



Global Advanced Research Journal of Medicine and Medical Sciences (ISSN: 2315-5159) Vol. 6(10) pp. 240-244, October, 2017  
Available online <http://garj.org/garjmms>  
Copyright © 2017 Global Advanced Research Journals

*Full Length Research Paper*

# Antibacterial activity of crude herbal extracts of *Zingiber officinale* and *Curcuma longa* against 13 reference bacterial species

Sami S. Ashgar

Microbiology Department, College of Medicine, Umm Al-Qura University, Saudi Arabia.  
E-mail – [ssashgar70@hotmail.com](mailto:ssashgar70@hotmail.com); Mobile: +966(0) 505 794 955

Accepted 09 October, 2017

Medicinal plants acquired great attention for their miracle properties against various human illnesses. In this laboratory-based study, Antibacterial effectiveness of *Curcuma longa* and *Zingiber officinale* (Ginger) were extracted in aqueous, 70% ethanol and ethyl acetate solvents and investigated against 13 various American type culture collection (ATCC) strains by the mean of determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Results demonstrated that ethyl acetate extract of *Curcuma longa* was superior to other solvents versus *Enterococcus faecalis*, *Enterococcus faecalis* (VRE), *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* with inhibition zones range (12-19mm) and exhibited killing concentration varies between 25-50 mg/ml. *Acinetobacter baumannii* expressed visible sensitivity to *Curcuma longa* extracted in aqueous, ethanol and ethyl acetate solvents. Bactericidal activity was experimentally constant at a concentration of 50mg/ml. Ethyl acetate extract of Ginger, on the other hand, was the only extract that showed antibacterial activity against three reference bacterial strains include *E. faecalis*, *S. aureus* and *Staphylococcus aureus* (MRSA), with inhibition zones range (12-19mm) and MBC value of 100, 12.5 and 25 mg/ml, respectively. In conclusion, demonstrated antibacterial activity of *Curcuma longa* and *Zingiber officinale* can be further invested as potential natural antibiotics against some multi-resistant pathogens.

**Keywords:** *Curcuma longa*, *Zingiber officinale*, MIC, MBC, ethyl acetate, ethanol, aqueous, ATCC strains.

## INTRODUCTION

Medicinal plants considered the main source of traditional medicine almost all over the world due to their accessibility, affordability and limited side effect. For centuries, medicinal plants used to improve human health (WHO, 2002). Furthermore, estimated 60-90% of the population in some developing countries such as Uganda and Ethiopia used medicinal plants extensively as a major component of traditional medicine and part of primary health care (WHO, 2002). Herbs are still used to treat a variety of health problems. The use of traditional medicine among the local population and the tribe

societies in Saudi Arabia measured as great part of the traditions. it started from ancient eras and still up to the present time (Al-Daihan et al., 2013). Medicinal plant ingredients have played an important role in traditional Western medicine, in 1984, at least 25% of the exported drugs in the United States and Canada were derived from natural plant products (Balunas and Kinghorn, 2005). Patrons of herbal medicines showed growing interest as they see the benefit of using herbs in treating physical health problems and inspired researchers to investigate the effect of various biological activities of the medicinal

plants both in the developed world as well as in the developing countries, in an attempt to defeat the global challenge of antibiotics resistances (Balunas and Kinghorn, 2005; Bibi et al., 2011; Vu et al., 2016; WHO, 2017). This study therefore, aimed to investigate the antibacterial efficacy of crude aqueous, ethanol 70% and ethyl acetate extracts of Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*), that are broadly imported to Saudi Arabia, and used extensively among locals, against 13 reference bacterial species including *Salmonella typhimurium*, *Klebsiella pneumonia* (ESBL), *Klebsiella pneumonia* (CRE), *Acinetobacter baumannii*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *E. coli*, *Enterococcus faecalis*, *Enterococcus faecalis* (VRE), *Staphylococcus aureus*, *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis*.

## MATERIALS AND METHODS

### Preparation of the crude herbal extracts

Two herbal samples are Turmeric (*Curcuma longa*) Ginger (*Zingiber officinale*) and were purchased and collected from herbal stores in Makah city and proceeded in microbiology research laboratory, faculty of medicine, Umm Al Qura University, Saudi Arabia. Fifty grams of each of the air-dried powdered plant materials were separately were ground and extracted with different solvents as distilled water, 70% ethanol, and ethyl acetate, using magnetic stirrer overnight. Extraction process repeated three times till complete exhaustion of the plant powder. The total collected extracts were separately filtered using Whitman paper then evaporated under vacuum using rotary evaporator till dryness to afford dry extracts. Extracts were then stored in an amber-glass vial in desiccator containing anhydrous calcium chloride till further use. For antimicrobial activities, an accurately weighed amount of the powder was dissolved in DMSO (dimethyl Sulfoxide) to give a concentration of 100 mg/mL (WHO, 2000).

