Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 4 (4): 173-177, 2009
ISSN 1816-496X
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Antimicrobial Activity of *Curcuma longa* Aqueous Extract

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Abstract: Ethnopharmacological relevance of *Curcuma longa* (Zingiberaceae) is known in many countries. The root of it was widely used as food ingredient and remedy. The present study aim to evaluate the antimicrobial activity of *C. longa* aqueous extract. The antimicrobial test was screened using agar diffusion method. The Minimum Inhibitory Concentration (MIC) were determined using agar dilution and confirm with broth macrodilution methods, while the Minimum Bactericidal Concentration (MBC). The aqueous extract of *C. longa* exhibited antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Krebsilla pneumoniae* ATCC 10031 and *Staphylococcus epidermidis* ATCC 12228 (MIC = 4-16 g L^{-1}; MBC = 16-32 g L^{-1}). In conclusion, the *C. longa* aqueous extract exhibited good antimicrobial activity against some of tested bacteria at low concentration. The results provide promising information for the potential use of *C. longa* aqueous extract in the treatment of infection.

Key words: *C. longa*, Zingiberaceae, antimicrobial, aqueous extract

INTRODUCTION

The bacterial infection diseases cause the problem for humankind beyond historical age. The researches to find antimicrobial medicine have been launch for over 50 years (Rudrappa and Bais, 2008). However, even we discovered many anti-biotic drug, we still facing multidrug resistance bacterial (Dowzicky and Park, 2008; Saonuam *et al*., 2008; Tillotson *et al*., 2008) and the side effect of antibiotic treatment for patients who allergic to its. There are the reports about the adverse effect of antibiotic treatment in children (Khotaei *et al*., 2008) and adults (Lin *et al*., 2009). Furthermore it has been reports about the decreasing of susceptibility in pathogenic bacteria (Dowzicky and Park, 2008; Saonuam *et al*., 2008). Therefore, the antibacterial research was become interesting to support the information for development of the anti-infection diseases remedy especially the development of folkloric medicine which has been used locally before (Nascimento *et al*., 2000; Tongson *et al*., 2005).

Curcuma longa or turmeric belongs to Zingiberaceae family. It roots part has traditionally been used as an insect repellant, antimicrobial (Rudrappa and Bais, 2008), antidiabetic (Mohamed *et al*., 2009) rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhoea and colic inflammatory disorders (Villegas *et al*., 2008). According to the folklore used of this plant extract it was interesting to investigated antibacterial activity of the plant root aqueous extract. This study aim to investigate antibacterial activity of *Curcuma longa* root aqueous extract against various bacteria.

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MATERIALS AND METHODS

Plant Collection Identification and Extraction

The plant material was brought from local market at Mahasarakham Province during September, 2008. The plant was identified by Department of Biology, Faculty of Sciences, Mahasarakham University, Thailand. The root of *C. longa* were washed with water and cut into small pieces and dried in oven at 40°C for 48 h. After the drying process *C. longa* was ground to powder. Ten gram powder of dried plant were boiled in 500 mL of distilled water and spray dried. The yields of extraction were in the range of 3.7-4% of dried weight plant powder. The extraction and antimicrobial activity test were conducted from November 2008-February 2009 at Department of Chemistry, Faculty of Sciences, Mahasarakham University, Thailand.

Microorganisms

Five strain of gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917) and five strains of gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Klebsiella pneumonia* ATCC 10031 *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9721) bacteria were used as test organisms.

Antimicrobial Susceptibility Test

Agar Diffusion Method

The agar diffusion method was conducted as standard method (Lorian, 1996) and as described in previous report (Sittiwet and Poungpronpitag, 2008). Briefly, the solution of plant extract were prepared the solution of plant extract was prepared at concentration 125, 250 and 500 g L^{-1} using sterile distilled water. The solutions were putted into the stainless cylinder (6 mm internal diameter and 10 mm height) which place on the inoculated Mueller-Hinton agar surface. After pre-incubation for 1 h the plates were incubated at 37°C for 19 h. The clear diameters of inhibition zones were observed.

Agar Dilution and Broth Macro Dilution Method

The Minimum Inhibitory Concentrations (MICs) were determined using agar dilution method and confirmed by result of broth macro-dilution method while Minimum Bactericidal Concentration (MBCs) were determined using broth macro-dilution method according to standard guideline (Lorian, 1996). The agar dilution method was using plant extract concentration in range of 0.5-256 g L^{-1} in Mueller-Hinton agar and spot with 0.5 McFarland bacterial suspension. The MICs of the plant was recorded by observed no growth of bacteria on the agar surface at each concentration after incubated at 37°C for 24 h. While broth macro-dilution method, plant solution were prepared in sterile water at concentration 256 g L^{-1} (range 0.5-256 g L^{-1}). Two fold serial dilutions in 3 mL of Mueller-Hinton broth were made and then 3 mL of bacterial suspension of was added to give final inoculums of 0.5×10^{6} cfu mL^{-1}. The solutions were incubated at 37°C for 24 h. The MICs were recorded by observed the lowest concentration that showed no visible growth of bacteria while MBCs were recorded as the lowest concentration that showed no growth of bacteria after subculture on agar medium.

