express as mean± stdev. * $p< 0.005$, ** $p< 0.0005$

Table 3: Antibacterial activity of ginger and metal salts combinations against Gram positive and Gram negative bacteria

Concentration of *Zingiber officinale*: 50mg/ml **Concentration of salts: 5mmol**

Name of organisms	*Ginger* /$CuSO_4$	*Ginger*/$ZnSO_4$	*Ginger*/$MnCl_2$	DMF.	Standard Ampicillin
S. aureus	26.06±0.60**	22.16±1.04**	19.5±0.5**	7.8±0.3	28.16±0.76**
B. subtilis	24.16±1.25**	22±1.32**	21.83±0.81**	8.06±0.25	29.8±0.3**
B. pumilis	23±0.28**	23.5±0.5**	22.8±0.80**	7.7±0.41	30.26±0.64**
E. coli	22.9±0.40**	23.5±0.5**	22.9±0.65**	8±0.5	28.5±0.5**
Ps. auroginosa	24.96±0.47**	22±0.5**	19.66±0.64**	7.7±0.32	31±0.77**
K. pneumoniae	21.16±1.04*	23±0.5*	25.5±0.4**	7.8±0.8	27.66±0.5**
Average	23.68±1.7*	22.69±0.72*	22.03±2.25*	7.85±0.15	29.23±1.3**

Results express as mean± stdev. * $p< 0.005$, ** $p< 0.0005$

Table-4: Minimum inhibitory concentration of *Ginger*/metal complexes.

	MIC mg/ml		
Name of Organisms	*Ginger* /$CuSO_4$	*Ginger*/$ZnSO_4$	*Ginger*/$MnCl_2$
S. aureus	.312	1.25	2.5
B.subtilis	0.625	1.25	1.25
B. pumilus	0.625	0.625	1.25
E.coli	1.25	0.625	1.25
P.auroginosa	0.625	1.25	2.5
K.pneumoniae	1.25	0.625	0.312

Experimental

Collection and Identification of Plant material

Roots of *Zingiber officinale* were purchased from local market and identified by taxonomist. Plant specimen was submitted having herbarium No. ZO-121-2011.

Preparation of Extract

The roots were washed with clean sterile distilled water and allowed to air-dry for one hour. Then the outer covering of roots were manually peeled off and again washed and dry. After complete drying powder was made and 100 g of powdered plant material was soaked in 100 ml of 70% ethanol, for 72 hours at room temperature. Mixture was stirred every 24 hours using a sterile glass rod. At the end of extraction, extract was passed through Whatman filter paper No. 1 (Whatman, UK). The filtrate obtained was concentrated in vacuo at 30°C and stored at 4°C until further use [25].

Preparation of Solution

Extract was dissolved in 6% dimethylformamide (DMF) to give strength of 10g/ml from which further dilutions were made in the same solvent. Ampicillin was used as reference standard (positive control) while 6% dimethylformamide (DMF) used as negative control.

Microorganisms used

The antibacterial activity was assessed against Gram positive (*Staphylococcus aureus, Bacillus subtilis, Bacillus pumilus,* and Gram negative (*Echrichia coli, Pseudomonas auroginosa and Klebsiela pneumoniae*.) microorganisms. All microorganisms used in the present study were clinically isolated. The clinical isolates were biochemical characterized by standard methods. All the organisms were maintained on tryptic soya agar slants at 4 °C prior to testing.

Inoculums Preparation by Direct Colony Suspension Method

A small volume of sterile water was poured inside a test tube to which general colonies of the test organisms taken directly from the plate were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard ($1x10^8$ CFU/ml) [26].

Screening Assay

Antibacterial Activity of Ethanol Extract of Zingiber officinale

Antimicrobial screening was carried out by agar plate diffusion method [27] using 0.5ml of the inoculums containing 10^8 bacterial cells. The inoculums were thoroughly mixed with 20 ml of molten sterile tryptic soya agar and poured into pre-sterilized Petri dishes respectively. All plates were left to set at 4^oC for 30 – 40 minutes. Holes of 6 mm diameter were made in the center of each seeded plates. Different dilutions of the plant extract prepared in the order of 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml and 50mg/ml. Exactly 0.02ml of each concentration was introduced into each hole on the medium and was allowed to stand at room temperature for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24 hours. Inhibition zones obtained were measured in millimeters.

Antibacterial Activity of Metal salts

All metal salts $CuSO_4$, $ZnSO_4$ and $MnCl_2$ were purchased from Sigma-Aldrich and dilutions of 5mmol concentration were prepared in the order of 5mg/ml, 10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml, and 50mg/ml in distilled water. Overnight cultures on tryptic soya agar were prepared. Exactly 0.02ml of each salt (5mmol) was introduced into each hole on the medium and was allowed to stand at room temperature for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24 hours. The sensitive bacteria grew everywhere except in areas around the holes in the medium. Inhibition zones obtained were measured in millimeters.

Antibacterial Activity of Ethanol Extract of Ginger and Metal Salts Combination

For antibacterial activity of ginger and metal complex, maximum concentration of ginger (50mg/ml) showing maximum antibacterial activity against all microorganisms was used. An equal volume of each metal salts (20ml) of 5mmol was added to equal volume of ginger (50mg/ml conc.). The mixture was stirred and continuously warmed for 2 hours. The resulting solution was then cooled, filtered and later reduced to a small volume (15 ml). Overnight cultures on tryptic soya agar were prepared as previously described. Same dilutions are prepared. Exactly 0.02ml of each concentration was introduced into each hole on the medium and was allowed to stand on the bench for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24 hours. The resulting inhibition zones obtained were measured in millimeters and recorded against the corresponding concentrations.

MIC Determination

The MIC values of ethanol extract of *Zingiber officinale* were determined against the Gram positive and Gram negative bacteria, (10^6 CFU/ml) by the serial dilution technique [28]. It was calculated from the concentration of that test tube which was showing visually no growth after 24 hours incubation period.

Statistical Calculation

The values are represented as Mean ± SD. The data was analyzed by Student s' *t* test and $p < 0.05$ was considered to be significant.

Conclusion

The present study indicated that the *Zingiber officinale* has significant antibacterial activity against gram positive and gram negative bacteria .This activity become most significant when *Zingiber officinale* is combined with metal salts complexes.i.e $CuSO_4$, $ZnSO_4$ and $MnCl_2$ .Among metal salts .$CuSO_4$ complex exhibited maximum antibacterial activity with *Zingiber* extrac.So it can be concluded from this study that antibacterial activity of *Zingiber Officinale* can be increased with metal salts.

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