### Preparation of tested organisms

A total of 13 bacterial species were used in this study, eight species were related to Gram-negative bacteria (reference strains: *Salmonella typhimurium* ATCC 12228, *Klebsiella pneumonia* (ESBL) ATCC 14028, *Klebsiella pneumonia* (CRE) ATCC 700603, *Acinetobacter baumannii* ATCC 1705, *Shigella sonnei* ATCC 19605, *Pseudomonas aeruginosa* ATCC 25931, *Proteus mirabilis* ATCC 27853 and *E. coli* ATCC 43071) and five species were related to Gram-positive bacteria (reference strains: *Enterococcus faecalis* ATCC 35218, *Enterococcus faecalis* (VRE) ATCC 29212, *Staphylococcus aureus* ATCC 51299, *Staphylococcus aureus* (MRSA) ATCC 43300, and *Staphylococcus epidermidis* ATCC 43300).

### Preparation of the McFarland standard

Tested bacterial strains were sub-cultured on Muller Hinton agar medium and incubated at 37°C for overnight and a single colony was obtained using a sterile loop and inoculated in 3 ml of Muller Hinton broth to form a homogenous suspension of the organism which was standardized to (0.5 McFarland) using calibrated VITEK 2 DENSICHEK.

### Antibacterial test of herbal extracts using well diffusion assay on Muller Hinton agar plates

As described in NCCLS manual (CLSI, 2015), The surface of the Mueller Hinton agar plate was streaking in three directions by the suspension of the testing organism, using sterile cotton swabs. Plates were allowed to dry for 10 minutes before cutting the wells (7mm) using sterile sharp glass rods. Wells were then filled with herbal extracts (150µL) of different solvents, distilled water, 70% ethanol and ethyl acetate. Vancomycin (30µg) and Amikacin (30µg) discs were used as a positive control for Gram-positive bacteria and Gram-negative bacteria, respectively. A single well in each plate was filled with DMSO (dimethyl Sulfoxide) as a negative control. All plates were incubated at 37°C for 24 hours. After the incubation period, the plates were examined and the diameter of each zone was measured for inhibition zone formed around the wells for each herbal extract and recorded.

### Determinations of MIC and MBC of herbal extract using microtitration plates

*Zingiber officinale* and *Curcuma longa* extracts with different solvents were prepared at 100 mg/mL DMSO. 200 µl of herbal extract at the highest concentration (100 mg/ml) was added to the first column in a microtiter plate and 100 µl Mueller Hinton broth to other wells. 100 µl herbal extract transferred from the 1st column to the next wells to produce a dilution series (50, 25, 12, 6, 3, 1.5, 0.75 and 0.32 mg/ml) of extracted Turmeric and Ginger. 50 µl of 0.5 adjusted McFarland bacterial suspension in Muller Hinton broth were added to dilution series, as well as a positive control well. Mueller Hinton broth only was used as negative control. Plates were incubated without agitation at 37°C for 24 hours. MIC was determined by wells with no visible growth which was sub-cultured using a 10 µl of the selected wells on Muller Hinton agar plates. MBC was recorded as the lowest concentration of the extracted *Curcuma longa* and Ginger that prevent any growth of an organism after sub-cultured on Muller Hinton agar plate according to clinical and laboratory standards institute (CLSI M26-A, 1998). For *Curcuma longa* versus *Acinetobacter baumannii* ATCC 1705, the same procedure was followed in addition to aqueous and ethanol extracts.