RESULTS

The root of *C. longa* has been used as remedy in many countries as an insect repellant, antimicrobial (Rudrappa and Bais, 2008), antidiabetic (Mohamed *et al*., 2009) rheumatism, bodyache,

Table 1: Inhibition zone diameters of *C. longa* root aqueous extract against various bacteria

Bacterias	Gram	Inhibition zone diameter of *C. longa* aqueous extract (mm) 500 (g L^{-1})	250 (g L^{-1})	125 (g L^{-1})	Gentamicin sulphate (10 mg L^{-1})
S. aureus ATCC 25923	+	15.5±0.6	nz	nz	20.3±1.35
S. epidermidis ATCC 12228	+	17.4±0.3	13.4±0.6	nz	20.1±0.42
M. luteus ATCC 9341	+	nz	nz	nz	16.9±0.67
B. subtilis ATCC 6633	+	nz	nz	nz	18.1±0.14
L. plantarum ATCC 14917	+	nz	nz	nz	20.7±0.32
E. coli ATCC 25922	-	18.2±0.3	14.3±0.6	nz	21.5±0.43
K. pneumoniae ATCC 10031	-	13.9±0.9	nz	nz	19.4±0.66
S. typhimurium ATCC 14028	-	nz	nz	nz	18.9±0.58
Ps. aeruginosa ATCC 9721	-	nz	nz	nz	19.9±0.63
P. vulgaris ATCC13315	-	nz	nz	nz	19.8±0.52

Data are Mean±SD (n = 3); nz: No inhibition zone

Table 2: The MICs and MBCs of *C. longa* root aqueous extract against various bacteria

Bacterias	*C. longa* aqueous extract (g L^{-1}) MIC	MBC	Gentamicin sulphate (mg L^{-1}) MIC	MBC
S. aureus ATCC 25923	6	32	<0.5	nd
S. epidermidis ATCC 12228	4	16	<0.5	nd
E. coli ATCC 25922	4	16	<0.5	nd
K. pneumoniae ATCC 10031	16	32	<0.5	nd

nd: Not determine

skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhoea and colic inflammatory disorders (Villegas *et al*., 2008). Curcumin is a major constituent of *C. longa* rhizomes. It has been reported about antimicrobial activity of *C. longa* against various bacteria such as *Pseumonas aeruginosa* (Rudrappa and Bais, 2008; Nagi *et al*., 1999), *Aeromonas hydrophila* (Harikrishnan and Balasundaram, 2008), *Helicobacer pylori* (Zaidi *et al*., 2009), *Escherichia coli* O157:H7 (Gupta and Ravishankar, 2005), *Listeria monocytogenes* and *Salmonella typhimurium* DT104 and methicillin-resistant *Staphylococcus aureus* (Kim *et al*., 2005).

In this study, the aqueous extract of root part of *C. longa* was evaluated. The results revealed that the aqueous extract of root part of *C. longer* showed inhibitory against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Krebsilla pneumoniae* ATCC and *Staphylococcus epidermidis* ATCC, respectively (Table 1). The MICs of aqueous extract of root part of *C. longer* were in the range of 4-16 g L^{-1}, while the MBCs were in the range of 16-32 g L^{-1} as shown in Table 2. The results give additional information for antimicrobial activity of *C. longa*. In comparison *C. longa* root aqueous extract exhibited the inhibitory effect against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *K. pneumoniae* ATCC 10031. The result did not showed inhibitory effect *Ps. aeruginosa* compared with previous reported by Rudrappa and Bais (2008) and Negi *et al*. (1999) maybe because of curcumin showed weak inhibitory effect against *Ps aeruginosa* and the inconsistency of natural abundance of curcumin in each season. However, in this study did not supply confirmation data for *L. monocytogenes*, *H. pylori* and *A. hydrophila*.

In conclusion, the aqueous extract of root part of *C. longa* showed good inhibitory effect on 4 out of 10 pathogenic bacteria which tested in this study.

DISCUSSION

As traditional medicine *C. longa* or turmeric has been extensively used for centuries to treat a diversity of disorders including antimicrobial (Rudrappa and Bais, 2008), antidiabetic

(Mohamed *et al*., 2009) rheumatism, bodyache, skin diseases, intestinal worms, diarrhea, intermittent fever, hepatic disorders, biliousness, urinary discharges, dyspepsia, inflammations, constipation, leukoderma, amenorrhoea, colic inflammatory disorders, colorectal cancer (Villegas *et al*., 2008) and diabetes (Mohamed *et al*., 2009).

The present study investigated the antibacterial activity of *C. longa* aqueous extract against various bacteria. The results indicate that the *C. longa* aqueous extract showed antimicrobial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Krebsilla pneumoniae* ATCC 10031 and *Staphylococcus epidermidis* ATCC 12228 at low concentration. Previous study show antimicrobial activity of *C. longa* in both essential oil which is non polar and aqueous extract which is polar compound. It has been reported the chemical composition of essential oil from *C. longa* consist of ar-turmerone (Martins *et al*., 2001) while aqueous extract mainly consist of curcumin (Rudrappa and Bais, 2008).

The antimicrobial activity of *C. longa* has been reported in previous study. It was found that the *C. longa* have inhibitory effect against *Pseumonas aeruginosa* (Rudrappa and Bais, 2008; Negi *et al*., 1999), *Aeromonas hydrophila* (Harikrishnan and Balasundaram, 2008), *Helicobacer pylori* (Zaidi *et al*., 2009), *Escherichia coli* O157:H7 (Gupta and Ravishankar, 2005), *Listeria monocytogenes* and *Salmonella typhimurium* DT104 and methicillin-resistant *Staphylococcus aureus* (Kim *et al*., 2005). In this study, the results showed antimicrobial activity of *C. longa* aqueous extract against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC25923, *Krebsilla pneumoniae* ATCC 10031 and *Staphylococcus epidermidis* ATCC 12228. The result from this study may supported the antimicrobial activity and somehow the confirmation of antimicrobial activity of *C. longa*. Moreover, it may support the use of *C. longa* for antimicrobial treatment disease or prevention of bacteria growth.

ACKNOWLEDGMENT

This study was partially financial supported from Faculty of Science, Mahasarakham University, Thailand.

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