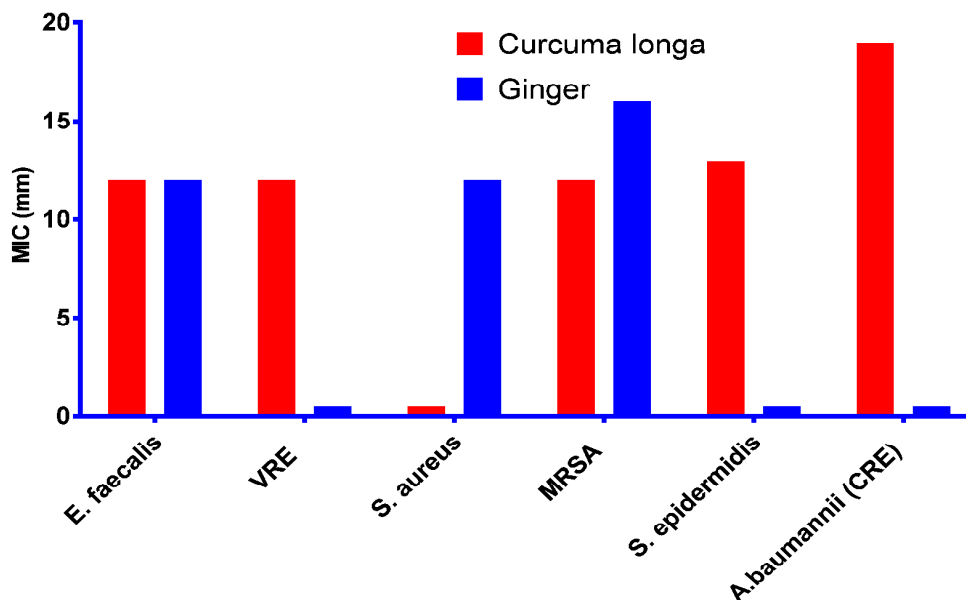


Figure 1. Antibacterial efficacy of *Curcuma longa* and Ginger on agar well diffusion (MIC).

Table 1. MIC and MBC activity of *Curcuma longa* and Ginger against reference strains.

Bacterial Strains	MIC (mm)		MBC (mg/ml)		Solvent
	<i>Curcuma longa</i>	Ginger	<i>Curcuma longa</i>	Ginger	
<i>E. faecalis</i>	12	12	50	100	ethyl acetate
VRE	12	0	50	-	ethyl acetate
<i>S. aureus</i>	0	12	-	12.5	ethyl acetate
MRSA	12	16	25	25	ethyl acetate
<i>S. epidermidis</i>	13	0	25	-	ethyl acetate
<i>A. baumannii</i> (CRE)	19	0	50	-	ethyl acetate
<i>A. baumannii</i> (CRE)	20	0	50	-	Aqueous
<i>A. baumannii</i> (CRE)	18	0	50	-	Ethanol

## RESULTS

### Antibacterial efficacy of *Curcuma longa* on agar well diffusion, and MBC

Examining antibacterial activity of *Curcuma longa*, extracted in ethyl acetate solvent, against thirteen ATCC reference bacterial strains showed noticeable antibacterial activity with only five strains include *E. faecalis*, *Enterococcus faecalis* (VRE), *Staphylococcus aureus* (MRSA), *S. epidermidis* and *A. baumannii* (CRE) with inhibition zones range (12-19mm). Furthermore, *Acinetobacter baumannii* expressed visible sensitivity to *Curcuma longa* extracted in aqueous and ethanol solvents with inhibition zone measured between 20mm and 18mm, respectively. No significant variations were detected when t-test was measured ( $p > 0.01$ ) for the three extracts against *A. baumannii* (Fig.1). Bactericidal activity was vastly effective (99.99%) against *E. faecalis*,

*Enterococcus faecalis* (VRE), *Staphylococcus aureus* (MRSA), *S. epidermidis* and *A. baumannii* (CRE) using ethyl acetate extract. Exhibited killing concentration varies between 25-50 mg/ml. *A. baumannii* bactericidal activity was experimentally constant at the concentration of 50mg/ml with *Curcuma longa* extracted in ethyl acetate, aqueous and ethanol (Table.1).

### Antibacterial efficacy of Ginger on agar well diffusion, and MBC

Ethyl acetate extracted Ginger was the only extract that showed antibacterial activity against three reference bacterial strains include *E. faecalis*, *S. aureus*, and *Staphylococcus aureus* (MRSA) (Fig.1). The zone of inhibition ranged between (12-16mm). MBC effectiveness was experimentally (plated out) measured at 100, 12.5 and 25 mg/ml versus *E. faecalis*, *S. aureus* and *Staphylococcus aureus* (MRSA), respectively (Table.1).

## DISCUSSION

Herbal remedy extensively used especially among people of developing countries. It has been projected that two-thirds or even more of populations considered medicinal plants as the primary choice of therapy against a variety of human illnesses. Likewise, individuals of Saudi Arabian local tribe relatively favor traditional medicine over biochemical synthetic drugs. In this lab-based experimental study, *Curcuma longa* and Ginger extracted with different solvents were investigated for their MIC and MBC effectiveness against thirteen ATCC bacterial strains, including Gram-negative (*Salmonella typhimurium*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Escherichia coli*) and Gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*), some counted as multidrug-resistant bacterial strains (VRE, MRSA, ESBL, and CRE) as shown previously in the material and methods. It appeared that crude extraction of *Curcuma longa* in ethyl acetate had potentially antibacterial effect than aqueous and ethanolic extracts, mainly against Gram-positive bacteria (*Enterococcus faecalis* (VRE), *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, but not versus *Staphylococcus aureus* with inhibition zones range (12-19mm). However, *Acinetobacter baumannii* expressed visible sensitivity to *Curcuma longa* extracted in aqueous, ethanol and ethyl acetate solvents with inhibition zone measured between 19mm, 20mm, and 18mm, respectively. Many the studies that investigated the antibacterial activities of extracted *Curcuma longa* and derivatives explored different results versus reference strains as well as clinical isolates of both Gram-negative and Gram-positive bacteria. A recent study examined the antimicrobial activity of methanol and chloroform extracted *Curcuma longa* against *Bacillus* spp, *S. aureus*, *P. aeruginosa* and *E. coli*. Result concluded that antibacterial effect of methanolic extract of the fresh and dry rhizome of *Curcuma longa* was superior to chloroform extract (Chauhan et al. 2017). Similarly, a study from Saudi Arabia evaluated the antibacterial activity of aqueous and methanolic of *Curcuma longa*, revealed that methanolic extract has higher antibacterial effect against *S. pyogenes* and *S. aureus* whereas aqueous extract inhibited the growth of *E. coli* and *P. aeruginosa* more than methanolic extract (Al-Daihan et al. 2013). Another study from India, observed the potent antimicrobial activity of ethanolic rhizome extract of *Curcuma longa* when compared with leaf extract against only *P. aeruginosa* and *Bacillus subtilis*, with noted MIC value of 6.25 mg/ml (Nikhil Singh, 2017). Aqueous extract of *Curcuma longa*, however, was proved over methanol, ethanol and acetone extracts in a previous study, against clinical isolates include *B. subtilis*, *K. pneumoniae*, and *S. aureus* (Chakraborty et al. 2014). A summary of the antibacterial broad-spectrum activity of *Curcuma longa*

was recently revised by two studies, both nominated *Curcuma longa* or its derivatives as a prospective antibiotic for their potential antibacterial activity especially against *S. aureus*, despite perceived cytotoxicity effect, weak solubility determined at high concentration (Kocaadam and Şanlıer, 2017; Teow et al. 2016). Comparatively, *Zingiber officinale* extract behaved differently. Aqueous extract in addition to ethanol extract presented no antibacterial effectiveness against tested organisms, though ethyl acetate extract showed efficacy only against *E. faecalis*, *S. aureus* and *Staphylococcus aureus* (MRSA) with an inhibition zone of 12, 12 and 16mm, respectively. Furthermore, MBC working concentration was detected at 100, 12.5 and 25mg/ml, respectively. This result almost stands in line with a recent study that showed an antimicrobial activity of ethanol extracted *Zingiber officinale* rhizome against *S. aureus* (Indrawati, 2017). The size of inhibition zone detected was 25mm at a concentration of 12.5%. Another study demonstrated the bioactivity of aqueous and ethanolic extracted *Zingiber officinale* against six pathogenic bacteria includes *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* (Akintobi et al. 2013). Results concluded that ethanolic extract reported inhibitory effect higher than aqueous extract versus *Proteus mirabilis*, *Salmonella typhi*, *S. aureus* and *Pseudomonas aeruginosa* but no effect on *E. coli* and *B. subtilis* was observed. Moreover, one comparative study of assorted extracts of *Zingiber officinale* observed that methanol extract was higher than another extract versus *B. subtilis* whereas, aqueous extract had a higher impact on *K. pneumoniae*, *P. mirabilis* and *S. aureus* (Chakraborty et al., 2014). Likewise, methanolic extract of *Zingiber officinale* was inhibiting the growth of *S. pyogenes*, *S. aureus*, and *E. Coli* more than aqueous extract but the reverse effect was noticed with *P. aeruginosa* (Al-Daihan et al., 2013). Contradictory, evaluated study revealed that aqueous extract of *Zingiber officinale* was superior to the ethanolic extract against *S. aureus*, with a zone of inhibition ranges between 9-19mm at concentration varies between 3-9%, respectively (Tambe et al., 2016). The disparity in the results could be due to extraction techniques differences, genetic variations of the organisms, bioactive compounds stability, media components interaction and sensitivity behave of organisms. Substantially, both *Curcuma longa* and *Zingiber officinale* showed potential antibacterial activity against some pathogenic bacteria especially those challenging exciting antibiotics. Thus further in vivo and in vitro studies on the bacterial inhibitory influence of *Curcuma longa* and *Zingiber officinale* herbs prior nominating them as possible prospective antibiotics is recommended. Also, due to various and dispersed investigations perhaps composing of review articles on antibacterial effectiveness particularly for *Zingiber officinale* is probably needed. As might concerned, this is

the first experimental study used aqueous, ethyl acetate and ethanolic extracts of crude *Curcuma longa* and *Zingiber officinale* rhizome against a wide range of bacterial strains as such.

## CONCLUSION

Based on obtained results, ethyl acetate extract of both *Curcuma longa* and Ginger owned the highest antibacterial activity versus selected bacterial reference strains, including some multi-drug resistance strains, except for *A. baumannii* where all the extracts expressed antibacterial activity. Both herbs retained potent antibacterial mediators and can be exploited as forthcoming antibiotics. Finally, it appeared that ethyl acetate has high affinity to extract natural bioactive compounds and thus can be utilized in further phytochemical extraction studies.

## ACKNOWLEDGEMENT

The author would like sincerely to thank Dr. Hamdy Alsayed for his help in presenting results and performing statistical analysis. Also Mr. Abdullah Abiyadh and Mr. Asim Abdul-Shakor for their assistance in providing essential materials and set up.

## REFERENCES

- Akintobi OA, Onoh CC, Ogele JO, Idowu AA, Ojo OV, Okonko IO (2013). Antimicrobial activity of *Zingiber officinale* (ginger) extract against some selected pathogenic bacteria. *Nature and science*. 11(1): 7-15.
- Al-Daihan S, Al-Faham M, Al-shawi N, Almayman R, Brnawi A, Zargar S, Bhat RS (2013). Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms. *J. King Saud Uni. Sci.* 25(2): 115-120.
- Balunas MJ, Kinghorn AD (2005). Drug discovery from medicinal plants. *Life Sciences*. 78(5): 431-441.
- Bibi Y, Nisa S, Chaudhary FM, Zia M (2011). Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Complement Altern Med.* 11: 52.
- Chakraborty B, Nath A, Saikia H, Sengupta M (2014). Bactericidal activity of selected medicinal plants against multidrug resistant bacterial strains from clinical isolates. *Asian Pacific J. Trop. Med.* 7(Supplement 1): S435-S441.
- Chauhan P, Keni K, Patel, R (2017). Investigation of phytochemical screening and antimicrobial activity of *Curcuma longa*, 4, 153-163 pp.
- CLSI (1998). *Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guidelin.* CLSI document M26-A [ISBN 1-56238-384-1].
- CLSI (2015). *Methods for dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, Approved Standard.* CLSI document M07-A10 (10th ed.).
- Indrawati IDA (2017). Antibacterial activity of ethanolic extracts of rhizome from three ginger varieties against acne isolated bacteria, 9.
- Kocaadam B, Şanlıer, N (2017). Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. *Critical Reviews in Food Science and Nutrition*, 57(13): 2889-2895.
- Nikhil Singh SG, Vaibhav Rathore (2017). Comparative Antimicrobial Study of Ethanolic Extract of Leaf and Rhizome of *Curcuma longa* Linn. *Pharmacogn J.* 9(2): 208-212.
- Tambe A, Deokar S, Pawar R (2016). Evaluation of Antimicrobial Activity and Phytochemical Analysis of *Zingiber officinale* (Ginger) Rhizome Extract. *Int. J. Allied Practice, Res. Rev.* 3(6).
- Teow SY, Liew K, Ali SA, Khoo AS, Peh SC (2016). Antibacterial Action of Curcumin against *Staphylococcus aureus*: A Brief Review. *J. Trop. Med.* 2016: 2853045.
- Vu TT, Kim H, Tran VK, Le Dang Q, Nguyen HT, Kim H, Kim IS, Choi GJ, Kim JC (2016). In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria. *BMC Complement Altern. Med.* 16: 32.
- WHO (2000). *General guidelines for methodologies on research and evaluation of traditional medicine.* p. 1-71.
- WHO (2002). *WHO Traditional medicine strategy 2002-2005.*
- WHO (2017). *Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis.*, Geneva.